CLINICAL GUIDELINES

Lab Management Program

Effective November 1, 2024

VERSION 2.0.2024

EviCore healthcare Clinical Decision Support Tool Diagnostic Strategies: This tool addresses common symptoms and symptom complexes. Requests for individuals with atypical symptoms or clinical presentations that are not specifically addressed will require physician review. Consultation with the referring physician, specialist and/or individual's Primary Care Physician (PCP) may provide additional insight.

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Clinical Use Guidelines

Confirmatory Genetic Testing

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Description

The Centers for Medicare and Medicaid Services (CMS) developed the Clinical Laboratory Amendments (CLIA) in order to help regulate laboratory tests. CMS intended to use this program as a way to ensure that quality laboratory testing was performed. Laboratories that receive reimbursement from Medicare or Medicaid must be CLIA certified.[1]

Most genetic or genomic tests are performed in a CLIA certified laboratory and used for a clear medical purpose. However, some genetic or genomic tests are performed in a research laboratory that is not CLIA certified or as part of a direct to consumer test that is not necessarily performed for a medical purpose.

When genetic testing is performed in a research laboratory or in a laboratory that is not CLIA certified, it is important to confirm any genetic change found prior to using this information to change an individual's medical treatment.

Criteria

Confirmatory single site genetic testing in a CLIA certified laboratory is medically necessary when the following criteria are met:

- A disease-causing genetic mutation was identified by a laboratory that is not CLIA certified (e.g. research lab), AND
- Healthcare providers can use the test results to directly impact medical care for the individual (e.g. change in surveillance or treatment plan)

Exclusions

- Confirmatory genetic testing is not considered medically necessary if the original testing was performed in a CLIA certified laboratory.
- Confirmatory genetic testing is not considered medically necessary if healthcare providers cannot use the test results to directly impact medical care for the individual.
- Confirmatory genetic testing is not considered medically necessary for variants of unknown significance (VUS).
- Tests that are considered not medically necessary (e.g., APOE for Alzheimer's risk assessment) or experimental, investigational, or unproven (e.g., MTHFR) per eviCore clinical guidelines are not eligible for confirmatory testing.

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References

1. Clinical Laboratory Improvement Amendments (CLIA). CMS.gov website. Available at: https://www.cms.gov/regulations-and-guidance/legislation/clia

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Genetic Presymptomatic and Predictive Testing for Adult-Onset Conditions in Minors

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Introduction

Genetic presymptomatic and predictive testing of minors for adult onset conditions is addressed by this guideline.

Description

Inherited disorders display a range of symptom onset, from congenital to adult. Some adult onset conditions have surveillance or medical intervention recommendations that are initiated in childhood, while for others there is no change in medical management. The National Society of Genetic Counselors (NSGC) states that individuals should be able to make the decision to have testing for themselves, after understanding and assessing the risks, benefits, and limitations of the test. In their 2018 position statement entitled “Genetic Testing of Minors for Adult-Onset Conditions,” NSGC “encourages deferring predictive genetic testing of minors for adult-onset conditions when results will not impact childhood medical management or significantly benefit the child.” [1]

According to the Genetics Home Reference, presymptomatic testing “can determine whether a person will develop a genetic disorder,” while predictive testing “can identify mutations that increase a person’s risk of developing disorders with a genetic basis.” [2] Predictive testing should be limited to disorders for which the genetic contribution is strong. Testing of minors for genetic variants that are not causative but confer susceptibility to disease is not medically necessary; and therefore, is not reimbursable.

Certain individual medical circumstances (such as consideration of a minor for organ/tissue donation or pregnancy in a minor with a family history of adult-onset disease) may present sufficient clinical utility to outweigh the criteria presented in this guideline. Such rare cases should be carefully considered on an individual basis.

Criteria

Introduction

Requests for genetic presymptomatic and predictive testing for adult-onset conditions in minors are reviewed using these criteria.

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Criteria: General Coverage Guidance

Predictive molecular testing of minors (members under the age of 18 years) for X-linked or autosomal dominant disorders is medically necessary when the following criteria have been met:

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous testing for the condition, and
  - A familial disease-causing mutation has been identified in a 1st or 2nd degree biological relative who is affected with an adult onset autosomal dominant or X-linked condition, AND
- Predictive Testing for Asymptomatic Individuals:
  - The minor is at risk for inheriting the familial disease-causing mutation, and
  - The condition may have onset in childhood, or
  - The condition has recommendations for surveillance that begin in childhood, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Note Testing of any minor who is symptomatic for a condition, regardless of typical circumstances of onset, is considered diagnostic testing and should be reviewed using *Genetic Testing to Diagnose Non-Cancer Conditions* or the appropriate test-specific guideline.

Limitations and Exclusions

Testing of minors for genetic variants that are not causative of inherited disease is not medically necessary; and therefore, is not reimbursable. Examples of mutations or variants that are not causative include:

- variants assessed by a testing laboratory to be of uncertain clinical significance
- variants that confer susceptibility for disease
- variants in genes of uncertain clinical significance.

Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests that may be requested for minors. For tests without a specific guideline, use the General Coverage Guidance above.

References

1. National Society of Genetic Counselors. Genetic testing of minors for adult-onset conditions. Adopted 2012; Updated: 2018. Available at: https://www.nsgc.org/Policy-Research-and-Publications/Position-Statements/Position-Statements/Post/genetic-testing-of-minors-for-adult-onset-conditions

2. What are the types of genetic tests? (Last Updated July 2021). In: MedlinePlus Genetics US National Library of Medicine (database online). Copyright, National Institutes of Health. 1993-2022. Available at: https://medlineplus.gov/genetics/understanding/testing/uses/

Genetic Testing by Multigene Panels

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Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Genomic Sequencing Procedures	81410-81471
Molecular Proprietary Laboratory Analyses (PLA)	Various Molecular* PLA codes (ending in U)
Tier 1 Molecular Pathology Procedures	81161-81383
Tier 2 Molecular Pathology Procedures	81400-81408
Unlisted Molecular Pathology Procedure	81479

What are multigene panels?

Definition

Various methodologies can be used to identify potential disease-causing gene mutations. Gene sequencing involves evaluating each DNA nucleotide along the length of a gene. Full gene sequencing is the best approach when many different mutations in the same gene can cause the disorder.

- There are two main ways to sequence a gene:
  - Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.[1]
  - Next generation sequencing (NGS), also called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence.[1]
- The efficiency of NGS has led to an increasing number of large, multigene testing panels.
  - NGS panels are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes.

  - Panels including genes associated with a high risk of a condition are of greatest value since these mutation-positive results often lead to changes in medical management.
  - Panels may also include genes believed to be associated with a particular condition, but with a more modest impact on risk. Results for such genes are of less clear value because there often are not clear management recommendation for mutation-positive individuals.

- Laboratories offer panel testing for multiple genes at the same time in an effort to increase the likelihood of finding a causative gene mutation in a more efficient manner. Such testing may be performed for diagnostic or predictive purposes.

  - Diagnostic testing is performed in patients with clinical signs or symptoms of a genetic condition. The genetic test may confirm or rule out a clinical diagnosis. However, many genetic conditions have overlapping features, which can make determining appropriate genetic testing difficult. The use of clinical and family history information may not always lead to a likely diagnosis for an individual. In some cases, many genes may be candidates for a person's symptoms. In these cases, testing one gene at a time may be time-consuming and costly.
  - Predictive genetic testing is performed in people known to be at increased risk of developing an inherited condition based on their family history. For some conditions, a positive genetic test predicts with certainty that the person will eventually develop signs and symptoms of a condition. For other conditions, a positive genetic test result indicates an increased risk (susceptibility) for a condition. Without a specific known mutation running in the family, a negative result rarely rules out a condition. Having test results may improve medical management through improved screening, preventive measures (e.g. prophylactic medication, surgery) and other means. In order to better define a person's risk, it is preferable to first test someone in the family who is affected.

Test information

- Multigene panel tests, even for similar clinical scenarios, vary considerably in the genes that are included and in technical specifications (e.g. depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis). Therefore, technologies used in multigene testing may fail to identify mutations that might be identifiable through single-gene testing.
- If high clinical suspicion remains for a particular syndrome after negative multigene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted.
- Results may be obtained that cannot be adequately interpreted based on the current knowledgebase. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a

variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.[2]

- Since genes can be easily added or removed from multigene tests over time by a given lab, medical records must document which genes were included in the specific multigene test used for each patient, and in which labs they were performed.
- Tests should be chosen to:
  - maximize the likelihood of identifying mutations in the genes of interest
  - contribute to alterations in patient management
  - minimize the chance of finding variants of uncertain significance.

Guidelines and evidence

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2021) revised technical standard for clinical NGS stated:[3]

- "Choosing an appropriate NGS-based test is the responsibility of the ordering health-care provider. Given the large number of tests (https://www.ncbi.nlm.nih.gov/gtr/) available to the clinician, the clinical laboratory often provides critical advice in test selection. Ordering providers must weigh considerations of sensitivity, specificity, cost, and turnaround time for each clinical situation."
- "Diagnostic gene panels are optimal for well-defined clinical presentations that are genetically heterogeneous (e.g., congenital hearing loss), for which pathogenic variants in disease-associated genes account for a significant fraction of cases. Secondary/ incidental findings should not be encountered, although broad panels (e.g., epilepsy, or pan-cancer panels) may identify clinically significant findings unrelated to the test indication. By limiting the test to those genes relevant to a given disease, the panel can be optimized to maximize coverage of relevant regions of the gene(s).[Bean et al. 2020]"
- "Test development must consider the variant types that will be detected in the genes or regions of the genome interrogated."

The ACMG (2020) technical standard on diagnostic gene panels stated:[4]

- "Gene panels developed by clinical molecular laboratories assess multiple potential genetic causes of a suspected disorder(s) simultaneously and reduce the cost and time of diagnostic testing. Gene panels are useful to diagnose disorders with genetic and clinical heterogeneity. Panels for phenotypically related disorders can increase the likelihood of identifying an underlying genetic cause and may be preferred to exome or genome sequencing to maximize target coverage and avoid secondary findings. [Klein et al, 2017; Bevilacqua et al, 2017]."

- "The goal of a diagnostic gene panel is to maximize clinical sensitivity and minimize the clinical burden from analysis of inappropriate or unnecessary genes that may result in variants of uncertain clinical significance (VUS)."
- "While it may be technically possible to sequence all genes related to a phenotype, the power of a gene panel is the ability to match a patient's specific clinical features to genes associated with that phenotype, thereby increasing clinical specificity and limiting the number of VUS."
- "While it is technically feasible to include genes with low-penetrance pathogenic variants on gene panels, the penetrance and the factors affecting penetrance are generally not known, thus limiting clinical utility. "

In an earlier Points to Consider document, ACMG (2012) offered general guidance on the clinical application of large-scale sequencing focusing primarily on whole exome and whole genome testing. However, some of the recommendations regarding counseling around unexpected results and variants of unknown significance and minimum requirements for reporting apply to many applications of NGS sequencing applications.[5]

Centers for Medicare and Medicaid Services

For laboratory procedures that include multiple molecular/genomic components the CMS National Correct Coding Initiative Policy Manual (CMS, updated 2022) provides the following coding guidance:[6]

- "If one laboratory procedure evaluates multiple genes using a next generation sequencing procedure, the laboratory shall report only one unit of service of one genomic sequencing procedure, molecular multianalyte assay, multianalyte assay with algorithmic analysis, or proprietary laboratory analysis CPT code. If no CPT code accurately describes the procedure performed, the laboratory may report CPT code 81479 (Unlisted molecular pathology procedure) with one unit of service or may report multiple individual CPT codes describing the component test results when medically reasonable and necessary. Procedures reported together must be both medically reasonable and necessary (e.g., sequencing of procedures) and ordered by the physician who is treating the beneficiary and using the results in the management of the beneficiary's specific medical problem."
- "All genomic sequencing procedures and molecular multianalyte assays (e.g., CPT codes 81410-81471), many multianalyte assays with algorithmic analyses (e.g., CPT codes 81490-81599, 0004M-XXXXM), and many Proprietary Laboratory Analyses (PLA) (e.g., CPT codes 0001U-XXXXU) are DNA or RNA analytic methods that simultaneously assay genes or genetic regions. A provider/supplier shall not additionally separately report testing for the same gene or genetic region by a different methodology (e.g., CPT codes 81105-81408, 81479, 88364-88377). CMS payment policy does not allow separate payment for multiple methods to test for the same analyte."

National Society of Genetic Counselors

The National Society of Genetic Counselors position statement on the use of multigene panels (NSGC, 2020) stated:[7]

- "The National Society of Genetic Counselors (NSGC) endorses the use of multi-gene panel tests when clinically warranted and appropriately applied. These tests can provide a comprehensive and efficient route to identifying the genetic causes of disease. Before ordering a multi-gene panel test, providers should thoroughly evaluate the analytic and clinical validity of the test, as well as its clinical utility. Additional factors to consider include, but are not limited to: clinical and family history information, gene content of the panel, limitations of the sequencing and informatics technologies, and variant interpretation and reporting practices."
- "Panels magnify the complexities of genetic testing and underscore the value of experts, such as genetic counselors, who can educate stakeholders about appropriate utilization of the technology to mitigate risks of patient harm and unnecessary costs to the healthcare system. NSGC supports straightforward and transparent pricing so that patients, providers, laboratories, and health plans can easily weigh the value of genetic testing in light of its cost."

Criteria

- This guideline applies to multigene panel testing, which is defined as any assay that simultaneously tests for more than one gene associated with a condition. The testing may focus on sequence variants and/or deletions/duplications of those genes. Panels vary in scope, such as:
  - Panels consisting of multiple genes that are associated with one specific genetic condition (e.g. Noonan syndrome, Stickler syndrome, etc.)
  - Panels consisting of multiple genes that are associated with a symptom or non-specific presentation (e.g. epilepsy, intellectual disability, hearing loss, retinal disorders, etc.)
- Coverage determinations generally rely on the medical necessity of the components of a panel. A panel approach to testing is most compelling when:
  - Multiple genes are known to cause the same condition and a limited subset of genes does not account for the majority of disease-causing mutations.
  - The clinical presentation is highly suspicious for a genetic disorder, but the constellation of findings in the personal or family history does not suggest a specific diagnosis or limited set of conditions.
- Multiple policies may apply, including test-specific policies where they exist or the following clinical use policies:
  - Genetic Testing to Diagnose Non-Cancer Conditions

  - Genetic Testing to Predict Disease Risk

- The following general principles apply:
  - Broad symptom-based panels (e.g. comprehensive ataxia panel) are not medically necessary when a narrower panel is available and more appropriate based on the clinical findings (e.g. autosomal dominant ataxia panel).
  - More than one multigene panel should not be necessary at the same time. Multigene panel testing should be performed in a tiered fashion with independent justification for each panel requested.
  - If more than ten units of any combination of procedure codes will be billed as part of a panel with no stated differential, the panel will be deemed excessive and not medically necessary.
  - Germline genetic testing is only medically necessary once per lifetime. Therefore, a single gene included in a panel or a multigene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- This guideline may not apply to multigene panel testing for indications that are addressed in test-specific guidelines.

Billing and reimbursement considerations

- All requested procedures must follow correct coding practices. Any procedure codes that do not meet these standards will not be reimbursable, even if medical necessity criteria for the associated test(s) are met. For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.
- If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- Panel coding and billing should reflect the efficiency gains for the laboratory in testing multiple candidate genes simultaneously. Currently, laboratories are billing for panels in a variety of ways. When a panel approach to testing is determined to be medically necessary, the following billing guidelines will apply:
  - If a panel is billed with multiple procedure codes representing individual genes analyzed, the panel will be redirected to an appropriate panel code. If the laboratory will not accept redirection to a single code, the medical necessity of each billed component procedure will be assessed independently. Only the individual panel components that meet medical necessity criteria as a first tier of testing will be reimbursed. The remaining individual components will not be reimbursable.

- Examples of appropriate panel codes include:
  - An appropriate proprietary laboratory analyses (PLA) code, or
  - An appropriate genomic sequencing procedure (GSP) code (if there are two different GSP codes to describe the sequencing and deletion/duplication analysis components of the test, both codes will be reimbursable as long as medical necessity is established for both methodologies), or
  - If no more specific code exists, the panel will be redirected to a single unit of the unlisted molecular pathology code 81479, which can be used to represent a panel in total.
- The billed amount should not exceed the list price of the test.

- If the member meets medical necessity, billing of the deletion/duplication portion of the panel with a microarray code (typically billed with 81228 or 81229) is allowed when at least 3 genes are included on the panel. Panels with less than 3 genes are more appropriately billed with individual CPT codes.

References

1. Roy S, LaFramboise WA, Nikiforov YE, et al. Next-generation sequencing informatics: challenges and strategies for implementation in a clinical environment. *Arch Pathol Lab Med*. 2016;140(9):958-975. doi: 10.5858/arpa.2015-0507-RA

2. Robson M. Multigene panel testing: planning the next generation of research studies in clinical cancer genetics. *J Clin Oncol*. 2014;32(19):1987-1989. doi: 10.1200/JCO.2014.56.0474

3. Rehder C, Bean LJH, Bick D, et al. Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(8):1399-1415. doi: 10.1038/s41436-021-01139-4

4. Bean LJH, Funke B, Carlston CM, et al. Diagnostic gene sequencing panels: from design to report—a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2020;22(3):453-461. doi: 10.1038/s41436-019-0666-z

5. ACMG Board of Directors. Points to consider in the clinical application of genomic sequencing. *Genet Med*. 2012 Aug;14(8):759-61. doi: 10.1038/gim.2012.74

6. CMS. NCCI Policy Manual for Medicare. Available at: https://www.cms.gov/medicare-medicaid-coordination/national-correct-coding-initiative-ncci/ncci-medicare/medicare-ncci-policy-manual

7. The National Society of Genetic Counselors. Position statement: Use of multi-gene panel tests. Released March 2017; Reaffirmed 2020. Available at: https://www.nsgc.org/Policy-Research-and-Publications/Position-Statements/Position-Statements/Post/use-of-multi-gene-panel-tests

Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes

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Description

Genetic testing for cancer susceptibility and hereditary cancer syndromes is performed in people with known risk factors for an inherited form of cancer. Testing may be used in people diagnosed with cancer when there are “red flags” in the individual’s personal medical and/or family history for a hereditary form. Predictive genetic testing may also be performed for this group of conditions, in people known to be at increased risk of developing an inherited condition based on their family history. This testing is generally limited to adult individuals; however, it may be considered for minors if the results will be of medical and/or psychosocial benefit.[1-3] A positive genetic test result increases the risk for cancer (types vary by the gene involved) and, therefore, impacts medical management decisions around screening, prevention, and treatment.

- For information on tests used to screen for or make a diagnosis of cancer, please refer to the guideline *Genetic Testing for the Screening, Diagnosis, and Monitoring of Cancer,* as this testing is not addressed here.
- For information on diagnostic or predictive testing for conditions other than hereditary cancer, please refer to the guideline *Genetic Testing to Diagnose Non-Cancer Conditions and Genetic Testing to Predict Disease Risk,* as this testing is not addressed here.

Criteria

Introduction

Genetic testing for cancer susceptibility and hereditary cancer syndromes are reviewed using the following criteria.

Criteria: General Coverage Guidance

Genetic testing for hereditary cancer syndromes is medically necessary when **ALL** of the following conditions are met:

- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.

- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the number of genes or tests necessary to establish carrier status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Genetic testing is medically necessary once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

Criteria: Special Circumstances

The following policies address a group of tests that are used for similar purposes. Because a variety of tests may be used, but the circumstances that justify testing are the same, individual test-specific policies are not necessary.

Predictive testing for at-risk people with known familial mutations

The genetic mutation(s) associated with a hereditary cancer syndrome can often be defined in an affected family member, allowing for testing of at-risk relatives for those specific mutations. Testing for known familial mutations is medically necessary when **ALL** of the following conditions are met:

- The mutation(s) in the family have been **clearly defined** by previous genetic testing and **information about those mutations can be provided** to the testing lab.
- **Technical and clinical validity**: The test must be accurate, sensitive and specific to the familial mutation(s).
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the known familial mutations when clinically possible.
- Predictive genetic testing is reimbursable once per lifetime per condition.
- Predictive genetic testing will be considered only for adult individuals (age 18 and over). Exceptions may be considered if there are medical management and/or significant psychosocial benefits to testing prior to adulthood.

Criteria: Test-specific Guidelines

Test-specific guidelines are available for some hereditary cancer syndrome tests. For tests without a specific guideline, use the General Coverage Guidance above.

References

Introduction

This guideline cites the following references.

1. Ross LF, Saal HM, David KL, Anderson RR. Technical report: ethical and policy issues in genetic testing and screening of children. *Genet Med.* 2013;15:234–245. Available at: http://www.nature.com/gim/journal/v15/n3/pdf/gim2012176a.pdf

2. National Society of Genetic Counselors Position Statement. Genetic testing of minors for adult-onset conditions. Adopted 2012. Updated April 2018. Available at: https://www.nsgc.org/Policy-Research-and-Publications/Position-Statements/Position-Statements/Post/genetic-testing-of-minors-for-adult-onset-conditions

3. Botkin, JR, Belmont JW, Berg JS, et al. Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. *Am J Hum Genet*. 2015;97:6-21.

Genetic Testing for Carrier Status

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Introduction

Carrier screening is performed to identify genetic risks that could impact reproductive decision-making for parents or prospective parents. Carriers are generally not affected but have an increased risk to have a child with a genetic condition.

Availability of genetic testing for carrier status

Carrier screening may be available for autosomal recessive conditions, X-linked conditions, and certain chromosome abnormalities. Ideally, carrier screening is performed prior to pregnancy so that a full range of reproductive options are available to an at-risk couple. However, in practice, it is often performed early in pregnancy when prenatal care is established.

Other applications of carrier testing

For information on prenatal screening and diagnostic testing, please refer to the guideline *Genetic Testing for Prenatal Screening and Diagnostic Testing,* as this testing is not addressed here.

For information on preimplantation genetic screening, please refer to the guideline *Preimplantation Genetic Screening and Diagnosis,* as this testing is not addressed here.

This guideline does not include testing that may identify carriers who have clinical signs and symptoms, such as cystic fibrosis testing for men with congenital absence of the vas deferens or fragile X genetic testing for women with premature ovarian failure. For information on this, please refer to the test specific guideline or *Genetic Testing to Diagnose Non-Cancer Conditions.*

Criteria

Introduction

Requests for carrier screening are reviewed using these criteria.

Criteria for general coverage guidance

Genetic testing for carrier screening is medically necessary when ALL of the following conditions are met:

- **Technical and clinical validity** — The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.

- **Clinical utility** — Healthcare providers can use the test results to provide significantly better medical care and/or assist individuals with reproductive planning.
- **Reasonable use** — The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits

- Testing will only be considered for the number of genes or tests necessary to establish carrier status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Carrier testing will be allowed once per lifetime. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- Carrier testing is indicated only in adults. Carrier screening in minor children is not indicated, except in the case of a pregnancy of the minor child.

Routine carrier screening

Individuals may be considered for routine carrier screening when testing is supported by evidence-based guidelines from governmental organizations and/or well-recognized professional societies in the United States.[1,2,3]

Carrier screening based on family history

Individuals may be considered for carrier screening based on a family history of a genetic condition when ALL of the following conditions are met in addition to the general criteria above:

- The diagnosis of a genetic condition in a family member is known.
- The parent(s) or prospective parent(s) are at-risk to be carriers of that condition based on the pattern of inheritance.
- The genetic condition is associated with potentially severe disability or has a lethal natural history.

Partner testing of known carrier or affected individuals

Individuals may be considered for carrier screening if their partners are known carrier or affected individuals when all of the following conditions are met in addition to the general criteria above:

- The diagnosis of a genetic condition or carrier status in the partner is known.
- The genetic condition is associated with potentially severe disability or has a lethal natural history.

Test-specific guidelines

Test-specific guidelines are available for some tests designed to predict carrier status. For tests without a specific guideline, use the General Coverage Guidance above.

References

Introduction

This guideline cites the following references.

1. ACOG Committee Opinion 690: Carrier screening in the age of genomic medicine. March 2017, reaffirmed 2023. Available at: https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/03/carrier-screening-in-the-age-of-genomic-medicine

2. ACOG Committee Opinion 691: Carrier screening for genetic conditions. March 2017, reaffirmed 2023. Available at: https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/03/carrier-screening-for-genetic-conditions

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Genetic Testing for Known Familial Mutations

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Introduction

Genetic Testing for Known Familial Mutations is addressed by this guideline.

Description

When genetic testing reveals the cause of an inherited disease in an affected family member, the genetic change is called a 'known familial mutation' (KFM). Relatives of the affected individual should generally have genetic testing that targets this disease-causing KFM rather than full sequencing of a gene or a multi-gene panel.

KFM testing is less expensive, less complex, and avoids finding variants of uncertain clinical significance (VUS) that have unclear medical management implications.

Presymptomatic or diagnostic testing for known familial mutations should only be offered when the variant is considered disease-causing, or classified as pathogenic or likely pathogenic per American College of Medical Genetics and Genomics (ACMG) variant classification guidelines.[1]

If there is a KFM in the family, testing for this mutation should be performed prior to any other genetic testing for the disease in an individual.[2,3]

Criteria

Introduction

Requests for genetic testing for Known Familial Mutations (KFM) are reviewed using the following criteria.

Criteria: General Coverage Guidance

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No previous genetic testing of the requested gene that would have included the KFM, AND
- Member is a 1st, 2nd, or 3rd degree biological relative of the family member with the KFM, AND
- KFM is disease-causing (classified as pathogenic or likely pathogenic), AND
- Diagnostic Testing in Symptomatic Individuals:

  - Member exhibits symptoms consistent with the disease caused by the KFM, OR
- Presymptomatic or Predictive Testing in Asymptomatic Adults:
  - Member is 18 years of age or older, AND
- Healthcare providers can use the test results to provide significantly better medical care for the individual, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Limits:

- Diagnostic or presymptomatic/predictive KFM testing will be allowed once per lifetime per condition.

Note For medical necessity criteria for presymptomatic/predictive testing of a known familial mutation in individuals younger than 18 years, see the guideline: *Genetic Presymptomatic and Predictive Testing for Adult-Onset Conditions in Minors*.

Billing and Reimbursement Considerations

- Once the mutation(s) that cause disease in the family have been identified, KFM testing is generally the only testing needed for that particular gene. As a result, if broad gene testing (for example, full gene sequencing or deletion/duplication analysis) is requested and a KFM has been identified in a family member, testing will be redirected to KFM testing.
- In rare circumstances, additional gene testing may be indicated following KFM testing, which will be assessed on a case-by-case basis.
- CPT codes specific for KFM testing (generally including language such as “known familial variant” in the code description) may not be used to bill for any other types of testing. There must be a documented KFM in the family. For example, the use of a KFM CPT code when billing part of a panel of genes, which is generally used as the initial step in identifying a disease-causing mutation in an individual, is not a correct use of these codes and is therefore not eligible for reimbursement.

Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests designed to assess known familial mutations. For tests without a specific guideline, use the General Coverage Guidance above.

References

Introduction

This guideline cites the following references.

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015; 17(5):405-24. doi: 10.1038/gim.2015.30.

2. U.S. Preventative Services Task Force. Final recommendation Statement BRCA-Related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing. August 20, 2019. Available at: https://www.uspreventiveservicestaskforce.org/uspstf/document/RecommendationStatementFinal/brca-related-cancer-risk-assessment-genetic-counseling-and-genetic-testing

3. NCCN Clinical Practice Guidelines: Genetic/Familial High-Risk Assessment: Colorectal. Version 2.2022. Available at: https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf.

Genetic Testing for Non-Medical Purposes

MOL.CU.111.A
v2.0.2024

Description

While most traditional genetic tests are used for clear medical purposes, advances in gene discovery and genetic testing technology allow laboratories to offer genetic testing for other uses. Testing for paternity, ancestry, and non-disease traits such as baldness and eye color may be highly accurate and interesting. However, because these kinds of tests are not useful for medical management in the vast majority of cases, they are typically excluded from consideration.

Non-medical tests are usually offered as direct-to-consumer products and do not require a clinical evaluation or order from a healthcare provider. Common providers of such tests may include:

- 23andMe
- Ancestry.com
- everlywell
- Invitae

Criteria

Criteria: General Coverage Guidance

Any genetic test that **DOES NOT** meet the following criteria is excluded from consideration:

- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Criteria:

The following types of testing are not considered medically necessary and therefore, not eligible for reimbursement:

- Genome-wide association studies (GWAS): testing a large number of genetic variations spread across the whole genome for disease associations, generally done for information outside of a specific clinical need or context
- Paternity testing: testing to establish biological relationships, often between a father and child(ren) but sometimes to determine other kinds of relationships (siblings, grandparents, etc.)
- Ancestry testing: testing that helps people discover more about the genetic make-up of their ancestors, generally used by genealogists and those interested in family history
- Nutritional testing: for variations in metabolism pathways that may suggest vitamin or other nutritional supplements.
- Athletic ability or fitness: Testing to predict athletic performance types.
- Genetic testing related to dating services.

Genetic Testing for Prenatal Screening and Diagnostic Testing

MOL.CU.112.A
v2.0.2024

Description

Prenatal screening and diagnostic testing is performed during pregnancy to identify fetuses at increased risk for or affected with genetic conditions and birth defects. Screening with ultrasound and maternal serum markers is routinely offered. Prenatal diagnosis by chorionic villus sampling or amniocentesis for chromosome abnormalities is available to all women. However, it is usually offered specifically to those at higher risk because of maternal age, a positive screen result, abnormal ultrasound findings, or known risk of a genetic condition based on family history. Investigations for fetal infection and blood antigen incompatibility may also be performed in the prenatal period. Results of testing are used to guide reproductive decision-making, pregnancy management and anticipatory management of the infant at birth.

Note For information on prenatal or preconception carrier screening or preimplantation genetic testing, please refer to the guidelines *Genetic Testing for Carrier Status* and *Preimplantation Genetic Screening and Diagnosis,* as this testing is not addressed here.

For information on fetal blood antigen incompatibility, please refer to the guideline *Human Platelet and Red Blood Cell Antigen Genotyping*, as this testing is not addressed here.

Criteria

Criteria: General Coverage Guidance

Genetic testing for prenatal screening and diagnostic testing is medically necessary when **ALL** of the following conditions are met:

- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care and/or assist patients with reproductive planning.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will only be covered for the number of genes or tests necessary to establish a prenatal diagnosis. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Prenatal diagnostic testing is medically necessary once per pregnancy. Exceptions may be considered if ambiguous results require retesting for clarification.
- If prenatal samples are studied concurrently with a maternal DNA sample to rule out prenatal analytic errors due to maternal cell contamination, a single unit of CPT code 81265 is reimbursable.

Criteria: Special Prenatal Diagnosis Circumstances

Each of the following policies addresses a group of tests that are used for similar purposes in pregnancy. Because a variety of tests may be used, but the circumstances that justify testing are the same, individual test-specific policies are not necessary.

Prenatal diagnostic testing based on family history

Prenatal genetic testing, generally by amniocentesis or CVS, for the diagnosis of a genetic condition is medically necessary when the following conditions are met:

- The pregnancy is at an increased risk for a genetic disease because of ANY of the following:
  - At least one parent is known or suspected to be a carrier of a genetic condition based on the family history and/or previous carrier testing results; or
  - One or both parent(s) are affected with a genetic condition; or
  - A sibling is affected with a genetic condition; AND
- The genetic condition is associated with potentially severe disability or has a lethal natural history.

Fetal infectious disease testing

Genetic testing may be used for the diagnosis of an infectious disease (e.g., cytomegalovirus, toxoplasmosis, parvovirus B19, and varicella zoster) in a fetus according to current guidelines from the American College of Obstetricians and Gynecologists (ACOG).[1] Prenatal testing, generally by amniocentesis or CVS, is reasonable when ANY of the following conditions are met:

- Clinical signs and symptoms of a current infection in the mother; OR
- Serologic evidence of a current or recent infection in the mother (with or without clinical signs); OR
- Fetal abnormalities identified on ultrasound indicating an increased risk for a congenital infection

Criteria: Test-specific Guidelines

- Test-specific guidelines are available for some prenatal screening tests and diagnostic tests. For tests without a specific guideline, use the General Coverage Guidance above.

References

1. ACOG Practice Bulletin. Cytomegalovirus, Parvovirus B19, Varicella Zoster, and Toxoplasmosis in Pregnancy. Number 151, June 2015 (reaffirmed 2020). *Obstet Gynecol.* 2015;125(6):1510-1525.

Genetic Testing for the Screening, Diagnosis, and Monitoring of Cancer

MOL.CU.113.A
v2.0.2024

Description

Genetic testing for screening, diagnosis and monitoring of cancer refers to molecular diagnostic tests whose purposes include identifying the possible presence of cancer in asymptomatic, average risk individuals; confirming the absence or presence of cancer; and monitoring the absence or presence of cancer after a prior diagnosis and treatment.

Screening

The goal of cancer screening is to identify the possible presence of cancer before symptoms appear. Screening tests cannot diagnose cancer, but typically determine if there is an increased chance cancer is present, and triages individuals for more invasive, diagnostic testing. Most cancer screening does not include genetic testing, but instead relies on physical exam, radiological exams, or non-genetic laboratory tests. Advances in human genetics, however, have identified several molecular diagnostic tests that may provide clues for early cancer detection.

Diagnosis

When cancer is suspected because of an abnormal screening test or symptoms, blood tests for tumor markers or molecular testing on tissue samples can aid in confirming a diagnosis of cancer. These tests may contribute information to helping the clinician understand prognosis and treatment options.

Monitoring

During treatment, or after an apparently successful treatment, active monitoring is often recommended to identify if the cancer is responding to treatment or has returned or spread, before any symptoms appear. Monitoring may include increased surveillance or routine blood tests for tumor markers, and increasingly, molecular genetic tests.

- For information on tests used to determine hereditary cancer risk, please refer to the guideline *Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes*, as this testing is not addressed here.
- For information on drug response to cancer or testing to determine which therapies to use, please refer to the guideline *Pharmacogenomic Testing for Drug Toxicity and Response*, as this testing is not addressed here.

- For information on molecular tumor marker testing in solid tumors, please refer to the guideline *Somatic Mutation Testing–Solid Tumors* and *Liquid Biopsy Testing,* as this testing is not addressed here.
- For information on diagnostic or predictive testing for conditions other than non-inherited cancer, please refer to the guideline *Genetic Testing to Diagnose Non-Cancer Conditions* and *Genetic Testing to Predict Disease Risk*, as this testing is not addressed here.

Criteria

Criteria: General Coverage Guidance

Genetic testing for screening, diagnosing, or monitoring cancer is medically necessary when **ALL** of the following conditions are met:

- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the number of genes or tests necessary. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- For tests that look for changes in germline DNA (i.e., not tumor DNA or viral DNA), testing is medically necessary once per lifetime per gene. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests designed to screen for, diagnose, or monitor cancer. For tests without a specific guideline, use the General Coverage Guidance above.

Genetic Testing for Variants of Uncertain Clinical Significance

MOL.CU.292.A
v2.0.2024

Introduction

Genetic testing for variants of uncertain clinical significance is addressed by this guideline.

Description

Genetic testing of an affected individual by gene sequencing or multi-gene panel testing can reveal genetic variants that have an unknown effect. These variants of uncertain clinical significance (VUS) may or may not cause disease in the individual; there is simply not enough known at the time of the report to call the variant disease-causing or benign.[1]

The accumulation of sufficient data to reclassify a VUS may take many years and require identification of the variant in multiple individuals. Pathogenicity of a variant is determined by labs through assessing:

- Disease-specific or gene-specific mutation databases
- Large population variant frequency databases
- In silico prediction tools
- Multi-species conservation assessment
- Peer reviewed literature
- Functional studies
- Family assortment studies

Family studies may be offered by the laboratory at no charge to the family, as the result may assist the lab in future classification of the variant. Testing relatives for a VUS may not always lead to reclassification of a variant to either disease-causing or benign, but it can be helpful in certain clinical scenarios, potentially contributing evidence that it is more or less likely to be disease-causing.

Targeted VUS Testing

Testing the parents of an affected child who has a VUS may be helpful in determining the clinical significance of that variant in some situations. For instance, if the condition is dominant and the VUS is not inherited from either parent (de novo), it is more likely to be disease-causing. If it is inherited from a healthy parent, it may be more likely to be benign.

Similarly, for an autosomal recessive condition, one or both of two potential disease-causing variants in a child may be called VUS. Testing parents should confirm whether one of the variants was inherited from each parent, and therefore fits the recessive pattern of inheritance.

If a VUS is identified in apparent homozygosity (2 copies), testing parents should determine copy number. A VUS that is inherited in two copies, one from each parent, would be consistent with the expected pattern of inheritance for recessive disease. If the VUS is only inherited from one parent, other mechanisms for pathogenicity (such as gene deletion or uniparental disomy) should be investigated.

Simply testing a relative for a VUS will not determine if that variant is disease causing or benign. This is especially true for adult onset conditions (e.g.: hereditary cancer syndromes) or conditions for which there is reduced or non-penetrance or highly variable expressivity. After targeted testing for a VUS, careful clinical and family history evaluation and correlation with the result are essential.

Genes of Uncertain Clinical Significance

Broader tests, such as whole exome sequencing or whole genome sequencing, may identify variants in genes that have an unknown effect. That is, for a gene of uncertain clinical significance (GUS) there is not enough known about the gene and its function to say whether it can cause the disease in question.[1]

Potential Outcomes of Targeted VUS testing

Results of testing and possible significance of testing.

Result of VUS testing	Possible significance
VUS is not inherited (de novo)	Increased likelihood of causing disease
VUS is inherited from affected parent	Increased likelihood of causing disease
VUS is inherited from unaffected parent	Decreased likelihood of causing disease
VUS is inherited with a disease-causing variant or VUS from the same parent	Decreased likelihood of causing disease
VUS that is apparently homozygous is not inherited from both parents	Alternate mechanisms should be investigated

Criteria

Introduction

Requests for genetic testing for variants of uncertain clinical significance are reviewed using these criteria.

Criteria: General Coverage Guidance

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No previous genetic testing of the requested gene, AND
- No known alternate genetic cause for the diagnosis in the family, AND
- Member is the biological parent of a child in whom a VUS was identified, AND
- VUS is in a gene that is
  - Known to be disease-associated, and
  - Consistent with the child's clinical diagnosis, AND
- Purpose of testing is to determine
  - Whether the VUS is inherited or de novo, or
  - Whether the VUS is present in homozygosity, AND
- Determination of the inheritance or copy number of the VUS will lead to treatment changes for the member or the member's child, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Limitations and Exclusions

- Testing of multiple affected and unaffected relatives to determine if a VUS assorts with symptoms in the family is not considered medically necessary; therefore, it is not reimbursable.
- Testing for variants in genes of uncertain clinical significance (GUS) is not considered medically necessary; therefore, it is not reimbursable.
- Each test request for VUS testing should be reviewed based on the medical information available for the member and the clinical utility and technical and clinical validity of the service requested.

Criteria: Test-specific Guidelines

Test-specific guidelines may be available for tests that could target a VUS. For tests without a specific guideline, use the General Coverage Guidance above.

References

Introduction

This guideline cites the following references.

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015; 17(5):405-24. doi: 10.1038/gim.2015.30.

Genetic Testing to Diagnose Non-Cancer Conditions

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v2.0.2024

Description

Diagnostic testing is performed in patients with clinical signs or symptoms of a non-cancer genetic condition. The genetic test may confirm or rule out a clinical diagnosis. In some cases, genetic testing is the gold standard for making a diagnosis based on evidence- or consensus-based guidelines. In others, it may be used to confirm a clinical diagnosis, offer prognostic information that impacts management, rule out a diagnosis in the differential, or confirm a positive newborn screening result. Often, diagnostic testing of an affected individual will offer results that are relevant to the testing of other family members.

- This guideline does not include risk assessment or predictive testing for at-risk, asymptomatic individuals. Please refer to *Genetic Testing to Predict Disease Risk* for that purpose.
- Diagnostic testing of a pregnancy or an embryo is addressed by guidelines on *Genetic Testing for Prenatal Screening and Diagnostic Testing* and *Preimplantation Genetic Screening and Diagnosis*, respectively.
- In addition, testing for hereditary cancer syndromes is addressed separately under *Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes*.

Criteria

Criteria: General Coverage Guidance

Diagnostic genetic testing is medically necessary when **ALL** of the following conditions are met:

- **Clinical signs and symptoms** in the individual are consistent with the diagnosis in question.
- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

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- Testing will be considered only for the number of genes or tests necessary to establish mutation status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Diagnostic genetic testing is medically necessary once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

Criteria: Special Circumstances

Diagnostic testing of an individual to inform reproductive planning and testing for parents or testing for siblings

Diagnostic genetic testing may be requested in a symptomatic individual with a known genetic condition. While diagnostic testing may not impact management of the affected individual, the information gained from genetic testing may be needed to perform accurate carrier testing in the parent(s), genetic diagnosis in a pregnancy, or genetic diagnosis in a sibling.*

In these circumstances, diagnostic genetic testing in a symptomatic individual is medically necessary when **ALL** of the following conditions are met:

- The diagnosis of the disease in the affected individual is **certain or highly probable** based on clinical signs and symptoms, history, imaging, and/or results of other laboratory testing.
- The results of the genetic test in the symptomatic individual must be **required** in order to perform accurate carrier testing in the parent(s), genetic diagnosis in a pregnancy, or genetic diagnosis in a sibling.
- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility**: Healthcare providers can use the test results to provide informative genetic testing for the sibling, parents, or for a current or future at-risk pregnancy.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be indicated only for the number of genes or tests necessary to establish the familial mutation(s). A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Diagnostic genetic testing is medically necessary once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

*Parent or sibling must also be a covered member under the same health plan.

Diagnostic testing of an individual to confirm newborn screening results

Newborn Screening (NBS) is state-mandated testing performed in the first days of life, using blood spots obtained from a heel stick. Biochemical studies are used, and often supplemented with molecular analysis, in order to screen for a number of different disorders. The goal of NBS is to identify affected infants before they become symptomatic, since these disorders may cause significant morbidity or mortality unless treatment is initiated in the neonatal period. Diagnostic genetic testing may be requested for infants with positive, borderline, or inconclusive results. The American College of Medical Genetics and Genomics (ACMG) ACT Algorithms contain an overview of the steps involved in determining a final diagnosis, and can be found here.

Diagnostic genetic testing in an individual for the purposes of confirming newborn screening results is medically necessary when the following conditions are met:

- The individual has had a newborn screening result that is positive, borderline, or inconclusive for a specific disorder for which confirmatory genetic testing is required, AND
- The requested testing has not been previously performed, AND
- The member will benefit from information provided by the requested gene testing based on at least one of the following:
  - All criteria are met from a test-specific guideline, if one is available, or
  - The ACMG ACT Algorithm associated with the suspected disorder includes genetic testing, and all preliminary studies recommended in the algorithm have been completed (however, the genetic test must not simply be listed as "optional", or as an intervention that may be considered), or
  - There is uncertainty in the diagnosis, despite further evaluation by an appropriate provider, and genetic testing is needed to clarify the diagnosis, or
  - An individual has a confirmed biochemical diagnosis of the disorder for which testing is requested, but healthcare providers can use the genetic test results to directly impact medical care for the individual (e.g. change in surveillance or treatment plan).

Limits:

- Testing will be indicated only for the number of genes or tests necessary to establish the diagnosis. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Diagnostic genetic testing is medically necessary once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests designed to diagnosis non-cancer conditions. For tests without a specific guideline, use the General Coverage Guidance above.

Genetic Testing to Predict Disease Risk

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Description

Predictive genetic testing is performed in people known to be at increased risk of developing an inherited non-cancer condition (for the purposes of this guideline) based on their family history. For some conditions, a positive genetic test predicts with certainty that the person will eventually develop signs and symptoms of a condition. For other conditions, a positive genetic test result indicates an increased risk (susceptibility) for a condition. A negative result may rule out a condition, or lower the risk significantly. Having test results may improve medical management through improved screening, preventive measures, prophylactic medication, and other means.

- For information on testing a symptomatic individual, please refer to the guideline *Genetic Testing to Diagnose Non-Cancer Conditions*.
- For information on predictive testing for hereditary cancer syndromes, please refer to the guideline *Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes*.
- For information on testing minors, please refer to the guideline *Genetic Presymptomatic and Predictive Testing for Adult-Onset Conditions in Minors*.

Criteria

Criteria: General Coverage Guidance

Predictive genetic testing is medically necessary when **ALL** of the following conditions are met:

- The individual is **known to be at-risk** for developing inherited condition because a parent, sibling, or child is affected by or known to be a carrier of a genetic disease.
- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

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- Testing will be considered only for the number of genes or tests necessary to establish carrier status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Predictive genetic testing is medically necessary once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- Predictive testing will be considered only for adult individuals (age 18 and over). Exceptions may be considered if there are medical management and/or significant psychosocial benefits to testing prior to adulthood.[1,2,3]

Criteria: Special circumstances

Testing for Known Familial Mutations

The genetic mutation(s) associated with a genetic disease can often be defined in an affected family member, allowing for testing of at-risk relatives for those specific mutations. Testing for known familial mutations (KFM) is medically necessary when the following conditions are met:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No previous genetic testing of the requested gene that would have included the KFM, AND
- KFM is disease-causing (classified as pathogenic or likely pathogenic), AND
- Member is a 1st, 2nd, or 3rd degree biological relative of the family member with the KFM, AND
- Member is 18 years of age or older, AND
- Healthcare providers can use the test results to provide significantly better medical care for the individual, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Limits:

- Testing will be considered only for the known familial mutations when clinically possible.
- Predictive genetic testing is medically necessary once per lifetime per condition.
- Predictive testing will be considered only for adult individuals (age 18 and over). Exceptions may be considered if there are medical management and/or significant psychosocial benefits to testing prior to adulthood.[1,2,3]

Note For medical necessity criteria for predictive testing of a known familial mutation in individuals younger than 18 years, see the guideline: *Genetic Presymptomatic and Predictive Testing for Adult-Onset Conditions in Minors*.

Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests designed to predict disease risk. For tests without a specific guideline, use the General Coverage Guidance above.

References

1. Ross LF, Saal HM, David KL, Anderson RR. Technical report: ethical and policy issues in genetic testing and screening of children. *Genet Med* 2013;15: 234–245. doi: 10.1038/gim.2012.176

2. National Society of Genetic Counselors Position Statement. Genetic testing of minors for adult-onset conditions. Adopted 2012. Updated 2018. Available at: https://www.nsgc.org/Policy-Research-and-Publications/Position-Statements/Position-Statements/Post/genetic-testing-of-minors-for-adult-onset-conditions

3. Botkin, JR, Belmont JW, Berg JS, et al. Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. *Am J Hum Genet*. 2015;97:6-21. doi: 10.1016/j.ajhg.2015.05.022

Hereditary (Germline) Testing After Tumor (Somatic) Testing

MOL.CU.246.A
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Introduction

Germline hereditary cancer testing following somatic tumor testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
APC Deletion/Duplication Analysis	81203
APC Known Familial Variants	81202
APC Sequencing	81201
ATM Sequencing	81408
BRCA1 Deletion/Duplication Analysis	81166
BRCA1 Sequencing	81165
BRCA2 Deletion/Duplication Analysis	81167
BRCA2 Sequencing	81216
BRCA1/2 185delAG, 5385insC, 617delT variants	81212
BRCA1/2 Deletion/Duplication Analysis	81164
BRCA1/2 Known Familial Variants	81215
BRCA1/2 Sequencing	81163
Chromosomal Microarray [BAC], Constitutional	81228
Chromosomal Microarray [SNP], Constitutional	81229

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Procedures addressed by this guideline	Procedure codes
Cytogenomic (genome-wide) Analysis for Constitutional Chromosomal Abnormalities; interrogation of genomic regions for copy number and loss-of-heterozygosity variants, low-pass sequencing analysis	81349
Hereditary Breast Cancer-related Disorders (e.g., hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11	81433
Hereditary Breast Cancer-related Disorders (e.g., hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53	81432
Hereditary Cancer Syndrome Gene Tests	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479

Procedures addressed by this guideline	Procedure codes
Hereditary Colon Cancer Disorders (e.g., Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11	81436
Hereditary Colon Cancer Disorders (e.g., Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11	81435
Hereditary Neuroendocrine Tumor Disorders (e.g., medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); duplication/deletion analysis panel, must include analyses for SDHB, SDHC, SDHD, and VHL	81438
Hereditary Neuroendocrine Tumor Disorders (e.g., medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); genomic sequence analysis panel, must include sequencing of at least 6 genes, including MAX, SDHB, SDHC, SDHD, TMEM127, and VHL	81437
MLH1 Deletion/Duplication Analysis	81294
MLH1 Known Familial Variants	81293
MLH1 Sequencing	81292
MSH2 Deletion/Duplication Analysis	81297
MSH2 Sequencing	81295
MSH2 Known Familial Variants	81296
MSH6 Deletion/Duplication Analysis	81300

Procedures addressed by this guideline	Procedure codes
MSH6 Known Familial Variants	81299
MSH6 Sequencing	81298
PMS2 Deletion/Duplication Analysis	81319
PMS2 Known Familial Variants	81318
PMS2 Sequencing	81317
PTEN Deletion/Duplication Analysis	81323
PTEN Known Familial Variants	81322
PTEN Sequencing	81321

What is germline hereditary cancer testing following somatic tumor testing?

Definition

Most cancer is sporadic and due to the acquisition of somatic mutations (also known as variants). About 5-10% of cancer has a hereditary etiology due to constitutional germline mutations.[1]

- In oncology, next generation sequencing (NGS) technology makes it feasible to catalog the DNA sequence mutations within a person's cancer (i.e., somatic mutation profiling). This helps define therapeutic targets which might improve outcomes through the use of specific medications directed at those mutations.[2] These genomic mutations can also serve as biomarkers of an individual's prognosis and aid in diagnosis.[3,4]
- Germline mutations can also be identified as an ancillary finding during primary tumor profiling to identify somatic mutations. "In the course of analyzing tumor DNA (without matched normal DNA), sequencing can identify potential constitutional (germline) DNA variations that are associated with disease or susceptibility to disease as well as carrier states for Mendelian disorders.[4] Centers may use matched tumor-normal sequencing to facilitate more accurate calling of somatic mutations by using the normal DNA to exclude germline variants from the tumor cells." [3,4]
    - In a study by Schrader et al, "Targeted tumor sequencing with a panel of 341 genes and matched normal DNA in 1566 individuals with advanced malignant neoplasms revealed presumed pathogenic germline variants (PPGVs) in about 16% of individuals. Most PPGVs (80.5%, 95% CI, 75.1%-85.0%) were in genes related to cancer susceptibility. The PPGVs in genes previously designated as clinically actionable cancer targets were seen in 5.0% (95% CI, 4.1%-6.2%) of individuals. Most cancer-susceptibility PPGVs were retained in the tumor

(91.9%; 95% CI, 87.3%-95.0%).[5] This study is in line with other published studies investigating the prevalence of incidental findings with somatic tumor profiling." [5-7]

- The debate continues regarding whether there is an obligation to test for and report these germline findings, which are secondary to the original purpose of somatic tumor profiling. In making this determination, pre-test informed consent is of utmost importance. "Honoring patient preferences requires oncology providers to communicate the potential for incidental and secondary germline information specific to the test being offered, the relevance and potential benefits of this information for patients and their relatives, and the limitations and risks of receiving incidental and secondary germline information" [2]

Test information

Introduction

Mutations detected on somatic testing may be indicative of a hereditary cancer syndrome due to a germline mutation. Thus, germline hereditary cancer testing following somatic tumor testing may be indicated in certain situations.

- Testing to investigate somatic and germline DNA mutations has become more common as sequencing technology has evolved from the more labor intensive Sanger sequencing to next generation sequencing (NGS). "NGS is a powerful technology that permits the characterization of large amounts of DNA sequence much quicker and at lower cost than traditional Sanger sequencing." [2]
- Laboratories performing somatic mutation profiling may include paired germline testing, not in an effort to identify hereditary etiologies, but to report pure somatic alterations, clarify interpretation, and identify mutations that are genetic "drivers" of the individual's malignancy.[4,5,8]
- Laboratories may also use bioinformatics to subtract the inherited mutations from the somatic tumor profiling findings. Germline mutations may be missed during this process without performing further analysis.[8-11]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to germline hereditary cancer testing following somatic tumor testing.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2020) stated the following regarding germline mutations in individuals undergoing somatic tumor testing:[12]

- “Individuals undergoing tumor testing should undergo informed consent of the possibility that a PGPV [presumed germline pathogenic variant] might be discovered. However, if there is clinical indicator for germline cancer predisposition, then dedicated germline testing should be ordered."
- “Patient choice and autonomy (opt-out of PGPV result return) should be respected."
- “When automated methods are used for pre- and post-testing education and counseling, clinicians with experience in cancer genetics should be available to answer specific questions."
- “Patients should be informed that discovery of a PGPV would prompt referral for genetic consultation and the possibility of confirmatory germline testing."
- “Confirmatory germline testing should be performed in a clinical laboratory that has adequate resources and expertise in conducting germline testing and interpreting and reporting the test results."
- “Positive germline test results should be returned by qualified and experienced clinicians (e.g., oncologists with genetics expertise, geneticists, and genetic counselors)."

European Society of Medical Oncology

The European Society for Medical Oncology (ESMO, 2019) published recommendations for germline analysis of tumor-only sequencing data.[13] Factors considered include the gene, tumor type, the age of the affected individual, and VAF to determine if germline testing is recommended. These guidelines were recently updated (ESMO, 2023) and stated:[14]

- "We analysed an expanded dataset including 49 264 paired tumour-normal samples. We applied filters to tumour-detected variants based on variant allele frequency, predicted pathogenicity and population variant frequency. For 58 cancer-susceptibility genes, we then examined the proportion of filtered tumour-detected variants of true germline origin [germline conversion rate (GCR)]. We conducted subanalyses based on the age of cancer diagnosis, specific tumour types and 'on-tumour' status (established tumour-gene association)."
- Forty genes were identified for potential germline follow-up testing.
- Four different approaches were provided for germline follow-up of tumor-only sequencing results:
    - "Permissive: germline follow-up for all 40 genes in all tumour types

  - Intermediate-permissive: germline follow-up for all 23 MA-CSGs/HA-CSGs [most-actionability cancer-susceptibility gene/high-actionability cancer-susceptibility gene] in all tumour types but germline follow-up only in 'associated' tumour types for 17 SA-CSGs [standard-actionability cancer-susceptibility gene].
  - Intermediate-conservative: germline follow-up in all tumour types for the 7 MA-CSGs but germline follow-up only in 'associated' tumour types for the other 33 HA-CSGs/SA-CSGs.
  - Conservative: germline follow-up only in 'associated' tumour types for all 40 genes"

- "Strategic filtering improves the GCR with minimal loss of true germline variants present in the tumour."
- "GCR of filtered tumour-detected variants is very high (>80%) for genes such as BRCA1, BRCA2 and PALB2."
- "GCR of filtered tumour-detected variants is very low (<2%) for genes such as APC, TP53 and STK11."
- "Germline follow-up should involve multidisciplinary expertise and follow expert guidance regarding tumour context."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2024) stated the following regarding germline testing following somatic tumor testing:[15]

- "Tumor profiling can be considered complementary to germline testing. However, the absence of a P/LP [pathogenic/likely pathogenic] variant for a given gene from tumor profiling does not rule out the possibility of a germline P/LP variant in that gene… Therefore, a variant interpreted as P/LP in the germline may be interpreted as normal or as a VUS in the tumor, if that variant has no clear clinical implications. In addition, the sensitivity of most tumor testing is lower (particularly for intermediate-sized deletions and duplications) than that for most dedicated germline tests, sometimes due to filtering out of germline findings reported in tumor sequencing results."
- “If a mutation is detected through tumor profiling that has clinical implications if identified in the germline, then germline testing for this variant is indicated."
- "Somatic P/LP variants seen in tumor specimens are common in some genes with germline implications (eg, TP53, STK11, PTEN) and may not indicate the need for germline testing unless the clinical/family history is consistent with a P/LP variant in the germline."
- "If a patient meets testing criteria for germline testing for a given gene, then confirmatory germline testing should be considered through a CLIA-approved lab despite tumor profiling results."

The National Comprehensive Cancer Network (NCCN, 2023) stated the following regarding interpreting information obtained from tumor-only profiling:[16]

- “Pathogenic/likely pathogenic variants reported by laboratories providing tumor-only profiling may be of somatic or germline origin. Although germline origin can sometimes be inferred with a high degree of confidence, confirmatory germline testing is indicated for pathogenic/likely pathogenic variants with a reasonable clinical suspicion of being of germline origin (based on patient/family history or clinical characteristics, presence of a founder mutation, and in some cases variant allele frequency)."
- "Somatic pathogenic/likely pathogenic variants in several genes with germline implications are common (e.g., TP53, STK11, PTEN, APC), and will rarely be indicative of a need for germline testing unless clinical/family history features suggest the possibility of a germline pathogenic/likely pathogenic variant."
- “It should be noted that the absence of reported pathogenic/likely pathogenic variants in a particular gene based on tumor testing does not rule out the possibility of a germline pathogenic/likely pathogenic variant in that gene. Clinically indicated germline testing is still appropriate for patients meeting testing guidelines regardless of tumor profiling results."

National Society of Genetic Counselors

The National Society of Genetic Counselors (NSGC, 2022) provided a "Somatic Research Task Force Incidental Findings Worksheet" which gave guidance for making decisions regarding the indications for germline testing after somatic testing. This stated the following:[17]

- First, determine if the gene with the mutation has an associated germline risk. If not, no further testing is indicated based on the somatic results. If so, then determine if the testing performed on the tumor was tumor paired with a normal sample such as blood or saliva. If it was paired testing, then determine if the mutation is a founder mutation or if the mutation is present in a relative to determine if confirmatory germline testing is necessary. Additionally, following-up with the testing laboratory to determine their germline confirmation policy may be necessary.
- If the testing was on tumor only, the following was stated:
    - If the following apply, then the mutation is likely somatic and no further testing may be indicated based on the somatic results:
        - The variant allele frequency is less 30%
        - The gene mutation(s) is/are associated with the tumor type
        - There is a lacking phenotype consistent with the gene mutation
        - The individual's age of diagnosis is not consistent with the gene mutation

- If any of the following apply and the mutation is classified as pathogenic/likely pathogenic when present in the germline, then confirmatory genetic testing is appropriate:
  - The variant allele frequency is 30% or greater
  - The phenotype matches the gene mutation
  - The individual's age at diagnosis is consistent with the gene mutation
  - Of note, if a mutation is not found in databases to confirm pathogenicity, confirmatory testing may still be indicated.
- If the gene change is classified as a variant of uncertain significance when present in the germline, confirmatory germline testing is generally not indicated however could be considered if:
  - Germline testing may be of benefit to the individual/family in the future
  - The individual/family are eligible for family or follow-up studies
  - There is clinical suspicion about the gene change
- If the gene change is classified as benign/likely benign when present in the germline, no further testing in indicated based on the somatic results.

Additionally points noted were:

- "Consider multigene panel testing rather than targeted variant testing based on personal/family history of cancer AND/OR other NCCN criteria met for germline testing."
- "Germline testing may be necessary despite paired tumor-normal report. Some somatic testing labs are not validated for germline analysis."

Selected Relevant Publications

There have been various peer-reviewed publications that reviewed pre- and post-test considerations for germline testing following somatic tumor testing.

- Pre-test considerations:
  - Somatic tumor-only NGS testing is used to guide treatment for an affected person. The testing is not designed to elucidate a hereditary etiology. A germline variant may not be detected (due to differences in coverage in the testing, cellularity of the sample, allelic loss of the germline mutation) or may not be reported by the somatic testing laboratory.[2,3,18,19]
  - Directed germline genetic testing can be ordered to identify a potential hereditary etiology for the person's tumor. Referrals to oncology genetic counselors or other specialized healthcare providers should occur if the individual's personal and/or family history meets established criteria to warrant a more detailed discussion.[13,18,20]

- Ancillary findings from somatic or germline testing may include variants in genes that cause a hereditary cancer syndrome, a non-oncologic hereditary syndrome, or identify carrier status for Mendelian disease. Specific findings are dependent on specific testing performed by the laboratory.[2,3,10,11,18]
- Many individuals undergoing somatic tumor profiling have advanced stage disease. Centers performing somatic tumor profiling should consider obtaining a surrogate individual to receive results in the event that the proband has passed away or is otherwise unable to receive the results.[2,3,18]

- Post-test considerations:
  - Clinicians must determine the technical specifications of the laboratory used for somatic tumor profiling and determine if this includes paired germline testing. Some laboratories may not report germline variants, include certain known germline variants on a panel, or be able to detect certain types of variants (such as copy number variants) depending on the assay methodology used.[2,3,21]
  - Somatic variant interpretation differs from the variant interpretation and classification process for germline variants. For example, a laboratory profiling a somatic tumor may classify a certain variant as pathogenic whereas a laboratory testing a germline mutation may classify that same variant as a variant of uncertain significance (VUS), or vice versa.[2,3,21] Resources, such as ClinVar, should be used by the provider to determine if a pathogenic variant classification provided by germline testing laboratories is consistent with independent assessments of that variant.[22]
  - Referrals to oncology genetic counselors or other specialized healthcare providers should occur if the individual's personal and/or family history meets established criteria to warrant a more detailed discussion, regardless of somatic tumor profiling results.[10,16,18] In individuals meeting criteria for germline DNA testing, analysis of the entire gene, as opposed to single site testing for the identified somatic variant, is recommended.[6]
  - Germline testing may also be considered in individuals when any of the following apply:
    - The individual does not meet published criteria for germline testing, but variant(s) within genes known to play a role in tumor biology and to cause an inherited cancer syndrome (including but not limited to TP53, APC, CDH1) are identified and the variant allele frequency in the tumor is at least 30%.[17,23-25]
    - One of the identified variants on tumor testing is a highly-recurrent or founder mutation (i.e., BRCA1 c185delAG, the recurrent inversion of MSH2 seen in some families with Lynch syndrome, the p.R337H TP53 mutation).[3,26]
    - The tumor profile shows thousands of somatic variants, suggesting a germline mutation in a DNA mismatch repair gene or in the POLE proofreading domain.[3,27]

- Two separate primary tumors are sequenced and both harbor the same genetic variant.[9]
- The individual's tumor harbors a mutation in BRCA1 or BRCA2.[15]

Criteria

Introduction

Requests for germline hereditary cancer testing following somatic tumor testing are reviewed using these criteria.

- Requests for single-site or full-gene sequence germline testing following somatic tumor analysis will be considered medically necessary when at least one of the following criteria is met:
  - The individual's personal or family history is suggestive of a germline mutation, a specific germline variation is identified by somatic tumor testing, and the individual meets the published test-specific criteria to test for that variant, OR
  - One of the identified variants is a highly-recurrent or founder mutation (i.e., BRCA1 c185delAG or the recurrent inversion of MSH2 seen in some families with Lynch syndrome, the p.R337H TP53 mutation), OR
  - The tumor profile shows thousands of somatic variants, suggesting a germline mutation in a DNA mismatch repair gene or in the POLE proofreading domain, OR
  - Two separate primary tumors are sequenced and both harbor the same genetic variant, OR
  - The individual's tumor harbors a mutation in BRCA1/2, OR
  - The individual does not meet published criteria for germline testing, but variant(s) within genes known to play a role in tumor biology and to cause an inherited cancer syndrome (including but not limited to TP53, APC, CDH1) are identified and the variant allele frequency in the tumor is at least 30%.

Exclusions and Other Considerations

- Germline testing of somatic variants of uncertain significance (VUS) is not considered medically necessary.
- Germline testing for asymptomatic individuals based solely on a family member's somatic testing result is not considered medically necessary.
- In individuals meeting criteria for germline DNA testing, analysis of the entire gene, as opposed to single site testing for the identified somatic variant, is recommended.
- Clinically indicated germline testing is still appropriate for individuals meeting testing guidelines regardless of tumor profiling results.

- Resources, such as ClinVar, should be used by the provider to determine if a pathogenic variant classification provided by germline testing laboratories is consistent with independent assessments of that variant.

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Colorectal, V2.2023 – October 30, 2023. ©2023 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guideline® and illustrations herein may not be reproduced in any form for any purpose without the express written permission of the NCCN. To view the most recent and complete version of the NCCN Guideline®, go online to NCCN.org.

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Experimental, Investigational, or Unproven Laboratory Testing

MOL.CU.117.A
v2.0.2024

Introduction

Experimental, investigational, or unproven (E/I/U) molecular and genomic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures address by this guideline	Procedure codes
9p21 Genotype	81479
AlloSure Heart	81479
AlloSure Lung	81479
AmHPR Helicobacter pylori Antibiotic Resistance Next Generation Sequencing Panel	0008U
Apolipoprotein E Genotype (APOE)	81401
Apolipoprotein L1 (APOL1) Renal Risk Variant Genotyping	0355U
ARISk Autism Risk Assessment Test	81479
AssureMDx	81479
Augusta Hematology Optical Genome Mapping	0331U
Augusta Optical Genome Mapping	0260U
Avantect Pancreatic Cancer Test	0410U
BBDRisk Dx	0067U
Bladder EpiCheck	81599
BluePrint Molecular Subtyping Profile	81479
BTG Early Detection of Pancreatic Cancer	0405U
CARDIO inCode Score (CIC SCORE)	0401U
CardioRisk+	0466U

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Procedures address by this guideline	Procedure codes
CELLSEARCH CTC Test	86152, 86153
ChemoFX	81535 81536
Chromosome Genome Mapping	0454U
Clarava	0319U
Clarifi ASD	0170U
CNGnome	0209U
Cologuard Plus	0464U
ColonAiQ	0453U
ColonSentry	81479
ColoScape Colorectal Cancer Detection	0368U
Colosense	0421U
Colvera	0229U
Crohn's Prognostic Test	81401
CyPath Lung	0406U
Decipher Bladder TURBT	0016M
DecisionDx Cutaneous Melanoma	81529
DecisionDx DiffDx - Melanoma	0314U
DecisionDx - SCC	0315U
DEPArray	0009U
DetermaRx	0288U
DH Optical Genome Mapping/Digital Karyotyping Assay	0413U
Digitization of pathology slides	0760T, 0761T, 0762T, 0763T, 0848T, 0849T, 0850T, 0851T, 0852T, 0853T
EarlyTect Bladder Cancer Detection (EarlyTect BCD)	0451U
Envisia Genomic Classifier	81554
Epi+Gen CHD	0439U
Epignostix CNS Tumor Methylation Classifier	0020M
EpiSign Complete	0318U
EpiSwitch CiRT	0332U

Investigational and Experimental

Procedures address by this guideline	Procedure codes
EpiSwitch Prostate Screening Test	0433U
ERA (Endometrial Receptivity Analysis)	0253U
EsoGuard	0114U
ESOPREDICT Barrett's Esophagus Risk Classifier Assay	0398U
ExoDx Prostate (IntelliScore)	0005U
FM/a fibromyalgia	81599
GPS Cancer	81479
HelioLiver Test	0333U
Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis	81451
HPV-SEQ Test	0470U
IBD sgi Diagnostic	81479, 82397, 83520, 86140, 86255, 88346, 88350
Insight TNBCtype	0153U
Invitae PCM MRD Monitoring	0307U
Invitae PCM Tissue Profiling and MRD Baseline Assay	0306U
IriSight CNV Analysis	0469U
IriSight Prenatal Analysis – Proband	0335U
IriSight Prenatal Analysis – Comparator	0336U
KawasakiDx	0389U
KIF6 Genotype	81479
Know error	81479, 81265, 81266
LactoTYPE	81400
LPA-Aspirin Genotype	81479
LPA-Intron 25 Genotype	81479
LungLB	0317U
LungOI	0414U

Investigational and Experimental

Procedures address by this guideline	Procedure codes
Lymph2Cx Lymphoma Molecular Subtyping Assay	0017M
Lymph3Cx Lymphoma Molecular Subtyping Assay	0120U
Macula Risk	81401, 81479
Mammostrat Breast Cancer Recurrence Assay	S3854
Mind.Px	0258U
MindX Blood Test - Longevity	0294U
MindX Blood Test - Memory/Alzheimer's	0289U
MindX Blood Test - Mood	0291U
MindX Blood Test - Pain	0290U
MindX Blood Test - Stress	0292U
MindX Blood Test - Suicidality	0293U
MindX One Blood Test – Anxiety	0437U
miR-31now	0069U
miR Sentinel Prostate Cancer Test	0343U
miR Sentinel Prostate Cancer Test	0424U
Molecular Microscope MMDx—Heart	0087U
Molecular Microscope MMDx—Kidney	0088U
mRNA CancerDetect	0296U
myPath Melanoma	0090U
MyProstateScore	81599 or 0113U
MyProstateScore 2.0	0403U
myPRS Myeloma Prognostic Risk Signature	81479
myTAIHEART	0055U
NavDx	0356U
OncobiotaLUNG	0395U
Oncomap ExTra	0329U
OncoSignal 7 Pathway Signal	0262U
OncoTarget/OncoTreat	0019U
OncotypeDx AR-V7 Nucleus Detect	81479

Procedures address by this guideline	Procedure codes
PAI-1 Testing for Cardiovascular Disease Risk Assessment	81400, 85415
PancreaSeq Genomic Classifier	0313U
PanGIA Prostate	0228U
Pathway Fit	81291, 81401, 81479
PCR Fungal Screen for Onychomycosis	87481, 87798
Percepta Genomic Sequencing Classifier	81479
Pharmaco-oncologic Algorithmic Treatment Ranking	0794T
POC (Products of Conception)	0252U
Praxis Optical Genome Mapping	0264U
Praxis Somatic Combined Whole Genome Sequencing and Optical Genome Mapping	0300U
Praxis Somatic Optical Genome Mapping	0299U
Praxis Somatic Transcriptome	0298U
Praxis Somatic Whole Genome Sequencing	0297U
Praxis Transcriptome	0266U
PreciseDx Breast Biopsy Test	0418U
PreciseDx Breast Cancer Test	0220U
PrecisionCHD	0440U
PredictSURE IBD Test	0203U
PrismRA	0456U
ProMark Proteomic Prognostic Test	81479
Prospera	81479
RadTox cfDNA test	0285U
RetnaGene AMD	81401, 81405, 81408, 81479, 81599
ROMA Risk of Ovarian Malignancy Algorithm	81500
Signatera	0340U
Single Cell Prenatal Diagnosis (SCPD) Test	0341U

Investigational and Experimental

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Procedures address by this guideline	Procedure codes
SMART PGT-A (Pre-implantation Genetic Testing - Aneuploidy)	0254U
SMASH	0156U
Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis	81449
Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis	81456
Spectrum PGT-M	0396U
Statin Induced Myopathy Genotype (SLCO1B1)	81328
Strata Select	0391U
Thyroid GuidePx	0362U
ToxLok	0079U
TruGraf Kidney	81479
Tuteva	0320U
Twin Zygosity, cell free fetal DNA	0060U
UriFind Blood Cancer Assay	0465U
UroAmp MRD	0467U
Vectra	81490
Viracor TRAC dd-cfDNA	0118U
Vita Risk	0205U
Investigational and experimental tests that make use of molecular and genomic technologies	81479, 84999, 81599, and others

What is E/I/U molecular and genomic testing?

Definition

An experimental, investigational, or unproven (E/I/U) procedure is the use of a service, supply, drug, or device that is not recognized as standard medical care for the condition, disease, illness, or injury. Treatment is determined by the health plan based on an independent, peer review of literature and scientific data. E/I/U molecular and genomic tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact.

Experimental, investigational, or unproven determinations

Molecular and genomic tests are routinely released to market that make use of novel technologies or have a novel clinical application. These tests are often available on a clinical basis long before the required evidence to support clinical validity and clinical utility are established. Typically, there is insufficient data to support that the test

- accurately assesses the outcome of interest, analytical and clinical validity
- significantly improves health outcomes, clinical utility, and
- performs better than an existing standard of care medical management option.

Because these tests are often proprietary, there may be no independent test evaluation data available in the early stages to support the laboratory's claims regarding test performance and utility.

As new molecular and genomic tests become commercially available, the evidence base is reviewed. Tests determined to be E/I/U by the Health Plan are addressed by this guideline or a test-specific guideline and are not eligible for reimbursement.

Food and Drug Administration (FDA) clearance

In the case of laboratory testing, FDA clearance is not a suitable standard given that the clearance assessment does not require evidence to support clinical utility. In addition, while the FDA has stated that it has the discretion to regulate laboratory developed tests (LDTs), it is currently only selectively exercising that discretion to take action against egregious practices.

Criteria

Introduction

This section catalogues some, but not all, molecular and genomic tests that have been determined to be experimental, investigational, or unproven (E/I/U). E/I/U tests may also be addressed in test-specific guidelines and the reader is referred to those documents for additional information. New E/I/U tests may not yet be specifically listed in this guideline, but such decisions will be made using the following criteria.

Criteria: general coverage guidance

Molecular and genomic tests are only eligible for reimbursement when ALL of the following conditions are met:

- Technical and clinical validity: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- Clinical utility: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- Reasonable use: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Experimental, investigational, or unproven molecular and genomic tests

The following tests do not meet the above criteria and are not eligible for reimbursement.

- 9p21 Genotype Test (rs10757278 and rs1333049 alleles) CPT: 81479
- AlloSure Heart [Proprietary non-invasive assay to screen for organ injury and rejection in heart transplant recipients through measurement of donor-derived cell-free DNA in recipient blood sample from CareDx] CPT: 81479
- AlloSure Lung [Proprietary non-invasive assay to screen for organ injury and rejection in lung transplant recipients through measurement of donor-derived cell-free DNA in recipient blood sample from CareDx] CPT: 81479
- AmHPR Helicobacter pylori Antibiotic Resistance Next Generation Sequencing Panel [Helicobacter pylori detection and antibiotic resistance, DNA, 16S and 23S rRNA, gyrA, pbp1, rdxA and rpoB, next generation sequencing, formalin-fixed paraffin embedded or fresh tissue, predictive, reported as positive or negative for resistance to clarithromycin, fluoroquinolones, metronidazole, amoxicillin, tetracycline and rifabutin from American Molecular Laboratories, Inc.] CPT: 0008U
- Apolipoprotein E Genotype (APOE) CPT: 81401
- Apolipoprotein L1 (APOL1) Renal Risk Variant Genotyping [APOL1 (apolipoprotein L1) (eg, chronic kidney disease), risk variants (G1, G2) from Quest Diagnostics] CPT: 0355U
- ARISk Autism Risk Assessment Test [Proprietary test from IntegraGen] CPT: 81479
- AssureMDx [Proprietary non-invasive assay that analyzes tumor markers in the urine of individuals with hematuria to identify those at low risk and high risk for bladder cancer by MDx Health] CPT: 81479
- Augusta Optical Genome Mapping [Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping from Bionano Genomics, Inc] CPT: 0260U

- Augusta Hematology Optical Genome Mapping [Oncology (hematolymphoid neoplasia), optical genome mapping for copy number alterations and gene rearrangements utilizing DNA from blood or bone marrow, report of clinically significant alternations from Georgia Esoteric and Molecular Labs] CPT: 0331U
- Avantect Pancreatic Cancer Test[Oncology (pancreatic), DNA, whole genome sequencing with 5-hydroxymethylcytosine enrichment, whole blood or plasma, algorithm reported as cancer detected or not detected from ClearNote Health] CPT: 0410U
- BBDRisk Dx [Oncology (breast), immunohistochemistry, protein expression profiling of 4 biomarkers (matrix metalloproteinase-1 [MMP-1], carcinoembryonic antigen-related cell adhesion molecule 6 [CEACAM6], hyaluronoglucosaminidase [HYAL1], highly expressed in cancer protein [HEC1]), formalin-fixed paraffin-embedded precancerous breast tissue, algorithm reported as carcinoma risk score from Silbiotech, Inc] CPT: 0067U
- Bladder EpiCheck [Proprietary non-invasive assay that analyzes methylation biomarkers in the urine of individuals with hematuria to identify those at low risk and high risk for bladder cancer or to monitor tumor recurrence from Nucleix Ltd] CPT: 81599
- BluePrint Molecular Subtyping Profile [Proprietary 80-gene expression signature to classify Basal-type, Luminal-type and ERBB2-type breast cancers from Agendia] CPT: 81479
- BTG Early Detection of Pancreatic Cancer [Oncology (pancreatic), 59 methylation haplotype block markers, next generation sequencing, plasma, reported as cancer signal detected or not detected from Breakthrough Genomics] CPT: 0405U
- CARDIO inCode Score (CIC SCORE) [Cardiology (coronary heart disease [CHD]), 9 genes (12 variants), targeted variant genotyping, blood, saliva, or buccal swab, algorithm reported as a genetic risk score for a coronary event from GENinCode U.S. Inc] CPT: 0401U
- CardioRisk+ [Cardiology (coronary artery disease [CAD]), DNA, genome-wide association studies (564856 single-nucleotide polymorphisms [SNPs], targeted variant genotyping), patient lifestyle and clinical data, buccal swab, algorithm reported as polygenic risk to acquired heart disease from Gene by Gene, Ltd] CPT: 0466U
- CELLSEARCH CTC Test [Immunologic selection of circulating tumor cells in individuals with metastatic breast, prostate, or colorectal cancer for purposes of assessing prognosis from Menarini Silicon Biosystems] CPT: 86152, 86153
- ChemoFX [Proprietary test from Helomics to assess chemosensitivity] CPT: 81535, 81536
- Chromosome Genome Mapping [Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping from Bionano Genomics, Inc] CPT: 0454U

- Clarava [Nephrology (renal transplant), RNA expression by select transcriptome sequencing, using pretransplant peripheral blood from Verici Dx, Inc] CPT: 0319U
- Clarifi ASD [Neurology (autism spectrum disorder [ASD]), RNA, next-generation sequencing, saliva, algorithmic analysis, and results reported as predictive probability of ASD diagnosis from Quadrant Biosciences] CPT: 0170U
- CNGnome [Cytogenomic constitutional (genome-wide) analysis, interrogation of genomic regions for copy number, structural changes and areas of homozygosity for chromosomal abnormalities from PerkinElmer Genomics] CPT: 0209U
- Cologuard Plus [Oncology (colorectal) screening, quantitative real-time target and signal amplification, methylated DNA markers, including LASS4, LRRC4 and PPP2R5C, a reference marker ZDHHC1, and a protein marker (fecal hemoglobin), utilizing stool, algorithm reported as a positive or negative result from Exact Sciences Laboratories, LLC] CPT: 0464U
- ColonAiQ [Oncology (colorectal cancer), cell-free DNA (cfDNA), methylation-based quantitative PCR assay (SEPTIN9, IKZF1, BCAT1, Septin9-2, VAV3, BCAN), plasma, reported as presence or absence of circulating tumor DNA (ctDNA) from Breakthrough Genomics/ Singlera Genomics, Inc] CPT: 0453U
- ColonSentry [Proprietary 7-gene signature to detect colorectal cancer from StageZero Life Sciences] CPT: 81479
- ColoScape Colorectal Cancer Detection [Oncology (colorectal cancer), evaluation for mutations of APC, BRAF, CTNNB1, KRAS, NRAS, PIK3CA, SMAD4, and TP53, and methylation markers (MYO1G, KCNQ5, C9ORF50, FLI1, CLIP4, ZNF132, and TWIST1), multiplex quantitative polymerase chain reaction (qPCR), circulating cell-free DNA (cfDNA), plasma, report of risk score for advanced adenoma or colorectal cancer from DiaCarta Clinical Lab] CPT: 0368U
- Colosense [Oncology (colorectal) screening, quantitative real-time target and signal amplification of 8 RNA markers (GAPDH, SMAD4, ACY1, AREG, CDH1, KRAS, TNFRSF10B, EGLN2) and fecal hemoglobin, algorithm reported as a positive or negative for colorectal cancer risk from Geneoscopy, Inc] CPT: 0421U
- Colvera [BCAT1 (Branched chain amino acid transaminase 1) and IKZF1 (IKAROS family zinc finger 1) (eg, colorectal cancer) promoter methylation analysis from Colvera] CPT: 0229U
- Crohn's prognostic test [NOD2/CARD15 gene variant testing] CPT: 81401
- CyPath Lung [Oncology (lung), flow cytometry, sputum, 5 markers (meso-tetra [4-carboxyphenyl] porphyrin [TCPP], CD206, CD66b, CD3, CD19), algorithm reported as likelihood of lung cancer from Precision Pathology Services, bioAffinity Technologies, Inc] CPT: 0406U
- Decipher Bladder TURBT [Oncology (bladder), mRNA, microarray gene expression profiling of 219 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as molecular subtype (luminal, luminal infiltrated, basal, basal claudin-low, neuroendocrine-like)] CPT: 0016M

- DecisionDx - Cutaneous Melanoma assay [Proprietary 31-gene signature to assess melanoma metastatic risk from Castle Biosciences] CPT: 81529
- DecisionDx DiffDx - Melanoma [Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 35 genes (32 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (ie, benign, intermediate, malignant) from Castle Biosciences, Inc] CPT: 0314U
- DecisionDx - SCC [Oncology (cutaneous squamous cell carcinoma), mRNA gene expression profiling by RT-PCR of 40 genes (34 content and 6 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical risk result (ie, Class 1, Class 2A, Class 2B) from Castle Biosciences, Inc] CPT: 0315U
- DEPArray [Oncology (breast cancer), ERBB2 (HER2) copy number by FISH, tumor cells from formalin fixed paraffin embedded tissue isolated using image-based dielectrophoresis (DEP) sorting, reported as ERBB2 gene amplified or non-amplified from PacificDx] CPT: 0009U
- DetermaRx [Oncology (lung), mRNA, quantitative PCR analysis of 11 genes (BAG1, BRCA1, CDC6, CDK2AP1, ERBB3, FUT3, IL11, LCK, RND3, SH3BGR, WNT3A) and 3 reference genes (ESD, TBP, YAP1), formalin-fixed paraffin-embedded (FFPE) tumor tissue, algorithmic interpretation reported as a recurrence risk score from Oncocyte Corporation] CPT: 0288U
- DH Optical Genome Mapping/Digital Karyotyping Assay [Oncology (hematolymphoid neoplasm), optical genome mapping for copy number alterations, aneuploidy, and balanced/complex structural rearrangements, DNA from blood or bone marrow, report of clinically significant alterations from The Clinical Genomics and Advanced Technology (CGAT) Laboratory at Dartmouth Health] CPT: 0413U
- Digitization of pathology slides CPT: 0760T, 0761T, 0762T, 0763T, 0848T, 0849T, 0850T, 0851T, 0852T, 0853T
- EarlyTect Bladder Cancer Detection (EarlyTect BCD) [Oncology (bladder), methylated PENK DNA detection by linear target enrichment-quantitative methylation-specific real-time PCR (LTE-qMSP), urine, reported as likelihood of bladder cancer from Promis Diagnostics, Inc] CPT: 0451U
- Envisia Genomic Classifier [Proprietary gene expression assay designed to aid in the diagnosis of idiopathic pulmonary fibrosis from Veracyte] CPT: 81554
- Epi+Gen CHD [Cardiology (coronary heart disease [CHD]), DNA, analysis of 5 single-nucleotide polymorphisms (SNPs) (rs11716050 [LOC105376934], rs6560711 [WDR37], rs3735222 [SCIN/LOC107986769], rs6820447 intergenic], and rs9638144 [ESYT2]) and 3 DNA methylation markers (cg00300879 [transcription start site {TSS200} of CNKSR1], cg09552548 [intergenic], and cg14789911 [body of SPATC1L]), qPCR and digital PCR, whole blood, algorithm reported as a 4-tiered risk score for a 3-year risk of symptomatic CHD from Cardio Diagnostics, Inc] CPT: 0439U

- Epignostix CNS Tumor Methylation Classifier [Oncology (central nervous system), analysis of 30000 DNA methylation loci by methylation array, utilizing DNA extracted from tumor tissue, diagnostic algorithm reported as probability of matching a reference tumor subclass from Heidelberg Epignostix] CPT: 0020M
- EpiSign Complete [Pediatrics (congenital epigenetic disorders), whole genome methylation analysis by microarray for 50 or more genes, blood from Greenwood Genetic Center] CPT: 0318U
- EpiSwitch CiRT (Checkpoint-inhibitor Response Test) [Oncology (pan-tumor), genetic profiling of 8 DNA-regulatory (epigenetic) markers by quantitative polymerase chain reaction (qPCR), whole blood, reported as a high or low probability of responding to immune checkpoint–inhibitor therapy from Next Bio-Research Services, LLC] CPT: 0332U
- EpiSwitch Prostate Screening Test [Oncology (prostate), 5 DNA regulatory markers by quantitative PCR, whole blood, algorithm, including prostate-specific antigen, reported as likelihood of cancer from Oxford BioDynamics, Inc] CPT: 0433U
- ERA (Endometrial Receptivity Analysis) [Reproductive medicine (endometrial receptivity analysis), RNA gene expression profile, 238 genes by next-generation sequencing, endometrial tissue, predictive algorithm reported as endometrial window of implantation (eg, pre-receptive, receptive, post-receptive) from Igenomix] CPT: 0253U
- EsoGuard [Gastroenterology (Barrett's esophagus), VIM and CCNA1 methylation analysis, esophageal cells, algorithm reported as likelihood for Barrett's esophagus from Lucid Diagnostics] CPT: 0114U
- ESOPREDICT Barrett's Esophagus Risk Classifier Assay [Gastroenterology (Barrett esophagus), P16, RUNX3, HPP1, and FBN1 DNA methylation analysis using PCR, formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as risk score for progression to high-grade dysplasia or cancer from Capsulomics, Inc d/b/a Previse] CPT: 0398U
- ExoDx Prostate (IntelliScore) [Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score from Exosome Diagnostics, Inc.] CPT: 0005U
- FM/a fibromyalgia [interleukin-6, interleukin-8, macrophage inflammatory protein-1 alpha and macrophage inflammatory protein-beta (IL-6, IL-8, MIP-1a and MIP-1b, supernatant of stimulated cell culture, immunoassay, multianalyte assay with algorithmic analysis, reported as a score from EpicGenetics, Inc] CPT: 81599
- GPS Cancer [Proprietary test using a tissue block sample of the highest carcinoma grade of a tumor and a sample of blood to compare an individual's normal DNA to the tumor DNA to be used as part of a precision medicine approach for individuals with cancer from NantHealth] CPT: 81479
- HelioLiver Test [Oncology (liver), surveillance for hepatocellular carcinoma (HCC) in high-risk patients, analysis of methylation patterns on circulating cell-free DNA (cfDNA) plus measurement of serum of AFP/AFP-L3 and oncoprotein des-gamma-

carboxy prothrombin (DCP), algorithm reported as normal or abnormal result from Fulgent Genetics] CPT: 0333U

- Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis CPT: 81451
- HPV-SEQ Test [Oncology (oropharyngeal), detection of minimal residual disease by next-generation sequencing (NGS) based quantitative evaluation of 8 DNA targets, cell-free HPV 16 and 18 DNA from plasma from Sysmex Inostics, Inc] CPT: 0470U
- IBD sgi Diagnostic [Proprietary test from Prometheus with genomic components including ATG16L1, STAT3, NKX2-3, and ECM1 gene variants.] CPT: 81479, 82397, 83520, 86140, 86255, 88346, 88350
- Insight TNBCtype [Oncology (breast), mRNA, gene expression profiling by next-generation sequencing of 101 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a triple negative breast cancer clinical subtype(s) with information on immune cell involvement from Insight Molecular Labs] CPT: 0153U
- Invitae PCM MRD Monitoring [Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD from Invitae Corporation] CPT: 0307U
- Invitae PCM Tissue Profiling and MRD Baseline Assay [Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient specific panel for future comparisons to evaluate for MRD from Invitae Corporation] CPT: 0306U
- IriSight CNV Analysis [Rare diseases (constitutional/heritable disorders), whole genome sequence analysis for chromosomal abnormalities, copy number variants, duplications/deletions, inversions, unbalanced translocations, regions of homozygosity (ROH), inheritance pattern that indicate uniparental disomy (UPD), and aneuploidy, fetal sample (amniotic fluid, chorionic villus sample, or products of conception), identification and categorization of genetic variants, diagnostic report of fetal results based on phenotype with maternal sample and paternal sample, if performed, as comparators and/or maternal cell contamination from Variantyx Inc] CPT: 0469U
- IriSight Prenatal Analysis – Proband [Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants from Variantyx, Inc] CPT: 0335U
- IriSight Prenatal Analysis – Comparator [Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions,

uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent) from Variantyx, Inc] CPT: 0336U

- KawasakiDx [Pediatric febrile illness (Kawasaki disease [KD]), interferon alpha-inducible protein 27 (IFI27) and mast cell-expressed membrane protein 1 (MCEMP1), RNA, using quantitative reverse transcription polymerase chain reaction (RT-qPCR), blood, reported as a risk score for KD from OncoOmicsDx Laboratory, mProbe] CPT: 0389U
- KIF6 Genotype Test CPT: 81479
- Know error [Proprietary test for DNA based specimen provenance confirmation from Strand Diagnostics] CPT: 81479, 81265, 81266
- LactoTYPE [Proprietary test from Prometheus that assesses the hypolactasia C/T genetic variant] CPT: 81400
- LPA-Aspirin Genotype Test (4399Met allele) CPT: 81479
- LPA-Intron 25 Genotype Test CPT: 81479
- LungLB [Oncology (lung cancer), four-probe FISH (3q29, 3p22.1, 10q22.3, 10cen) assay, whole blood, predictive algorithm generated evaluation reported as decreased or increased risk for lung cancer from LungLife AI] CPT: 0317U
- LungOI [Oncology (lung), augmentative algorithmic analysis of digitized whole slide imaging for 8 genes (ALK, BRAF, EGFR, ERBB2, MET, NTRK1-3, RET, ROS1), and KRAS G12C and PD-L1, if performed, formalinfixed paraffin-embedded (FFPE) tissue, reported as positive or negative for each biomarker from Imagene] CPT: 0414U
- Lymph2Cx Lymphoma Molecular Subtyping Assay, [Oncology (diffuse large B-cell lymphoma [DLBCL]), mRNA, gene expression profiling by fluorescent probe hybridization of 20 genes, formalin-fixed paraffin embedded tissue, algorithm reported as cell of origin from Mayo Clinic] CPT: 0017M
- Lymph3Cx Lymphoma Molecular Subtyping Assay, [Oncology (B-cell lymphoma classification), mRNA, gene expression profiling by fluorescent probe hybridization of 58 genes (45 content and 13 housekeeping genes), formalin-fixed paraffin-embedded tissue, algorithm reported as likelihood for primary mediastinal B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL) with cell of origin subtyping in the latter from Mayo Clinic] CPT: 0120U
- Macula Risk [SNP-based assay to assist in the selection of eye supplement formulations for individuals diagnosed with intermediate dry age-related macular degeneration from ArcticDx, Inc] CPT: 81401, 81479
- Mammostrat Breast Cancer Recurrence Assay [Proprietary immunohistochemical (IHC) assay of 5 proteins in individuals with early stage breast cancer to assess recurrence risk from Clarient, Inc.] CPT: S3854

- MethylDetox Profile [The MethylDetox Profile test is a testing panel that assesses genes in the methylation pathway to provide "more actionable information than MTHFR testing alone" and provides "suggestions for specific nutrient needs" based on test findings from Cell Science Systems] CPT: none; no insurance billing
- Mind.Px [Autoimmune (psoriasis), mRNA, next-generation sequencing, gene expression profiling of 50-100 genes, skin-surface collection using adhesive patch, algorithm reported as likelihood of response to psoriasis biologics from Mindera Corporation] CPT: 0258U
- MindX Blood Test - Longevity [Longevity and mortality risk, mRNA, gene expression profiling by RNA sequencing of 18 genes, whole blood, algorithm reported as predictive risk score from MindX Sciences Inc] CPT: 0294U
- MindX Blood Test - Memory/Alzheimer's [Neurology (Alzheimer disease), mRNA, gene expression profiling by RNA sequencing of 24 genes, whole blood, algorithm reported as predictive risk score from MindX Sciences Inc] CPT: 0289U
- MindX Blood Test - Mood [Psychiatry (mood disorders), mRNA, gene expression profiling by RNA sequencing of 144 genes, whole blood, algorithm reported as predictive risk score from MindX Sciences Inc] CPT: 0291U
- MindX Blood Test - Pain [Pain management, mRNA, gene expression profiling by RNA sequencing of 36 genes, whole blood, algorithm reported as predictive risk score from MindX Sciences Inc] CPT: 0290U
- MindX Blood Test - Stress [Psychiatry (stress disorders), mRNA, gene expression profiling by RNA sequencing of 72 genes, whole blood, algorithm reported as predictive risk score from MindX Sciences Inc] CPT: 0292U
- MindX Blood Test - Suicidality [Psychiatry (suicidal ideation), mRNA, gene expression profiling by RNA sequencing of 54 genes, whole blood, algorithm reported as predictive risk score from MindX Sciences Inc] CPT: 0293U
- MindX One Blood Test – Anxiety [Psychiatry (anxiety disorders), mRNA, gene expression profiling by RNA sequencing of 15 biomarkers, whole blood, algorithm reported as predictive risk score from MindX Sciences] CPT: 0437U
- miR-31now [Oncology (colorectal), microRNA, RT-PCR expression profiling of miR-31-3p, formalin fixed paraffin-embedded tissue, algorithm reported as an expression score from GoPath Laboratories] CPT: 0069U
- miR Sentinel Prostate Cancer Test [Oncology (prostate), exosome-based analysis of 442 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chainreaction (RT-qPCR), urine, reported as molecular evidence of no-, low-, intermediate- or high-risk of prostate cancer from miR Scientific, LLC] CPT: 0343U
- miR Sentinel Prostate Cancer Test [Oncology (prostate), exosome-based analysis of 53 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as no molecular evidence, low-, moderate- or elevated-risk of prostate cance from miR Scientific, LLC] CPT: 0424U

- Mitomic Prostate Test [Proprietary test using mitochondrial DNA to detect prostate cancer not identified by standard biopsy pathology from MDNA Life Sciences] CPT: none; research use only
- Molecular Microscope MMDx—Heart [Transplantation medicine (heart allograft rejection), microarray gene expression profiling of 1283 genes, utilizing transplant biopsy tissue, algorithm reported as a probability score for rejection from Kashi Clinical Laboratories] CPT: 0087U
- Molecular Microscope MMDx—Kidney [Transplantation medicine (kidney allograft rejection), microarray gene expression profiling of 1494 genes, utilizing transplant biopsy tissue, algorithm reported as a probability score for rejection from Kashi Clinical Laboratories] CPT: 0088U
- mRNA CancerDetect [Oncology (oral and/or oropharyngeal cancer), gene expression profiling by RNA sequencing at least 20 molecular features (eg, human and/or microbial mRNA), saliva, algorithm reported as positive or negative for signature associated with malignancy from Viome Life Sciences, Inc] CPT: 0296U
- Myeloma Prognostic Risk Signature (myPRS) [Proprietary gene expression assay that is designed to predict an individual's risk of early relapse of multiple myeloma from Quest Diagnostics] CPT: 81479
- myPath Melanoma [Proprietary 23-gene expression assay to assess the risk of malignant melanoma when a result cannot be obtained by clinical assessment and/or histopathology alone from Castle Biosciences, Inc] CPT: 0090U
- MyProstateScore [urine analysis of TMPRSS2:ERG and PCA3 genes combined with blood PSA levels for early detection of prostate cancer from Lynx Dx] CPT: 81599 or 0113U
- MyProstateScore 2.0, [Oncology (prostate), mRNA, gene expression profiling of 18 genes, first catch post-digital rectal examination urine (or processed first-catch urine), algorithm reported as percentage of likelihood of detecting clinically significant prostate cancer from LynxDX] CPT: 0403U
- myTAIHEART CPT: 0055U
- NavDx [Oncology (oropharyngeal or anal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence from Naveris] CPT: 0356U
- OncobiotaLUNG [Oncology (lung), multi-omics (microbial DNA by shotgun next-generation sequencing and carcinoembryonic antigen and osteopontin by immunoassay), plasma, algorithm reported as malignancy risk for lung nodules in early-stage disease from Micronoma] CPT: 0395U
- Oncomap ExTra [Oncology (neoplasia), exome and transcriptome sequence analysis for sequence variants, gene copy number amplifications and deletions, gene rearrangements, microsatellite instability and tumor mutational burden utilizing DNA and RNA from tumor with DNA from normal blood or saliva for subtraction, report of clinically significant mutation(s) with therapy associations from Exact Sciences] CPT: 0329U

- OncoSignal 7 Pathway Signal [Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score from Protean BioDiagnostics] CPT: 0262U
- OncoTarget/OncoTreat [Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents from Columbia University Department of Pathology and Cell Biology, Darwin Health] CPT: 0019U
- OncotypeDx AR-V7 Nucleus Detect [Proprietary test designed to detect AR-V7 proteins in the nucleus of CTCs to determine response to AR-targeted therapies from Genomic Health] CPT: 81479
- PAI-1 Testing for Cardiovascular Disease Risk Assessment CPT: 81400, 85415
- PancreaSeq Genomic Classifier [Oncology (pancreas), DNA and mRNA next-generation sequencing analysis of 74 genes and analysis of CEA (CEACAM5) gene expression, pancreatic cyst fluid, algorithm reported as a categorical result (ie, negative, low probability of neoplasia or positive, high probability of neoplasia) from Molecular and Genomic Pathology Laboratory, University of Pittsburgh Medical Center] CPT: 0313U
- PanGIA Prostate [Oncology (prostate), multianalyte molecular profile by photometric detection of macromolecules adsorbed on nanosponge array slides with machine learning, utilizing first morning voided urine, algorithm reported as likelihood of prostate cancer from Genetics Institute of America] CPT: 0228U
- Pathway Fit [Proprietary test from Pathway Genomics that focuses on metabolism, diet, and exercise traits] CPT: 81291, 81401, 81479
- PAULA [Proprietary panel of four proteins designed to detect lung cancer in asymptomatic individuals at high risk from Genesys Biolabs] CPT: none; no insurance billing
- PCR Fungal Screen for Onychomycosis [Molecular tests for onychomycosis (e.g. Bako Diagnostics Onychodystrophy DNA Test)] CPT: 87481, 87798
- Percepta Genomic Sequencing Classifier [Proprietary gene expression assay designed to assess the risk of malignancy of lung nodules from Veracyte] CPT: 81479
- Pharmaco-oncologic Algorithmic Treatment Ranking [Patient-specific, assistive, rules-based algorithm for ranking pharmaco-oncologic treatment options based on the patient's tumor-specific cancer marker information obtained from prior molecular pathology, immunohistochemical, or other pathology results which have been previously interpreted and reported separately from CureMatch] CPT: 0794T
- POC (Products of Conception) [Fetal aneuploidy short tandem–repeat comparative analysis, fetal DNA from products of conception, reported as normal (euploidy), monosomy, trisomy, or partial deletion/duplication, mosaicism, and segmental aneuploidy from Igenomix] CPT: 0252U

- Praxis Optical Genome Mapping [Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping from Praxis Genomics, LLC] CPT: 0264U
- Praxis Somatic Combined Whole Genome Sequencing and Optical Genome Mapping [Oncology (pan tumor), whole genome sequencing and optical genome mapping of paired malignant and normal DNA specimens, fresh tissue, blood, or bone marrow, comparative sequence analyses and variant identification from Praxis Genomics LLC] CPT: 0300U
- Praxis Somatic Optical Genome Mapping [Oncology (pan tumor), whole genome optical genome mapping of paired malignant and normal DNA specimens, fresh frozen tissue, blood, or bone marrow, comparative structural variant identification from Praxis Genomics LLC] CPT: 0299U
- Praxis Somatic Transcriptome [Oncology (pan tumor), whole transcriptome sequencing of paired malignant and normal RNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and expression level and chimeric transcript identification from Praxis Genomics LLC] CPT: 0298U
- Praxis Somatic Whole Genome Sequencing [Oncology (pan tumor), whole genome sequencing of paired malignant and normal DNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and variant identification from Praxis Genomics LLC] CPT: 0297U
- Praxis Transcriptome [Unexplained constitutional or other heritable disorders or syndromes, tissue-specific gene expression by whole-transcriptome and next-generation sequencing, blood, formalin-fixed paraffin-embedded (FFPE) tissue or fresh frozen tissue, reported as presence or absence of splicing or expression changes from Praxis Genomics, LLC] CPT: 0266U
- PreciseDx Breast Biopsy Test [Oncology (breast), augmentative algorithmic analysis of digitized whole slide imaging of 8 histologic and immunohistochemical features, reported as a recurrence score from PreciseDx, Inc] CPT: 0418U
- PreciseDx Breast Cancer Test [Oncology (breast cancer), image analysis with artificial intelligence assessment of 12 histologic and immunohistochemical features, reported as a recurrence score from PreciseDx] CPT: 0220U
- PrecisionCHD [Cardiology (coronary heart disease [CHD]), DNA, analysis of 10 single-nucleotide polymorphisms (SNPs) rs710987 [LINC010019], rs1333048 [CDKN2B-AS1], rs12129789 [KCND3], rs942317 [KTN1-AS1], rs1441433 [PPP3CA], rs2869675 [PREX1], rs4639796 [ZBTB41], rs4376434 [LINC00972], rs12714414 [TMEM18], and rs7585056 [TMEM18]) and 6 DNA methylation markers (cg03725309 [SARS1], cg12586707 [CXCL1, cg04988978 [MPO], cg17901584 [DHCR24-DT], cg21161138 [AHRR], and cg12655112 [EHD4]), qPCR and digital PCR, whole blood, algorithm reported as detected or not detected for CHD from Cardio Diagnostics, Inc] CPT: 0440U

- PredictSURE IBD Test [Autoimmune (inflammatory bowel disease), mRNA, gene expression profiling by quantitative RT-PCR, 17 genes (15 target and 2 reference genes), whole blood, reported as a continuous risk score and classification of inflammatory bowel disease aggressiveness from KSL Diagnostics, PredictImmune Ltd] CPT: 0203U
- PrismRA [Autoimmune (rheumatoid arthritis), next-generation sequencing (NGS), gene expression testing of 19 genes, whole blood, with analysis of anti-cyclic citrullinated peptides (CCP) levels, combined with sex, patient global assessment, and body mass index (BMI), algorithm reported as a score that predicts nonresponse to tumor necrosis factor inhibitor (TNFi) therapy from Scipher Medicine] CPT: 0456U
- ProMark Proteomic Prognostic Test [Proprietary proteomic assay designed to assess the risk of aggressive prostate cancer from Metamark] CPT: 81479
- Prospera [Proprietary non-invasive assay that uses a single-nucleotide polymorphism (SNP)-based technology to evaluate active allograft rejection by measuring the DNA derived from transplanted donor kidneys; from Natera] CPT: 81479
- RadTox cfDNA test [Oncology, response to radiation, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported as a radiation toxicity score from DiaCarta Inc] CPT: 0285U
- RetnaGene AMD [Proprietary test from Sequenom CMM to predict risk of wet AMD progression] CPT: 81401, 81405, 81408, 81479, 81599
- ROMA Risk of Ovarian Malignancy Algorithm [Proprietary test using the combination of CA125 + HE4 antigens to assess the likelihood of malignancy before surgery; test kit from Fujirebio Diagnostics, Inc. and offered by several reference laboratories] CPT: 81500
- Signatera [Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease-burden correlation, if appropriate from Natera, Inc] CPT: 0340U
- Single Cell Prenatal Diagnosis (SCPD) Test [Fetal aneuploidy DNA sequencing comparative analysis, fetal DNA from products of conception, reported as normal (euploidy), monosomy, trisomy, or partial deletion/duplication, mosaicism, and segmental aneuploid from Luna Genetics, Inc] CPT: 0341U
- SMART PGT-A (Pre-implantation Genetic Testing - Aneuploidy) [Reproductive medicine (preimplantation genetic assessment), analysis of 24 chromosomes using embryonic DNA genomic sequence analysis for aneuploidy, and a mitochondrial DNA score in euploid embryos, results reported as normal (euploidy), monosomy, trisomy, or partial deletion/duplication, mosaicism, and segmental aneuploidy, per embryo tested from Igenomix] CPT: 0254U
- SMASH [Copy number (eg, intellectual disability, dysmorphology), sequence analysis from Marvel Genomics] CPT: 0156U

- Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis CPT: 81449
- Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis CPT: 81456
- Spectrum PGT-M [Obstetrics (pre-implantation genetic testing), evaluation of 300000 DNA single-nucleotide polymorphisms (SNPs) by microarray, embryonic tissue, algorithm reported as a probability for single-gene germline conditions from Natera, Inc.] CPT: 0396U
- Statin Induced Myopathy Genotype (SLCO1B1) CPT: 81328
- Strata Select [Oncology (solid tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded (FFPE) tissue, 437 genes, interpretive report for single nucleotide variants, splice-site variants, insertions/deletions, copy number alterations, gene fusions, tumor mutational burden, and microsatellite instability, with algorithm quantifying immunotherapy response score from Strata Oncology, Inc] CPT: 0391U
- Thyroid GuidePx [Oncology (papillary thyroid cancer), gene-expression profiling via targeted hybrid capture-enrichment RNA sequencing of 82 content genes and 10 housekeeping genes, fine needle aspirate or formalin-fixed paraffin embedded (FFPE) tissue, algorithm reported as one of three molecular subtypes from Protean BioDiagnostics] CPT: 0362U
- ToxLok [Comparative DNA analysis using multiple selected single-nucleotide polymorphisms (SNPs), urine and buccal DNA, for specimen identity verification from InSource Diagnostics] CPT: 0079U
- TruGraf Kidney [gene expression profile of mRNA from 107 inflammatory pathway genes to rule out subclinical rejection in renal transplant patients from Eurofins Transplant Genomics] CPT: 81479
- Tuteva [Nephrology (renal transplant), RNA expression by select transcriptome sequencing, using posttransplant peripheral blood, algorithm reported as a risk score for acute cellular rejection from Verici Dx, Inc] CPT: 0320U
- Twin zygosity [genomic targeted sequence analysis of chromosome 2, using circulating cell-free fetal DNA in maternal blood from Natera] CPT: 0060U
- UriFind Blood Cancer Assay [Oncology (urothelial carcinoma), DNA, quantitative methylation-specific PCR of 2 genes (ONECUT2, VIM), algorithmic analysis reported as positive or negative from DiaCarta, Inc, AnchorDx] CPT: 0465U
- UroAmp MRD [Oncology (bladder), DNA, next-generation sequencing (NGS) of 60 genes and whole genome aneuploidy, urine, algorithms reported as minimal residual disease (MRD) status positive or negative and quantitative disease burden from Convergent Genomics, Inc] CPT: 0467U

- Vectra [Proprietary panel of 12 biomarkers that yields a rheumatoid arthritis disease activity score from LabCorp] CPT: 81490
- Viracor TRAC dd-cfDNA [Transplantation medicine, quantification of donor-derived cell-free DNA using whole genome next-generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA in the total cell-free DNA from Viracor Eurofins] CPT: 0118U
- Vita Risk [Ophthalmology (age-related macular degeneration), analysis of 3 gene variants (2 CFH gene, 1 ARMS2 gene), using PCR and MALDI-TOF, buccal swab, reported as positive or negative for neovascular age-related macular-degeneration risk associated with zinc supplements from Arctic Medical Laboratories] CPT: 0205U

Medically Necessary Laboratory Testing

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Description

All delegated lab service procedure codes are subject to this guideline. Refer to the specific Health Plan's procedure code list for management requirements.

Background

Laboratory testing represents approximately 4% of healthcare expenditures.[1] While a relatively small contributor to overall healthcare expense, laboratory testing is a high volume service commonly performed during healthcare encounters with a critical role in informing downstream medical decisions.[2,3] Therefore, inappropriate over- or under-utilization of laboratory tests presumably also influences the medical costs associated with those medical services informed by test results.[1]

Laboratory tests are imperfect due to the overlap between disease and health as well as the fact that laboratory errors can occur in any phase of the laboratory process from specimen collection through specimen reporting and interpretation.[4] Even under ideal testing conditions, approximately 5% of healthy patients will have results outside of the reference range simply due to the method used to calculate most reference ranges for laboratory tests. Most reference ranges represent the central 95% of the results (e.g. the mean +/- two standard deviations) for a population of reasonably healthy individuals.[5] The individuals used for a reference range calculation are often people who are accepted as blood donors. When a result occurs outside the reference range in a healthy individual, that result is a setup for an erroneous interpretation, such as a false positive, which can lead to a false diagnosis. False diagnoses can lead to low value healthcare in the form of unnecessary interventions that can be dangerous and expensive.

Excessive testing

Testing that is unfocused, not indicated for routine prevention, and not specific to a patient's symptoms has an increased likelihood of false positives. As the number of tests ordered increases, so does the likelihood that at least one result will fall outside the reference range in a healthy individual. Therefore, large wellness panels in asymptomatic individuals or individuals with nonspecific signs and symptoms associated with daily life will nearly always lead to false positive tests and a potentially expensive medical diagnostic odyssey.

Appropriate test use

Laboratory tests are routinely used to screen for common disease, diagnose disorders in patients with signs or symptoms, inform effective treatment plans, and monitor therapies. Thus, correct test choice and interpretation is critical.

For individuals with suspected or diagnosed disease, appropriate laboratory testing may be defined in guidelines issued by the professional societies that guide care for those individuals. However, a substantial number of tests and indications will not be addressed in clear evidence-based guidelines, therefore requiring ongoing evaluation of the primary literature.

Laboratory testing is considered medically necessary when proven to be clinically useful for routine preventive screening or to diagnose, treat, monitor, or otherwise manage significant illness, infirmity, disability, or suffering.

Guidelines and Evidence

Introduction

This section includes relevant guidelines and evidence pertaining to medically necessary laboratory testing.

U.S Preventive Task Force (USPSTF)

The U.S. Preventive Services Task Force, with the support of the Agency for Healthcare Research and Quality, develops evidence-based preventive service recommendations, including laboratory screening tests, that are generally accepted as the standard of care in screening otherwise healthy individuals. USPSTF describes its scope as follows:[6]

- "The recommendations apply only to people who have no signs or symptoms of the specific disease or condition under evaluation, and the recommendations address only services offered in the primary care setting or services referred by a primary care clinician."

Choosing Wisely

Choosing Wisely is an initiative that started in 2012 with a mission to: "promote conversations between clinicians and patients by helping patients choose care that is:

- Supported by evidence
- Not duplicative of other tests or procedures already received
- Free from harm
- Truly necessary"[7]

Choosing Wisely includes over 90 recommendations related to lab testing issued by tens of professional societies that tend to address the most egregious, obvious, or easily addressed issues in lab overutilization.[1]

Criteria

Introduction

Requests for medically necessary laboratory testing are reviewed using these criteria.

Criteria: General Coverage Guidance

In order for a test to be considered medically necessary, the following criteria must be met:

- Be a preventive service as defined by the U.S. Preventive Services Task Force, Centers for Disease Control and Prevention, or other widely recognized preventive service guideline authors, OR
- Be necessary for the member's indication based on strong evidence-based professional society practice guidelines, OR
- Meet ALL of the following criteria:
    - Clinical signs, symptoms, treatment or monitoring needs are consistent with the test being performed, and
    - Technical and clinical validity: The test must be accurate, precise, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test, and
    - Clinical utility: Healthcare providers can use the test results to provide significantly better medical care for the individual, and
    - Reasonable use: The test is cost-effective when compared with equally acceptable alternatives and its usefulness is not significantly offset by negative factors, AND
- Testing must be ordered by a qualified healthcare provider who is actively managing the member's medical care, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other considerations

- Tests should not be duplicative or overlap in clinical intent with other performed services.
- Tests should not be repeated more often than is recommended and necessary.

- Direct-to-consumer lab testing is not eligible for reimbursement. This includes laboratory services supported by physicians serving in the role of ordering provider without having an active role in managing the member's healthcare.
- Expanded health and wellness panels that exceed routine preventive care services are not eligible for reimbursement.

References

Introduction

These references are cited in this guideline.

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2. Ngo A, Gandhi P, Miller WG. Frequency that laboratory tests influence medical decisions. *JALM*. 2017;1(4):410-414. Available at: http://jalm.aaccjnls.org/content/1/4/410
3. Zhi M, Ding EL, Theisen-Toupal J, Whelan J, Arnaout R. The landscape of inappropriate laboratory testing: a 15-year meta-analysis. *PLoS One*. 2013 Nov 15;8(11).
4. Astion M. The Google Factor: Are the Worried Well Making Healthcare Sick. *Clin Lab*. 2014;40(1).
5. Henry's Clinical Diagnosis and Management by Laboratory Methods, 23rd edition. McPherson RA and Pincus MR, eds. Elsevier. Amsterdam, Netherlands, 2016.
6. U.S. Preventive Services Task Force. About the USPSTF. Available at: https://www.uspreventiveservicestaskforce.org/Page/Name/about-the-uspstf
7. Choosing Wisely. Our Mission. Available at: https://www.choosingwisely.org/our-mission/

Pharmacogenomic Testing for Drug Toxicity and Response

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Introduction

Pharmacogenomic testing for drug toxicity and response is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
5HT2C Serotonin Receptor (HTR2C) Gene Variants	81479
5-Fluorouracil (5-FU) Toxicity and Chemotherapeutic Response	81232 81346
BRACAnalysis CDx Germline Companion Diagnostic Test	81162 81479
Catechol-O-Methyltransferase (COMT) Genotype	0032U
CNT (CEP72, TPMT and NUDT15) genotyping panel	0286U
COMT (Catechol Methyl Transferase) Gene Variants	81479
CYP1A2 Genotyping	81479
CYP2C9 Genotyping	81227
CYP2C19 Genotyping	81225
CYP4F2 Genotyping	81479
CYP2D6 Genotyping for Drug Response	81226
CYP2D6 Common Variants and Copy Number	0070U
CYP2D6 Full Gene Sequencing	0071U

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Procedures addressed by this guideline	Procedure codes
CYP2D6-2D7 Hybrid Gene Targeted Sequence Analysis	0072U
CYP2D7-2D6 Hybrid Gene Targeted Sequence Analysis	0073U
CYP2D6 trans-duplication/ multiplication nonduplicated gene targeted sequence analysis	0074U
CYP2D6 5’ gene duplication/ multiplication targeted sequence analysis	0075U
CYP2D6 3’ gene duplication/ multiplication targeted sequence analysis	0076U
CYP3A4 Gene Analysis	81230
CYP3A5 Gene Analysis	81231
Cytochrome P450 1A2 (CYP1A2) Genotype	0031U
DPYD Genotyping	81232
Drug metabolism (eg, pharmacogenomics) genomic sequence analysis panel, must include testing of at least 6 genes, including CYP2C19, CYP2D6, and CYP2D6 duplication/deletion analysis	81418
EffectiveRX Comprehensive Panel	0438U
Focused Pharmacogenomics Panel	0029U
G6PD Common Variants	81247
G6PD Full Gene Sequencing	81249
GeneSight Psychotropic	0345U
Genomind Pharmacogenetics Report - Full	0423U
Genomind Professional PGx Express	0175U
HLA-B*1502 Genotyping	81381
HLA-B*5701 Genotyping	81381
IDgenetix	0411U
IFNL3 rs12979860 Gene Variant	81283
INFINITI Neural Response Panel	0078U

Procedures addressed by this guideline	Procedure codes
Medication Management Neuropsychiatric Panel	0392U
Mental Health DNA Insight	81225 81226 81479
MTHFR Gene Variants	81291
NT (NUDT15 and TPMT) Genotyping Panel	0169U
NUDT15 Genotyping	81306
Pain Medication DNA Insight	81225 81226 81227 81291 81479
PersonalisedRX	0380U
Psych HealthPGx Panel	0173U
RightMed Comprehensive Test	0349U
RightMed Comprehensive Test Exclude F2 and F5	0348U
RightMed Gene Report	0350U
RightMed Gene Test Exclude F2 and F5	0434U
RightMed Oncology Gene Report	0460U
RightMed Oncology Medication Report	0461U
RightMed PGx16 Test	0347U
Serotonin Receptor Genotype (HTR2A and HTR2C)	0033U
Tempus nP	0419U
Thiopurine Methyltransferase (TPMT) and Nudix Hydrolase (NUDT15) Genotyping	0034U
TPMT Genotyping	81335
TYMS Genotyping	81346
UGT1A1 Targeted Variant Analysis	81350

Procedures addressed by this guideline	Procedure codes
VKORC1 Genotyping	81355
Warfarin Response Genotype	0030U
Warfarin responsiveness testing by genetic technique using any method	G9143
Pharmacogenomic tests that make use of molecular and genomic technologies	81479, 81599, and others

What are pharmacogenomic tests?

Definition

For the purposes of this guideline, pharmacogenomic tests are those germline tests performed to predict or assess an individual's response to therapy as well as the risk of toxicity from drug treatment.

Testing may be performed prior to treatment in order to determine if the individual has genetic variants that could affect drug response and/or increase the risk for adverse drug reactions. Testing may also be performed during treatment to assess whether an individual is having an adequate response or investigate the cause of an unexpected or adverse reaction.

Companion Diagnostics

Companion diagnostics are assays that help determine whether a drug may be safe or effective for a particular individual. Companion assays are evaluated as part of the Food & Drug Administration's (FDA's) development and approval process for the new drug. According to the FDA, "A companion diagnostic is a medical device, often an in vitro device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product. The test helps a health care professional determine whether a particular therapeutic product's benefits to patients will outweigh any potential serious side effects or risks." [1] Although specific companion diagnostic tests may be identified in the FDA label for a new drug approval, similar laboratory-developed tests (LDTs) performed by a CLIA-certified laboratory are generally accepted as alternatives that can typically provide the required information.

Complementary Diagnostics

Complementary diagnostics are assays that were developed and in use prior to the FDA's approval of a new drug. They are not evaluated through the FDA's development and approval process for new drugs. Complementary diagnostics are used to help provide additional information about how a drug might be used, or whether someone should receive a certain class of drugs. These tests are not

specifically required for the safe and effective use of a drug, which is part of what differentiates them from companion diagnostics. As with companion diagnostics, LDTs that are similar to the defined complementary diagnostic, when performed by a CLIA-certified laboratory, are able to provide the same information.[2]

An international consortium called the Clinical Pharmacogenetics Implementation Consortium (CPIC) develops and maintains detailed gene/drug practice guidelines to assist healthcare providers in the interpretation of pharmacogenomic test results when they are available; however, the consortium does not make specific recommendations about whether these tests should be performed.[3]

Test information

Introduction

Pharmacogenomic testing involves testing single nucleotide polymorphisms (SNPs) within genes that affect an individual's metabolism and response to certain medications.

Test Methods

For pharmacogenomic testing, one of the following testing strategies is typically employed:

- **Targeted testing:** assesses SNPs in a single gene or narrow subset of genes focused on response to one particular medication that is currently prescribed or under consideration for an individual.
- **Multi-gene panels:** assess SNPs in multiple genes to determine general drug response or response to a broad category of medications (e.g., psychotherapeutic, cardiovascular, etc.). Some laboratories apply a proprietary algorithm in order to classify the suitability of various medications based on the results. In contrast to targeted testing, multi-gene panels do not require that a particular medication be prescribed or under consideration, and the test may identify variants in many genes with no current impact on the individual's clinical care.

Criteria

Criteria: General Coverage Guidance

Pharmacogenomic tests are considered medically necessary when ALL of the following conditions are met:

- The individual is currently taking or considering treatment with a drug potentially affected by a known mutation that can be detected by a corresponding test.
- Technical and clinical validity: The test must be accurate, sensitive, and specific, based on sufficient, quality scientific evidence to support the claims of the test.

- Clinical utility: Healthcare providers can use the test results to guide changes in drug therapy management that will improve patient outcomes.
- Reasonable use: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social, or ethical challenges.

Criteria: Targeted Pharmacogenomic Tests

Targeted testing of gene variants associated with response to a specific medication (i.e. genotyping) will be considered medically necessary when the following criteria are met:

- Requested testing is performed in a CLIA-certified laboratory, AND
- Testing of the requested gene has not been previously performed, AND
- Healthcare providers can use the test results to directly impact medical care for the individual, AND
- At least one of the following criteria is met:
    - Documentation is provided that the requested testing is required to obtain health plan coverage for the medication being considered for treatment, or
    - A medication's FDA label requires results from the genetic test to effectively or safely use the therapy in question, with specific actions recommended based on the tested genotype (e.g., the label states the drug is contraindicated, recommends consideration of an alternative therapy, and/or provides dosage adjustments), or
    - The member meets criteria for one of the following tests covered without FDA label requirements:
        - DPYD testing for genetic variants DPYD*2A (rs3918290), DPYD*13 (rs55886062), and rs67376798 A (on the positive chromosomal strand) is requested for an individual considering or currently on therapy with any 5-FU containing drug including, but not limited to: 5-fluorouracil (Fluorouracil®, Adrucil®), capecitabine (Xeloda®), or fluorouracil topical formulations (Carac®, Efudex®, Fluoroplex®).

Targeted testing will be covered only for the number of genes or tests necessary to establish drug response.

- When available and cost-efficient, a tiered approach to testing, with reflex to more detailed testing and/or different genes, is recommended.
- When requested with a single procedure code, all components of the test must individually meet the above medical necessity criteria in order to be considered for reimbursement (e.g., CYP2D6 tests denoted by CPT codes 0071U–0076U, which test for other CYP2D6 findings in addition to the common gene variants, are typically not medically necessary).

Targeted Pharmacogenomic Tests Considered Not Medically Necessary

The following tests and specific indications (i.e. gene/drug interactions) are considered not medically necessary.[4-25] This list is not intended to be all-inclusive.*

- CNT (CEP72, TPMT and NUDT15) genotyping panel from RPRD Diagnostics for response to thiopurines and/or vincristine CPT: 0286U
- CYP450 gene variants (including, but not limited to CYP1A2, CYP2D6, CYP2C9, CYP2C19, CYP3A4, CYP3A5) for general drug response or response to broad categories of drugs (e.g., psychotherapeutic, cardiovascular, etc.) rather than a specific drug CPT: 81225, 81226, 81227, 81230, 81231, 81479
- CYP2C19 testing for the management of H. pylori CPT: 81225
- CYP2C9, VKORC1, and/or CYP4F2 testing for warfarin response CPT: 81227, 81355, 81479
- CYP2D6 testing for tamoxifen response CPT: 81226
- Warfarin Response Genotype from Mayo Clinic CPT: 0030U

Note *Please note that some targeted tests and procedure codes in this list may be coverable for other indications. When a test is requested for the purposes of assessing response to a drug/indication not listed here, please see Criteria: Targeted Pharmacogenomic Tests above.

Targeted Pharmacogenomic Tests Considered Experimental, Investigational, or Unproven

The following pharmacogenomic tests have not demonstrated clinical utility for any indication at the time this guideline was updated and are considered experimental, investigational, or unproven, and therefore not eligible for reimbursement. This list is not intended to be all-inclusive.†

- 5HT2C (Serotonin Receptor; HTR2C) gene variants CPT: 81479
- COMT (Catechol Methyl Transferase) gene variants CPT: 81479
- Catechol-O-Methyltransferase (COMT) Genotype from Mayo Clinic CPT: 0032U
- Cytochrome P450 1A2 (CYP1A2) Genotype from Mayo Clinic CPT: 0031U
- IFNL3 rs12979860 gene variant CPT: 81283
- MTHFR gene variants CPT: 81291
- Serotonin Receptor Genotype (HTR2A and HTR2C) from Mayo Clinic CPT: 0033U
- TYMS gene variants CPT: 81346

Note †Please note that some targeted tests in this list may be coverable at the time of request due to an update in the FDA drug label. When a test is requested for the purposes of assessing response to a specific drug, please see Criteria: Targeted Pharmacogenomic Tests above.

Criteria: Pharmacogenomic Panel Tests

Multi-gene pharmacogenomic panels that assess general drug response or response to broad categories of medications (e.g., psychotherapeutic, cardiovascular, etc.), regardless of how they are billed, are considered experimental, investigational, or unproven (E/I/U) and therefore not eligible for reimbursement. The following are examples of panels that are considered E/I/U. This list is not intended to be all-inclusive.

- Drug metabolism (eg, pharmacogenomics) genomic sequence analysis panel, must include testing of at least 6 genes, including CYP2C19, CYP2D6, and CYP2D6 duplication/deletion analysis CPT 81418
- EffectiveRX Comprehensive Panel [Drug metabolism (adverse drug reactions and drug response), buccal specimen, gene-drug interactions, variant analysis of 33 genes, including deletion/duplication analysis of CYP2D6, including reported phenotypes and impacted gene-drug interactions from RCA Laboratory Services] CPT: 0438U
- Focused Pharmacogenomics Panel from Mayo Clinic CPT: 0029U
- GeneSight Psychotropic [Psychiatry (eg, depression, anxiety, attention deficit hyperactivity disorder [ADHD]), genomic analysis panel, variant analysis of 15 genes, including deletion/duplication analysis of CYP2D6 from Myriad Genetics] CPT: 0345U
- Genomind Pharmacogenetics Report – Full [Psychiatry (eg, depression, anxiety), genomic analysis panel, including variant analysis of 26 genes, buccal swab, report including metabolizer status and risk of drug toxicity by condition from Genomind, Inc] CPT: 0423U
- Genomind Professional PGx Express CPT: 0175U
- IDgenetix [Psychiatry (eg, depression, anxiety, attention deficit hyperactivity disorder [ADHD]), genomic analysis panel, variant analysis of 15 genes, including deletion/duplication analysis of CYP2D6 from Castle Biosciences, Inc] CPT: 0411U
- INFINITI® Neural Response Panel [Pain management (opioid-use disorder) genotyping panel, 16 common variants (ie, ABCB1, COMT, DAT1, DBH, DOR, DRD1, DRD2, DRD4, GABA, GAL, HTR2A, HTTLPR, MTHFR, MUOR, OPRK1, OPRM1), buccal swab or other germline tissue sample, algorithm reported as positive or negative risk of opioid-use disorder from Prescient Medicine Holdings, Inc.] CPT: 0078U
- Medication Management Neuropsychiatric Panel [Drug metabolism (depression, anxiety, attention deficit hyperactivity disorder [ADHD]), gene-drug interactions, variant analysis of 16 genes, including deletion/duplication analysis of CYP2D6,

reported as impact of gene-drug interaction for each drug from RCA Laboratory Services LLC d/b/a GENETWORx] CPT: 0392U

- Mental Health DNA Insight [Proprietary test from Pathway Genomics] CPT: 81225, 81226, 81479
- Pain Medication DNA Insight [Proprietary test from Pathway Genomics] CPT: 81225, 81226, 81227, 81291, 81479
- PersonalisedRX [Proprietary test from Lab Genomics, LLC] CPT: 0380U
- RightMed Comprehensive Test [Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 27 gene report, with variant analysis including reported phenotypes and impacted gene-drug interactions from OneOme, LLC] CPT: 0349U
- RightMed Comprehensive Test Exclude F2 and F5 [Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 25 gene report, with variant analysis and reported phenotypes from OneOme, LLC] CPT: 0348U
- RightMed Gene Report [Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 27 gene report, with variant analysis and reported phenotypes from OneOme, LLC] CPT: 0350U
- RightMed Gene Test Exclude F2 and F5 [Drug metabolism (adverse drug reactions and drug response), genomic analysis panel, variant analysis of 25 genes with reported phenotypes from OneOme LLC] CPT: 0434U
- RightMed Oncology Gene Report [Oncology, whole blood or buccal, DNA single-nucleotide polymorphism (SNP) genotyping by real-time PCR of 24 genes, with variant analysis and reported phenotypes from OneOme LLC] CPT: 0460U
- RightMed Oncology Medication Report [Oncology, pharmacogenomic analysis of single-nucleotide polymorphism (SNP) genotyping by real-time PCR of 24 genes, whole blood or buccal swab, with variant analysis, including impacted gene-drug interactions and reported phenotypes from OneOme LLC] CPT: 0461U
- RightMed PGx16 Test [Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 16 gene report, with variant analysis and reported phenotypes from OneOme, LLC] CPT: 0347U
- Tempus nP [Neuropsychiatry (eg, depression, anxiety), genomic sequence analysis panel, variant analysis of 13 genes, saliva or buccal swab, report of each gene phenotype from Tempus Labs, Inc] CPT: 0419U

Other Considerations

For pharmacogenomic tests that look for changes in germline DNA (i.e., not tumor DNA or viral DNA), testing will be allowed once per lifetime per gene. Exceptions may be considered if technical advances in testing or the discovery of novel genetic variants demonstrate significant advantages that would support a medical need to retest.

Testing performed in a CLIA-certified laboratory will be considered for coverage. The use of a specific FDA approved companion diagnostic is not necessary for coverage to be considered.

Test-specific guidelines are available for some pharmacogenomic tests. Please refer to the guidelines manual for a list of test-specific guidelines (for example: *GeneSight Psychotropic Test*). For tests without a specific guideline, use the above criteria.

For information on somatic mutation testing in solid tumor tissue or hematological malignancies, please refer to the guideline, *Somatic Mutation Testing*, as this testing is not addressed here.

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Preimplantation Genetic Screening and Diagnosis

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Introduction

Preimplantation genetic screening and diagnosis is addressed by this guideline.

Definition

Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) are used to detect genetic conditions, chromosome abnormalities, and fetal sex during assisted reproduction with in vitro fertilization (IVF). PGD refers to embryo testing that is performed when one or both parents have a known genetic abnormality. This includes single-gene mutations and chromosome rearrangements. PGS refers to screening an embryo for aneuploidy when both parents are chromosomally normal. Genetic testing is performed on cells from the developing embryo prior to implantation. Only those embryos not affected with a genetic condition are implanted. PGD may allow at-risk couples to avoid a pregnancy affected with a genetic condition. The Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine have published joint practice committee opinions to address the safety, accuracy, and overall efficacy of PGD and PGS.[1,2]

- For information on prenatal and preconception carrier screening, please refer to the guideline *Genetic Testing for Carrier Status,* as this testing is not addressed here.
- For information on prenatal genetic testing, please refer to the guideline *Genetic Testing for Prenatal Screening and Diagnostic Testing,* as this testing is not addressed here.

Terminology for preimplantation genetic testing has recently been updated, with terms for various clinical testing indications:

- PGT-M: testing performed when the embryo is at an increased risk for a monogenic disorder[3]
- PGT-SR: testing performed when the embryo is at increased risk for a structural chromosome rearrangement[3]
- PGT-A: testing performed to screen an embryo for aneuploidy when both parents are chromosomally normal[3]
- PGT-P: testing performed to screen an embryo for polygenic disorders using polygenic risk score analyses[4]

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to PGD and/or PGS.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2023) published a points to consider statement regarding the clinical application of preimplantation polygenic risk score (PRS) testing.[5] This statement provided several general considerations regarding PRS testing for healthcare providers. Regarding preimplantation PRS testing, they stated: "The ACMG's position is that preimplantation PRS testing is not yet appropriate for clinical use and should not be offered at this time."

American College of Obstetrics and Gynecology

The American College of Obstetrics and Gynecology (ACOG, 2020) stated the following:[6]

- Confirmation of results from PGT-M and PGT-SR should be offered. This confirmation is completed through chorionic villus sampling or amniocentesis.
- For PGT-A, "traditional diagnostic testing or screening for aneuploidy should be offered to all patients who have had preimplantation genetic testing-aneuploidy, in accordance with recommendations for all pregnant patients."

American Society of Reproductive Medicine

The American Society of Reproductive Medicine (ASRM, 2023) published a committee opinion for the indications and management of preimplantation genetic testing for monogenic conditions.[7]

Initially, PGT-M was utilized for "severe, untreatable, or life-threatening childhood-onset conditions". However, the technology can be used for a variety of conditions with a broad range of symptoms including a mild to moderate phenotype, later age of onset, and/or reduced penetrance. Testing for some conditions is controversial. Additionally, there are also some conditions for which PGT-M is "not technically feasible". The committee opinion of ASRM stratified PGT-M indications into four categories on the "basis of age of onset, condition severity, penetrance, and the expected impact of PGT-M on overall risk reduction".

- "Traditional/Pediatric Indications: childhood-onset, lethal, and/or severe conditions that lack effective treatment. Most providers agree that PGT-M should be available for these conditions."
- "Serious Adult-Onset Conditions: ... [ASRM] has issued a statement generally supporting the use of the technology for such conditions "when the conditions are

serious and when there are no known interventions...or the available interventions are either inadequately effective or significantly burdensome."

- "Mild Conditions or Indications of Limited/Questionable Risk Reduction: ... These include cases in which the risk of offspring is very low or not increased above that of the general population, conditions of very low penetrance or mild severity, and variants of uncertain significance (VUSs). ... Whether or not to offer PGT for a VUS may depend on a variety of factors including how the VUS was identified, supporting classification evidence, whether it tracks with the condition in the patient and family, associated recurrence risks, supporting clinical documentation, and the patient's risk tolerance."
- "Indications for Which PGT-M is not Recommended: ... Autosomal recessive carrier status without manifestations of symptoms; combination of variants not associated with disease; pseudodeficiency alleles; somatic only variants."

The committee also stated PGT-M should be optional, individuals should have access to genetic counseling to discuss all reproductive options and individuals may benefit from genetic counseling to discuss PGT-M results. Additionally, there are technical limitations with PGT-M and thus, prenatal testing should be offered for pregnancies conceived using PGT-M. Prenatal testing may include confirmation of the PGT-M results and also testing for other fetal conditions unrelated to the reason for PGT-M.

Society for Assisted Reproductive Technology and American Society for Reproductive Medicine

In a joint practice committee opinion, the Society for Assisted Reproductive Technology (SART, 2008) and the American Society for Reproductive Medicine (ASRM, 2008) stated the following:[8]

- "PGD is indicated for couples at risk for transmitting a specific genetic disease or abnormality to their offspring."
- "Due to the risk for conceiving a child with a genetic disease or other abnormality, counseling for couples considering PGD is required..."
- Suggested key points of genetic counseling include IVF and embryo biopsy-related risks, natural history of the tested condition, other reproductive options, limitations of preimplantation testing, and prenatal follow-up options.

In a joint practice committee opinion, the Society for Assisted Reproductive Technology (SART, 2018) and the American Society for Reproductive Medicine (ASRM, 2018) stated the following:[2]

- "The value of PGT-A as a universal screening test for all IVF patients has yet to be determined."
- There is currently insufficient evidence to recommend the use of PGT-A in all individuals undergoing IVF.

Criteria

Introduction

Requests for preimplantation genetic diagnosis (PGD) are reviewed using the following criteria.

Criteria: General Coverage Guidance

Preimplantation genetic diagnosis is medically necessary when **ALL** of the following conditions are met:

- **Technical and clinical validity**: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test. In the case of PGD, the mutation(s) or translocation(s) to be tested in the embryo should first be well-characterized in the parent(s) AND the embryonic test results must be demonstrated to be highly accurate.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care and/or assist individuals with reproductive planning.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

AND THE FOLLOWING APPLY:

- The couple is known to be at-risk to have child with a genetic condition because of ANY of the following:
    - Both parents are known carriers of a recessive genetic condition and the specific gene mutation has been identified in each parent; OR
    - One parent is affected by or known to be a carrier of a dominant condition and the specific gene mutation has been identified; OR
    - The female contributing the egg is known to be a carrier of an X-linked condition and the specific gene mutation has been identified; OR
    - One or both parents are carriers of a structural chromosome rearrangement (e.g., translocation or inversion); OR
    - One or both parents have a known chromosome microdeletion (e.g. 22q11 deletion – DiGeorge syndrome, 7q11.23 deletion – Williams syndrome);

AND

- The genetic condition is associated with potentially severe disability or has a lethal natural history.

Note This guideline ONLY addresses the genetic testing component of PGS or PGD. Coverage of any procedures, services, or tests related to assisted reproduction is subject to any applicable plan benefit limitations.

Criteria: Special Circumstances

Sex determination

- PGD for sex (X and Y chromosome testing) is medically necessary only for identification of potentially affected embryos for gender-related conditions.

HLA typing

- PGD for human leukocyte antigen (HLA) typing for transplant donation is medically necessary only if:
    - A couple has child with a bone marrow disorder needing a stem cell transplant; AND
    - The only potential source of a compatible donor is an HLA-matched sibling

Chromosome abnormality screening

- PGS for de novo chromosome abnormalities is not medically necessary. This includes the following indications:
    - Maternal age alone
    - To improve in vitro success rates
    - For recurrent unexplained miscarriage and/or recurrent implantation failures

Variants of Unknown Significance (VUS)

- PGD for variants of unknown significance is not medically necessary.

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Test Specific Guidelines

AlloMap Gene Expression Profiling for Heart Transplant Rejection

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Introduction

AlloMap Gene Expression Profiling is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
AlloMap	81595

Criteria

Introduction

Requests for AlloMap Gene Expression Profiling are reviewed using these criteria.

Criteria

AlloMap is considered medically necessary when ALL of the following criteria are met:

- Medical records indicate that member has been under the care of the ordering provider within the past 30 days, and
- Member is not acutely symptomatic, and
- Member does not have recurrent rejection (defined as having a documented prior rejection and currently having signs/symptoms of rejection), and
- Member is not currently receiving 20 mg or more of daily oral prednisone, and
- Member has not received high-dose intravenous corticosteroids or myeloablative therapy in the past 21 days, and
- Member has not received blood products or hematopoietic growth factors in the past 30 days, and
- Member is not pregnant, and
- Member is at least 2 months post-transplant, and
- Member is less than 5 years post-transplant, and

- Member is at least 15 years of age

Recommended frequency of AlloMap testing

This table describes the recommended frequency of AlloMap testing.

Months post-transplant	Frequency of AlloMap testing
2 to 6 months	every 2 to 4 weeks
6 to 12 months	every 2 months
12 to 24 months	every 3 months
24 months to 60 months	every 6 months
greater than 60 months	every 12 months

Exceptions to testing frequency

AlloMap may be used as a substitute for endomyocardial biopsy in surveillance of stable individuals. Exceptions to the above testing frequencies may be considered as warranted by an individual's clinical presentation. AlloMap testing is not routinely medically necessary in individuals greater than 5 years post-transplant. Requests for exceptions to this criteria will be evaluated on a case by case basis.

Other indications

The use of AlloMap for prognostic purposes is not medically necessary. Studies on the ability of the test to predict future clinical events do not provide enough evidence to warrant coverage at this time.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- The use of AlloMap for prognostic purposes is not reimbursable.

What is AlloMap?

Definition

AlloMap is a non-invasive blood test that is designed to help identify heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection at the time of testing.[1]

Current uses

AlloMap is designed to help providers obtain this information without the use of endomyocardial biopsy. While endomyocardial biopsy is currently the standard of care for heart transplant recipients, it is an invasive procedure with associated risks.

Description

The AlloMap assay interrogates a panel of 20 genes. The assay uses gene expression of RNA isolated from peripheral blood mononuclear cells.[1]

Results

Using data from the gene expression of these genes, an AlloMap score is calculated. The lower the score, the lower the probability of acute cellular rejection at the time of testing.[1]

Intended use

AlloMap is intended for use in heart transplant recipients with the following characteristics:

- 15 years of age or older[1]
- not currently pregnant[2]
- at least 2 months but not more than 5 years post-transplant[1-3]
- not acutely symptomatic[2]
- not in recurrent rejection[2] (defined as having a documented prior rejection and currently having signs/symptoms of rejection)
- not currently receiving oral prednisone (20 mg or more daily)[2]
- have not received high-dose intravenous corticosteroids or myeloablative therapy in the past 21 days[2]
- have not received blood products or hematopoietic growth factors in the past 30 days[2]

Exceptions may be made as needed for individual clinical presentation.[2,3,4]

Test information

Introduction

The AlloMap assay measures the RNA gene expression of 20 genes: 11 of these genes are thought to be informative for the assay, while the remaining 9 are used for quality control.[1] The test is intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate or severe acute cellular rejection at the time of testing, in conjunction with standard clinical assessment. The AlloMap assay was developed against the phenotype of acute cellular rejection only, thus neither antibody-mediated nor chronic rejection can be ruled out using AlloMap.[2]

Risk score

The data collected from these genes is translated into a risk score. Scores range from 0-40 and are compared to post-transplant individuals in the same post-transplant period. The lower the score, the lower the probability of acute cellular rejection at the time of testing.[1]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to AlloMap testing.

International Society of Heart and Lung Transplantation

The International Society of Heart and Lung Transplantation (ISHLT, 2010) stated:[5]

"Gene Expression Profiling (AlloMap) can be used to rule out of the presence of acute cellular rejection (ACR) of grade 2R or greater in appropriate low risk patients, between 6 months and 5 years after HT."

Class IIa

Class IIa: Weight of evidence/opinion is in favor of usefulness/efficacy.

Level of evidence: B – data derived from a single randomized clinical trial or large non-randomized studies.

U.S. Food and Drug Administration

In 2008, the U.S. Food and Drug Administration (FDA, 2008) cleared AlloMap as a Class II Medical Device. [3]

Selected Relevant Publications

A number of peer-reviewed, expert-authored studies that evaluated the clinical validity and utility of the AlloMap test are available. These studies were designed to identify

heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection at the time of testing.[4,6-15] Most of these studies demonstrated the potential for the assay to help cardiologists rule out acute cellular rejection in low-risk individuals and reduce risks associated with endomyocardial biopsy.

Limitations were noted across the studies and included inconsistent thresholds for defining a positive AlloMap test and few cases of allograft rejection which may have contributed to imprecision when computing diagnostic accuracy. Results were conflicting across the available studies regarding the appropriate frequency of testing intervals. Some studies reported frequency of testing (which did not include testing in consecutive months), while other studies did not. Several studies evaluating outcomes across multiple centers stated that each center or physician was responsible for determining the frequency of interval testing. Additionally, several studies were limited for failing to adequately represent the intended study populations.

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AlloSure for Kidney Transplant Rejection

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Introduction

AlloSure for kidney transplant rejection (AlloSure Kidney) is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
AlloSure Kidney	81479

Criteria

Introduction

Requests for AlloSure Kidney testing for allograft kidney transplant rejection are reviewed using the following criteria.

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What Is Kidney Transplant Rejection?

Definition

Kidney disease is a loss of renal function which, without treatment, leads to eventual build-up of waste and other toxic substances in the blood.[1] Treatment of advanced kidney disease, called end-stage kidney disease, consists of dialysis or renal transplant. Transplant rejection can be acute or chronic.

Incidence and Prevalence

According to the National Kidney Foundation, 97% of kidney transplants are functioning 1 year after transplant, and about 80% of kidneys from living donors are functioning after 5 years.[2] Deceased-donor kidneys have lower success rates.[2]

Symptoms

Kidney transplant rejection can be acute (occurring suddenly and progressing quickly) or chronic (occurring slowly over time), and is typically immune system mediated. Symptoms of transplant rejection include fever and flu-like symptoms, decreased urinary output, weight gain, fatigue, and pain over the transplanted organ.[3]

Acute rejection of the donated kidney is thought to lead to tissue injury, including increased cell death in the allograft, which then leads to increased donor-derived cell free DNA (dd-cfDNA) in the bloodstream. Other investigators have reported that the fraction of cell-free DNA (cfDNA) originating from the organ grafts is approximately less than 1% and during rejection, level of dd-cfDNA increase.[4-6]

Cause

Transplanted kidneys can fail for multiple reasons:[7]

- Blood clot in the vessels leading to the kidney
- Infection
- Medication side effects
- Non-compliance with post-transplant medications and other post-surgical care
- Recurrence of the original medical problem that caused the kidney transplant
- Acute or chronic rejection caused by immune-mediated donor kidney damage

Diagnosis

Rise in creatinine levels is currently used to initially diagnose graft rejection, and the gold standard for initial diagnosis is histological analysis based on needle biopsy of the organ.[4-5] However, organ biopsy is invasive and often associated with complications, patient discomfort, and inconvenience. Biopsy is also prone to sampling error. Serum creatinine is one of the main markers used to monitor allograft functioning, but has been shown to lack sensitivity and specificity for graft injury and may change too late to allow prompt clinical management decisions.[8,9]

Alternatively, donor-derived cell-free DNA (dd-cfDNA) (as a fraction of the total cell-free DNA [cfDNA]) has been proposed as a noninvasive marker for detecting graft rejection and measuring allograft damage among recent kidney transplant patients.

Treatment

Renal transplantation has been shown to increase the survival and quality of life (QOL) of patients with end stage renal disease (ESRD), and is often considered the preferred treatment option for these patients.[10] When a transplanted kidney is rejected, dialysis is performed until another organ can be procured for transplant.

Survival

If the kidneys fail completely, survival is a few months without treatment.[1] After transplant, long-term survival is still limited, and acute rejection is a frequent complication and associated with reduced graft survival.[1]

Test Information

Introduction

AlloSure Kidney is an assay designed to detect allograft rejection in kidney transplant recipients.

Description and Purpose

According to the manufacturer of AlloSure Kidney (Care Dx, Inc), the test is intended to non-invasively measure donor DNA in the blood for kidney transplant surveillance of active donor graft injury and rejection.[11] Active rejection as defined by the manufacturer includes “T cell–mediated rejection [TCMR], “acute/active” antibody-mediated rejection [ABMR], and “chronic, active” ABMR)”.[11] The test has been primarily studies in adult transplant recipients.

Test Targets

AlloSure Kidney is a targeted next-generation sequencing assay that uses 266 single-nucleotide polymorphisms (SNPs) to quantify dd-cfDNA in transplant patients.[11]

Result

The test reports the percent of donor derived DNA in the patient's blood sample along with quality control cut-off values.[11]

Interpretation of test results:[11]

- Low risk of rejection: <0.5%
- Likely graft injury: 0.5-1.0%
- High risk of rejection: 1.0-2.9%

In addition, the relative change of dd-cfDNA over time can provide additional information:[11]

- "Increases in AlloSure results over 61% exceed biological variation"
- "A median increase of 149% between serial results is indicative of graft injury"

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to AlloSure for Kidney Transplant Rejection.

American Society of Transplant Surgeons

The American Society of Transplant Surgeons (ASTS, 2023) issued a position statement on the use of dd-cfDNA in transplant recipients that stated:[12]

- "We recommend that clinicians measure dd-cfDNA levels in kidney transplant recipients with acute allograft dysfunction to exclude the presence of rejection, particularly antibody-mediated rejection (ABMR)."
- "We strongly recommend ongoing further clinical studies to clarify the scenarios in which molecular diagnostic studies should be utilized."
- "We specifically recommend that studies be carried out to evaluate the potential role of dd-cfDNA surveillance in kidney transplant recipients to improve long term allograft survival."

The Renal Association

The Renal Association Clinical Practice Guideline Post-Operative Care in the Kidney Transplant Recipient (RA, 2017, Reviewed 2022) was endorsed by the British Transplantation Society and the National Institute for Health and Care Excellence. The guideline stated:[13]

- "We recommend that a transplant renal biopsy should be carried out before treating an acute rejection episode unless this will substantially delay treatment or pose a significant risk to the patient. (1C)"
- “We suggest that two cores of renal tissue should be obtained at transplant biopsy since this will increase the sensitivity of the investigation. (2C)”
- “We recommend that a protocol transplant renal biopsy, defined as a biopsy performed in a stable graft without clinical evidence of acute rejection, be considered in the setting of persisting delayed graft function. (1C)”

The Transplantation Society

The Transplantation Society, via the Kidney Disease: Improving Global Outcomes (KDIGO, 2009) Transplant Work Group, states the following regarding acute rejection, renal allograft function, and renal allograft biopsy:[14]

Treatment of Acute Rejection

- "6.1: We recommend biopsy before treating acute rejection, unless the biopsy will substantially delay treatment. (1C)"
- "6.2: We suggest treating subclinical and borderline acute rejection. (2D)"

Kidney Allograft Biopsy

- “9.1: We recommend kidney allograft biopsy when there is a persistent, unexplained increase in serum creatinine. (1C)"
- “9.2: We suggest kidney allograft biopsy when serum creatinine has not returned to baseline after treatment of acute rejection. (2D)"
- “9.3: We suggest kidney allograft biopsy every 7–10 days during delayed function. (2C)”
- “9.4: We suggest kidney allograft biopsy if expected kidney function is not achieved within the first 1–2 months after transplantation. (2D)"
- “9.5: We suggest kidney allograft biopsy when there is"
    - "new onset proteinuria (2C)"
    - "unexplained proteinuria ≥3.0 g/g creatinine or ≥3.0 proteinuria >3.0g/g creatinine or >3.0g per 24 hours. (2C)"

Selected Relevant Publications

The available studies evaluating AlloSure Kidney provide limited evidence regarding the validity of the test for detecting renal graft rejection.[15-25] Several studies have shown an association between levels of donor derived cell-free DNA (dd-cfDNA) and kidney function, donor specific antibodies, non-immune injury, and rejection. However, these studies were hampered by several limitations including observational study designs,

small sample sizes, lack of blinding, and overlapping patient populations. Additionally, the diagnostic threshold has not been definitively established, nor has the importance of absolute percentage of dd-cfDNA compared to relative changes in dd-cfDNA over time. Evidence of clinical utility for AlloSure Kidney is lacking, thus the impact of testing on clinically relevant outcomes and clinical decision-making remains unclear. Further studies are needed that demonstrate the safety of forgoing biopsies based on AlloSure Kidney results, or that demonstrate the use of AlloSure Kidney ultimately leads to improved survival outcomes.

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Alpha-1 Antitrypsin Deficiency Testing

MOL.TS.124.A
v2.0.2024

Introduction

Alpha-1 antitrypsin deficiency (AATD) testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Protease Inhibitor (PI) Typing	82104
SERPINA1 Sequencing	81479
SERPINA1 Targeted Mutation Analysis	81332

Criteria

Introduction

Requests for alpha-1 antitrypsin deficiency (AATD) testing are reviewed using these criteria.

Protease Inhibitor Typing or SERPINA1 Targeted Mutation Analysis

Protease inhibitor (PI) typing or SERPINA1 targeted mutation analysis (*S, *Z) is considered medically necessary in individuals who meet the following criteria:

- Abnormally low (less than 120mg/dL) or borderline (90-140mg/dL) alpha-1 antitrypsin (AAT) levels; AND
- At least one of the following:
    - Symptomatic adults with emphysema, chronic obstructive pulmonary disease (COPD), or asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators; or
    - Individuals of any age with unexplained liver disease (including obstructive liver disease in infancy); or

- Asymptomatic individuals with persistent obstruction on pulmonary function tests who have identifiable risk factors (e.g., cigarette smoking, occupational exposure); or
- C-ANCA positive vasculitis; or
- Adults with necrotizing panniculitis; or
- Siblings of an individual with AATD, AND

- Render laboratory is a qualified provider of service per the Health Plan policy.

SERPINA1 Sequence Analysis

Sequencing of the SERPINA1 gene is considered medically necessary in individuals who meet the following criteria:

- There are discrepancies between clinical presentation, serum alpha-1 antitrypsin quantification, targeted mutation analysis, and/or PI typing; OR
- The presence of rare variants or null alleles (which cannot be identified by other methods) is suspected, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is alpha-1 antitrypsin deficiency?

Definition

Alpha-1 antitrypsin deficiency (AATD) is an inherited condition which may cause chronic obstructive pulmonary disease (COPD) and liver dysfunction. This condition is also referred to as AAT Deficiency and A1AT Deficiency.

Prevalence

It is estimated that 1 in 5000 to 1 in 7000 people in North America have AATD. AATD commonly affects individuals of Northern European heritage. This disorder is most common in Scandinavia, occurring in approximately 1 in 1500 to 1 in 3000 individuals there.[1] However, AATD is an under-recognized condition, with estimates that only 10% of those affected are actually diagnosed.[2]

Symptoms

The most common clinical manifestation is COPD, particularly emphysema.[1-3] Smoking is a major environmental risk factor for lung disease in AATD.[1,3]

AATD also increases the risk for neonatal or childhood liver disease, manifested by obstructive jaundice and hyperbilirubinemia, and early onset adult liver disease, usually cirrhosis and fibrosis.[1] Individuals are also at increased risk for panniculitis (tender skin

nodules which may be inflammatory and may ulcerate) and C-ANCA positive vasculitis.[1]

Cause

AATD results from mutations in the SERPINA1 gene, which codes for the enzyme alpha-1 antitrypsin (AAT).[1]

Inheritance

AATD is an autosomal recessive disorder.[1]

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

AATD may first be suspected based on reduced serum levels of AAT. Confirmatory testing includes either protease inhibitor typing or genetic testing for common mutations.[1] Sequence analysis may be indicated in certain situations.[1]

SERPINA1 targeted mutation analysis tests for the two common mutations in the gene (Z and S), which make up greater than 95% of the mutations.[1] The Z allele is by far the most common and more severe variant.[3]

SERPINA1 sequencing is available, but only appropriate in limited situations. The proportion of individuals with AATD that have a mutation identified by sequencing is unknown.[1]

Management

Individuals with COPD are treated with standard therapy. Individuals with emphysema may be treated with periodic human serum AAT by intravenous infusion. For individuals with end-stage lung disease, lung transplantation may be considered. Liver transplant may be considered as treatment for those with severe disease. “Dapsone or doxycycline therapy is used for panniculitis; if refractory to this, high-dose intravenous AAT augmentation therapy is indicated.”[1] Individuals are strongly encouraged to avoid exposure to active and passive smoking, environmental pollutants, and excessive alcohol use. Surveillance includes periodic pulmonary and liver function tests.[1]

Survival

The prognosis for individuals with AATD is dependent on the severity of the disease and lifestyle factors. Individuals with AATD may have a normal lifespan; however, those with exposure to cigarette smoke may experience earlier and faster progression of lung disease.

Test information

Introduction

Testing for AATD may include protease inhibitor typing, targeted mutation analysis, and/or next generation sequencing.

Protease Inhibitor Typing

Protease Inhibitor (PI) typing by isoelectric focusing to determine phenotype (PI*Z, PI*S).[1] PI typing can detect normal as well as variant alleles, but cannot detect null alleles[1,2]

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to AATD testing.

American Thoracic Society and European Respiratory Society

The American Thoracic Society and the European Respiratory Society stated that testing for AATD is recommended for the following indications:[3]

- "symptomatic adults with emphysema, chronic obstructive pulmonary disease (COPD), or asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators
- individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly
- asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors, examples include cigarette smoking and occupational exposure
- adults with necrotizing panniculitis, and
- siblings of an individual with AATD."

Selected Relevant Publications

The following selected relevant publications outlined recommendations for the diagnosis of AATD. When ambiguous results are obtained between quantification, genotype or phenotype assays, gene sequencing can identify rare variants or null alleles that would otherwise be missed.

Prins et al. (2008)[5]

Prins et al. (2008) sequenced exons 2, 3, and 5 of the SERPINA1 gene from 66 individuals with AAT concentration less than or equal to 1.0 g/L. They predicted that up to 22% of the disease-associated AATD alleles could be missed by S and Z genotyping or by phenotyping. They also identified rare alleles $M_{procida}$, $M_{palermo}$, $M6_{passau}$, $M_{wurzburg}$, $M_{heerlen}$ and the previously undescribed null alleles $Q0_{Soest}$ and $Q0_{amersfoort}$.

They found pathogenic mutations in 22% of those who had negative PI and targeted mutation testing. The authors recommended direct sequencing of the coding regions of the SERPINA1 gene for individuals with suspected AATD based on a serum AAT concentration ≤1.0 g/L.

Graham et al. (2015)[6]

Graham et al. (2015) found pathogenic mutations with sequencing after PI and targeted mutation analysis were performed. They supported full gene sequencing when there are discrepancies between clinical presentation and genotyping after PI and targeted mutation analysis.

Sandhaus et al. (2016)[7]

Sandhaus et al. (2016) provided recommendations for the diagnosis of AATD based on systematic review and expert scientist and clinician appraisal. For diagnostic

testing of symptomatic individuals, the authors recommended “genotyping for at least the S and Z alleles. Advanced or confirmatory testing should include Pi-typing, AAT level testing, and/or expanded genotyping.” The authors also recommended that the following groups be tested for AATD.

- “All individuals with COPD, regardless of age or ethnicity”
- “All individuals with unexplained chronic liver disease”
- “All individuals with necrotizing panniculitis, granulomatosis with polyangiitis (GPA, formerly Wegener’s granulomatosis), or unexplained bronchiectasis”

In addition, the authors recommended that “adult siblings of individuals identified with an abnormal gene for AAT, whether heterozygote or homozygote, should be provided with genetic counseling and offered testing for AATD”.

Balderacchi et al. (2021)[8]

Balderacchi et al. (2021) reviewed various diagnostic algorithms described in the literature, with particular concern for false negatives. Inclusion of C-reactive protein levels, a marker of inflammation reported to impact observed AAT levels, can decrease the rate of false negative results in individuals with intermediate deficiency. They found the highest sensitivity by using an approach that evaluated all individuals for AAT levels, serum CRP levels, and genotyping of the S and Z alleles.

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Amyotrophic Lateral Sclerosis (ALS) Genetic Testing

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Introduction

Amyotrophic lateral sclerosis (ALS) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
ALS Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
ALS Known Familial Mutation Analysis	81403
Genetic Testing for ALS	S3800
ALS Multigene Panel	81479

Criteria

Introduction

Requests for amyotrophic lateral sclerosis (ALS) genetic testing are reviewed using these criteria.

400 Buckwalter Place Boulevard, Bluffton, SC 29910 (800) 918-8924

Known Familial Mutation Testing

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for ALS that would detect the familial mutation, AND
- Diagnostic Testing for Symptomatic or Presymptomatic Individuals:
  - Genetic ALS known familial mutation identified in a 1st, 2nd, or 3rd degree biological relative(s), and
  - Age 18 years or older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Multigene Panel Testing for ALS

When a multi-gene panel is being requested and will be billed with an appropriate CPT panel code, (e.g. 81479), the panel will be considered medically necessary when the following criteria are met:

- Previous Genetic Testing:
  - No previous ALS multi-gene panel testing, and
  - No previous C9orf72 or SOD1 testing performed, and
  - No known ALS-related mutation in the member's family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Family history of ALS in a first degree relative (i.e., parent, sibling, child), and
  - Evidence of lower motor neuron degeneration (e.g., clinical exam, electrophysiological, muscle or nerve biopsy), and
  - Evidence of upper motor neuron degeneration (e.g., clinical exam, imaging), and
  - Progressive spread of signs within a region or to other regions, and
  - Lower and upper motor neuron disease cannot be explained by another condition, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other Considerations

For information on ALS genetic testing to determine eligibility for targeted treatment, please refer to the guideline *Pharmacogenomic Testing for Drug Toxicity and Response*, as this testing is not addressed here.

Gene panels that are specific to ALS will be considered for medical necessity according to the criteria outlined in this guideline. Panels must include, at minimum, analysis of all of the following genes: C9orf72, SOD1, FUS, TARDBP.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81479*).
        - Gene panels that are specific to ALS and include all of the following genes will be eligible for reimbursement according to the criteria outlined in this guideline: C9orf72, SOD1, FUS, TARDBP.
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
    - When an ALS multi-gene panel is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
        - C9orf72
        - SOD1

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is amyotrophic lateral sclerosis?

Definition

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disease that involves the brain and spinal cord.[1]

Prevalence

Between 4 and 8 out every 100,000 people develop ALS.[2] About 10% of individuals with ALS have at least one other family member affected with ALS.[1] About 85% of ALS occurs in individuals with no family history of ALS.[1]

Symptoms

While ALS historically has been described as primarily affecting motor neurons, additional areas within the frontal and temporal lobes are involved to varying degrees in a subset of individuals.[1] Systems outside the nervous system may also be involved, such as bone (Paget disease of the bone) and muscle (inclusion body myopathy). The clinical picture includes motor decline, and may also include cognitive and behavioral symptoms, based on the location and extent of the degeneration in an individual.[1]

The average age of ALS onset is 55 years in males, and mid 60s in females.[1] Earlier onset of symptoms is seen in individuals with genetic forms of ALS.[1] There are infantile and juvenile onset forms that should also prompt consideration of a genetic etiology.[1]

Cause

Traditionally, a diagnosis of "familial ALS" indicated that two or more close relatives were known to be affected with ALS and "sporadic ALS" indicated that no other relatives are known to have ALS. However, evolving genetic research in ALS and an increase in the clinical use of genetic testing has resulted in new terminology. "Genetic ALS" refers to ALS caused by a pathogenic mutation in a known ALS gene, regardless of family history and "ALS of unknown cause" refers to ALS in which a pathogenic mutation in a known ALS gene has not been identified, also regardless of family history.[1]

Thirty genes have been implicated with varying degrees of certainty to cause genetic ALS and the condition demonstrates genetic overlap with frontotemporal dementia (FTD). Genetic testing for many of the genes is clinically available.[1,4-7]

A pathogenic mutation can be identified in 70% of cases of ALS when there is a family history of the disease.[8] Mutations in SOD1, C9orf72, TARDBP (TDP-43), and FUS account for the greatest number of cases, while the remaining genes are relatively rare causes of the disorder.[1,4-10] The majority of combined ALS/FTD cases with a family history of either disorder are caused by C9orf72 repeat expansions, particularly in Caucasian populations, while the percentage of cases attributed to this gene is somewhat lower in China.[5,10] Many other candidate genes have been identified and are still pending further validation studies.[7]

Inheritance

Genetic ALS can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner.[1] The mode of inheritance is based on family history and molecular genetic testing.

Genes commonly associated with genetic ALS

Some of the most common genetic causes of genetic ALS are summarized below. The remaining genes are relatively rare causes of the disorder. Genetic testing for many of the genes is available clinically.[1,4-9,11,12]

Gene symbol	% of ALS with family history	% of simplex ALS	Inheritance
C9orf72	39%-45%	3%-7%	Autosomal dominant
SOD1	15%-20%	3%	Autosomal dominant, Autosomal recessive
FUS	~4%-8%	Very Rare	Autosomal dominant
TARDBP/TDP43	1%-4%	Unknown	Autosomal dominant

Diagnosis

Most cases of suspected ALS are diagnosed based on a unique combination of symptoms and the exclusion of similar disorders. The Escorial Criteria were developed in 2000 to standardize the clinical diagnosis of ALS.[3] These criteria include:

- the presence of upper and lower motor neuron deterioration
- the progressive spread of symptoms, and
- no clinical evidence of other diseases with similar symptoms.

Management

Treatment for individuals with ASL is palliative. There are three FDA-approved drugs available, including a gene-specific treatment for individuals with ALS due to a SOD1 mutation.[1] "Many individuals benefit from care by a multidisciplinary team that includes a neurologist, specially trained nurses, pulmonologist, speech therapist, physical therapist, occupational therapist, respiratory therapist, nutritionist, psychologist, social worker, and genetic counselor."[1]

Survival

ALS is fatal. Disease duration is variable and can range from months to several decades. Approximately half of affected individuals die within five years of symptom onset.[1] Treatment focuses on slowing progression with medication and therapy.[1]

Test information

Introduction

Testing for genetic forms of ALS may include known familial mutation testing, targeted expansion analysis of C9orf72, or next generation sequencing of a single gene or in multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutation analysis can provide predictive information about the risk to develop genetic ALS. It can also be used to diagnose ALS when the individual does not yet meet the full ALS diagnostic criteria.[13]

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Expansions of the hexanucleotide repeat non-coding region of the open reading frame C9orf72 (a protein as yet uncharacterized) are the most frequent cause of genetic ALS and can be assessed through targeted analysis.[1,8] Although estimation of the repeat size is typically accurate, there is disagreement as to the normal and pathogenic repeat

size ranges.[14] In general, more than 30 hexanucleotide repeats are considered pathogenic and Southern blot is considered the gold standard for clinical testing.[11]

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to ALS genetic testing.

European Federation of Neurological Societies

A European Federation of Neurological Societies Task Force (EFNS, 2012) addressed presymptomatic testing in its diagnosis and management guidelines: “Presymptomatic genetic testing should only be performed in first-degree adult blood relatives of patients with a known gene mutation. Testing should only be performed on a strictly voluntary basis as outlined (see Table 7 in the original guideline document) and should follow accepted ethical principles.” [15]

The EFNS (2012) stated the following regarding molecular testing for ALS:[15]

- “Clinical DNA analysis for gene mutations should only be performed in cases with a known family history of ALS, and in sporadic ALS cases with the characteristic phenotype of the recessive D90A mutation.”
- “Clinical DNA analysis for gene mutations should not be performed in cases with sporadic ALS with a typical classical ALS phenotype.”

- "In familial or sporadic cases where the diagnosis is uncertain, SMN, androgen receptor, or TARDBP, FUS, ANG, or SOD1 DNA analysis may accelerate the diagnostic process."
- "Before blood is drawn for DNA analysis, the patient should receive genetic counseling. Give the patient time for consideration. DNA analysis should be performed only with the patient's informed consent."

The EFNS (2011) addressed the molecular diagnosis of ALS and other neurogenetic disorders:[16]

- "Currently molecular diagnosis mainly has implications for genetic counseling rather than for therapy. However, when more directed causal therapies become available in the future, establishing a correct genetic diagnosis in a given patient will be essential. Despite the rather low prevalence, sequencing of the small SOD1 gene should be considered in patients with ALS with dominant inheritance to offer presymptomatic or prenatal diagnosis, if this is requested by the family (Level B)."

World Federation of Neurology Research Group on Motor Neuron Diseases

The World Federation of Neurology Research Group on Motor Neuron Diseases (WFNALS, 2015) revised the El Escorial criteria:[17]

- These revised criteria did not specify when genetic testing should be done, but stated "If a pathogenic mutation in a disease-causing gene is found in the patient and segregates with the disease the term hereditary or primary genetic ALS (HALS/GALS) should be used. The finding of a pathogenic mutation in a known gene can substitute for either lower or upper motor neuron signs, so that diagnosis of ALS can be made on the basis of UMN or LMN signs in one body region, associated with a positive genetic test."
- "ALS can be defined as Mendelian in inheritance if a disease-causing gene variant can be shown to segregate within a family. In such cases the genetic variant can serve as a substitute for upper motor neuron deficits or a second limb or region (rule of two)."

Consensus guidelines from the WFNALS (2000) revised the El Escorial criteria to improve ALS diagnostic sensitivity.[3] This group didn't specify when genetic testing should be done, but stated, "The demonstration of the presence of a pathogenetically relevant gene mutation can assist in the diagnosis of ALS (such as SOD1)".

These criteria set a lower threshold for diagnosis when an ALS-causing mutation is known in the family. For example, an individual may be diagnosed as "Clinically Definite Familial ALS — Laboratory-supported" with evidence of only upper or lower motor neuron disease in one region; whereas a definite diagnosis without genetic test results requires upper and lower motor neuron disease in three regions.

Selected Relevant Publications

An ALS Expert Panel (2023) developed evidence-based, consensus guidelines for care of individuals with ALS that stated:[18]

- "...all persons with ALS should be offered single-step genetic testing, consisting of a C9orf72 assay, along with sequencing of SOD1, FUS, and TARDBP at a minimum."
- In addition, gene panels should include "Any gene for which the Food and Drug Administration (FDA) approves a targeted therapy" and "Genes rated as 'strong' or 'definitively' associated with ALS by ClinGen".
- The guideline stated that pretest genetic counseling should be provided. This should include pedigree analysis, risk assessment, discussion of genetic heterogeneity, penetrance, and inheritance patterns, and a review of the possible test results such as positive, negative, and variant of uncertain significance. Furthermore, pretest genetic counseling should also "prepare individuals for possible personal, psychological, and economic impacts of testing on themselves and their family members."
- "All persons with ALS who have genetic testing should receive posttest counseling." The points to review in the posttest counseling sessions are outlined in the consensus guideline.
- The consensus guideline also provided information for commercial laboratories on testing methods and reporting of the C9orf72 mutation and other genes.

An expert recommendation (2016) for predictive genetic counseling and testing for genetic ALS stated:[19]

- Testing should be voluntary and include informed consent.
- Psychosocial readiness to undergo presymptomatic testing should be assessed.
- Genetic counseling should be provided and at least two counseling sessions should be performed. "These may include predecision counseling as well as pretest and posttest counseling." Specific points to review during each of these sessions were discussed in detail.
- Individuals have the option to not undergo testing or may decide to not receive the results.
- The rendering laboratory should be a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory.

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Angelman Syndrome Genetic Testing

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Introduction

Angelman syndrome genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Chromosomal Microarray [BAC], Constitutional	81228
Chromosomal Microarray [CGH], Constitutional	S3870
Chromosomal Microarray [SNP], Constitutional	81229
Chromosome 15 Uniparental Disomy	81402
Cytogenomic (genome-wide) Analysis for Constitutional Chromosomal Abnormalities; Interrogation of Genomic Regions for Copy Number and Loss-of-heterozygosity Variants, Low-pass Sequencing Analysis	81349
FISH Analysis for 15q11-q13 Deletion	88271
Imprinting Center Defect Analysis	81479
Imprinting Center Known Familial Mutation Analysis	81403
SNRPN/UBE3A Methylation Analysis	81331
UBE3A Deletion/Duplication Analysis	81479
UBE3A Known Familial Mutation Analysis	81403
UBE3A Sequencing	81406

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Criteria

Introduction

Requests for Angelman syndrome testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous UBE3A sequencing or imprinting center defect analysis testing that would detect the familial mutation, AND
- Family History:
  - Known familial UBE3A mutation in a blood relative, or
  - Known familial imprinting center defect mutation in a blood relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

SNRPN/UBE3A Methylation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous SNRPN/UBE3A methylation analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Some combination of the following:
    - Movement or balance disorder, typically with ataxia, or
    - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements),or hypermotoric behavior, or
    - Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Deletion Analysis (FISH for 15q11-q13 Deletion or Chromosomal Microarray)

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous chromosomal microarray, and
  - No previous 15q11-q13 deletion analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Some combination of the following:
    - Movement or balance disorder, typically with ataxia, or
    - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements),or hypermotoric behavior, or
    - Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Chromosome 15 Uniparental Disomy (UPD)

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN/UBE3A methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - No previous chromosome 15 UPD studies, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Meets clinical criteria for SNRPN/UBE3A methylation analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Imprinting Center Defect Analysis

- Genetic Counseling:

  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN/UBE3A methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - Previous chromosome 15 UPD testing is negative, and
  - No previous imprinting center (IC) analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Meets clinical criteria for SNRPN/UBE3A methylation analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

UBE3A Sequencing

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN/UBE3A methylation analysis results are normal, and
  - No previous sequencing of UBE3A, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Meets clinical criteria for SNRPN/UBE3A methylation analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

UBE3A Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN/UBE3A methylation analysis results are normal, and
  - Normal UBE3A sequencing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Meets clinical criteria for SNRPN/UBE3A methylation analysis, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Angelman syndrome?

Definition

Angelman syndrome (AS) is a genetic disorder that can cause intellectual disability, severe speech impairment, tremors, seizures, microcephaly, and decreased need for sleep.

Prevalence

The prevalence of AS in the population is one in 12,000-24,000.[1]

Symptoms

Clinical features of Angelman syndrome (quoted directly):[2]

A. Consistent (100%)

- Developmental delay, functionally severe
- Movement or balance disorder, usually ataxia of gait, and/or tremulous movement of limbs. Movement disorder can be mild. May not appear as frank ataxia but can be forward lurching, unsteadiness, clumsiness, or quick, jerky motions
- Behavioral uniqueness: any combination of frequent laughter/smiling; apparent happy demeanor; easily excitable personality, often with uplifted hand-flapping, or waving movements; hypermotoric behavior
- Speech impairment, none or minimal use of words; receptive and non-verbal communication skills higher than verbal ones

B. Frequent (more than 80%)

- Delayed, disproportionate growth in head circumference, usually resulting in microcephaly (2 SD of normal OFC) by age 2 years. Microcephaly is more pronounced in those with 15q11.2-q13 deletions
- Seizures, onset usually <3 years of age. Seizure severity usually decreases with age but the seizure disorder lasts throughout adulthood
- Abnormal EEG, with a characteristic pattern, as mentioned in the text. The EEG abnormalities can occur in the first 2 years of life and can precede clinical features, and are often not correlated to clinical seizure events

C. Associated (20%-80%)

- Flat occiput
- Occipital groove
- Protruding tongue
- Tongue thrusting; suck/swallowing disorders
- Feeding problems and/or truncal hypotonia during infancy
- Prognathia
- Wide mouth, wide-spaced teeth
- Frequent drooling
- Excessive chewing/mouthing behaviors
- Strabismus
- Hypopigmented skin, light hair, and eye color compared to family, seen only in deletion cases
- Hyperactive lower extremity deep tendon reflexes
- Uplifted, flexed arm position especially during ambulation
- Wide-based gait with pronated or valgus-position ankles
- Increased sensitivity to heat
- Abnormal sleep-wake cycles and diminished need for sleep
- Attraction to/fascination with water, fascination with crinkly items such as certain papers and plastics
- Abnormal food related behaviors
- Obesity (in older child)
- Scoliosis
- Constipation

Causes

Features of Angelman syndrome are caused by a missing or defective UBE3A gene inherited from the individual's mother.[3]

A missing or defective UBE3A gene can be caused by a gene deletion, gene mutation, uniparental disomy (two copies of paternal chromosome), imprinting defect, or a chromosome rearrangement.[3,4]

Diagnosis

The diagnosis of AS is established in an individual who has findings on molecular genetic testing that are consistent with deficient expression or function of the maternally inherited UBE3A allele.[1,2,5,6]

Genetic testing is recommended when an individual has all of the clinical findings in sub-bullets A and B listed above under "symptoms" and whose developmental history is as follows:[2]

- Unremarkable prenatal and birth history. The neonate does not present with an abnormal head circumference or major birth defects although feeding difficulties may be evident.
- At 6-12 months of age, developmental delays become evident and there may be low muscle tone of the trunk. Differences in limb movements and/or increased smiling may be noticed.
- There is no regression but there is delayed development in progression of skills.
- Metabolic, hematologic, and chemistry profiles are normal.
- Overall normal brain MRI or CT although there may be "mild cortical atrophy or dysmyelination".
- The authors note that "these findings are useful as inclusion criteria but deviations should not exclude diagnosis"

Determination of recurrence risk following a diagnosis of AS may require genetic testing of one or both parents depending on the identified molecular cause.[5,6]

Management

“Anti-seizure medication for seizures. Accommodation for hypermotoric behaviors and disruptive nighttime wakefulness. Behavior modification can be effective for disruptive or self-injurious behaviors. Physical therapy, occupational therapy, and speech therapy with an emphasis on nonverbal methods of communication, including augmentative communication aids (e.g., picture cards, communication boards) and signing. Individualization and flexibility in school settings. Routine management of gastroesophageal reflux, feeding difficulties, constipation, and strabismus. Thoraco-lumbar jackets and/or surgical intervention for scoliosis. Bracing or surgery as needed for subluxed or pronated ankles or tight Achilles tendons."[1]

Other recommendations include the following:

- Sleep disturbance: Sleep concerns may require consideration of effect on other aspects of the individual's health, etiological investigations, behavioral interventions, medication trials, and evaluation by a sleep specialist.[6]
- "Surveillance: Evaluation of older children for obesity associated with an excessive appetite. Annual clinical examination for scoliosis; ophthalmology examination in the first year if strabismus is present; ophthalmology exam at age two years with follow up per ophthalmologist; clinical examination for scoliosis annually."[1]
- "Agents/circumstances to avoid: Overtreatment with sedating medications in order to reduce hyperexcitable and hypermotoric behavior. Overtreatment with antiepileptic drugs when movement abnormalities are mistaken for seizures and/or when EEG abnormalities persist even as seizures are controlled."[1]

Test information

Introduction

Testing for Angelman syndrome may include known familial mutation analysis, SNRPN/UBE3A methylation analysis, chromosomal microarray, FISH analysis for 15q11-q13 deletion, chromosome 15 uniparental disomy (UPD), imprinting center defect analysis, or UBE3A sequencing and deletion testing.

Known Familial Mutation Analysis: Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing. Analysis for known familial mutations typically includes only the specific mutation identified in the family, but if available, a targeted mutation panel that includes the familial mutation(s) may be performed.

SNRPN/UBE3A Methylation Analysis: This test is typically the first test in the evaluation of both Angelman syndrome (AS) and Prader-Willi syndrome (PWS). It will detect about 80% of individuals with AS and greater than 99% of individuals with PWS. However, DNA methylation analysis does not identify the underlying cause, which is important for determining the risk to future siblings. This risk ranges from less than 1% to up to 50%, depending on the genetic mechanism. Follow-up testing for these causes may be appropriate.

Chromosomal Microarray or FISH Analysis for 15q11-q13 Deletion: If DNA methylation analysis for AS or PWS is abnormal, deletion analysis is typically the next step. Approximately 70% of cases of both AS and PWS have a deletion in one copy of chromosome 15 involving the 15q11.2-q13 region. FISH (fluorescence in situ hybridization) analysis and chromosomal microarray (CMA, array CGH) can detect such deletions. If CMA has already been done, FISH is not likely to be necessary.

Chromosome 15 Uniparental Disomy (UPD): If DNA methylation analysis is abnormal but deletion analysis is normal, UPD analysis may be an appropriate next step for evaluation of both AS and PWS. About 28% of PWS cases are due to maternal UPD (both chromosome 15s are inherited from the mother). About 7% of cases of AS are due to paternal UPD (both chromosome 15s are inherited from the father). Both parents must be tested to diagnose UPD.

Imprinting Center Defect Analysis: This test may be considered in the evaluation of AS and PWS when methylation is abnormal, but FISH (or array CGH) and UPD studies are normal. Individuals with such results are presumed to have an imprinting defect. An abnormality in the imprinting process has been described in a minority of cases. However, imprinting center deletions may be familial, and if familial, the recurrence risk can be up to 50%.

UBE3A Sequencing

If DNA methylation analysis is normal, UBE3A gene mutations should be suspected. Such mutations are found in 11% of individuals with Angelman syndrome and can only be detected by sequencing the entire gene.[1] These mutations can be carried by the mother of an affected individual and pose up to a 50% risk of recurrence in her other children, and an increased risk to other family members.

UBE3A Gene-Targeted Deletion/Duplication Analysis

"Gene-targeted deletion/duplication analysis detects deletions or duplications in intragenic or other targeted regions…CMA usually detects large 15q11.2-q13 deletions, but in rare instances has detected UBE3A multiexon or whole-gene deletions."[1]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Angelman syndrome genetic testing.

The Angelman Syndrome Foundation

The Angelman Syndrome Foundation (ASF, 2023) recommended the following genetic testing strategy:[4,7]

- UBE3A methylation analysis
  - If normal, consider UBE3A gene sequencing.
  - If abnormal (only paternal alleles are present), a diagnosis of Angelman Syndrome is confirmed. Consider the following to identify the underlying molecular cause for recurrence risk counseling.
- Deletion analysis (chromosomal microarray or FISH for 15q11-q13)
  - If deletion testing is abnormal, FISH testing on the mother should be done to rule out an inherited chromosome abnormality (rare).
  - If deletion testing is normal, consider UPD analysis.
- Uniparental Disomy (UPD) analysis of chromosome 15 to determine whether the proband inherited both copies of chromosome 15 from the father.
- If deletion analysis and UPD analysis are normal, an imprinting center mutation is a likely cause and should be evaluated (which may carry a higher recurrence risk than other causes). A portion of individuals (around 10%) with a clinical diagnosis of Angelman syndrome will not have a molecular cause identified.

Selected Relevant Publications

An expert-authored review (2021) commented on the appropriate diagnostic testing strategy and the utility of familial testing analysis:[1]

Diagnostic Testing:

- DNA methylation testing is usually the first tier test. If methylation analysis is abnormal, additional analysis is needed to identify the molecular cause.

- If methylation analysis is normal, UBE3A sequencing should be considered, followed by deletion/duplication analysis.

Familial Testing:

- Individuals with an imprinting center (IC) deletion can have a phenotypically normal mother who also has an IC deletion. If a proband's mother has a known IC deletion, the risk to the sibs is 50%.
- UBE3A pathogenic variants can be inherited or de novo. Cases of somatic and germline mosaicism for a UBE3A pathogenic variant have been noted. If a proband's mother has a UBE3A pathogenic variant, the risk to the sibs is 50%.
- "If a proband's mother is heterozygous for a known imprinting center deletion or UBE3A pathogenic variant, the mother's sibs are also at risk of having the imprinting center deletion or the UBE3A pathogenic variant. Each child of the unaffected heterozygous sister is at a 50% risk of having AS. Unaffected maternal uncles of the proband who are heterozygous are not at risk of having affected children, but are at risk of having affected grandchildren through their unaffected daughters who inherited the imprinting center deletion or UBE3A pathogenic variant from them."

References

Introduction

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Ashkenazi Jewish Carrier Screening

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Introduction

Ashkenazi Jewish carrier screening is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Ashkenazi Jewish Genetic Disorders Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Ashkenazi Jewish Genetic Disorders Sequencing	81412
ASPA Targeted Mutation Analysis	81200
BCKDHB Targeted Mutation Analysis	81205
BLM Targeted Mutation Analysis	81209
CFTR Targeted Mutation Analysis	81220
FANCC Targeted Mutation Analysis	81242
G6PC Targeted Mutation Analysis	81250
GBA Targeted Mutation Analysis	81251
HEXA Targeted Mutation Analysis	81255
IKBKAP Targeted Mutation Analysis	81260

Procedures addressed by this guideline	Procedure codes
MCOLN1 Targeted Mutation Analysis	81290
SMPD1 Targeted Mutation Analysis	81330

Criteria

Introduction

Requests for Ashkenazi Jewish carrier screening are reviewed using these criteria.

Single Ashkenazi Jewish Genetic Diseases Carrier Screening Tests

Carrier screening may be considered for a single Ashkenazi Jewish disease if any of the following are met:

- The individual is of Ashkenazi Jewish ancestry, OR
- The individual has a family history of the condition for which testing is being requested, OR
- The individual's partner is a known carrier or affected with the condition for which testing is being requested

Ashkenazi Jewish Genetic Diseases Carrier Screening Panels

Carrier screening may be considered for all or any desired subset of the Ashkenazi Jewish genetic diseases eligible for coverage per the Coverage Guidance table when the following criteria are met:

- The individual is planning a pregnancy or currently pregnant, AND
- At least one partner of a couple is Ashkenazi Jewish (NOTE: Detection rates for testing are higher in people with Ashkenazi Jewish ancestry. If only one partner of a couple is Ashkenazi Jewish, testing should start in that person when possible.)

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will

not be reimbursable as billed. Any claim may require submission of medical records for post service review.

When testing is otherwise reimbursable, the following limitations apply:

- If an Ashkenazi Jewish carrier screening panel was previously performed and an updated, larger panel is being requested, only testing of previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- If testing will be billed using procedure code 81412 to represent all tests performed for the assessment of carrier status based on Ashkenazi Jewish ancestry, no additional tests for this purpose will be reimbursed for the same date of service.
- If testing will be billed for separate genes because the panel code is not more appropriate (e.g., fewer than the 9 stated genes will be assessed or a different methodology is used), reimbursement for individual gene tests will be assessed based on the guidance provided in the Criteria section above and in the Table: *Coverage Guidance for Genes Included in Ashkenazi Jewish Carrier Screening Tests*.

Table: Coverage Guidance for Genes Included in Ashkenazi Jewish Carrier Screening Tests

Condition, Gene, CPT Code, Required Claim Code, Guideline ID

Condition	Gene	CPT	Required Claim Code	Guideline ID
Bloom syndrome	BLM	81209	NONE	MOL.TS.129
Canavan disease	ASPA	81200	NONE	MOL.TS.129
Cystic fibrosis	CFTR	81220	NONE	MOL.TS.129
Dihydrolipoamide dehydrogenase deficiency	DLD	81406	DLD	MOL.TS.129
Familial dysautonomia	ELP1	81260	NONE	MOL.TS.129
Familial hyperinsulinism	ABCC8	81401	ABCC8	MOL.TS.129
Fanconi anemia, type C	FANCC	81242	NONE	MOL.TS.129
Gaucher disease, type 1	GBA	81251	NONE	MOL.TS.129

Condition	Gene	CPT	Required Claim Code	Guideline ID
Glycogen storage disease, type 1A	G6PC	81250	NONE	MOL.TS.129
Joubert syndrome, type 2	TMEM216	81479	TMEM216	MOL.TS.129
Maple syrup urine disease, type 1b	BCKDHB	81205	NONE	MOL.TS.129
Mucolipidposis, type IV	MCOLN1	81290	NONE	MOL.TS.129
Nemaline myopathy, type 2	NEB	81400	NEB	MOL.TS.129
Niemann-Pick disease, type A	SMPD1	81330	NONE	MOL.TS.129
Tay-Sachs disease	HEXA	81255	NONE	MOL.TS.129
Usher syndrome, type 1F	PCDH15	81400	PCDH15	MOL.TS.129
Usher syndrome, type 3	CLRN1	81400	CLRN1	MOL.TS.129

Note Other tests may be eligible for coverage under the above criteria if the condition is associated with significant morbidity and mortality, the allele frequency is >1% in the Ashkenazi Jewish population, and the selected test method has >90% detection rate for disease-causing mutations.

What is Ashkenazi Jewish carrier screening?

Definition

Ashkenazi Jewish carrier screening is available for certain genetic conditions that are either more common or for which there are higher mutation detection rates in the Ashkenazi Jewish population. “Ashkenazi” refers to someone whose Jewish ancestors originally came from Central or Eastern Europe, such as Russia, Poland, Germany,

Hungary, Lithuania. Most Jewish people in the US are of Ashkenazi descent. There are regional differences in the number and types of tests commonly offered. Individuals and providers may choose all or a subset of these conditions.[1-3]

Inheritance

These Jewish genetic diseases are inherited in an autosomal recessive manner. An affected individual must inherit a gene mutation from both parents.[1,2]

- Individuals who inherit only one mutation are called carriers. Carriers usually do not show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children.
- Two carriers of the same disease have a 25% chance, with each pregnancy, of having a child with the disorder.

Prevalence

While these genetic diseases are individually rare, the overall chance for an individual of Ashkenazi Jewish descent to be a carrier for one of these genetic diseases is 1 in 4 to 1 in 5.[2,3] An individual can also be a carrier of more than one condition.

People from other ethnic backgrounds can be carriers of these conditions, but it is generally less common. The test is typically not as effective at identifying carrier status in individuals of non-Ashkenazi Jewish descent.

Test information

Introduction

Ashkenazi Jewish carrier screening can be offered to couples or individuals of Ashkenazi Jewish descent when they are planning a pregnancy (preconceptional) or during a pregnancy (prenatal).[1-3]

One member of couple is Jewish

If only one member of the couple is Ashkenazi Jewish, carrier screening should start with the Ashkenazi Jewish partner. In general, both parents must be carriers to have an affected child, so reproductive partners of known carriers should also be offered testing even if not Jewish. In some cases, full gene sequencing would be most appropriate for testing of a non-Jewish partner.

Purpose of test

Carrier screening generally looks for a small number of gene mutations that are particularly common in the Ashkenazi Jewish population, although an increasing number of full gene sequencing panels are becoming available.

In addition, enzyme analysis is particularly effective for Tay-Sachs disease and is generally preferred to mutation testing.

Detection rate

The carrier detection rate is greater than 95% in the Ashkenazi Jewish population for most diseases.[3]

The detection rate for these tests in the non-Ashkenazi population is unknown for most conditions, but generally low. Exceptions include cystic fibrosis and Tay-Sachs enzyme analysis, which each have good detection rates in non-Jewish populations.

A negative test result in one or both partners significantly lowers the chance of an affected child, but does not eliminate it.[2]

Commonly tested conditions

The genes included in carrier screening panels vary widely between laboratories. The following table includes the most commonly tested conditions.

Ashkenazi Jewish genetic disease	Ashkenazi carrier frequency	What the test looks for	Chance of correctly finding an Ashkenazi Jewish carrier
Bloom syndrome[3]	1/107	1 mutation (2281del6ins7)	Greater than 99%
Canavan disease[3]	1/41	2 mutations (E285A, Y231X)	97.4%
Cystic fibrosis[2]	1/29	23 most common mutations in several ethnic groups	97%
Dihydrolipoamide dehydrogenase deficiency[4]	1/107	2 mutations (G229C and Y35X)	Greater than 95%
Familial dysautonomia[3]	1/31	2 mutations (2507+6TtoC, R696P)	Greater than 99%
Familial hyperinsulinism[4]	1/68	2 mutations (c.3989-9G>A and Phel1387del)	90%
Fanconi anemia group C[3]	1/89	1 mutation (IVS4+4AtoT)	Greater than 99%
Gaucher disease[3]	1/18	4 mutations (N370S, 84GG, L444P, IVS2+1GtoA)	Up to 94.6%

Ashkenazi Jewish genetic disease	Ashkenazi carrier frequency	What the test looks for	Chance of correctly finding an Ashkenazi Jewish carrier
Glycogen storage disease type 1A (GSD1A)[5]	1/71	1 mutation (R83C)	93% to 100%
Joubert syndrome 2[6]	1/92	1 mutation (R12L)	99%
Maple syrup urine disease (MSUD)[7,8]	1/80	3 mutations (R183P, G278S, E372X)	About 99%
Mucolipidosis IV[3]	1/127	2 mutations (IVS3–2AtoG, Del6.4kb)	95%
Nemaline myopathy[4]	1/168	1 mutation (R2478_D2512del)	Greater than 95%
Niemann-Pick disease type A[3]	1/90	3 mutations (R496L, L302P, fsP330)	97%
Tay-Sachs disease[3]	1/90	Mutation analysis: 3 mutations (1278insTATC, 1421+1GtoC, G269S) OR	92-94%
		Hexosaminidase A enzyme analysis	About 98%
Usher syndrome III[4]	1/120	1 mutation (N48K)	Greater than 95%

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Ashkenazi Jewish carrier screening.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2008) guidelines outlined criteria for adding disorders to carrier screening in the Ashkenazi Jewish population:[3]

- the natural history must be well understood
- people affected with the disorder must have significant morbidity and/or mortality, and

- the test must have greater than 90% detection OR the allele frequency must be at least 1%.

Conditions that meet ACMG criteria

The following conditions meet these criteria:

- cystic fibrosis
- Canavan disease
- familial dysautonomia
- Tay-Sachs disease
- Fanconi anemia (group C)
- Niemann-Pick (type A)
- Bloom syndrome
- mucolipidosis IV
- Gaucher disease
- dilipoamide dehydrogenase deficiency[4]
- familial hyperinsulinism[4]
- glycogen storage disease type 1a[5]
- Joubert syndrome 2[6]
- maple syrup urine disease [7,8]
- nemaline myopathy,[4] and
- Usher syndrome type III.[4]

ACMG (2021) released an educational practice resource on carrier screening.[9] This consensus statement asserted that general population carrier screening should be ethnicity and family history agnostic. To accomplish this, screening all individuals in the prenatal/preconception period for autosomal recessive and X-linked conditions with a carrier frequency of >1/200 was suggested. ACMG generated a list of 113 genes meeting these criteria.

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2017; reaffirmed 2023) Committee on Genetics issued an opinion that "ethnic-specific (e.g. Ashkenazi Jewish), panethnic, and expanded carrier screening are acceptable strategies for pre-pregnancy and prenatal carrier screening."[10]

If providers choose to offer ethnic-specific screening to individuals of Ashkenazi Jewish ancestry, ACOG recommended that screening include Canavan disease, cystic fibrosis, familial dysautonomia, Tay-Sachs disease, Bloom syndrome, familial

hyperinsulinism, Fanconi anemia, Gaucher disease, glycogen storage disease type I, Joubert syndrome, maple syrup urine disease, mucolipidosis type IV, Niemann-Pick disease, and Usher syndrome.[2]

Regardless of screening strategy chosen by the provider and regardless of the individual's ethnicity, ACOG recommended that all individuals who are considering pregnancy or are already pregnant be "...offered carrier screening for cystic fibrosis and spinal muscular atrophy, as well as a complete blood count and screening for thalassemias and hemoglobinopathies. Fragile X premutation carrier screening is recommended for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome, or women with a personal history of ovarian insufficiency."[10]

National Society of Genetic Counselors

The National Society of Genetic Counselors (NSGC, 2023) issued a practice guideline on carrier screening in support of an expanded panel approach that is ethnicity and family history agnostic. They recommended expanded carrier screening be made available for all individuals considering reproduction and all pregnant reproductive pairs. "The final decision to pursue carrier screening should be directed by shared decision-making, which takes into account specific features of patients as well as their preferences and values."[6]

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Ataxia-Telangiectasia Genetic Testing

MOL.TS.130.A
v2.0.2024

Introduction

Ataxia-telangiectasia genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
ATM Deletion/Duplication Analysis	81479
ATM Known Familial Mutation Analysis	81403
ATM Sequencing	81408

Criteria

Introduction

Requests for ataxia-telangiectasia (A-T) genetic testing are reviewed using these criteria.

ATM Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation(s), AND
- Carrier Screening Individuals:
  - Known family mutation in ATM in 1st, 2nd, or 3rd degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - ATM mutations identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

ATM Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous ATM gene sequencing, and
  - No known ATM mutation in family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Elevated alpha-fetoprotein (AFP) levels, or
  - Decreased ATM protein detected by immunoblotting, and
  - Progressive cerebellar ataxia, or
  - Truncal and gait ataxia, or
  - Oculomotor apraxia, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1st, 2nd, or 3rd, degree relative diagnosed with Ataxia-Telangiectasia clinical diagnosis, family mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for ATM mutation, and
  - Has living children with this partner, or
  - Has the potential and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

ATM Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous deletion/duplication analysis of ATM, and
  - No mutations detected in full sequencing, or
  - Heterozygous for mutation and individual is expected to be affected (eg, elevated alpha-fetoprotein levels, decreased ATM protein detected by immunoblotting (if performed), other features of disorder are present), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Ataxia-telangiectasia?

Definition

Ataxia-telangiectasia (A-T) is a progressive neurological disorder. Individuals with A-T also have an increased risk for immunodeficiency, frequent infections, and malignancy. Additionally, they are unusually sensitive to ionizing radiation.[1]

Prevalence

The prevalence of A-T is approximately 1 in 40,000 to 1 in 100,000 live US births.[1-3] The estimated pan-ethnic carrier frequency of mutations in the ATM gene is approximately 1% in the general population.[4,5]

Symptoms

The onset of symptoms of A-T is typically between the ages of 1 and 4 years.[1,3] Signs and symptoms of A-T include[1,6]

- progressive cerebellar atrophy and dysfunction, which can present with the following symptoms at a young age:
  - truncal and gait ataxia,
  - ocular apraxia,
  - slurred speech, and
  - head tilting, after the age of 6 months;
- conjunctival telangiectasias;
- immunodeficiencies and frequent non-opportunistic infections;
- malignancies, especially leukemias and lymphomas; and
- radiation sensitivity.

Cause

A-T is caused by biallelic mutations in the ATM gene.

Inheritance

A-T is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each

pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

The diagnosis may be suspected based on clinical symptoms and preliminary laboratory data.[1] Individuals with A-T often have an elevated serum alpha-fetoprotein (AFP) level and immunoblotting may demonstrate reduce or absent ATM protein.[1] A diagnosis of A-T is established in an individual with characteristic clinical features and/or biallelic pathogenic mutations in ATM.

Sequence analysis of the ATM gene can identify 90-95% of A-T mutations in affected individuals.[1]

Deletion and duplication analysis of the ATM gene can identify another 1-2% of mutations.[1]

Management

Individuals with A-T are best cared for by a multidisciplinary team. Management and treatment includes addressing the neurological and immunodeficiency symptoms while also monitoring for malignancy.[1]

Survival

Although individuals with A-T live to adulthood, they are at an increased risk for early death. Currently, most individuals live beyond 25 years, with some surviving into their 50s.[1] Cause of death is associated with A-T associated cancers, infection, and pulmonary failure.[7]

Related Conditions

Individuals with a single ATM mutation are carriers. ATM carriers may be at an increased risk for breast cancer, especially women with a strong family history of breast cancer.[2,4,5,7,8] Epidemiological data has also suggested an increased risk for cardiovascular disease in carriers.[5,7] Therefore, the detection of carriers can have medical management implications for breast cancer and cardiovascular disease screening.

Test information

Introduction

Testing for A-T may include known familial mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to A-T genetic testing.

International Workshop on A-T

The Eighth International Workshop on Ataxia-telangiectasia (A-T) was convened in 1999. The workshop described ATM mutations and cancer risk in carriers, and potential therapeutic approaches. Genetic testing strategies were not described.[9] A subsequent workshop in 2012 provided updated information about the cancer risks and potential treatment options, but still did not address genetic testing strategies.[10]

References

Introduction

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Autism, Intellectual Disability, and Developmental Delay Genetic Testing

MOL.TS.269.A
v2.0.2024

Introduction

Autism, intellectual disability, and developmental delay genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures covered by this guideline	Procedure codes
AFF2 gene analysis; evaluation to detect abnormal (eg, expanded) alleles	81171
AFF2 gene analysis; characterization of alleles (eg, expanded size and methylation status)	81172
Autism Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Autism Known Familial Mutation Analysis	81403

400 Buckwalter Place Boulevard, Bluffton, SC 29910 (800) 918-8924

Procedures covered by this guideline	Procedure codes
Developmental Delay Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Developmental Delay Known Familial Mutation Analysis	81403
Intellectual Disability Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Intellectual Disability Known Familial Mutation Analysis	81403
X-linked Intellectual Disability Duplication/ Deletion Analysis Panel	81471
X-linked Intellectual Disability Sequence Analysis Panel	81470

Criteria

Introduction

Requests for Autism Spectrum Disorder (ASD), Intellectual Disability (ID), and Developmental Delay testing are reviewed using the following clinical criteria.

Known Familial Mutation Testing

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for the known familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Known family mutation in a causative gene in 1st, 2nd, or 3rd degree biological relative, OR
- Prenatal Testing for At-Risk Pregnancies:
  - Known familial disease-causing mutation identified in both biological parents (if recessive), or a single biological parent or an affected sibling of the pregnancy (if dominant), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Autism, Intellectual Disability and Developmental Delay Single Gene Diagnostic Tests (Sequencing and Deletion/Duplication)

- The member has a formal diagnosis of ASD/autism, intellectual disability, and/or developmental delay as made by an appropriate health care professional, AND
- The member has a condition that will benefit from information provided by the requested gene testing based on the following:
  - The member displays at least one clinical feature (in addition to autism, intellectual disability, and/or developmental delay) of the suspected condition for which testing is being requested, AND
    - The member's medical management would be significantly altered by the genetic diagnosis, or
    - A particular treatment is being considered for the member that requires a genetic diagnosis, OR
  - The member meets all criteria in a test-specific guideline, if available (see the Table below for a list of genes, associated conditions, and applicable guidelines), AND

- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Autism, Intellectual Disability, and Developmental Delay Multi-Gene Panels

Multi-gene panels for individuals with a primary medical diagnosis of ASD, ID, and/or GDD (global developmental delay) have not demonstrated a high diagnostic yield and are not likely to lead to a change in treatment. Comprehensive ASD and/or ID/GDD panels, regardless of panel size, are experimental, investigational, or unproven (E/I/U). However, separate clinical guidelines may apply to panel testing and exome sequencing for members who have findings in addition to ASD/ID/GDD, such as seizures or multiple congenital anomalies.

Other considerations

- ASD, ID, and/or GDD testing may be performed as part of a chromosomal microarray, exome sequence, or genome sequence. For information on these tests, please refer to the guidelines *Chromosomal Microarray Testing For Developmental Disorders (Prenatal and Postnatal)*, *Exome Sequencing*, or *Whole Genome Sequencing*, as these tests are not addressed here.
- Genetic testing is only medically necessary once per lifetime. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- This guideline may not apply to genetic testing for indications that are addressed in test-specific guidelines. Please see the test-specific list of guidelines for a complete list of test-specific panel guidelines.

Table: Common neurodevelopmental disorder genes, associated conditions, and applicable guidelines

This list is not all-inclusive.

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
15q11.2	81331	Prader-Willi Syndrome, Angelman Syndrome	Prader-Willi Syndrome testing; Angelman Syndrome Testing.10059	MOL.TS.217; MOL.TS.126

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
AFF2	81171 81172	Fragile X Syndrome 2 (FRAXE)	Autism, Intellectual Disability, and Developmental Delay Genetic Testing	MOL.TS.269
BRAF	81406	Noonan Syndrome, Cardiofaciocutaneous Syndrome	Autism, Intellectual Disability, and Developmental Delay Genetic Testing	MOL.TS.269
CHD7	81407	CHARGE Syndrome	CHARGE Syndrome Genetic Testing	MOL.TS.324
FMR1	81243 81244	Fragile X Syndrome	FMR1-Related Disorders (Fragile X) Genetic Testing	MOL.TS.172
MECP2	81302	Classic Rett Syndrome, Preserved Speech Variant Rett Syndrome, MECP2-Related Epileptic Encephalopathy (males), X-Linked ID	Rett Syndrome Testing 10629	MOL.TS.224
NF1	81408	Neurofibromatosis 1	Neurofibromatosis type 1 Genetic Testing	MOL.TS.301
PTEN	81321	PTEN Hamartoma Tumor Syndromes	PTEN Hamartoma Tumor Syndrome Genetic Testing 10192	MOL.TS.223

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
PTPN11	81406	Noonan Syndrome	Autism, Intellectual Disability, and Developmental Delay Genetic Testing	MOL.TS.269
UBE3A	81406	Angelman Syndrome	Angelman Syndrome Testing 10059	MOL.TS.126

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- Broad Autism Spectrum Disorder panels, Intellectual Disability/Developmental Delay panels, and Neurodevelopmental Disorder panels, regardless of how they are billed, are not reimbursable.

What are Autism Spectrum Disorders, Intellectual Disability, and Global Developmental Delay?

Definition

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in communication and social interaction, as well as restricted, repetitive patterns of behavior, interests, or activities. Intellectual disability (ID, formerly referred to as mental retardation) is "a disability characterized by significant limitations in both intellectual functioning and in adaptive behavior as expressed in conceptual, social and practical adaptive skills."[1] Global developmental delay (GDD) categorizes younger children (typically less than 5 years of age) who have significant delay (characterized as performance two standard deviations or more below the mean on

age-appropriate, standardized, normal-referenced testing) in two or more developmental domains, including gross or fine motor, speech and language, cognitive, social and personal, and activities of daily living.[2]

Incidence

ASD affects approximately 1/54 children.[3] ID affects 1-3% of the population worldwide.[2] The incidence of GDD is estimated to be comparable to ID.[1,4] All three neurodevelopmental disorders are more common in males.[2,4-7]

Symptoms

ASD was previously divided into categories that included autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder- not otherwise specified (PDD-NOS). With current diagnostic criteria, these categories were subsumed under the diagnosis of ASD.

Symptom onset is in early childhood (typically before 3 years of age).[6,7] ASD is often accompanied by intellectual disability, behavioral difficulties, and/or sensory abnormalities.

ID and GDD may present in infancy or early childhood. ID is assessed in three domains: intelligence (IQ), adaptive behavior, and systems of supports the individual requires.[1] Children with GDD have significant delay in two or more developmental domains. Young children with GDD may later be diagnosed with ID and/or ASD.[2,4] There are both syndromic and non-syndromic forms of inherited ASD, ID, and GDD. The constellation of associated findings is highly dependent on the underlying etiology. Clinical information (e.g. presence of specific congenital malformations, dysmorphic features, and other symptoms) may be used in some cases to help narrow down the suspected cause. In these cases, it may be possible to identify a narrow subset of genes that may be responsible for an individual's neurodevelopmental concerns.

Cause

ASD, ID, and GDD can develop secondary to head injury, birth complication, endocrine disorder (e.g., hypothyroidism), toxic exposure (e.g., fetal alcohol syndrome), inborn error of metabolism (e.g., phenylketonuria), and central nervous system infection.[2,6,7]

There are also many known genetic conditions that are associated with an increased risk for ASD, ID, and GDD. A thorough clinical genetics evaluation is estimated to result in an identified cause in 30–40% of affected individuals with ASD.[6] Chromosome microarray analysis was previously thought to have the highest diagnostic yield of any single test for these disorders, with an estimated detection rate of at least 10-20% for ASD, ID, and GDD (often grouped together as neurodevelopmental disorders, or NDDs).[4,6,8,9] Whole exome and genome sequencing have more recently been demonstrated to have diagnostic yields of up to 35% for those with NDDs and potentially higher for those with other comorbidities such as epilepsy or congenital anomalies.[9]

Inheritance

Inheritance patterns differ between the various syndromes associated with ASD, ID, GDD. Inherited forms of these disorders can show autosomal dominant, autosomal recessive, X-linked, or mitochondrial patterns of inheritance.

Diagnosis

ASD, ID, and GDD are diagnosed through the evaluation of an individual's development and behaviors by an appropriate specialist (such as neurodevelopmental pediatrician or developmental-behavioral pediatrician). Medical tests such as hearing screening, vision screening, and neurological evaluations may also be performed.[2,5] A diagnosis of ASD and/or ID is often difficult to establish in infants and very young children, as the standardized methods used for diagnosis are less reliable in children under the age of 5 years; the term "global developmental delay" is thus used to categorize these individuals.[2] Identifying an underlying genetic etiology for an individual's NDD cannot provide a diagnosis of ASD versus ID versus a specific learning disability.

Management

Management for ASD includes behavioral interventions such as applied behavioral analysis (ABA) therapy, structured educational interventions, and in some cases, pharmacotherapy.[6,7] NDDs are also managed with therapies and educational intervention plans tailored to the individual's needs. In a limited number of cases (mostly metabolic disorders), knowing the genetic mutation that is responsible for a neurodevelopmental disorder can help to guide management. Identifying a genetic syndrome may also alert the healthcare team to potential comorbidities for which evaluation and surveillance may be needed.

Survival

While life expectancy in autism may be reported as reduced, this is often secondary to accidents such as drowning.[3] With the exception of individuals with multiple disabilities (such as Down syndrome), the life expectancy of individuals with intellectual disability is now similar to that of the general population.[10] Comorbid conditions can also affect survival in these disorders.

Test information

Introduction

Testing for ASD, ID, and GDD may include known familial mutation analysis, single gene sequence analysis, single gene deletion/duplication analysis, or multi-gene panels of various sizes.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

ASD, ID and GDD multi-gene panels include a wide variety of genes: from a few to hundreds or even thousands. These disorders may also be grouped together in broad "neurodevelopmental" panels. Multi-gene panels may also include genes believed to be associated with disease (e.g. "susceptibility" genes), but with a lower impact on risk than recognized syndromes. Results for such genes are of less clear value because there often are not clear management recommendations for mutation-positive individuals.

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to testing for ASD, ID, and GDD.

American Academy of Child and Adolescent Psychiatry

The American Academy of Child and Adolescent Psychiatry (AACAP, 2014) stated that as a clinical standard, clinicians should coordinate an appropriate multidisciplinary assessment of children with ASD to include:[5]

- "All children with ASD should have a medical assessment, which typically includes physical examination, a hearing screen, a Wood's lamp examination for signs of tuberous sclerosis, and genetic testing, which may include G-banded karyotype, fragile X testing, or chromosomal microarray."
- "Unusual features in the child (e.g., history of regression, dysmorphology, staring spells, family history) should prompt additional evaluations… Genetic or neurologic consultation, neuroimaging, EEG, and additional laboratory tests should be obtained when relevant, based on examination or history (e.g., testing for the MECP2 gene in cases of possible Rett's disorder)."

American Academy of Child and Adolescent Psychiatry

The American Academy of Child and Adolescent Psychiatry (AACAP, 2020) recommended a diagnostic genetic testing algorithm for youth with developmental disorders (autism spectrum disorder, intellectual disability, or global developmental delay):[11]

- If there is a recognized genetic syndrome, targeted testing is recommended first. This could include a karyotype if Down syndrome is suspected.
- In the absence of a recognized syndrome, or if testing is unrevealing, then chromosomal microarray and Fragile X testing are recommended as the next step.
- "Microarray is currently the genetic test with the highest diagnostic yield in children with unexplained ID/IDD, with an abnormal result reported in 7.8% of subjects with GDD/ID/IDD and in 10.6% of those with syndromic features, on average."

American Academy of Pediatrics

The American Academy of Pediatrics (AAP, 2014, Reaffirmed 2019) recommended a clinical genetics evaluation for all individuals with ID, regardless of degree of severity.[4]

- "If a specific diagnosis is suspected, arrange for the appropriate diagnostic studies to confirm including single-gene tests or chromosomal microarray test."
- "If diagnosis is unknown and no clinical diagnosis is strongly suspected, begin the stepwise evaluation process:

  - Chromosomal microarray should be performed in all.
  - Specific metabolic testing should be considered and should include serum total homocysteine, acyl-carnitine profile, amino acids; and urine organic acids, glycosaminoglycans, oligosaccharides, purines, pyrimidines, GAA/creatine metabolites.
  - Fragile X genetic testing should be performed in all."

- "If no diagnosis is established:

  - Male gender and family history suggestive X-linkage, complete XLID panel that contains genes causal of nonsyndromic XLID and complete high-density X-CMA. Consider X-inactivation skewing in the mother of the proband.
  - Female gender: complete MECP2 deletion, duplication, and sequencing study."

The American Academy of Pediatrics (AAP, 2020) recommended the following for the evaluation of children with ASD:[7]

- "Families should be offered genetic evaluation, including chromosomal microarray and fragile X testing, with consideration of other cytogenetic and molecular testing, as indicated. Consultation with a pediatric geneticist may be warranted."

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2013) recommended a genetic evaluation, with a tiered approach, for all individuals with diagnosed ASD:[6]

- "Several well-described single-gene disorders have been reported for which ASDs can be seen as part of the expanded phenotype associated with changes in that gene...For a selected few of such conditions, there is adequate evidence to suggest testing for changes in these genes in patients with ASDs with no other identifiable etiology. These would include fragile X syndrome, methyl-CPG-binding protein 2 (MECP2) spectrum disorders, and phosphatase and tensin homolog (PTEN)–related conditions."
- First tier:

  - Three-generation family history with pedigree analysis.
  - Initial evaluation to identify known syndromes or associated conditions:

    - Examination with special attention to dysmorphic features
    - If specific syndromic diagnosis is suspected, proceed with targeted testing
    - If appropriate clinical indicators present, perform metabolic and/ or mitochondrial testing (alternatively, consider a referral to a metabolic specialist)

  - Chromosomal microarray: oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array.
  - DNA testing for fragile X (to be performed routinely for males and in females if indicators are present - e.g., family history and phenotype).

- Second tier:
  - MECP2 sequencing to be performed for all females with ASDs
  - MECP2 duplication testing in males, if phenotype is suggestive
  - PTEN testing only if the head circumference is >2.5 SD above the mean
  - Brain magnetic resonance imaging only in the presence of specific indicators (e.g., microcephaly, regression, seizures, and history of stupor/coma)

- “When a family history is consistent with X-linked inheritance and the patient has cognitive impairments, an “X-linked intellectual disability gene panel” is a consideration. Several X-linked genes are known to present as either ASD or intellectual disability. Another disorder to consider is the X-linked creatine transporter defect (SCL6A8 gene). Patients with this condition have been reported with neurobehavioral changes in the ASD spectrum, along with hypotonia and seizures. Currently, no studies have been reported on the diagnostic yield of such panels in persons with ASDs."
- The following are genetic tests “that have been suggested in the etiologic evaluation of ASDs, but currently with insufficient evidence to recommend routine testing:” CDKL5 testing, NSD1 testing, chromosome 15 methylation/UBE3A gene testing, methylation/epigenetic testing, mitochondrial gene sequencing/oligoarray, and metabolic studies.

The American College of Medical Genetics and Genomics (ACMG, 2021) developed an evidence-based clinical practice guideline for the use of exome and genome sequencing (ES/GS) in the care of children with one or more congenital anomalies (CA) with onset prior to age one year, or development delay (DD) or ID with onset prior to 18 years.[12]

- ES/GS is strongly recommended as a first- or second-tier test for children with CA/DD/ID.
- "Consistent with existing guidelines/recommendations/position statements, patients with clinical presentations highly suggestive of a specific genetic diagnosis should undergo targeted testing first. This may include patients with suspicion of a chromosomal disorder, known family history of a disorder, or strong clinical suspicion of a diagnosis in which sequencing may not be diagnostic, such as Prader–Willi/Angelman related methylation abnormality or fragile X syndrome."
- "Isolated autism without ID or congenital malformation is formally out of scope for this recommendation."

The National Institute for Health and Clinical Excellence

The National Institute for Health and Clinical Excellence (NICE, 2017) stated the following regarding medical investigations following diagnosis of an ASD: “Do not routinely perform any medical investigations as part of an autism diagnostic assessment, but consider the following in individual circumstances and based on physical examination, clinical judgment, and the child or young person's profile:[13]

- Genetic tests, as recommended by your regional genetics center, if there are specific dysmorphic features, congenital anomalies and/or evidence of intellectual disability.
- Electroencephalography if there is suspicion of epilepsy."

Selected Relevant Publications

A 2017 peer reviewed article assessed the clinical utility of a targeted gene panel (101-237 genes) in 100 well-phenotyped individuals with ASD, and found:[14]

- 12% diagnostic yield for chromosomal microarray
- 0% diagnostic yield for targeted gene panel (11 pathogenic variants identified; all assessed as non-causative by clinicians based on clinical evaluation of the individuals, allele frequency in the study population, or conflicting data in the literature on causation)
- If the individual does not fit a syndromic diagnosis, the authors suggested ACMG recommended tests followed by whole exome sequencing in individuals with ASD plus
    - Severe disability
    - Congenital abnormalities
    - Comorbid conditions (eg: seizure disorder)
    - Abnormal head size

A 2019 meta-analysis published the diagnostic yield of exome sequencing compared to chromosomal microarray for neurodevelopmental disorder (NDD, defined as GDD, ID, and/or ASD) and found:[8]

- The yield of exome sequencing overall was 36%, markedly greater than previous studies of chromosomal microarray (15-20%).
- The diagnostic yield in individuals with isolated NDD was 31% and 53% for individuals with NDD plus associated conditions (such as Rett-like features).

A 2021 systematic review published results of clinical sequencing studies utilizing targeted gene panel sequencing and exome sequencing in individuals with epilepsy, ASD, or ID.[15] Of the 103 studies included, 73 utilized targeted gene panels and 36

used exome sequencing.

- The overall diagnostic yield was 23.7% (17.1% for ASD, 24% for epilepsy, and 28.2% for ID).
- Although not statistically significant, the diagnostic yield for exome sequencing was higher than for panel sequencing (27.2% vs 22.6%, P = .071).

A 2022 peer-reviewed article assessed different genetic testing strategies for individuals with ID and/or NDD.[9] Three cohorts of individuals underwent testing. The three strategies included chromosomal microarray with or without FMR1 analysis (421 individuals), genome sequencing as a secondary testing (129 individuals), and genome sequencing first (100 individuals).

- The diagnostic yield was 11% for individuals who underwent chromosomal microarray / FMR1 analysis, 26% for individuals who underwent genome sequencing as a secondary test, and 35% for individuals who underwent genome sequencing as a first test.

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BCR-ABL Negative Myeloproliferative Neoplasm Genetic Testing

MOL.TS.240.A
v2.0.2024

Introduction

BCR-ABL negative myeloproliferative neoplasm (MPN) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
ASXL1 Mutation Analysis	81175
CALR Exon 9 Mutation Analysis	81219
DNMT3A Targeted Mutation Analysis	81403
EZH2 Common Variant(s) (e.g. codon 646)	81237
EZH2 Full Gene Sequencing	81236
IDH1 Common Variants	81120
IDH2 Common Variants	81121
JAK2 Exons 12 to 15 Sequencing	0027U
JAK2 Mutation	0017U
JAK2 Targeted Mutation Analysis (e.g exons 12 and 13)	81279
JAK2 V617F Mutation Analysis	81270
MPL Common Variants (e.g. W515A, W515K, W515L, W515R)	81338
MPL Mutation Analysis, Exon 10	81339
SF3B1 Common Variants (e.g. A672T, E622D, L833F, R625C, R625L)	81347
SRSF2 Common Variants (e.g. P95H, P95L)	81348
TET2 Mutation Analysis	81479

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Procedures addressed by this guideline	Procedure codes
U2AF1 Common Variants (e.g. S34F, S34Y, Q157R, Q157P)	81357
Targeted Genomic Sequence Analysis Panel, Hematolymphoid Neoplasm or Disorder	81450

Criteria

Introduction

Requests for genetic testing for BCR-ABL negative myeloproliferative neoplasm (MPN) are reviewed using these criteria.

JAK2 V617F Mutation Analysis

- Member does not meet WHO criteria for BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms, AND
- Member meets at least ONE of the following diagnostic criteria for MPN:
    - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF, overt primary myelofibrosis (PMF), essential thrombocythemia (ET), or polycythemia vera (PV), or
    - Platelet count ≥ 450 x 10^9/L, or
    - Hemoglobin > 16.5 g/dL in men, > 16.0 g/dL in women, or
    - Hematocrit >49% in men, >48% in women, or
    - Increased red cell mass (RCM), defined as >25% above the mean normal predicted value, or
    - A combination of two of the following symptoms:
        - Anemia not attributed to a comorbid condition, or
        - Leukocytosis ≥ 11 x 10^9/L, or
        - Palpable splenomegaly, or
        - LDH increased to above upper normal limit of institutional reference range, or
        - Leukoerythroblastosis, OR
- MPN is being considered in the differential diagnosis with the member meeting both of the following:

  - Variable lab abnormalities, including erythrocytosis, thrombocytosis and leukocytosis, which are not otherwise assigned an etiology, and
  - Constitutional symptoms, including fatigue, pruritus, weight loss and symptoms of splenomegaly, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

JAK2 Exon 12 Analysis

- Member does not meet WHO criteria for BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2 V617F mutation analysis is negative, AND
- Member meets at least ONE of the following diagnostic criteria for PV:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for PV, or
  - Hemoglobin > 16.5 g/dL in men, > 16.0 g/dL in women, or
  - Hematocrit >49% in men, >48% in women, or
  - Increased red cell mass (RCM), defined as >25% above the mean normal predicted value, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CALR Exon 9 and MPL Mutation Analysis

- Member does not meet WHO criteria for BCR-ABL1+ CML, PV, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2 V617F mutation analysis is negative, AND
- Member meets at least ONE of the following diagnostic criteria for ET or PMF:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF, overt PMF, or ET, or
  - Platelet count ≥ 450 x 10^9/L, or
  - A combination of two of the following symptoms:
    - Anemia not attributed to a comorbid condition, or
    - Leukocytosis ≥ 11 x 10^9/L, or
    - Palpable splenomegaly, or
    - LDH increased to above upper normal limit of institutional reference range, or
    - Leukoerythroblastosis, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Analysis of ASXL1, DNMT3A, EZH2, TET2, IDH1, IDH2, SRSF2, And/Or SF3B1

- Member does not meet WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2, CALR, and MPL mutation analyses are all negative, AND
- Member meets at least ONE of the following diagnostic criteria for PMF:
    - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF or overt PMF, or
    - A combination of two of the following symptoms:
        - Anemia not attributed to a comorbid condition, or
        - Leukocytosis ≥ 11 x 10^9/L, or
        - Palpable splenomegaly, or
        - LDH increased to above upper normal limit of institutional reference range, or
        - Leukoerythroblastosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

If requested, gene panels that include the following genes will be eligible for reimbursement according to the criteria outlined in this guideline: ASXL1, DNMT3A, EZH2, TET2, IDH1, IDH2, SRSF2, and SF3B1. This sequencing panel will only be considered for reimbursement when billed with the appropriate panel CPT code: 81450.

What are BCR-ABL Negative Myeloproliferative Neoplasms?

Definition

Myelofibrosis (MF), polycythemia vera (PV) and essential thrombocythemia (ET) are a group of heterogeneous disorders of the hematopoietic system collectively known as Philadelphia chromosome-negative myeloproliferative neoplasms (MPN).

Prevalence

The following table describes the prevalence of Philadelphia chromosome-negative MPNs in the U.S.[1]

Disorder	Prevalence in the U.S.
MF	13,000
ET	134,000
PV	148,000

Symptoms

Symptoms vary among the subtypes, but generally include

- constitutional symptoms
- fatigue
- pruritus
- weight loss
- symptoms of splenomegaly, and
- variable lab abnormalities, including
    - erythrocytosis
    - thrombocytosis, and
    - leukocytosis.[1]

Risks

Individuals with MPNs are at risk of the condition transforming into acute myeloid leukemia (AML), which is associated with a poor response to therapy and short survival. These disorders are also associated with an increased risk of major bleeding and thrombosis or thromboembolism compared to the general population.[1]

Diagnosis

The diagnosis and management of individuals with MPN has evolved since the identification of mutations that activate the JAK pathway, including JAK2, CALR, and

MPL. The development of targeted therapies has resulted in significant improvements in disease-related symptoms and quality of life.[1] In a minority of individuals, recurrent mutations in other genes contribute to initiation or progression of disease. These mutations may serve as markers of clonality in cases where mutations in JAK2, MPL or CALR are not detected.[2]

- **JAK2 V617F mutations** — JAK2 V617F mutations account for the majority of individuals with PV (greater than 90%), ET or MF (60%). Most of the mutations occur in exon 14 with rare insertions/deletions in exon 12.[1]
- **JAK2 exon 12 mutations** — JAK2 exon 12 mutations have been seen in approximately 2-3% of individuals with PV.[1] Individuals "with JAK2 exon 12-mutated PV exhibit younger age, increased mean hemoglobin/hematocrit, and lower mean WBC [white blood cell] and platelet counts at diagnosis compared to those with JAK2 V617F-mutated PV. However, both JAK2 mutations are associated with similar rates of thrombosis, evolution to MF or leukemia, and death."[1]
- **MPL mutations** — MPL mutations have been reported in 5-8% of individuals with MF and 1-4% of individuals with ET. "MPL mutations are associated with lower hemoglobin levels at diagnosis and increased risk of transfusion dependence in patients with MF."[1]
- **CALR mutations** — CALR frameshift mutations in exon 9 are reported in approximately 20-35% of individuals with ET and MF, accounting for approximately 60-80% of individuals with JAK2/MPL-negative ET and MF. CALR deletion mutations are more commonly seen in individuals with MF, and CALR insertion mutations are associated with ET. CALR-mutated ET is associated with a lower hemoglobin level, lower WBC count, higher platelet count and lower incidence of thrombosis than JAK2-mutated ET.[1]

Test information

Introduction

Testing for BCR-ABL negative MPN may include cytogenetic testing, single gene mutation analysis, or multi-gene panel testing.

Types of tests

There are various methods used to test for the cytogenetic and molecular abnormalities associated with MPN.[1,3] Tests for the cytogenetic and molecular abnormalities include:

- bone marrow (BM) cytogenetics: karyotype, with or without FISH
- single gene mutation analysis for JAK2, MPL, and CALR, and
- panel testing using next generation sequencing for somatic mutations in genes associated with MPN.

This guideline only addresses single gene mutation analysis and multi-gene panel testing.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to BCR-ABL negative MPN genetic testing.

International Consensus Classification

The International Consensus Classification of myeloid neoplasms and acute leukemias (ICC, 2022) revised and updated established diagnostic criteria for these conditions.[4]

- The major MPN categories remain unchanged compared to other society guidelines, but "new molecular data and improved understanding of morphology have sharpened the proposed diagnostic criteria." The differences in classifications between ICC and other societies are minor and unlikely to markedly impact MPN categorizations.
- "The classical BCR::ABL1-negative MPN subtypes include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The principal objective in the classification of these cases it to reduce diagnostic uncertainty especially in initial/early disease stages presenting with elevated platelet counts and to optimize clinical management of patients. The integration of molecular findings with BM morphology and blood counts remains the cornerstone of diagnosis. Importantly, morphologic diagnosis should not only focus on megakaryocytic atypia but has to consider characteristic patterns of other features like age-related cellularity, changes in erythropoiesis, and neutrophil granulopoiesis in context with the grade of BM fibrosis."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) evidence and consensus-based guidelines recommended the following initial laboratory evaluations for individuals suspected to have MPN:[1]

- “Laboratory evaluations should include complete blood count (CBC) with differential, microscopic examination of the peripheral smear, comprehensive metabolic panel with serum uric acid, serum LDH, liver function tests, serum EPO level, and serum iron studies.”
- “Fluorescence in situ hybridization (FISH) or a multiplex reverse transcriptase polymerase chain reaction (RT-PCR), if available, on peripheral blood to detect BCR-ABL1 transcripts and exclude the diagnosis of CML [chronic myelogenous leukemia] is especially recommended for patients with left-shifted leukocytosis and/or thrombocytosis with basophilia.”

- "Molecular testing on blood or bone marrow for JAK2 V617F mutations is recommended as part of initial workup for all patients. If JAK2 V617F mutation testing is negative, molecular testing for CALR and MPL mutations should be performed for patients with suspected ET and MF; molecular testing for the JAK2 exon 12 mutation should be done for those with suspected PV and negative for the JAK2 V617F mutation."
- “Alternatively, molecular testing using the multi-gene NGS [next generation sequencing] panel that includes JAK2, CALR, and MPL can be used as part of initial workup for all patients.”
- "Once an MPN diagnosis is confirmed, NGS is recommended for mutational prognostication. The application of an NGS-based 28-gene panel in patients with MPN identified significantly more mutated splicing genes (SF3B1, SRSF2, and U2AF1) in patients with PMF compared to those with ET, and no mutations in splicing genes were found in patients with PV. NGS may also be useful to establish the clonality in selected circumstances (eg, triple-negative MPN with non-mutated JAK2, MPL, and CALR). It can also identify second, third, and fourth mutations that may hold prognostic relevance."
- “Bone marrow aspirate with iron stain and biopsy with trichrome and reticulin stains and bone marrow cytogenetics (karyotype, with or without FISH; peripheral blood for FISH, if bone marrow is inaspirable) are necessary to accurately distinguish the bone marrow morphological features between the disease subtypes (early or prefibrotic PMF, ET and masked PV).”

World Health Organization: PMF

The World Health Organization (WHO, 2016; reaffirmed 2022) established diagnostic criteria for PMF.[3,5]

PMF, early/prefibrotic stage (pre-PMF) [Diagnosis requires meeting all 3 major criteria, and at least 1 minor criterion]	PMF, overt fibrotic stage [Diagnosis requires meeting all 3 major criteria, and at least 1 minor criterion]
Major criteria: • Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis • Not meeting WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms • Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of reactive BM reticulin fibrosis	Major criteria: • Megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3 • Not meeting WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms • Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of reactive BM myelofibrosis
Minor criteria: Presence of at least one of the following, confirmed in 2 consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 x 10^9/L • Palpable splenomegaly • LDH increased to above upper normal limit of institutional reference range	Minor criteria: Presence of at least one of the following, confirmed in 2 consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 x 10^9/L • Palpable splenomegaly • LDH increased to above upper normal limit of institutional reference range • Leukoerythroblastosis

Absence of 3 major clonal mutations

In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations help determine the clonal nature of the disease.[2] Examples of frequent accompanying mutations include:

- ASXL1
- DNMT3A

- EZH2
- TET2
- IDH1
- IDH2
- SRSF2
- SF3B1

World Health Organization: PV

The World Health Organization (WHO, 2022) updated diagnostic criteria for PV.[5]

Polycythemia Vera (PV)
[Diagnosis requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion]
Major criteria: • Hemoglobin > 16.5 g/dL in men, > 16.0 g/dL in women OR Hematocrit >49% in men, >48% in women • Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size) • Presence of JAK2 V617F or JAK2 exon 12 mutation Minor criteria: • Subnormal serum EPO level

Bone marrow biopsy not required in some cases

A bone marrow biopsy may not be required in cases with sustained absolute erythrocytosis; hemoglobin levels >18.5 g/dL in men (hematocrit, >55.5%) or >16.5 g/dL in women (hematocrit, >49.5%) if 3 major criterion and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV PMF).

World Health Organization: ET

The World Health Organization (WHO, 2016; reaffirmed 2022) established diagnostic criteria for ET.[3,5]

Essential Thrombocythemia (ET)
[Diagnosis requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion]
Major criteria: • Platelet count ≥ 450 x 10^9/L • Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers • Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms • Presence of JAK2, CALR, or MPL mutation Minor criteria: • Presence of a clonal marker or absence of evidence for reactive thrombocytosis

References

Introduction

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Bloom Syndrome Genetic Testing

MOL.TS.132.A
v2.0.2024

Introduction

Bloom syndrome genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
BLM Deletion/Duplication Analysis	81479
BLM Known Familial Mutation Analysis	81403
BLM Sequencing	81479
BLM Targeted Mutation Analysis	81209
Sister Chromatid Exchange	88245

Criteria

Introduction

Requests for Bloom syndrome genetic testing are reviewed using these criteria.

Sister Chromatid Exchange (Chromosome Analysis for Breakage Syndromes)

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous sister chromatid exchange analysis performed, and
  - No previous BLM full sequencing, or BLM sequencing performed and only one mutation identified, and
  - No known BLM mutation in biologic relative, and
  - If Ashkenazi Jewish, targeted mutation analysis performed and no mutation detected or one mutation detected, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Unexplained severe intrauterine growth deficiency (less than 10th percentile) that persists throughout infancy and childhood, or
  - An individual with moderate-to-severe growth deficiency who develops erythematous skin lesions in the “butterfly area” of the face after sun exposure, or
  - An individual with moderate-to-severe growth deficiency who develops a malignancy OR
- Prenatal Testing for At-Risk Pregnancies:
  - Known increased risk due to affected first-degree relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

BLM Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing of BLM that would detect the familial mutation, AND
- Carrier Screening:
  - Known family mutation in BLM identified in 1st, 2nd, or 3rd degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - BLM mutation identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

BLM Targeted Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous BLM genetic testing, including Ashkenazi Jewish screening panels containing targeted mutation analysis for blm^{Ash}, AND
- Carrier Screening:

- Ashkenazi Jewish descent, and
- Have the potential and intention to reproduce, OR

- Diagnostic Testing for Symptomatic Individuals:
  - Unexplained severe intrauterine growth deficiency (less than 10th percentile) that persists throughout infancy and childhood, or
  - An individual with moderate-to-severe growth deficiency who develops erythematous skin lesions in the "butterfly area" of the face after sun exposure, or
  - An individual with moderate-to-severe growth deficiency who develops a malignancy AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

BLM Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous BLM full sequencing, and
  - No known BLM mutation in biologic relative, and
  - If Ashkenazi Jewish, targeted mutation analysis performed and no mutation detected or one mutation detected, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Unexplained severe intrauterine growth deficiency (less than 10th percentile) that persists throughout infancy and childhood, or
  - An individual with moderate-to-severe growth deficiency who develops erythematous skin lesions in the “butterfly area” of the face after sun exposure, or
  - An individual with moderate-to-severe growth deficiency who develops a malignancy, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1st, 2nd, or 3rd degree biologic relative with Bloom syndrome clinical diagnosis, family mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for BLM mutation, and
  - Have the potential and intention to reproduce, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

BLM Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous BLM deletion/duplication testing, and
  - Previous BLM full sequencing, and no mutations or only one mutation detected, and
  - Meets criteria for BLM full sequencing, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Bloom syndrome?

Definition

Bloom syndrome is an autosomal recessive disorder resulting from biallelic pathogenic mutations in the BLM gene which encodes the BLM DNA helicase. Pathogenic mutations in BLM lead to genomic instability where the chromosomes contain gaps and breaks that impair normal cell activities.[1,2]

Prevalence

Fewer than 300 cases of Bloom syndrome have been reported since the disease was first described over 50 years ago. Approximately one third are of Ashkenazi Jewish descent due to founder alleles.[1,3-5]

Symptoms

Affected individuals are usually smaller than average and may have a variety of symptoms.[1-3]

- Pre- and post-natal growth deficiency
- Short stature
- Long, narrow face, small lower jaw, and prominent nose and ears
- Sensitivity to sunlight: Exposure to sunlight causes a characteristic butterfly-shaped rash on the face
- Chronic lung problems, insulin resistance, and immune deficiencies
- Gastroesophageal reflux

- Decreased fertility in males
- Skin lesions that develop over time
- Cancer predisposition (including, but not limited to, gastrointestinal, genital and urinary tract, lymphoma, acute lymphoblastic leukemia, acute myeloid leukemia (AML), sarcoma, Wilms tumor, medulloblastoma, retinoblastoma)
- Learning disabilities

Cause

Bloom syndrome is caused by biallelic mutations in the BLM gene.[1,2,4-6]

The BLM gene encodes the BLM DNA helicase, a member of the RECQ family and is essential to maintaining the stability of chromosomes during DNA replication and cell division.[1,4-6]

Pathogenic mutations in the BLM gene lead to mistakes during cellular replication.[4-6]

Individuals with Bloom syndrome have multiple breaks, gaps, and genetic rearrangements in their chromosomes, leading to a unique combination of signs and symptoms. Cells from individuals with Bloom syndrome with absent BLM activity demonstrate a 10 times higher rate of sister chromatid exchange.[1,4,5]

Inheritance

Bloom syndrome is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

A diagnosis of Bloom syndrome is established in an individual with characteristic clinical features and/or biallelic pathogenic mutations in BLM. Increased frequency of sister-chromatid exchange and exclusion of RMI1, RMI2, and TOP3A-related disorders may be helpful in establishing the diagnosis in those with characteristic clinical features who do not have biallelic pathogenic mutations in BLM.[4,5]

Targeted mutation testing analyzes for the pathogenic BLM mutation most often found in Ashkenazi Jewish individuals, called blm^{Ash}.[5] The detection rate of this mutation in Ashkenazi Jewish individuals is greater than 93%.[5]

Next generation sequencing analyzes for mutations across the entire gene, and can identify at least 87% of disease-causing mutations in individuals with non-Jewish ancestry and greater than 99% of disease-causing mutations in Ashkenazi Jewish individuals.[5] It is typically used only for diagnosis of an affected individual or carrier testing of a non-Ashkenazi Jewish individual when the partner is a known carrier.

Deletion/duplication testing may be performed when there is a high suspicion for disease but targeted mutation analysis and next generation sequencing did not identify biallelic mutations.[5]

Management

There is no cure for Bloom syndrome. Treatment involves continuous monitoring by multiple physicians and specialists.[2,5,6] Treatment and surveillance may include the following:[5]

- Skin protection
- Nutrition and developmental services and therapies as needed
- Insulin resistance and hyperglycemia are treated as in type 2 diabetes
- Modification of chemotherapy as needed with cautious use of ionizing radiation or alkylating agents
- Gamma globulin infusions in individuals with recurrent infections
- Surveillance includes:
    - Abdominal ultrasound: completed every 3 months until 8 years
    - Whole body MRI: beginning at 12-13 years and completed every 1-2 years
    - Colonoscopy: beginning at 10-12 years and completed annually
    - Fecal immunochemical testing: beginning at 10-12 years and completed every 6 months
    - Breast MRI: beginning at 18 years in women and completed annually
    - Fasting blood glucose, hemoglobin A1C, serum TSH with reflex to T4, and lipid profile: beginning at 10 years and completed annually

Survival

Lifespan is limited. No individuals have been reported to survive past 50 years. The most common cause of death is from cancer.[6,7]

Test information

Introduction

Testing for Bloom syndrome may include sister chromatid exchange, known familial mutation analysis, targeted mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Sister Chromatid Exchange

Sister chromatid exchange (SCE) testing involves exposing an individual's cells to bromodeoxyuridine (BrdU), a compound that helps identify which cells contain chromosomes with unusually large numbers of rearrangements, or "exchanges." Individuals with Bloom syndrome will have a substantially higher number of these exchanges compared with unaffected individuals.[4,8] Increased SCE may be helpful in situations where BLM mutation analysis is inconclusive but SCE analysis alone is not sufficient to confirm a diagnosis of Bloom syndrome because increased SCEs are observed in other disorders (such as RMI1, RMI2, and TOP3A).[5]

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Bloom syndrome genetic testing.

Diagnostic testing strategy

A 2019 expert-authored review suggested the following related to Bloom syndrome diagnosis:[5]

“The diagnosis of BSyn [Bloom syndrome] is established in a proband by identification of biallelic pathogenic variants in BLM on molecular genetic testing.”

Carrier testing strategy

The following was stated regarding carrier testing:

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2021)[9] recommended offering carrier testing for a number of disorders, including Bloom syndrome, to all pregnant individuals and those planning a pregnancy. Male partners of pregnant women should be offered carrier screening "when carrier screening is performed simultaneously with their female partner."

American College of Obstetrics and Gynecologists

The American College of Obstetrics and Gynecologists (ACOG, 2017)[10] supported offering carrier testing for Bloom syndrome to individuals of Ashkenazi Jewish descent that are pregnant or are planning a pregnancy.

- This guideline supported testing individuals of Ashkenazi Jewish descent, even when their partner is non-Ashkenazi Jewish. In this situation, testing would start with the individual who is Jewish.[9] However, concurrent screening of both partners "is suggested if there are time constraints for decisions about prenatal diagnostic evaluation."
- If one or both partners are found to be carriers of Bloom syndrome, genetic counseling should be provided and prenatal testing offered, if appropriate.

Prenatal testing strategy

A 2019 expert-authored review stated:[5]

- "Once the BLM pathogenic variants have been identified in an affected family member, prenatal diagnosis (by amniocentesis or chorionic villus sampling [CVS]) and preimplantation genetic diagnosis are possible."

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Introduction

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BRCA Analysis

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Introduction

Germline BRCA analysis is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
BRCA1 Full Duplication/Deletion Analysis	81166
BRCA1 Full Sequencing	81165
BRCA1 Known Familial Mutation Analysis	81215
BRCA2 Full Duplication/Deletion Analysis	81167
BRCA2 Full Sequencing	81216
BRCA2 Known Familial Mutation Analysis	81217
BRCA1/2 Full Duplication/Deletion Analysis	81164
BRCA1/2 Full Sequence Analysis	81163
BRCA1/2 Full Sequencing and Duplication/Deletion Analysis (Combined)	81162
BRCA1 and BRCA2 Ashkenazi Jewish Founder Mutation Analysis	81212

Criteria

Introduction

Requests for BRCA analysis are reviewed using these criteria.

Known Familial Mutation Analysis

- Genetic Counseling:

400 Buckwalter Place Boulevard, Bluffton, SC 29910 (800) 918-8924

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, and
  - Known family mutation in BRCA1/2 identified in 1st, 2nd, or 3rd degree relative(s), AND
- Age 18 years or older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Ashkenazi Jewish Founder Mutation Testing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous full sequence testing, and
  - No previous deletion/duplication analysis, and
  - No previous Ashkenazi founder mutation testing, AND
- Age 18 years or older, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Ashkenazi Jewish descent, and
    - Epithelial ovarian, fallopian tube, or primary peritoneal cancer diagnosis at any age, or
    - Male or female breast cancer diagnosis at any age, or
    - Personal history of exocrine pancreatic cancer, or
    - Personal history of a confirmed diagnosis of prostate cancer at any age, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Ashkenazi Jewish descent, and
  - A first or second degree relative who is Ashkenazi Jewish and meets at least one of the following:
    - Epithelial ovarian, fallopian tube, or primary peritoneal cancer diagnosis at any age, or
    - Male or female breast cancer diagnosis at any age, or

  - Exocrine pancreatic cancer, or
  - A confirmed diagnosis or prostate cancer at any age, and
  - The affected relative is deceased, unable, or unwilling to be tested†, or
  - Close blood relative (1st, 2nd, or 3rd degree) with a known Ashkenazi Jewish founder mutation in BRCA 1/2 gene, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

†Testing of unaffected individuals should only be considered when an affected family member is unavailable for testing due to the significant limitations in interpreting a negative result.

Full Sequence Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous full sequencing of BRCA1/2, and
  - No known mutation identified by previous BRCA analysis, AND
- Age 18 years or older, AND
- Ancestry:
  - Member is of non-Ashkenazi Jewish descent, or
  - Member is of Ashkenazi Jewish descent and is negative for founder mutation testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Female with breast cancer diagnosis 50 years of age or younger, and/or
  - Diagnosed with two or more primary breast cancers at any age, and/or
  - Diagnosed at any age with estrogen receptor negative (ER-), progesterone receptor negative (PR-), and human epidermal growth factor receptor negative (HER2-) (i.e. triple negative) breast cancer, and/or
  - Male with breast cancer at any age, and/or
  - Epithelial ovarian, fallopian tube, or primary peritoneal cancer diagnosis at any age, and/or
  - Prostate cancer at any age with metastatic (radiographic evidence of or biopsy-proven disease), intraductal/cribriform histology, high-risk, or very-high-risk group, and/or

- Exocrine pancreatic cancer, OR

- Personal & Family History Combination
  - Initial breast cancer diagnosis at any age and one or more of the following:
    - Breast cancer in at least 1 close blood relative (first-, second-, or third-degree) occurring at 50 years of age or younger, and/or
    - Epithelial ovarian, fallopian tube, or primary peritoneal cancer in at least 1 close blood relative (first-, second-, or third- degree) at any age, and/or
    - At least three breast cancer diagnoses at any age in patient and close blood relatives (first-, second-, or third- degree on same side of family), and/or
    - Male close blood relative (first- or second-degree) with breast cancer, and/or
    - Metastatic (radiographic evidence of or biopsy proven disease) or intraductal/cribriform histology, high- or very-high risk prostate cancer in at least 1 close blood relative (first-, second-, or third- degree) at any age, and/or
    - Pancreatic cancer in at least 1 close blood relative (first-, second-, or third-degree), and/or
    - A close blood relative (first- or second-degree) with a triple negative breast cancer (ER-, PR-, HER2-) at any age, and/or
    - At least two close blood relatives (on the same side of the family) with either breast cancer or a confirmed diagnosis of prostate cancer at any age, and/or
  - Personal history of a confirmed diagnosis of prostate cancer at any age with ≥1 close blood relatives (on the same side of the family) with ovarian cancer at any age, pancreatic cancer at any age, metastatic (radiographic evidence of or biopsy proven disease) or intraductal/cribriform prostate cancer at any age, breast cancer occurring at 50 years of age or younger, or male breast cancer, and/or
  - Personal history of a confirmed diagnosis of prostate cancer at any age with two or more close blood relatives (on the same side of the family) with breast or prostate cancer (any grade) at any age, OR

- Predisposition Testing for Presymptomatic/Asymptomatic Individuals
  - The member has a first or second degree relative who meets any of the “Diagnostic Testing for Symptomatic Individuals” or “Personal & Family History Combination” criteria above, with the exception of an affected relative with pancreatic or prostate cancer. A member will meet criteria if the affected relative with pancreatic cancer or prostate cancer (metastatic, intraductal/cribriform, or high- or very-high-risk group per NCCN) is a first-degree relative. If the relative with prostate or pancreatic cancer is a second-degree relative, additional family history is needed to support testing of the member, and

  - Unaffected member is the most informative person to test and an affected family member cannot proceed with testing. If the member is not the most informative person to test, documentation must be provided by the ordering physician's office clearly documenting that it is impossible to test the most informative family member and describing the reason the unaffected member is being tested at this time, OR

- BRCA 1/2 mutation detected by tumor profiling in the absence of a germline mutation analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous BRCA deletion/duplication analysis, and
  - Meets criteria for full sequence analysis of BRCA1/2, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other Considerations

- Family history terminology used in the above criteria is defined as follows:
  - First-degree relatives: parents, siblings, children
  - Second-degree relatives: aunts, uncles, grandparents, grandchildren, nieces, nephews and half-siblings
  - Third-degree relatives: great-grandparents, great-aunts, great-uncles, and first cousins
  - Relatives "on the same side of the family" are defined as individuals who share a common ancestor and are thus related to each other by blood (e.g., a member's maternal grandmother and maternal grandfather are not considered to be on the same side of the family if they are only related by marriage).
- For information on BRCA genetic testing to determine eligibility for targeted treatment (e.g., BRCAnalysis CDx), please refer to the guidelines *Pharmacogenomic Testing for Drug Toxicity and Response* or *Somatic Mutation Testing-Solid Tumors*, as this testing is not addressed here.
- BRCA1/2 testing may be performed as part of a multigene, multisyndrome panel. For information on multigene, multisyndrome panel testing, please refer to the

guideline *Hereditary Cancer Syndrome Multigene Panels*, as this testing is not addressed here.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - If BRCA1/2 deletion/duplication analysis will be performed concurrently with BRCA1/2 gene sequencing, CPT code 81162 is likely most appropriate.

What is hereditary breast and ovarian cancer?

Definition

Hereditary breast and ovarian cancer (HBOC) is an inherited form of cancer.

Prevalence

About 1 in 400-500 people in the general population has a BRCA1 or BRCA2 mutation. The prevalence of mutations is higher in people of Norwegian, Dutch, Inuit from Ammassalik (Greenland), or Icelandic ethnicity.[1,2]

The prevalence of BRCA mutations varies among African Americans, Hispanics, Asian Americans, and non-Hispanic whites.[2]

Ashkenazi Jewish ancestry

About 1 in 40 people of Ashkenazi Jewish ancestry has a BRCA1 or BRCA2 mutation. The majority of the risk in the Ashkenazi Jewish population is associated with three common founder mutations, two of which are in the BRCA1 gene and one in the BRCA2 gene.[1,3,4] These three mutations account for 99% of identified mutations in the Ashkenazi Jewish population.[1]

Signs of HBOC

Individuals and/or families with HBOC may have the following histories of cancer or other characteristics:[1,3,5]

- breast cancer at a young age, typically under age 50
- multiple breast primaries in one individual and/or family members (on the same side of the family)
- triple negative breast cancer (ER-, PR-, HER2-)
- ovarian, fallopian tube, or primary peritoneal cancer
- metastatic (radiographic evidence of or biopsy-proven disease), intraductal/cribriform histology, high-risk, or very-high-risk group prostate cancer as defined by NCCN
- male breast cancer
- exocrine pancreatic cancer
- multiple cases of breast and/or ovarian cancer in a family or one individual with breast and ovarian cancer
- a confirmed diagnosis of prostate cancer and a family history of ovarian, breast, prostate, or pancreatic cancer
- previously identified germline BRCA1 or BRCA2 mutation in the family, or
- any of the above with Ashkenazi Jewish ancestry.

Cancer Risks

People with a BRCA mutation have an increased risk of various types of cancer.[1] These risks vary based on whether the mutation is in the BRCA1 or BRCA2 gene.

Type of cancer	Risk for malignancy with a BRCA1 mutation	Risk for malignancy with a BRCA2 mutation
Breast cancer	55-72% by age 70	45-69%
Ovarian cancer	39-44%	11-17%
Male breast cancer	1-2%	6-8%
Prostate cancer	21% by age 75	27% by age 70
Pancreatic cancer	1-3%	3-5% by age 70
Melanoma	N/A	Elevated

Note The risk for breast and ovarian cancer varies among family members and between families.

Cause

Up to 10% of all breast cancer and 15% of all ovarian cancer is associated with an inherited gene mutation, with BRCA1 and BRCA2 accounting for about 20-25% of all hereditary cases.[1,2,6,7]

Inheritance

HBOC due to a mutation in BRCA1 or BRCA2 is an autosomal dominant disorder.[1]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

BRCA2 mutations inherited in an autosomal recessive manner (mutations in both copies of the gene) cause Fanconi Anemia. BRCA1 mutations inherited in an autosomal recessive manner usually end in miscarriage, however, rare reports of individuals with Fanconi Anemia due to biallelic mutations in BRCA1 have been reported. For more information on testing for Fanconi Anemia, please refer to the guideline *Inherited Bone Marrow Failure Syndrome (IBMFS) Testing*, as this testing is not addressed here.

Diagnosis

The diagnosis is established by the identification of a pathogenic mutation in a gene associated with HBOC.

Management

Screening and prevention options are available to specifically address the increased risk of these cancers in a person with a BRCA mutation.[1]

Special Considerations

Other inherited cancer syndromes that can include breast cancer are Li-Fraumeni syndrome10417 (TP53), Cowden syndrome10192 (PTEN), Hereditary Diffuse Gastric Cancer10317 (CDH1), and Peutz-Jeghers syndrome10643 (STK11). Additionally, other genes that can increase the risk for breast cancer are ATM, BARD1, CHEK2, NF1, and PALB210690.[1,3,8,9]

Test information

Introduction

BRCA testing may include known familial mutation analysis, Ashkenazi Jewish founder mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

This test is appropriate for those who have a known BRCA mutation in the family and are not Ashkenazi Jewish.

Ashkenazi Jewish Founder Mutation Testing

This test is appropriate for those who have a known BRCA mutation in the family and are not Ashkenazi Jewish.[3,4]

Ashkenazi Jewish founder mutation testing includes the three mutations most commonly found in the Ashkenazi Jewish population:

- 185delAG and 5382insC in BRCA1, and
- 6174delT in BRCA2

Testing for these mutations detects up to 99% of mutations in those with Ashkenazi Jewish ancestry.

Founder mutation testing may be appropriate for those with Ashkenazi Jewish ancestry, even with a known familial mutation, since these mutations are common enough that multiple mutations can be found in the same Ashkenazi Jewish individual or family. If the familial mutation is not one of the three Ashkenazi Jewish mutations, the known familial mutation analysis for that mutation should be performed in addition to the founder mutation panel.[1,3]

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Full sequence testing is typically appropriate as an initial test for people who meet criteria and do NOT have Ashkenazi Jewish ancestry.[1,3]

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to BRCA analysis.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2019) issued a statement regarding BRCA1/2 testing in all individuals with breast cancer:[10]

- “With the advances in sequencing technologies and increasing access to and expanding indications for genetic testing, it remains critical to ensure that implementation of testing is based on evidence. Currently, there is insufficient evidence to recommend genetic testing for BRCA1/2 alone or in combination with multi-gene panels for all breast cancer patients.”

American Society of Breast Surgeons

The American Society of Breast Surgeons (ASBrS, 2019) published a consensus guideline on genetic testing for hereditary breast cancer. They stated the following:[11]

- "Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling and make recommendations to their patients regarding genetic testing and arrange testing. When the patient’s history and/or test results are complex, referral to a certified genetic counselor or genetics professional may be useful. Genetic testing is increasingly provided through multi-gene panels. There are a wide variety of panels available, with different genes on different panels. There is a lack of consensus among experts regarding which genes should be tested in different clinical scenarios. There is also variation in the degree of consensus regarding the understanding of risk and appropriate clinical management of mutations in some genes."
- "Genetic testing should be made available to all patients with a personal history of breast cancer. Recent data support that genetic testing should be offered to each patient with breast cancer (newly diagnosed or with a personal history). If genetic

testing is performed, such testing should include BRCA1/BRCA2 and PALB2, with other genes as appropriate for the clinical scenario and family history. For patients with newly diagnosed breast cancer, identification of a mutation may impact local treatment recommendations (surgery and potentially radiation) and systemic therapy. Additionally, family members may subsequently be offered testing and tailored risk reduction strategies."

- "Patients who had genetic testing previously may benefit from updated testing. Every patient being seen by a breast surgeon, who had genetic testing in the past and no pathogenic variant was identified, should be re-evaluated and updated testing considered. In particular, a patient who had negative germline BRCA1 and 2 testing, who is from a family with no pathogenic variants, should be considered for additional testing. Genetic testing performed prior to 2014 most likely would not have had PALB2 or other potentially relevant genes included and may not have included testing for large genomic rearrangements in BRCA1 or BRCA2."
- "Genetic testing should be made available to patients without a history of breast cancer who meet NCCN guidelines. Unaffected patients should be informed that testing an affected relative first, whenever possible, is more informative than undergoing testing themselves. When it is not feasible to test the affected relative first, then the unaffected family member should be considered for testing if they are interested, with careful pre-test counseling to explain the limited value of “uninformative negative” results. It is also reasonable to order a multi-gene panel if the family history is incomplete (i.e., a case of adoption, patient is uncertain of exact type of cancer affecting family members, among others) or other cancers are found in the family history, as described above."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) evidence and consensus-based guidelines addressed test indications for BRCA testing. These guidelines included recommendations related to unaffected individuals with a family history of cancer, those with a known mutation in the family, those with a personal history of breast cancer, exocrine pancreatic cancer, ovarian cancer, a confirmed diagnosis of prostate cancer, and men with breast cancer. They take into consideration age of diagnosis, tumor pathology, degree of relationship, and Ashkenazi Jewish ancestry.[3]

These recommendations are Category 2A, defined as "lower-level evidence" with "uniform NCCN consensus that the intervention is appropriate" and are frequently updated.[3]

Testing unaffected individuals

NCCN stated "[t]he testing of the unaffected individual (or of unaffected family members) is reasonable when no affected family member is available for testing." They cautioned that the significant limitations in interpreting results from unaffected relatives must be discussed.[3]

National Society of Genetic Counselors

The National Society of Genetic Counselors (NSGC, 2021) guidelines stated: "[f]or families with a known P/LPV, cascade testing refers to the process of counseling and testing at-risk family members. Relatives who do not carry the variation can avoid unnecessary medical interventions, whereas those who do can pursue surveillance and prevention measures aimed at reducing morbidity and mortality."[8]

U.S. Preventive Services Task Force

The U.S. Preventive Services Task Force (USPSTF, 2019) recommendations addressed women with a personal and/or family history of breast cancer and/or ovarian, tubal, or primary peritoneal cancer. The USPSTF guideline recommended:[12]

- When a woman's personal or family history of cancer is consistent with a BRCA1/2 mutation: “that primary care clinicians assess women with a personal or family history of breast, ovarian, tubal, or peritoneal cancer or who have an ancestry associated with breast cancer susceptibility 1 and 2 (BRCA1/2) gene mutations with an appropriate brief familial risk assessment tool. Women with a positive result on the risk assessment tool should receive genetic counseling and, if indicated after counseling, genetic testing.” (Evidence grade: B)
- When a woman's personal or family history is not consistent with a BRCA1/2 mutation: “recommends against routine risk assessment, genetic counseling, or genetic testing for women whose personal or family history or ancestry is not associated with potentially harmful BRCA1/2 gene mutations.” (Evidence grade: D)
- "Genetic risk assessment and BRCA1/2 mutation testing is a multistep process that begins with identifying patients with family or personal histories of breast, ovarian, tubal, or peritoneal cancer; family members with known harmful BRCA1/2 mutations; or ancestry associated with harmful BRCA1/2 mutations. Risk for clinically significant BRCA1/2 mutations can be further evaluated with genetic counseling by suitably trained health care clinicians, followed by genetic testing of selected high-risk individuals and posttest counseling about results.”
- "The type of mutation analysis required depends on family history. Individuals from families with known mutations or from ancestry groups in which certain mutations are more common (eg, Ashkenazi Jewish founder mutations) can be tested for these specific mutations."

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Breast Cancer Index for Breast Cancer Prognosis

MOL.TS.248.A
v2.0.2024

Introduction

Breast Cancer Index for breast cancer prognosis is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
Breast Cancer Index	81518

Criteria

Introduction

Requests for Breast Cancer Index testing are reviewed using these criteria.

Criteria

- For prognostic testing for adjuvant chemotherapy decision making
  - No previous gene expression assay on the same tumor when a prognostic result was previously successfully obtained, AND
  - Required Clinical Characteristics at Initial Diagnosis:
    - Primary invasive breast cancer meeting all of the following criteria:
    - Unilateral tumor
      - Tumor size >0.5cm (5mm) in greatest dimension (T1b-T3), and
      - Hormone receptor positive (ER+ or PR+), and
      - Human epidermal growth factor receptor 2 (HER2) negative, AND
  - Individual has no regional lymph node metastasis (pN0) or only micrometastases (pN1mi, malignant cells in regional lymph node(s) not greater than 2.0 mm), and

- Adjuvant endocrine systemic chemotherapy is a planned treatment option for the individual or results from this Breast Cancer Index test will be used in making adjuvant chemotherapy treatment decision, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

- For predictive testing for extended endocrine therapy decision making
  - No previous gene expression assay on the same tumor when a predictive result was previously successfully obtained, AND
  - Required Clinical Characteristics at Initial Diagnosis:
    - Primary invasive breast cancer meeting all of the following criteria:
    - Unilateral tumor:
      - Hormone receptor positive (ER+ or PR+), and
      - Human epidermal growth factor receptor 2 (HER2) negative, AND
  - Individual has involvement of 0-3 ipsilateral axillary lymph nodes, and
  - Extended endocrine therapy beyond five years is a treatment option for the individual and results from this Breast Cancer Index test will be used in making extended endocrine therapy treatment decisions, AND
  - Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other Considerations

Testing Multiple Samples:

- When more than one ipsilateral breast cancer primary is diagnosed, testing should be performed on the tumor with the most aggressive histologic characteristics. If an exception is requested, the following criteria will apply:
  - There should be reasonable evidence that the tumors are distinct (e.g., different quadrants, different histopathologic features, etc.), AND
  - There should be no evidence from either tumor that chemotherapy is indicated (e.g., histopathologic features or previous Breast Cancer Index result of one tumor suggest chemotherapy is indicated), AND
  - If both tumors are to be tested, both tumors must independently meet the required clinical characteristics

What is Breast Cancer Index for breast cancer prognosis?

Definition

Breast Cancer Index® (BCI) is a commercial multigene expression profiling assay designed to assess prognosis in individuals with early-stage breast cancer.[1]

Breast Cancer Recurrence

A large percentage of individuals with breast cancer (ER+/LN-) treated with endocrine therapy alone are free of disease 10+ years after initial diagnosis, and could forgo chemotherapy and its toxic side effects. Furthermore, a meta-analysis (n=~35,000 patients) reported a rate of recurrence of ~2% per year for individuals with breast cancer (ER+/LN-) receiving only tamoxifen.[2] Consequently, accurate prediction of the risk of breast cancer recurrence is important for establishing the most optimal course of treatment with endocrine therapy, adjuvant chemotherapy, or both for individuals with early-stage breast cancer.

Risk Assessment

Conventional methods of risk assessment including using the following clinicopathologic factors

- tumor size
- involvement of regional lymph nodes
- histologic grade
- expression of hormone receptors (estrogen and progesterone), and
- human epidermal growth factor receptor 2 (HER2) amplification.

These may not be sufficiently accurate to identify those subgroups of individuals who are at low risk of recurrence and who are unlikely to benefit from extended endocrine therapy or adjuvant chemotherapy.[3]

As a result, alternative biomarker prognostic tests have been developed to more accurately predict individual risk of cancer recurrence and to better inform clinicians making treatment decisions for individuals with early-stage breast cancer, including

- determining appropriate chemotherapy regimens
- decreasing treatment-associated complications, and
- avoiding unnecessary treatment.[4]

Intended Use

According to the manufacturer, "The Breast Cancer Index (BCI) Risk of Recurrence & Extended Endocrine Benefit Test is indicated for use in women diagnosed with hormone receptor-positive (HR+), lymph node-negative (LN-) or lymph node positive (LN+; with 1-3 positive nodes) early-stage, invasive breast cancer, who are distant

recurrence-free. The BCI test provides: 1) a quantitative estimate of the risk for both late (post-5 years from diagnosis) distant recurrence and of the cumulative distant recurrence risk over 10 years (0-10y) in patients treated with adjuvant endocrine therapy (LN- patients) or adjuvant chemoendocrine therapy (LN+ patients), and 2) prediction of the likelihood of benefit from extended (>5 year) endocrine therapy.

- A quantitative estimate of the risk for both late (post-5 years from diagnosis) distant recurrence and of the cumulative distant recurrence risk over 10 years (0-10y) in patients treated with adjuvant endocrine therapy (LN- patients) or adjuvant chemoendocrine therapy (LN+ patients), and
- Prediction of the likelihood of benefit from extended (>5 year) endocrine therapy BCI results are adjunctive to the ordering physician’s workup; treatment decisions require correlation with all other clinical findings."[1]

Test information

Introduction

The test is intended to provide risk information beyond standard predictive and prognostic factors and identify those individuals unlikely to benefit from extended endocrine therapy or adjuvant chemotherapy.[1]

Breast Cancer Index

The Breast Cancer Index assay is an algorithmic gene expression-based signature, which combines 2 independent biomarkers (HOXB13:IL17BR [H:I or H/I] and the 5-gene molecular grade index (MGI) to evaluate estrogen-mediated signaling and tumor grade.[2]

As a risk stratification tool, BCI attempts to stratify individuals with early-stage estrogen-receptor positive (ER+), lymph-node negative (LN-) individuals into three different risk groups, as well offer a continuous evaluation of an individual's risk of distant recurrence.[2]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Breast Cancer Index testing.

American Society of Clinical Oncology

The American Society of Clinical Oncology (ASCO, 2022) published a clinical practice guideline regarding the use of biomarkers to guide clinical decision-making on adjuvant systemic therapy among individuals with early-stage invasive breast cancer. Based on

a review of the peer-reviewed scientific evidence, the following recommendations were published:[5]

- "If a patient has node-negative or node-positive breast cancer with 1-3 positive nodes and has been treated with 5 years of primary endocrine therapy without evidence of recurrence, the clinician may offer the BCI test to guide decisions about extended endocrine therapy with either tamoxifen, an AI, or a sequence of tamoxifen followed by AI (Type: evidence-based; Evidence quality: intermediate; Strength of recommendation: moderate)."
- "If a patient has node-positive breast cancer with 4 or more positive nodes and has been treated with 5 years of primary endocrine therapy without evidence of recurrence, there is insufficient evidence to use the BCI test to guide decisions about extended endocrine therapy with either tamoxifen, an AI, or a sequence of tamoxifen followed by AI (Type: evidence-based; Evidence quality: intermediate; Strength of recommendation: strong)."
- "If a patient has HER2-positive breast cancer or TNBC [triple negative breast cancer], the clinician should not use multiparameter gene expression or protein assays (Oncotype DX, EndoPredict, MammaPrint, BCI, Prosigna, Ki67, or IHC4) to guide decisions for adjuvant endocrine and chemotherapy (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: strong)."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) Clinical Practice Guidelines for Breast Cancer provided evaluations of various multigene assays used to determine whether adjuvant systemic chemotherapy should be added to adjuvant endocrine therapy.[6] With regard to prognostic use of the Breast Cancer Index (BCI) assay, the NCCN stated the following (with evidence level of category 2A):[6] BCI is listed as predictive of benefit of extended adjuvant endocrine therapy and as prognostic.

- "For patients with T1 and T2 HR-positive, HER2-negative, and pN0 tumors, a BCI (H/I) in the low-risk range (0-5), regardless of T size, places the tumor into the same prognostic category as T1a-T1b, N0, M0. Patients with BCI (H/I) low demonstrated a lower risk of distant recurrence (compared to BCI [H/I] high) and no significant improvement in DFS [disease free survival] or OS [overall survival] compared to control arm in terms of extending endocrine therapy duration."
- "For patients with T1 HR-positive, HER2-negative, and pN0 tumors, a BCI (H/I) high (5.1-10) demonstrated significant rates of late distant recurrence. In secondary analyses of the MA.17, Trans-aTTom, and IDEAL trials, patients with HR-positive, T1-T3, pN0 or pN+ who had a BCI (H/I) high demonstrated significant improvements in DFS when adjuvant endocrine therapy was extended, compared to the control arm. In contrast, BCI (H/I) low patients derived no benefit from extended adjuvant therapy."

Ontario Health (Cancer Care Ontario) Program in Evidence-Based Care

The Ontario Health (Cancer Care Ontario) Program in Evidence-Based Care (PEBC, 2022) conducted a systematic review of the literature to serve as the basis of their clinical practice guideline. The clinical practice guideline for the clinical utility of multigene profiling assays in early-stage invasive breast cancer stated the following regarding BCI:[7]

- "In patients with early-stage estrogen receptor (ER)-positive/human epidermal growth factor 2 (HER2)-negative breast cancer, clinicians should consider using multigene profiling assays (i.e., Oncotype DX, MammaPrint, Prosigna, EndoPredict, and the Breast Cancer Index) to help guide the use of systemic therapy.
- In patients with early-stage node-negative ER-positive/HER2-negative disease, clinicians may use a low-risk result from Oncotype DX, MammaPrint, Prosigna, EndoPredict/EPclin, or Breast Cancer Index assays to support a decision not to use adjuvant chemotherapy.
- The evidence to support the use of molecular profiling to select the duration of endocrine therapy is evolving. In patients with ER-positive disease, clinicians may consider using a Breast Cancer Index (H/I) high assay result to support a decision to extend adjuvant endocrine therapy if the decision is supported by other clinical, pathological, or patient-related factors."

St. Gallen International Expert Consensus

The St. Gallen International Expert Consensus (2017) stated the following:

- “The Panel did not recommend the use of gene expression signatures for choosing whether to recommend extended adjuvant endocrine treatment, as no prospective data exist and the retrospective data were not considered sufficient to justify the routine use of genomic assays in this setting.”[8]

Selected Relevant Publications

Several retrospective and prospective-retrospective studies, published by the manufacturer, have assessed the clinical validity of the BCI test for individuals with early stage breast cancer (ER+/LN-) to guide clinical decision making regarding adjuvant therapy (prognostic) or regarding treatment response (predictive).[2,9-17] Results of clinical validity are generally consistent across these studies, reporting that individuals classified by the BCI test into higher risk categories tend to have worse rates of distant recurrence, and individuals in lower risk categories have better rates of distant recurrence.

There is evidence that Breast Cancer Index is predictive of extended endocrine therapy benefit. Two retrospective studies evaluating subsets of individuals from the IDEAL and ATAC trials found that Breast Cancer Index was significantly associated with extended letrozole benefit.[10,15] Two retrospective analyses of individuals from the Trans-aTTom trial, both by the same author, assessed Breast Cancer Index for predicting extended

tamoxifen benefit.[14,17] The first study of a small subset of individuals who were node positive and postmenopausal found that the test was associated with individuals who experienced a benefit from extended therapy. The second study included individuals with varying nodal (32% node positive) and menopausal statuses (86% postmenopausal). Notably, the overall and node negative cohorts were underpowered due to low even rates. In the node positive group, Breast Cancer Index results were significantly associated with a benefit from extended therapy. Several individual study limitations were identified across the evidence for the predictive use of the test including: limited numbers of premenopausal individuals, wide confidence intervals, potential selection bias, and retrospective study designs.

The evidence for the use of BCI as a prognostic test in node positive patients is sparse and of low quality. Additional well designed clinical trials are needed that evaluate the prognostic performance of BCI in large populations of node positive women currently receiving endocrine therapy and adjuvant chemotherapy.[18,19]

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CADASIL Genetic Testing

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v2.0.2024

Introduction

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
NOTCH3 Deletion/Duplication Analysis	81479
NOTCH3 Known Familial Mutation Analysis	81403
NOTCH3 Targeted Sequencing	81406

Criteria

Introduction

Requests for CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) genetic testing are reviewed using these criteria.

Known Familial Mutation Testing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for NOTCH3 mutations that would detect the familial mutation, AND
- Predictive Testing:
  - Member has a first-degree relative (i.e. parent, sibling, child) with an identified NOTCH3 gene mutation, and
  - Member is at least 18 years of age, OR

- Diagnostic Testing for Symptomatic Individuals:
  - Member has a first-degree relative (i.e. parent, sibling, child) with an identified NOTCH3 gene mutation, and
  - High index of suspicion for CADASIL diagnosis based on clinical findings, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

NOTCH3 Targeted Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic sequencing for NOTCH3 mutations, AND
- Diagnostic Testing for Symptomatic Individuals:
  - High index of suspicion for CADASIL diagnosis based on clinical findings, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

NOTCH3 Deletion/Duplication Analysis

- Member meets the above criteria for NOTCH3 targeted sequencing, AND
- NOTCH3 targeted sequencing performed and detected no mutations, AND
- No previous NOTCH3 deletion/duplication analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is CADASIL?

Definition

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is an adult-onset form of cerebrovascular disease. There are no generally accepted clinical diagnostic criteria for CADASIL and symptoms vary among affected individuals.

Prevalence

CADASIL is a rare disease.[1-3] The exact prevalence is unknown. CADASIL is probably still underdiagnosed. The minimum prevalence is estimated to be between 2-5 per 100,000 based on multiple small and national registries.[1,3] More recent "reports suggest

that the prevalence of NOTCH3 cysteine-altering pathogenic variants is substantially higher, and may be as high as 1 in 300 worldwide."[2] A founder effect has been reported for Finnish individuals and individuals in the Marche region of Italy.[1]

CADASIL is the most prevalent inherited cause of cerebral small-vessel disease.[4]

Symptoms

Typical signs and symptoms include[1,3,5]

- transient ischemic attacks and ischemic stroke, occurs at a mean age of 47 years (age range 20-70 years), in most cases without conventional vascular risk factors
- cognitive disturbance, primarily affecting executive function, may start as early as age 35 years
- psychiatric or behavioral abnormalities
- migraine with aura, occurs with a mean age of onset of 30 years (age range 6-48 years), and

Less common symptoms include:

- recurrent seizures with onset in middle age, usually secondary to stroke
- acute encephalopathy, with a mean age of onset of 42 years

Cause

CADASIL is caused by mutations in the NOTCH3 gene.

To date, NOTCH3 is the only gene in which mutations are known to cause CADASIL.[1] NOTCH3 has 33 exons. CADASIL pathogenic variants occur in exons 2–24, which encode the 34 epidermal growth factor repeats (EGFR).[1,6] The majority of pathogenic variants occur in exons 2-6.[3] NOTCH3 encodes a transmembrane receptor that is primarily expressed in vascular smooth-muscle cells, preferentially in small arteries.[1] "In CADASIL, the extracellular domain of the Notch3 receptor accumulates within blood vessels. Accumulation takes place at the cytoplasmic membrane of VSMCs [vascular smooth muscle cells] and pericytes in close vicinity to the granular osmiophilic deposits (GOM) that characterize the disease. NOTCH3 recruits other proteins into the extracellular deposits, among them vitronectin and tissue inhibitor of metalloproteinase-3 (TIMP3), which may be relevant for disease pathogenesis."[3] There is a hypothesis that structural abnormalities in the vascular smooth-muscle protein NOTCH3 trigger arterial degeneration, vascular protein accumulation, and cerebrovascular failure.[4]

No clear genotype-phenotype correlations exist for individuals with CADASIL.[7,8] Some studies describe phenotype-genotype correlations. "There is reasonably strong evidence that pathogenic variants in the first six epidermal growth factor-like repeat domains (EGFR 1 to 6) of the Notch3 protein are associated with an earlier age of stroke onset, a more severe phenotype, and lower survival compared with pathogenic variants in EGFR 7 to 34."[3] However, there can be significant intrafamilial variability with the age of onset, disease severity, and disease progression. The genotype cannot

be used to predict the phenotype.[1,4] NOTCH3 cysteine-altering pathogenic variants are associated with a broad phenotypic spectrum which includes classic CADASIL, mild small vessel disease, and non-penetrance.[3]

Inheritance

CADASIL is an autosomal dominant disorder.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Diagnosis

Brain Magnetic Resonance Imaging (MRI) findings include T2-signal-abnormalities in the white matter of the temporal pole and T2-signal-abnormalities in the external capsule and corpus callosum.[1,3]

CADASIL is suspected in an individual with the clinical signs and MRI findings. A positive family history for stroke or dementia is also indicative of disease in symptomatic individuals. However, a negative family history should not exclude the diagnosis, as de novo mutations have been reported, and affected family members are frequently misdiagnosed.[1,7]

Sequencing of all NOTCH3 exons encoding EGF–like domains fails to identify a mutation in up to 4% of individuals with CADASIL. Therefore, skin biopsy with histopathologic evaluation for characteristic GOM deposits is appropriate for individuals with a high index of clinical suspicion for CADASIL and negative genetic testing.[2,3]

For a firm diagnosis of CADASIL, at least one of the following is required:

- Documentation of a typical NOTCH3 mutation by genetic analysis.[1,3,7]
    - NOTCH3 mutation detection may reach >95% in individuals with strong clinical suspicion of CADASIL[1].
- Documentation of characteristic GOM deposits within small blood vessels by skin biopsy.[1,3,7]

Management

A correct diagnosis of CADASIL is important because the clinical course of disease is different from individuals with other types of cerebral small-vessel disease and proven therapies for stroke have not been validated in individuals with CADASIL.[7] However, no specific disease-modifying treatments for CADASIL exist. Management and treatment of individuals is generally symptomatic and supportive.[1,3,5,7,9]

Patients with CADASIL should avoid anticoagulants, angiography, and smoking to avoid disease-related complications, so clinical utility is represented.[1,7] Because of the risk for cerebral hemorrhage, use of antiplatelets rather than anticoagulants is considered for prevention of ischemic attacks. Evidence against the use of intravenous tissue plasminogen activator (IV tPA) has been suggested due to the possibility of hemorrhage; however, this is not conclusive.[10] Statins are used for treatment of hypercholesterolemia and antihypertensive drugs are used for hypertension and hypertension treatment may have an additional benefit.[3] Management of neurologic events (migraines, depression, psychiatric manifestations) by a neurologist or neuropsychiatrist can be beneficial; pregnancy and postpartum periods are potential risk factors.[1] The American Heart Association issued a scientific statement summarizing the current recommendations for the diagnosis and management of CADASIL.[11]

Survival

"In a retrospective analysis of 411 patients with CADASIL, the median age at death was 65 years in men and 71 years in women."[2]

Test information

Introduction

Testing for CADASIL may include genetic testing (known familial mutation analysis, sequence analysis, or deletion/duplication analysis) and/or skin biopsy.

Skin biopsy

A pathognomonic characteristic of CADASIL is the finding of characteristic GOM within the vascular media and increased NOTCH3 staining of the arterial wall, which can be evaluated in a skin biopsy.[1] Specificity of skin biopsy findings is high, as the characteristic deposits have not been documented in any other disorder. Sensitivity has been reported to range from 45%-100%. Sensitivity and specificity can be maximized to >90% by immunostaining for NOTCH3 protein.[7] When interpreted by an experienced (neuro) pathologist, combined analysis by electron microscopy and immunohistochemistry usually allows for a conclusive CADASIL diagnosis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Sequence analysis

To date, all mutations in NOTCH3 causing CADASIL have been in exons 2-24, including intron-exon boundaries.[1] In the United States, laboratories offering CADASIL testing appear to perform, at minimum, next-generation sequencing (NGS) of exons 2-24 at the time of this review.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Large deletions and duplications in the NOTCH3 gene have not been reported.[3] Molecular testing approaches can include deletion/duplication analysis if sequencing analysis of NOTCH3 is unrevealing.[1]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to CADASIL testing. No evidence-based U.S. testing guidelines have been identified.

American Heart Association

The American Heart Association (AHA, 2023) published a scientific statement with information on the management of inherited cerebral small vessel disease (CSVD), including CADASIL.[11] They stated the following regarding genetic testing for CADASIL.

- "Several approaches have been developed to help clinicians prioritize gene testing. For at least two decades, it has been recognized that anterior temporal polar WMH [white matter hyperintensities] on brain MRI is a marker of CADASIL with good sensitivity and specificity. The Pescini scale ranges from 0 to 25 (>14 points suspicious of CADASIL) and uses clinical features like stroke or transient ischemic attack onset before 50 years of age."
- "Strong consideration should be given to genetic counseling to allow discussion of the ramifications of obtaining genetic test results on the individual patient and their family." The authors provided additional considerations of pre-test genetic counseling including addressing possible discrimination, the possible "negative psychological consequences", and the general consensus against predictive testing for minors.

- "Posttest counseling can help with interpretation, especially of variants of unclear significance, and navigating grief or guilt from positive, negative, or equivocal results."
- "A known pathogenic NOTCH3 mutation within a family simplifies testing because only a single mutation needs to be investigated. When the mutation is unknown or unavailable, the laboratory must undertake a more complex analysis." The genetic analysis outlined when a known familial mutation has not been identified may include targeted sequencing or full gene sequencing. If multiple inherited CVSDs are in the differential, a multigene panel may be considered.
- "Although used less frequently, there is still a role for skin biopsy to look for pathognomonic granular osmophilic material on electron microscopy that may clarify the clinical significance of a mutation of uncertain or unknown significance. The accuracy of skin biopsy is enhanced by the use of immunohistochemistry."

European Academy of Neurology

The European Academy of Neurology (EAN, 2020) consensus panel stated:[9]

- "CADASIL can only be definitively confirmed by genetic testing, revealing a NOTCH3 mutation altering the number of cysteines in one of the 34 EGFr domains of the NOTCH3 protein."
- A diagnosis of CADASIL can be established by skin biopsy with electron microscopy showing GOM, but genetic testing should be the first diagnostic line of investigation.
- "In the case of a NOTCH3 variant of unknown significance, CADASIL can be confirmed using a skin biopsy for electron microscopy and/or NOTCH3 immunostaining."
- "All or almost all variants leading to CADASIL result in a loss or gain of a cysteine in EGFr repeats. Some non-cysteine-changing variants have been reported but the consensus was that the vast majority of these variants are not pathogenic. In such cases, electron microscopy revealing GOM can be a useful diagnostic tool."
- "The diagnosis of CADASIL should be considered in any patient with unexplained symmetrical periventricular WMHs and a positive family history of migraine with aura, stroke, mood disorders or dementia."

Selected Relevant Publications

The following publications addressed CADASIL testing.

Guey et al (2021)

Guey et al (2021) stated that due to the phenotypic overlap between CADASIL and other more recently characterized hereditary cerebral small vessel diseases (e.g., CARASIL, HTRA1/CADASIL type 2, COL4A1-related small vessel disease) as well as the lack of highly specific or sensitive clinical features, a multigene panel which

includes genes associated with these related inherited conditions may be preferred when offering genetic testing to a symptomatic proband.[12]

Pescini et al (2012)

Pescini et al (2012) published a scale to help guide clinicians in selecting individuals for NOTCH3 genetic analysis due to a high probability of a CADASIL genetic diagnosis. This scale assigns weighted scores to common features of CADASIL. The authors state that their scale is accurate, demonstrating optimal sensitivity (96.7%) and specificity (74.2%). At the time of publication, results needed to be confirmed and further validated.[13]

Choi et al (2010)

A two-center cohort study found that blood pressure and hemoglobin A1c levels were associated with cerebral mini bleeds in individuals with CADASIL.[7] Therefore, controlling blood pressure and glucose levels may improve the clinical course of the disease. It is also reasonable to control for high cholesterol and high blood pressure given the high rate of ischemic stroke seen in CADASIL.[7]

Tikka et al (2009)

Evidence from a 2009 retrospective cohort study suggested that an adequate skin biopsy for analysis of GOM is a cost effective way to determine a diagnosis of CADASIL in symptomatic individuals.[14]

The authors suggest that biopsy results can be used to guide the decision for who should have genetic testing, particularly in individuals with no known familial mutation or from ethnic populations with no evidence of founder mutations.[14]

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CADASIL Testing

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Canavan Disease Genetic Testing

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Introduction

Canavan disease genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
ASPA Deletion/Duplication Analysis	81479
ASPA Known Familial Mutation Analysis	81403
ASPA Sequencing	81479
ASPA Targeted Mutation Analysis	81200

Criteria

Introduction

Requests for Canavan disease genetic testing are reviewed using these criteria.

ASPA Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Carrier Screening for Asymptomatic Individuals:
  - Known family mutation in ASPA in 1st, 2nd, or 3rd degree biologic relative, OR
- Prenatal Testing for At-Risk Pregnancies:
  - ASPA mutations identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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ASPA Targeted Mutation Analysis for Common Mutations

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous ASPA genetic testing, including Ashkenazi Jewish screening panels containing targeted mutation analysis for Canavan disease, AND
- Diagnostic Testing or Carrier Screening:
  - Ashkenazi Jewish descent, regardless of disease status and N-acetylaspartic acid (NAA) levels, OR
- Prenatal Testing for At-Risk Pregnancies:
  - ASPA Ashkenazi mutations identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

ASPA Sequence Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous ASPA gene sequencing, and
  - No known ASPA mutation in family, and
  - No mutations or one mutation detected by common mutation panel, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Increased levels of N-acetylaspartic acid (NAA) in urine, and
  - An individual age three to five months of age with a triad of hypotonia, macrocephaly and head lag, or
  - Failure to attain independent sitting, walking or speech, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1st, 2nd, or 3rd degree biologic relative with Canavan disease clinical diagnosis, family mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for ASPA mutation, and
    - Have the potential and intention to reproduce, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy

What is Canavan disease?

Definition

Canavan disease is a genetic disorder leading to progressive damage to the brain's nerve cells.[1,2]

Prevalence

Canavan disease is most often found in Ashkenazi Jewish populations.[1,2]

- Between 1 in 40 and 1 in 82 people of Ashkenazi Jewish descent are carriers for Canavan disease.[2] Because of this relatively high carrier rate, population based screening in the Ashkenazi Jewish population is available.
    - For information on Ashkenazi Jewish carrier screening, please refer to the guideline *Ashkenazi Jewish Carrier Screening*, as this testing is not addressed here.
- Between 1 in 6,400 and 1 in 13,500 Ashkenazi Jews have the disease.[1]

Canavan disease occurs in all ethnic groups, and the prevalence among the general population is significantly lower than that in the Ashkenazi Jewish population.[2]

Symptoms

Signs and symptoms of Canavan disease usually begin in infancy and include:[1]

- developmental delays including motor skills, learning disabilities, or problems sleeping
- weak muscle tone (hypotonia)
- large head size (macrocephaly)
- abnormal posture
- leukodystrophy on neuroimaging, and
- seizures.

Cause

Canavan disease is caused by changes, or mutations, in the ASPA gene.[1] ASPA helps make an enzyme called aspartoacylase.[1]

This enzyme is essential to maintain the health of myelin, the nerve cells' protective covering, by breaking down harmful compounds that would otherwise degrade myelin.[1]

The most significant of these compounds that breaks down myelin is called N-acetylaspartic acid (NAA).

In the absence of aspartoacylase, the myelin protective covering of the nerve is eventually destroyed. Without this protective covering, nerve cells malfunction and die.[1]

Inheritance

Canavan disease is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

Canavan disease is suspected when an individual presents with classic signs and symptoms. Diagnosis is confirmed by biochemical testing, genetic testing, or both.[2] Biochemical tests analyze either NAA levels or aspartoacylase enzyme activity in someone with suspected Canavan disease.

- Affected individuals will have elevated levels of NAA because they cannot break it down; therefore, NAA accumulates in the blood or urine.
- Affected individuals will have severely reduced or nonexistent aspartoacylase enzyme activity.

Molecular genetic testing can be used for confirmation of the diagnosis and to help family planning by identifying individuals at risk of being carriers.[2]

- Targeted mutation analysis is the most common genetic test for Canavan disease. The panel analyzes for up to four of the most common mutations in the ASPA gene linked to Canavan disease, including the Glu285Ala and Tyr231X mutations, which account for 98% of all Ashkenazi Jewish cases.[2,3] The panel also includes the p.Ala305Glu mutation, which accounts for between 30% and 60% of all non-Ashkenazi Jewish cases.[2,3]
- Sequence analysis analyzes for mutations across the entire coding region of the ASPA gene. In addition to the more common mutations found in the Ashkenazi Jewish population, sequencing is also able to find less common mutations found in non-Ashkenazi Jews.[2,3] Sequence analysis has a detection rate of about 99% in all populations.[2]

- Large deletions in the ASPA gene have been reported but are believed to be uncommon.[2] Therefore, deletion/duplication analysis is unlikely to be indicated in most cases.

Management

Symptomatic infants need supportive care such as ensuring adequate nutrition, addressing infections, and providing protection for their airway. Physical therapy may be helpful in addition to programs to facilitate communication. Antiepileptic medications are used for those with seizures. Hospice care can be a valuable resource as well.[2]

Survival

Canavan disease does not usually allow survival beyond childhood.[1]

Test information

Introduction

Testing for Canavan disease may include known familial mutation analysis, targeted mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Canavan disease genetic testing.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2008) supported offering carrier testing for Canavan disease to individuals of Ashkenazi Jewish descent for the two common mutations. It is anticipated that the detection rate will be ~97%. This test should be offered to individuals of reproductive age, preferentially prior to pregnancy, with genetic counseling performed by a geneticist or genetic counselor. ACMG supports the testing of individuals of Ashkenazi Jewish descent, even when their partner is non-Ashkenazi Jewish. In this situation, testing would start with the individual who is Ashkenazi and reflex back to the partner if necessary.[4]

ACMG (2021) released an educational practice resource on carrier screening.[5] This consensus statement asserted that general population carrier screening should be ethnicity and family history agnostic. To accomplish this, screening all individuals in the prenatal/preconception period for autosomal recessive and X-linked conditions with a carrier frequency of >1/200 was suggested. ACMG generated a list of 113 genes, which included the ASPA gene, meeting these criteria.

American College of Obstetricians and Gynecologists

Consensus guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2023) stated:[6]

- "A number of clinically significant, autosomal recessive disease conditions are more prevalent in individuals of Ashkenazi Jewish (Eastern European and Central European) descent…When only one partner is of Ashkenazi Jewish descent, that individual should be offered screening first. If it is determined that this individual is a carrier, the other partner should be offered screening."
- “The American College of Obstetricians and Gynecologists has previously recommended offering carrier screening for four conditions in the Ashkenazi population.” Canavan disease is one of the four conditions listed.

Selected Relevant Publication

A 2018 expert-authored review stated the following regarding molecular genetic testing for diagnostic purposes:[2]

- The targeted mutation panel may be used to confirm a clinical diagnosis, biochemical diagnosis, or both.
- “Targeted analysis for the pathogenic variants p.Glu285Ala, p.Tyr231Ter, and p.Ala305Glu can be performed first in individuals of Ashkenazi Jewish ancestry.”
- “Targeted analysis for the pathogenic variant p.Ala305Glu can be performed first in individuals of non-Ashkenazi Jewish ancestry.”
- “Sequence analysis of ASPA detects small intragenic deletions/insertions and missense, nonsense, and splice site variants; typically, exon or whole-gene deletions/duplications are not detected. Perform sequence analysis first. If only one or no pathogenic variant is found perform gene-targeted deletion/duplication analysis to detect intragenic deletions or duplications.”

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Cardiomyopathy and Arrhythmia Genetic Testing

MOL.TS.410.A
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Introduction

Genetic testing for non-syndromic cardiomyopathy and arrhythmia is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
4q25-AF Risk Genotype	81479
Arrhythmia Single Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Arrhythmia Known Familial Mutation Analysis	81403
Brugada Syndrome Genetic Testing (SCN5A and Variants)	S3861

Procedures addressed by this guideline	Procedure codes
Cardiomyopathy Single Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Cardiomyopathy Known Familial Mutation Analysis	81403
Cardiac Ion Channelopathies Sequencing Panel (at least 10 channelopathy-related genes, including ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, and SCN5A)	81413
Cardiac Ion Channelopathies Deletion/Duplication Panel (at least 2 channelopathy-related genes, including KCNH2 and KCNQ1)	81414
Genomic Unity Cardiac Ion Channelopathies Analysis	0237U
Hereditary Cardiomyopathy Sequencing Panel (at least 5 cardiomyopathy-related genes)	81439

Criteria

Introduction

Requests for cardiomyopathy and arrhythmia genetic testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic or Predisposition Testing:*
  - Known familial mutation in a 1st or 2nd degree biological relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Note Since symptoms may occur in childhood, testing of children who are at-risk for a pathogenic mutation may be appropriate, but requires genetic counseling and careful consideration of ethical issues related to genetic testing in minors.

Single Gene Tests (Sequencing and Deletion/Duplication Analysis)

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous analysis of the requested gene, and
  - No known mutation in the family that would explain the member's clinical features, AND
- Diagnostic Testing in Symptomatic Individuals:
  - Clinical history points to the specific gene requested, and
  - Single gene analysis is appropriate due to one or more of the following:
    - The requested gene is the only gene that has a confirmed association with the member's cardiac subtype (e.g., SCN5A for individuals with an established or suspected diagnosis of Brugada syndrome), or
    - Analysis of other genes associated with the member's cardiac subtype was previously completed and was not diagnostic, and
  - Non-genetic causes have been ruled out (e.g., hypokalemia for arrhythmia; sarcoidosis, endomyocardial fibrosis, infection, or toxin exposure for cardiomyopathy), or clinical suspicion for a gene mutation remains high even in the presence of a potential non-genetic cause, and

  - The results of the test will directly impact the diagnostic and treatment options that are recommended for the individual, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy

Multi-Gene Sequencing Panels

Subtype-specific panels or comprehensive panels with multiple cardiomyopathy and/or arrhythmia subtypes are considered medically necessary when the criteria below are met.

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous gene sequencing for the suspected condition, and
  - No known pathogenic or likely pathogenic mutation in the family that would explain the member's clinical features, AND
- Diagnostic Testing for Symptomatic Individuals:
  - The member meets subtype-specific criteria (see below) for one or more of the following cardiac subtypes:
    - Arrhythmogenic cardiomyopathy (ACM), or
    - Catecholaminergic polymorphic ventricular tachycardia (CPVT), or
    - Dilated cardiomyopathy (DCM), or
    - Hypertrophic cardiomyopathy (HCM), or
    - Long QT syndrome (LQTS) with or without signs of Jervell and Lange-Nielson Syndrome (JLNS), or
    - Progressive cardiac conduction disease or cardiac conduction disease (PCCD/CCD), or
    - Restrictive cardiomyopathy (RCM), or
    - Short QT syndrome (SQTS), and
  - No personal or family history of extra-cardiac features that are highly suggestive of an underlying multi-systemic syndrome for which syndrome-specific genetic testing is available and appropriate (see table titled *Select Cardiac Syndromes, Associated Genes, and Applicable Guidelines*), and
  - Non-genetic causes have been ruled out (e.g., hypokalemia for arrhythmia; sarcoidosis, endomyocardial fibrosis, infection, or toxin exposure for cardiomyopathy), or clinical suspicion for a gene mutation remains high even in the presence of a potential non-genetic cause, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Arrhythmogenic Cardiomyopathy (ACM) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: DSC2, DSG2, DSP, JUP, PKP2 (plus FLNC if the left ventricle is affected), AND
- The member meets at least one of the following:
  - Arrhythmogenic right ventricular cardiomyopathy (ARVC) Task Force criteria are met for at least possible ARVC (defined as having at least one major or two minor criteria) based on electrocardiogram, echocardiogram, MRI, and/or angiogram findings, or
  - Clinical documentation is provided supporting a diagnosis or clinical suspicion of ARVC, arrhythmogenic left ventricular cardiomyopathy (ALVC), or bi-ventricular arrhythmogenic cardiomyopathy (BiVACM) and the presence of one or more of the following:
    - One or more first- or second-degree relatives with a diagnosis of cardiomyopathy, or
    - A suspicious family history including a first- or second-degree relative with sudden death or cardiac event at <50 years of age.

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: RYR2 and CASQ2, AND
- The member has an established or suspected diagnosis of CPVT based on at least one of the following:
  - A CPVT diagnostic score ≥3.5, or
  - All of the following features are present:
    - A structurally normal heart, and
    - Normal resting ECG, and
    - Exercise- or emotion-induced bidirectional or polymorphic ventricular tachycardia (VT).

Dilated Cardiomyopathy (DCM) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: BAG3, FLNC, LMNA, MYH7, RBM20, SCN5A, TNNT2, and TTN, AND
- The member meets at least one of the following:
  - Diagnosis of idiopathic DCM (IDCM) based on the following findings from appropriate imaging and/or electrophysiology modality (e.g. echocardiogram, electrocardiogram, MRI, angiogram):
    - Left ventricular (LV) enlargement with end-diastolic dimensions or volumes >2 z-scores above population mean values corrected for body size, sex, and/or age (i.e. in adults, LV end-diastolic diameter >58mm in males and >52 mm in females and an LVEDV index of ≥75 mL/m2 in males and ≥62 mL/m2 in females), and
    - Left ventricular systolic dysfunction, (defined as an ejection fraction of less than 50%), and
    - Absence of abnormal loading conditions (severe hypertension and valve disease) or coronary artery disease sufficient to cause the above features, or
  - Clinical documentation is provided supporting a diagnosis of DCM (with or without abnormal loading conditions or coronary artery disease) and at least one of the following:
    - One or more first- or second-degree relatives with a diagnosis of DCM, peripartum cardiomyopathy, or alcoholic cardiomyopathy, or
    - A suspicious family history including a first- or second-degree relative with sudden death or cardiac/thromboembolic event at <50 years of age, or
  - Mildly affected individual (defined as having dilated left ventricle but normal ejection fraction, or left ventricular systolic dysfunction without dilatation) with a known diagnosis of IDCM in a first- or second-degree relative who is deceased or otherwise unavailable for testing.

Hypertrophic Cardiomyopathy (HCM) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2, and TPM1, AND
- The member meets at least one of the following:

- Diagnosis of HCM based on the following findings from appropriate imaging (e.g., echocardiogram or MRI):
  - Left ventricular hypertrophy without obvious cause (valvular disease, hypertension, infiltrative or neuromuscular disorder), and
  - Maximum myocardial wall thickness meeting one of the following parameters:
    - ≥15mm (1.5cm) in adults without a family history of HCM, or
    - ≥13mm (1.3 cm) in adults with a first- or second-degree relative with a known diagnosis of HCM who is deceased or otherwise unavailable for testing, or
    - >2 standard deviations for age in children, or
- Pathognomonic histopathologic features of HCM on endomyocardial biopsy (e.g. myocyte disarray, hypertrophy, increased myocardial fibrosis).

Long QT Syndrome (LQTS) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: KCNQ1, KCNH2, and SCN5A (or KCNQ1 and KCNE1 if Jervell and Lange-Nielson syndrome is suspected), AND
- The member has an established or suspected diagnosis of LQTS based on at least one of the following:
  - Schwartz criteria score ≥1.5, or
  - Confirmation of prolonged QTc or T-wave abnormalities [>460ms (prepuberty) or >480ms (adults) on serial 12-lead ECGs] on exercise or ambulatory ECG, or during pharmacologic provocation testing, or
  - A prolonged or borderline prolonged QT interval on ECG or Holter monitor, or
  - Profound congenital bilateral sensorineural hearing loss and prolonged QTc.

Progressive Cardiac Conduction Disease (PCCD/CCD) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: SCN5A and TRPM4, AND
- Clinical documentation is provided supporting a diagnosis of PCCD/CCD (e.g., complete right bundle branch block, complete left bundle branch block, left anterior fascicular block/hemiblock or left posterior hemiblock, prolonged PR interval or complete atrioventricular block with broad QRS complexes), AND

- The member has one or more of the following:
  - PCCD/CCD diagnosed at <50 years of age, or
  - A first- or second-degree relative with PCCD/CCD.

Restrictive Cardiomyopathy (RCM) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2, TPM1, and TTR, AND
- Clinical documentation is provided supporting a diagnosis of RCM, AND
- The member has one or more of the following:
  - Left ventricular hypertrophy and/or hypertrophic cardiomyopathy (HCM), or
  - A first- or second-degree relative with cardiomyopathy (e.g., RCM, HCM) and/or left ventricular hypertrophy.

Short QT Syndrome (SQTS) Specific Criteria

- The member meets the above general criteria for multi-gene panel sequencing, AND
- The panel includes, at minimum, the following genes: KCNH2 and KCNQ1, AND
- The member has an established or suspected diagnosis of SQTS based on at least one of the following:
  - An SQTS diagnostic score ≥4, or
  - A QTc ≤330ms, or
  - A QTc <360ms with survival of a ventricular tachycardia/fibrillation episode in the absence of heart disease, or
  - A QTc <360ms with family history of SQTS or sudden death at age ≤40.

Diagnostic criteria, scoring systems, and their associated references are summarized in the background section of this guideline, under "Diagnosis".

Multi-Gene Deletion/Duplication Panels

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:

  - No previous gene deletion/duplication testing for the suspected condition, and
  - A multi-gene sequencing panel was previously performed for the suspected condition, with one of the following results:
    - No pathogenic or likely pathogenic mutation identified, or
    - One pathogenic or likely pathogenic mutation identified in a gene associated with an autosomal recessive condition (e.g., Jervell and Lange-Nielson Syndrome), AND

- Diagnostic Testing for Symptomatic Individuals:
  - Meets clinical criteria for multi-gene sequencing panels, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Exclusions and Other Considerations

- Genetic testing (including single-gene or multi-gene panels) for the following conditions, in isolation, is considered experimental, investigational, or unproven:
  - Left ventricular non-compaction (LVNC)
  - The following types of arrhythmia: atrial fibrillation (including the 4q25 risk genotype), early repolarization syndrome, sinus node dysfunction ('sick sinus syndrome') and Wolff-Parkinson-White syndrome
- Due to low test yield and lack of clinical utility for genes other than SCN5A, multi-gene panel testing for Brugada syndrome (BrS) is considered experimental, investigational, or unproven.
- This guideline may not apply to genetic testing for indications that are addressed in other test-specific guidelines (e.g., testing for multi-system syndromes that may include cardiomyopathy or arrhythmia as a feature). For these indications, please refer to applicable test-specific guidelines in the table titled *Select Cardiac Syndromes, Associated Genes, and Applicable Guidelines*, or the general guideline, *Genetic Testing to Diagnose Non-Cancer Conditions*.
- Genetic testing for cardiomyopathies and/or arrhythmias is only medically necessary once per lifetime. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest (e.g., additional genes are being tested that account for >1% of cases of the member's cardiac subtype and have a definitive association with the subtype according to the ClinGen Gene-Disease Validity Curation).

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
    - A single gene included in a multi-gene panel may not be reimbursed if testing has been performed previously.
    - If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81439, 81413/81414 or 81479)*.
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
    - When the test is billed with multiple stacked procedure codes, only the following genes may be considered for reimbursement, based on the cardiac subtype:
        - Arrhythmogenic right ventricular cardiomyopathy: DSC2, DSG2, DSP, JUP, PKP2, TMEM43
        - Catecholaminergic polymorphic ventricular tachycardia: RYR2, CASQ2
        - Dilated cardiomyopathy: TTN, TNNT2, MYH7, SCN5A, MYBPC3, LMNA
        - Hypertrophic cardiomyopathy: MYH7, MYBPC3, TNNT2, TNNI3
        - Long QT syndrome: KCNQ1, KCNH2, SCN5A (if Jervell and Lange-Nielson syndrome is suspected: KCNQ1 and KCNE1)
        - Progressive cardiac conduction disease: SCN5A, LMNA, TRPM4
        - Restrictive cardiomyopathy: ACTC1, MYH7, TNNI3, TTN, TTR
        - Short QT syndrome: KCNH2, KCNQ1

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are Cardiomyopathy and Arrhythmia?

Definition

Cardiomyopathy is a disease of the heart muscle that compromises heart function. The most relevant subtypes for genetic testing include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy (ACM), and restrictive cardiomyopathy (RCM). ACM is further divided into the following, based on which ventricles are involved: arrhythmogenic right ventricular cardiomyopathy (ARVC), arrhythmogenic left ventricular cardiomyopathy (ALVC), and bi-ventricular arrhythmogenic cardiomyopathy (BiVAC). Left ventricular non-compaction (LVNC) is now more often considered a phenotypic trait rather than a primary cardiomyopathy; it may occur alongside other cardiac subtypes or as an isolated finding seen in athletes, pregnant individuals, and healthy adult populations.[1-4]

In addition to non-syndromic forms of cardiomyopathy, more than 100 syndromes have cardiomyopathy as a feature, including various muscular dystrophies and storage disorders.[1,3,4] It is beyond the scope of this guideline to provide detailed descriptions of these syndromes (see table titled *Select Cardiac Syndromes, Associated Genes, and Applicable Guidelines* for eviCore guidelines that address some of them more specifically).

Arrhythmias (sometimes called channelopathies) are heart rhythm disturbances that may be detected on electrocardiogram (ECG). Genetic arrhythmias are primarily ventricular arrhythmias that occur due to abnormalities in the ion currents that drive the electrical activity of the heart.[5] Subtypes of arrhythmia that are most likely to prompt a genetic evaluation include long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), short QT syndrome (SQTS) and progressive cardiac conduction disease (PCCD). Extra-cardiac features may be present, particularly with certain types of LQTS. Syndromic forms of arrhythmia are included in the table titled *Select Cardiac Syndromes, Associated Genes, and Applicable Guidelines*. Other more common arrhythmias include atrial fibrillation, early repolarization syndrome (ERS), sinus node dysfunction (SND; also called 'sick sinus syndrome') and Wolff-Parkinson-White (WPW) syndrome. These arrhythmias may occur in conjunction with some of the cardiac subtypes listed above; however, isolated cases are generally acquired.[1]

Table: Select Cardiac Syndromes, Associated Genes, and Applicable Guidelines

Syndromes that may include cardiomyopathy and/or arrhythmia, along with their associated clinical features, genes, and eviCore guidelines. Note: Some genes may be associated with both syndromic and non-syndromic forms of disease. .

Syndrome	Clinical Features	Genes	Applicable Guideline Name	Applicable Guideline Number
Anderson-Tawil Syndrome (ATS or LQTS type 7)[6]	Prominent U waves, prolonged QTc or QUc interval, or bidirectional and/or polymorphic PVCs/VT; characteristic dysmorphic features; periodic paralysis	KCNJ2	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114
Barth syndrome[1,7]	DCM; LVNC; neutropenia; muscle weakness; growth delay; infantile/early-childhood onset	TAFAZZIN (TAZ)	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114
Danon disease[1,7,8]	In males: HCM; DCM; LVNC; CCD; skeletal myopathy; retinal dystrophy; learning disability. Females may present with isolated cardiac features.	LAMP2	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114

Syndrome	Clinical Features	Genes	Applicable Guideline Name	Applicable Guideline Number
Duchenne & Becker muscular dystrophy[1,7]	In males: DCM; CCD; muscle weakness, increased serum creatine kinase (CK); loss of ambulation. Females may present with isolated cardiac features.	DMD	Duchenne and Becker Muscular Dystrophy Testing	MOL.TS.161
Emery-Dreifuss muscular dystrophy[1,7,8]	DCM; HCM; conduction system disease, and/or arrhythmias; joint contractures; increased serum CK; muscle weakness	EMD FHL1 LMNA	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114
Fabry disease[1,8]	HCM; RCM; CCD; periodic pain crises; angiokeratomas; hypohidrosis; ocular abnormalities (cornea verticillatata); hearing loss; proteinuria and renal dysfunction	GLA	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114

Syndrome	Clinical Features	Genes	Applicable Guideline Name	Applicable Guideline Number
Friedreich ataxia[1,8]	HCM; slowly progressive ataxia at <25 years; dysarthria; muscle weakness	FXN	Friedreich Ataxia Genetic Testing	MOL.TS.309
Glycogen storage disease of the heart, lethal congenital[1,8]	HCM; conduction system disease (e.g., sinus node disease, atrial fibrillation, etc.); neonatal hypoglycemia; vacuolar myopathy; facial dysmorphism and/or macroglossia	PRKAG2	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114
Hereditary transthyretin amyloidosis[1,8]	HCM; RCM; heart failure and/or aortic stenosis at ≥65 years; peripheral sensorimotor neuropathy and autonomic neuropathy; vitreous opacities, central nervous system amyloidosis	TTR	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114

Syndrome	Clinical Features	Genes	Applicable Guideline Name	Applicable Guideline Number
HFE hemochromatosis[1,7]	DCM; non-dilated and/or infiltrative cardiomyopathy; cirrhosis; diabetes; hypermelanotic pigmentation; increased serum iron & ferritin	HFE	HFE Hemochromatosis Genetic Testing	MOL.TS.183
Laing distal myopathy[7,8]	DCM; HCM; facial weakness; childhood-onset weakness of ankles, great toes, finger extensors, & neck flexors	MYH7	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114
Mitochondrial disorders[1,7]	Complex phenotypes including DCM and/or CCD; focal segmental glomerulosclerosis; Kearns-Sayre syndrome (KSS); ptosis; progressive external ophthalmoplegia; ataxia	mtDNA	Mitochondrial Disorders Genetic Testing	MOL.TS.266

Syndrome	Clinical Features	Genes	Applicable Guideline Name	Applicable Guideline Number
Myotonic dystrophy type 1[1,7]	DCM; CCD; adults may present with muscle weakness (especially distal leg, hand, neck & face); myotonia; posterior subcapsular cataracts. Neonates: hypotonia; facial muscle weakness; generalized weakness; clubfoot; respiratory insufficiency.	DMPK	Myotonic Dystrophy Type 1 Genetic Testing	MOL.TS.312
Pompe disease[8]	HCM with onset in first few months of life; poor feeding; macroglossia; motor delay; hypotonia; muscle weakness; respiratory difficulty	GAA	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114

Syndrome	Clinical Features	Genes	Applicable Guideline Name	Applicable Guideline Number
RASopathies (Noonan syndrome, cardiofaciocutaneous syndrome, Costello syndrome, Noonan syndrome with multiple lentigines)[8]	HCM with infant or childhood onset; congenital heart defects; characteristic facies; short stature; developmental delay; broad, webbed neck; unusual chest shape	BRAF HRAS KRAS LZTR1 MAP2K1 MAP2K2 NRAS PTPN11 RAF1 RASA2 RRAS2 RIT1 SOS1 SOS2	Noonan Spectrum Disorder Genetic Testing	MOL.TS.371
Timothy Syndrome (LQTS type 8)[9]	Prolonged QT interval (QTc >480 ms); cardiovascular malformations; cutaneous syndactyly of the fingers/toes; neurological findings (autism, seizures, intellectual disability, and/or hypotonia); facial anomalies	CACNA1C	Genetic Testing to Diagnose Non-Cancer Conditions	MOL.CU.114

Prevalence

Prevalence of cardiomyopathy varies by subtype, and is estimated to be 0.2% for HCM, 0.036-0.400% for DCM, and 0.078% for ARVC in adult populations.[4] Childhood prevalence for these cardiomyopathies is 0.029% for HCM, 0.026% for DCM, and

unknown for ARVC.[4] The true prevalence of RCM is unknown; it is considered the rarest cardiomyopathy subtype.[4,10]

Genetic arrhythmias have a prevalence of 1:2,500 for LQTS, 1:1000 to 1:10,000 for BrS (more common in Southeast Asia than in other regions) and 1:10,000 for CPVT.[1,11-15]

Symptoms

The severity of cardiomyopathy ranges from a lifelong asymptomatic course to thromboembolism, arrhythmia, progressive heart failure, and sudden cardiac death (SCD).[5,16] Affected individuals may present with signs and symptoms of heart failure, including peripheral edema, fatigue, orthopnea, dyspnea, syncope, and cardiac ischemia.[16] Sometimes the symptoms may suggest a particular subtype of cardiomyopathy.[4,16]

Cardiac channelopathies can be largely asymptomatic. Symptoms of ventricular arrhythmias may include palpitations, either skipped or extra beats or sustained palpitations, shortness of breath, chest pain, dizziness, near syncope, and syncope.[12] Consideration of a channelopathy is warranted when there is recurrent syncope, aborted cardiac arrest, ventricular fibrillation, or sudden death in a child or young adult.[11]

Variable expressivity and reduced penetrance have been reported for cardiomyopathy and arrhythmia syndromes, and disease onset can span all ages, from the prenatal period to late adulthood.[3,4,11,17] SCD can be the presenting symptom in some cases.[8,13,15,18]

Cause

Cardiomyopathies and arrhythmias may be inherited or acquired disorders.

Non-syndromic cardiomyopathies are "mainly caused by pathogenic variants in genes encoding the structural components of cardiomyocytes."[5] Acquired causes of cardiomyopathy can include myocarditis, stress, and/or tachycardia. Cardiomyopathy may also be associated with pregnancy and delivery.[16,19] Secondary systemic etiologies are extensive in variety, and include but are not limited to, sarcoidosis, endomyocardial fibrosis, autoimmune disease, toxin exposure, and underlying cardiac diseases such as hypertension.[3,16,19] While the percentage varies by subtype, cardiomyopathy is thought to have a genetic etiology in up to 60% of cases.[1,4,5,7,8,20] The presence of an acquired cause does not always preclude the possibility of a genetic etiology; for example, 10-15% of individuals with chemotherapy-induced, alcoholic, or peripartum DCM will have a causative gene variant.[4.]

Genetic arrhythmias are "generally caused by defects in genes encoding cardiac ion channel macromolecular complexes and associated regulatory proteins."[5] Inherited cardiac channelopathies may present with similar clinical and ECG features to other causes for these cardiac symptoms, including heart-rhythm altering drugs, hypokalemia, syndromic genetic disorders, stroke, and structural heart disease. When a genetic etiology is suspected based on initial investigations, but a clear diagnosis

cannot be established, molecular testing may help to clarify a cause.[11,14] The yield of genetic testing is highest for LQTS (70-85%) and somewhat lower for other subtypes.[1,11,14]

Inheritance

Non-syndromic cardiomyopathies and arrhythmias most commonly follow an autosomal dominant inheritance pattern.[3-5] Autosomal recessive inheritance is often associated with childhood onset, more severe cardiac disease and/or extra-cardiac manifestations.[3,7,11,20] X-linked and mitochondrial inheritance are rare and typically seen only in syndromic cases.[3,7]

Diagnosis

Diagnosis of a cardiomyopathy or arrhythmia can often be confirmed with cardiac imaging (e.g., echocardiography, cardiac magnetic resonance imaging [CMR]) and/or electrocardiogram (ECG). Cases of arrhythmia may be further evaluated with the use of exercise testing, toxicology, and blood testing.[13,21] Endomyocardial biopsy (EMB) may aid in the diagnosis of a cardiomyopathy in some cases.[4,10] A detailed clinical history and evaluation should aim to exclude acquired and secondary causes when an isolated genetic etiology is under consideration.

Consensus clinical diagnostic criteria have been developed for most recognized subtypes of cardiomyopathy and arrhythmia.

- **Arrhythmogenic cardiomyopathy (ACM):** Task force diagnostic criteria for ARVC were last revised by Marcus et al in 2010.[18] As summarized in an expert-authored review, these criteria use findings from cardiac imaging, EMB, ECG, family history, and/or genetic testing to classify individuals as having a definite, borderline, or possible diagnosis of ARVC.[20] Updated criteria, which further incorporated ALVC and BiVAC, were proposed in 2020, but have not yet been validated in larger studies.[20,22]
- **Brugada Syndrome (BrS):** A clinical diagnosis of BrS is suspected in an individual with a type 1, type 2, or type 3 ECG pattern and at least one of the following: recurrent syncope, ventricular fibrillation, self-termination polymorphic ventricular tachycardia, cardiac arrest, or family history of SCD. The diagnosis is established clinically in the majority of cases, although genetic testing may help confirm it.[14]
- **Catecholaminergic polymorphic ventricular tachycardia (CPVT):** CPVT can be diagnosed clinically based on cardiac findings and/or the presence of a pathogenic or likely pathogenic mutation.[15,23] A scoring system has also been developed to categorize the pretest probability of CPVT, with a score of 3.5 or greater indicating a high probability of having the condition.[1]
- **Dilated cardiomyopathy** (**DCM):** The diagnosis of DCM is established in individuals having the following findings on echocardiogram or cardiac MRI: left ventricular enlargement and systolic dysfunction.[7]

- **Hypertrophic cardiomyopathy (HCM):** The diagnosis of HCM is typically established with cardiac imaging (echocardiogram and/or cardiac MRI), and is defined by the presence of unexplained left ventricular hypertrophy (LVH) with a maximum wall thickness ≥15 mm in adults or a z-score >3 in children. In individuals with a family history of HCM, a maximum left ventricular wall thickness of ≥13 mm supports the diagnosis.[8]
- **Long QT syndrome (LQTS):** Schwartz et al developed a clinical diagnostic scoring system for this condition, which was last updated in 2011.[24] As described in an expert-authored review, this score is used to calculate the risk of having LQTS, with a score of 1.5-3 indicating an intermediate risk, and a score of ≥3.5 indicating a high risk.[11] A diagnosis of LQTS can be made with a Schwartz diagnostic score ≥3.5, a pathogenic mutation in an LQTS gene, or specific ECG findings.[23]
- **Progressive cardiac conduction disease (PCCD):** A diagnosis of PCCD can be made in individuals with unexplained progressive conduction abnormalities at a young age (<50 years), structurally normal hearts, and absence of skeletal myopathies, especially when there is a family history of PCCD.[23]
- **Restrictive cardiomyopathy (RCM):** Consensus diagnostic criteria for RCM are currently lacking.[10,25] A 2022 expert-authored review proposed an updated strategy for the diagnosis of this condition, which includes identification of a restrictive pathophysiology confirmed in repeated evaluations, absence of ventricular dilatation, and investigation of red flags for specific conditions among clinical, ECG, and imaging findings.[25]
- **Short QT syndrome (SQTS):** A diagnosis of SQTS can be made in the presence of a QTc <330 ms or with a QTc <360 ms when at least one of the following is present: a pathogenic mutation, family history of SQTS, family history of sudden death at age ≤40, or survival of a ventricular tachycardia/fibrillation episode in the absence of heart disease.[23]

The yield of genetic testing varies by subtype, and the presence of a family history usually increases the likelihood of identifying a causative mutation.[1,3-5,26] Genetic testing can be useful to confirm a diagnosis of inherited cardiomyopathy or arrhythmia in persons with cardiac symptoms and has been incorporated into the diagnostic criteria for ARVC, CPVT, LQTS, and SQTS.[1,4,11,15,20,23] Post-mortem genetic testing may be performed after a sudden death when an inherited cardiomyopathy or arrhythmia is suspected in order to aid in the risk assessment of family members.[1,5,13,21]

Once a disease-causing mutation is identified, at-risk relatives can have reliable genetic testing to define their risk and screening needs. Identifying a gene mutation significantly changes medical management in symptomatic individuals without a clinical diagnosis and may improve life expectancy.[3,4,7,13,17] For relatives who are not found to have the familial pathogenic mutation, it may be possible to eliminate the need for ongoing clinical surveillance and other medical expenditures.[1,3,4,17] Clinical screening is recommended for family members when genetic testing was not performed in the affected individual or failed to identify a causative mutation.[1,12]

Management

Treatment of cardiomyopathies and arrhythmias is focused on controlling and preventing symptoms. Management may include therapy and/or screening for heart failure, activity restriction, pharmacologic therapy (including beta blockers), catheter ablation, consideration of a pacemaker or implantable cardioverter defibrillator (ICD), and heart transplantation.[4,13,19] The identification of a genetic variant may provide prognostic information, guide treatment strategies, and allow for earlier intervention.[1,3,5,13] Genotype-phenotype correlation exists for a subset of genes, and management recommendations have been developed for certain genes/mutations.[1,13,17,20,27] Identification of a syndromic form may also facilitate surveillance and treatment for associated extra-cardiac manifestations.[3,4,13]

Survival

Cardiomyopathy and arrhythmia are both associated with a higher mortality rate than is seen in the general population.[13,19] Some factors that may affect the survival rate include age at diagnosis, presence of symptoms, etiology, and cardiac subtype.[13,19] Diagnosis and appropriate treatment has led to a decrease in the mortality rate for some of these disorders.[3,11]

A significant proportion of SCD is attributed to genetic arrhythmias and, to a lesser extent, cardiomyopathies, especially in individuals under the age of 50 years.[1,13] SCD can occur in all subtypes of cardiomyopathy and arrhythmia.[13] The overall risk, age distribution, and triggering factors vary by subtype.[13]

Test information

Introduction

Testing for cardiomyopathies may include known familial mutation analysis, single gene sequencing or deletion/duplication analysis, or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis

detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Panels may be subtype-specific (e.g., long QT syndrome panel, hypertrophic cardiomyopathy panel, etc.) or broad panels that address multiple cardiomyopathy and/or arrhythmia subtypes. Due to overlapping clinical features and associated genes, panels that include genes for multiple cardiac subtypes are increasingly employed, and often include syndromes with important medical management implications.[3]

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to cardiomyopathy and arrhythmia genetic testing.

American College of Cardiology, American Heart Association Task Force, and Heart Rhythm Society

A guideline from the American College of Cardiology, the American Heart Association Task Force on Clinical Practice Guidelines, and the Heart Rhythm Society (ACC/AHA/HRS, 2017) on the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death (SCD) made the following recommendations regarding genetic testing for these indications:[12]

- "In young patients (<40 years of age) with unexplained SCA [sudden cardiac arrest], unexplained near drowning, or recurrent exertional syncope, who do not

have ischemic or other structural heart disease, further evaluation for genetic arrhythmia syndromes is recommended." (Class I, Level B)

- "In first-degree relatives of SCD victims who were 40 years of age or younger, cardiac evaluation is recommended, with genetic counseling and genetic testing performed as indicated by clinical findings." (Class I, Level B)
- "In patients and family members in whom genetic testing for risk stratification for SCA or SCD is recommended, genetic counseling is beneficial." (Class I, Level C)

The ACC/AHA/HRS 2017 guideline also made the following recommendations regarding genetic testing for specific cardiomyopathies and arrhythmias:[12]

- Nonischemic Cardiomyopathy (NICM):
  - "In patients with NICM [nonischemic cardiomyopathy] who develop conduction disease or LV dysfunction at less than 40 years of age, or who have a family history of NICM or SCD in a first-degree relative (<50 years of age), genetic counseling and genetic testing are reasonable to detect a heritable disease that may clarify prognosis and facilitate cascade screening of relatives." (Class IIa, Level C)
- Arrhythmogenic Right Ventricular Cardiomyopathy:
  - "In selected first-degree relatives of patients with arrhythmogenic right ventricular cardiomyopathy, clinical screening for the disease is recommended along with genetic counseling and genetic testing, if the proband has a disease causing mutation." (Class I, Level B)
  - "In patients with clinically diagnosed or suspected arrhythmogenic right ventricular cardiomyopathy, genetic counseling and genetic testing can be useful for diagnosis and for gene-specific targeted family screening." (Class IIa, Level B)
- Hypertrophic Cardiomyopathy (HCM):
  - "In first-degree relatives of patients with HCM due to a known causative mutation, genetic counseling and mutation-specific genetic testing are recommended." (Class I, Level B).
  - "In patients with clinically suspected or diagnosed HCM, genetic counseling and genetic testing are reasonable." (Class IIa, Level B)
- Long QT Syndrome (LQTS):
  - The authors highlighted the ability to stratify risk based on genotype in LQTS and stated, "In patients with clinically diagnosed long QT syndrome, genetic counseling and genetic testing are recommended." (Class I, Level B)
- Catecholaminergic Polymorphic Ventricular Tachycardia:

- "In patients with catecholaminergic polymorphic ventricular tachycardia and with clinical VT or exertional syncope, genetic counseling and genetic testing are reasonable." (Class IIa, Level B)

- Brugada Syndrome:
  - "In patients with suspected or established Brugada syndrome, genetic counseling and genetic testing may be useful to facilitate cascade screening of relatives." (Class IIb, Level C)
- Short QT Syndrome:
  - "In patients with short QT syndrome, genetic testing may be considered to facilitate screening of first-degree relatives." (Class IIb, Level C)
- Early Repolarization Syndrome:
  - "In patients with early repolarization pattern on ECG, genetic testing is not recommended." (Class III: No Benefit, Level B)

In a guideline for the evaluation and management of patients with bradycardia (including sinus node disease) and cardiac conduction delay authored by the American College of Cardiology, the American Heart Association Task Force, and the Heart Rhythm Society (ACC/AHA/HRS, 2018), genetic testing was not included in the diagnostic algorithms for these conditions, and the authors acknowledged that these disorders are usually acquired. However, they made the following recommendations:[28]

- "In patients in whom a conduction disorder-causative mutation has been identified, genetic counseling and mutation-specific genetic testing of first-degree relatives is recommended to identify similarly affected individuals." (Class I, Level C)
- "In patients with inherited conduction disease, genetic counseling and targeted testing may be considered to facilitate cascade screening of relatives as part of the diagnostic evaluation." (Class IIb, Level C)

American College of Cardiology, American Heart Association Task Force, American College of Clinical Pharmacy, and Heart Rhythm Society

In a guideline for the management of patients with atrial fibrillation (AF), the American College of Cardiology, American Heart Association Task Force, American College of Clinical Pharmacy and Heart Rhythm Society (ACC/AHA/ACCP/HRS, 2023) stated the following regarding genetic testing for this condition:[29]

- "In patents with an onset of AF before 45 years of age without obvious risk factors for AF, referral for genetic counseling, genetic testing for rare pathogenic variants, and surveillance for cardiomyopathy or arrhythmia syndromes may be reasonable." (Class IIb, Level B)

American College of Cardiology and American Heart Association

A joint committee guideline from the American College of Cardiology and American Heart Association (ACC/AHA, 2020) made the following class 1 recommendations for HCM:[30]

- "When performing genetic testing in an HCM proband, the initial tier of genes tested should include genes with strong evidence to be disease-causing in HCM."
- "In first-degree relatives of patients with HCM, both clinical screening (ECG and 2D echocardiogram) and cascade genetic testing (when a pathogenic/likely pathogenic variant has been identified in the proband) should be offered."
- “In patients with an atypical clinical presentation of HCM or when another genetic condition is suspected to be the cause, a work-up including genetic testing for HCM and other genetic causes of unexplained cardiac hypertrophy ('HCM phenocopies') is recommended.”

Asia Pacific Heart Rhythm Society and Heart Rhythm Society

The Asia Pacific Heart Rhythm Society and the Heart Rhythm Society (APHRS/HRS, 2020) made the following recommendations in regards to sudden cardiac arrest (SCA) and sudden unexplained death (SUD):[21]

- "Genetic evaluation of SCA survivors is recommended for those with a diagnosed or suspected genetic cardiac disease phenotype when the results are likely to influence diagnosis, management, or family screening." (Class 1, Level B)
- "When genetic evaluation is performed in an SCA survivor with a suspected or diagnosed genetic cardiac disease phenotype, it is recommended that evaluations include only genes where there is robust gene–disease association." (Class 1, Level B)
- "Genetic testing in SCA survivors with a well-established nongenetic cause of SCA is not recommended." (Class 3: No benefit, Level C)
- "Family screening should include genetic testing and clinical evaluation when genetic testing of a proband with SUD detects a pathogenic or likely pathogenic variant." (Class 1, Level B)
- "If a pathogenic or likely pathogenic variant that fits the phenotype has been identified in an SUD proband, first-degree relatives should be offered DNA testing, with ongoing clinical evaluation for those testing positive." (Class 1, Level C)
- "It is recommended that genetic testing in families where an SUD or resuscitated SCA due to a heritable cause is suspected is performed only with appropriate genetic counseling." (Class 1, Level C)

European Heart Rhythm Association, Asia Pacific Heart Rhythm Society, Heart Rhythm Society, and Latin American Heart Rhythm Society

An expert consensus statement from the European Heart Rhythm Association, Heart Rhythm Society, Asia Pacific Heart Rhythm Society, and Latin American Heart Rhythm Society (EHRA/HRS/APHRS/LAHRS, 2022) addressed the utility and appropriateness of genetic testing for inherited cardiovascular conditions.[1] The consensus statements were categorized as follows:

- Supported by strong observational evidence and author's consensus
- Some evidence and general agreement favor the usefulness/ efficacy of a test
- There is evidence or general agreement not to recommend a test

Regarding the choice of genetic testing, EHRA/HRS/APHRS/LAHRS (2022) stated the following:[1]

- Genetic testing should occur with genetic counseling. [Supported by strong observational evidence and authors' consensus]
- If an individual has a clear phenotype, it is appropriate to analyze genes with definite/strong evidence support disease causation [Supported by strong observational evidence and author's consensus] and may be appropriate to analyze genes with moderate evidence for disease causation. [Some evidence and general agreement favor the usefulness/ efficacy of a test]
- In some cases with a clear phenotype and negative genetic testing of genes with definite/strong evidence for disease causation, broader genetic testing may be considered. [Some evidence and general agreement favor the usefulness/ efficacy of a test]
- "Genetic testing for genes with (i) limited, (ii) disputed, or (iii) refuted evidence should not be performed in patients with a weak (non-definite) phenotype in the clinical setting." [There is evidence or general agreement not to recommend a test]
- When a likely pathogenic or pathogenic variant has been identified, genetic counseling should be offered. The inheritance pattern, penetrance, and associated risks can be discussed. Additionally, cascade testing for relatives should be facilitated. [Supported by strong observational evidence and author's consensus]
- Some affected individuals may have had previous genetic testing that was not a comprehensive, such as prior to the use of next generation sequencing or with an incomplete testing panel. Repeat testing should be considered in these cases. [Supported by strong observational evidence and author's consensus]

Regarding genetic testing for specific cardiomyopathies and arrhythmias, EHRA/HRS/APHRS/LAHRS (2022) stated the following:[1]

- Long QT Syndrome (LQTS):
    - Genetic testing for genes with a definitive disease association was recommended for all patients with a high probability of LQTS (Schwartz Score ≥

3.5) [Supported by strong observational evidence and author's consensus]. Testing of less definitive genes could also be considered in these individuals [Some evidence and general agreement favor the usefulness/ efficacy of a test].

- Genetic testing of definitive disease-associated genes should also be offered to "all patients with acquired LQTS who experienced drug-induced TdP [torsades de pointes], are aged below 40 years and have a QTc >440 ms (males) and >450 ms (females) in the absence of culprit drug" [Supported by strong observational evidence and author's consensus].
- Targeted gene analysis was recommended in patients with Jervell and Lange-Nielsen syndrome, Timothy syndrome, Andersen–Tawil syndrome, and suspected triadin knockout syndrome" [Supported by strong observational evidence and author's consensus].

- Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT):
  - Genetic testing for genes with definite/strong evidence of disease association is recommended for individuals meeting diagnostic criteria for CPVT ("Class 1 clinical diagnosis or CPVT diagnostic score >3.5") [Supported by strong observational evidence and author's consensus].
  - Genetic testing can also be considered for individuals with a modest CPVT phenotype ("i.e. CPVT diagnostic score ≥ 2 but < 3.5") [Some evidence and general agreement favor the usefulness/ efficacy of a test].
  - For individuals meeting CPVT diagnostic criteria, genetic testing could also be considered for CPVT phenocopies (e.g., pathogenic variants in the KCNJ2, SCN5A, and PKP2 genes) [Some evidence and general agreement favor the usefulness/ efficacy of a test].
- Short QT Syndrome (SQTS):
  - Genetic testing for genes with definite/strong evidence of disease association was recommended for individuals meeting diagnostic criteria for SQTS ("Class 1 clinical diagnosis or SQTS diagnostic score >4") [Supported by strong observational evidence and author's consensus].
  - Testing of the KCNJ2 and SLC4A3 genes could be considered in individuals with an SQTS diagnostic score ≥4. [Some evidence and general agreement favor the usefulness/ efficacy of a test].
- Brugada Syndrome (BrS):
  - "Genetic testing with sequencing of SCN5A is recommended for an index case diagnosed with BrS with a type I ECG in standard or high precordial leads occurring either (i) spontaneously, or (ii) induced by sodium-channel blockade in presence of supporting clinical features or family history" [Supported by strong observational evidence and author's consensus].

- "Rare variants in genes with a disputed or refuted gene-disease clinical validity should not be reported routinely for BrS genetic testing in a diagnostic setting" [There is evidence or general agreement not to recommend a test].

- Progressive Cardiac Conduction Disease (PCCD/CCD):
  - "Targeted genetic testing is recommended as part of the diagnostic evaluation for index patients with isolated cardiac conduction disease (CCD/PCCD) or with concomitant structural heart disease or extracardiac disease, when there is early age of diagnosis or a suspicion of laminopathy, especially when there is documentation of a positive family history of CCD/PCCD" [Supported by strong observational evidence and author's consensus]. Such testing could also be considered for individuals "with isolated cardiac conduction disease (CCD/PCCD) or with concomitant structural heart disease or extracardiac disease, especially in the setting of a positive family history" [Some evidence and general agreement favor the usefulness/ efficacy of a test].
- Other Arrhythmias:
  - The authors stated that targeted genetic testing could be considered for individuals with familial atrial fibrillation (AF at age <60), unexplained cardiac arrest survivors with a clinical diagnosis of early repolarization syndrome (ERS), and for individuals "with familial or isolated, but otherwise unexplained sinus node dysfunction (SND)" [Some evidence and general agreement favor the usefulness/ efficacy of a test].
  - No recommendations were made regarding genetic testing for Wolff-Parkinson-White (WPW) syndrome, as the authors concluded "only in the presence of the combination of pre-excitation and HCM and/or progressive CCD is genetic testing pertinent."
- Hypertrophic cardiomyopathy (HCM):
  - Comprehensive genetic testing was recommended for all individuals with HCM, using a first tier of genes with a definitive/strong disease association [Supported by strong observational evidence and author's consensus]. According to the authors, the inclusion of genes with moderate evidence of pathogenicity should also be considered [Some evidence and general agreement favor the usefulness/ efficacy of a test].
  - The authors also recommended genetic testing in "patients with atypical clinical presentation of HCM, or when another genetic condition associated with unexplained hypertrophy is suspected (e.g. HCM phenocopy)." [Supported by strong observational evidence and author's consensus]
- Dilated cardiomyopathy (DCM):
  - Comprehensive genetic testing was recommended for all individuals with DCM with a family history of DCM, using a first tier of genes with a definitive/strong disease association [Supported by strong observational evidence and author's

consensus]. The authors stated that the inclusion of genes with moderate evidence of pathogenicity could also be considered in these individuals [Some evidence and general agreement favor the usefulness/ efficacy of a test].

  - Genetic testing could be considered in individuals with apparently sporadic DCM, or "patients with DCM related to an acquired or environmental cause that may overlap with a genetic cause (such as peripartum or alcoholic cardiomyopathy)." [Some evidence and general agreement favor the usefulness/ efficacy of a test].

- Arrhythmogenic cardiomyopathy (ACM):
  - Comprehensive testing of definitive disease-associated genes was recommended for all individuals with features of (ACM) [Supported by strong observational evidence and author's consensus].
  - Genetic testing could also be considered in individuals with a borderline ACM phenotype [Some evidence and general agreement favor the usefulness/ efficacy of a test].

- Left Ventricular Non-compaction (LVNC):
  - Genetic testing could be considered for individuals with LVNC in whom a cardiologist has established a diagnosis based on clinical history, family history, and electrocardiographic/echocardiographic/MRI phenotype. [Some evidence and general agreement favor the usefulness/ efficacy of a test]
  - "Genetic testing should not be performed in isolated (incidental) LVNC with normal LV function, no associated syndromic features and no family history." [There is evidence or general agreement not to recommend a test]

- Restrictive Cardiomyopathy (RCM):
  - Genetic testing could be considered for individuals with RCM in whom a cardiologist has established a diagnosis based on clinical history, family history, and electrocardiographic/echocardiographic/MRI phenotype. [Some evidence and general agreement favor the usefulness/ efficacy of a test]
  - Genetic testing of the TTR gene was specifically recommended for patients with RCM and a clinical diagnosis of cardiac TTR amyloidosis. [Supported by strong observational evidence and author's consensus]

Lastly, EHRA/HRS/APHRS/LAHRS (2022) made recommendations regarding genetic testing in survivors of unexplained cardiac arrest (UCA) or relatives of individuals with sudden cardiac death (SCD):[1]

- "In selected UCA survivors with idiopathic VF [ventricular fibrillation], genetic testing for founder variants, where relevant, should be considered." [Supported by strong observational evidence and authors' consensus]

- "In relatives of UCA survivors or SCD decedents, clinical evaluation of 1st degree family members should be performed, and targeted to the index case's phenotype if present."
- "In decedents with SCD or survivors with cardiac arrest in whom a non-genetic cause has been identified, genetic testing of the index case and clinical evaluation of relatives should not be performed." [There is evidence or general agreement not to recommend a test]

European Society of Cardiology

In their 2023 guidelines for the management of cardiomyopathies, the European Society for Cardiology (ESC, 2023) made the following genetic testing recommendations:[4]

- "Genetic counselling, provided by an appropriately trained healthcare professional and including genetic education to inform decision-making and psychosocial support, is recommended for families with an inherited or suspected inherited cardiomyopathy, regardless of whether genetic testing is being considered." (Class I, Level B)
- "It is recommended that genetic testing for cardiomyopathy is performed with access to a multidisciplinary team, including those with expertise in genetic testing methodology, sequence variant interpretation, and clinical application of genetic testing, typically in a specialized cardiomyopathy service or in a network model with access to equivalent expertise." Also, "pre- and post-test genetic counselling is recommended in all individuals undergoing genetic testing for cardiomyopathy." (Class I, Level B)
- "Genetic testing is recommended in patients fulfilling diagnostic criteria for cardiomyopathy in cases where it enables diagnosis, prognostication, therapeutic stratification, or reproductive management of the patient, or where it enables cascade genetic evaluation of their relatives who would otherwise be enrolled into long-term surveillance." (Class I, Level B)
- "Genetic testing may be considered in patients fulfilling diagnostic criteria for cardiomyopathy when it will have a net benefit to the patient, considering the psychological impact and preference, even if it does not enable diagnosis, prognostication, or therapeutic stratification, or cascade genetic screening of their relatives." (Class IIb, Level C)
- "Genetic testing in patients with a borderline phenotype not fulfilling diagnostic criteria for a cardiomyopathy may be considered only after detailed assessment by specialist teams." (Class IIb, Level C)
- "It is recommended that cascade genetic testing, with pre- and post-test counselling, is offered to adult at-risk relatives if a confident genetic diagnosis (i.e. a P/LP variant) has been established in an individual with cardiomyopathy in the family (starting with first-degree relatives if available, and cascading out sequentially)." (Class I, Level B)

- "Cascade genetic testing with pre- and post-test counselling should be considered in paediatric at-risk relatives if a confident genetic diagnosis (i.e. a P/LP variant) has been established in an individual with cardiomyopathy in the family (starting with first-degree relatives, if available, and cascading out sequentially), considering the underlying cardiomyopathy, expected age of onset, presentation in the family, and clinical/legal consequences." (Class IIa, Level B)
- "Testing for the presence of a familial variant of unknown significance, typically in parents and/or affected relatives, to determine if the variant segregates with the cardiomyopathy phenotype should be considered if this might allow the variant to be interpreted with confidence." (Class IIa, Level C)
- "Diagnostic genetic testing is not recommended in a phenotype-negative relative of a patient with cardiomyopathy in the absence of a confident genetic diagnosis (i.e. a P/LP variant) in the family." (Class III, Level C)

2022 European Society of Cardiology guidelines (ESC, 2022), which were endorsed by the Association for European Paediatric and Congenital Cardiology (AEPC, 2022), addressed the management of individuals with ventricular arrhythmias [VA] and the prevention of sudden cardiac death [SCD]. They stated the following regarding genetic testing for these indications:[13]

- "Genetic testing is recommended when a condition is diagnosed in a living or deceased individual with a likely genetic basis and a risk of VA and SCD." (Class I, Level B)
- "When a putative causative variant is first identified, evaluation for pathogenicity is recommended using an internationally accepted framework." (Class I, Level C)
- "When a Class IV or Class V variant has been identified in a living or deceased individual with a condition that carries a risk of VA and SCD, genetic testing of first-degree and symptomatic relatives and obligate carriers is recommended." (Class I, Level C)
- "It is recommended that genetic testing and counselling on its potential consequences should be undertaken by an expert multidisciplinary team." (Class I, Level C)
- "It is recommended that Class III (variants of uncertain significance) and Class IV variants should be evaluated for segregation in families where possible, and the variant re-evaluated periodically." (Class I, Level C)
- "It is not recommended to undertake genetic testing in index patients with insufficient evidence of a genetic disease." (Class III, Level C)

The 2022 ESC guidelines also included the following recommendations regarding testing for specific arrhythmias and cardiomyopathies:[13]

- Long QT Syndrome (LQTS):
    - "In patients with clinically diagnosed LQTS, genetic testing, and genetic counselling are recommended." (Class I, Level C)

- "It is recommended that LQTS is diagnosed in the presence of a pathogenic mutation, irrespective of the QT duration." (Class I, Level C) The guideline also noted that genetic testing is useful in providing genotype-specific risks and, in some cases, genotype-specific treatment.
- "Genetic testing is recommended in patients with suspected Anderson-Tawil syndrome." (Class I, Level C)

- Brugada Syndrome (BrS):
  - "Genetic testing for SCN5A gene is recommended for probands with BrS." (Class I, Level C)
  - The authors also noted: "The yield of genetic testing in BrS patients is approximately 20%, with the SCN5A gene the only gene with evidence of association for clinical testing purposes."
- Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT):
  - "Genetic testing and genetic counselling are indicated in patients with clinical suspicion or clinical diagnosis of CPVT." (Class I, Level C)
  - "It is recommended that CPVT is diagnosed in patients who are carriers of a mutation in disease-causing genes." (Class I, Level C)
- Short QT Syndrome (SQTS):
  - "Genetic testing is indicated in patients diagnosed with SQTS." (Class I, Level C)
  - "It is recommended that SQTS is diagnosed in the presence of a QTc ≤360ms and one or more of the following: (a) a pathogenic mutation, (b) a family history of SQTS, (c) survival from a VT/VF episode in the absence of heart disease." (Class I, Level C)
- Early repolarization syndrome (ERS):
  - "Genetic testing in ERS patients may be considered." (Class IIb, Level C)
- Dilated Cardiomyopathy (DCM):
  - "Genetic testing (including at least LMNA, PLN, RBM20, and FLNC genes) is recommended in patients with DCM/HNDCM [hypokinetic non-dilated cardiomyopathy] and AV [atrioventricular] conduction delay at <50 years, or who have a family history of DCM/HNDCM or SCD in a first-degree relative (at age <50 years)." (Class I, Level B)
  - "Genetic testing (including at least LMNA, PLN, RBM20, and FLNC genes) should be considered for risk stratification in patients with apparently sporadic DCM/HNDCM, who present at young age, or with signs suspicious for an inherited aetiology." (Class IIa, Level C)
- Arrhythmogenic right ventricular cardiomyopathy (ARVC):

- "In patients with a suspected or definite diagnosis of ARVC, genetic counselling and testing are recommended." (Class I, Level B)

- Hypertrophic cardiomyopathy (HCM):
  - "Genetic counselling and testing are recommended in HCM patients." (Class I, Level B)

Heart Failure Society of America and American College of Medical Genetics and Genomics

The Heart Failure Society of America in collaboration with the American College of Medical Genetics and Genomics (HFSA/ACMG, 2018) stated the following regarding cardiomyopathy genetic testing:[3]

- "Guideline 4: Genetic testing is recommended for patients with cardiomyopathy" (Level of evidence A for HCM, DCM, ARVC, and cardiomyopathies associated with extracardiac manifestations; evidence level B for RCM)
  - "4a: Genetic testing is recommended for the most clearly affected family member."
  - "4b: Cascade genetic testing of at-risk family members if recommended for pathogenic and likely pathogenic variants."
  - "4c: In addition to routine newborn screening tests, specialized evaluation of infants with cardiomyopathy is recommended, and genetic testing should be considered."
- "Genetic testing is recommended to determine if a pathogenic variant can be identified to facilitate patient management and family screening."
- "Testing should ideally be initiated on the person in a family with the most definitive diagnosis and most severe manifestations. This approach would maximize the likelihood of obtaining diagnostic results and detecting whether multiple pathogenic variants may be present and contributing to variable disease expression or severity."
- "Molecular genetic testing for multiple genes with the use of a multigene panel is now the standard of practice for cardio-vascular genetic medicine. Furthermore, multigene panel genetic testing is recommended over a serial single-gene testing approach owing to the genetically heterogeneous nature of cardiomyopathy. Genetic testing and cascade screening have been shown to be cost-effective."
- "[T]he LVNC phenotype may be observed in conjunction with all other cardiomyopathy phenotypes, so considerations related to genetic testing should always be directed by findings of a cardiomyopathy (or other cardiovascular) phenotype. Genetic testing is not recommended when the LVNC phenotype is identified serendipitously in asymptomatic individuals with otherwise normal cardiovascular structure and function."

The American College of Medical Genetics and Genomics (ACMG, 2018) published a practice resource on genetic testing for cardiomyopathies.[31] This practice resource was an abbreviated version of the Heart Failure Society of America (HFSA) guideline above, on which the ACMG collaborated.

Heart Rhythm Society

In a consensus statement focused on arrhythmogenic cardiomyopathy (ACM), the Heart Rhythm Society (HRS, 2019) stated the following:[32]

- "For individuals and decedent with either a clinical or necropsy diagnosis of ACM, genetic testing of the established ACM-susceptibility genes is recommended." (Class I, Level C)
- "For genetic testing of the established ACM-susceptibility genes, comprehensive analysis of all established genes with full coverage is recommended." (Class I, Level C)

Heart Rhythm Society, European Heart Rhythm Association, and Asia Pacific Heart Rhythm Society

An expert consensus statement from the Heart Rhythm Society, the European Heart Rhythm Association, and the Asia Pacific Heart Rhythm Society (HRS/EHRA/APHRS, 2013) for the diagnosis and management of inherited primary arrhythmia established diagnostic criteria for multiple arrhythmia syndromes and recommended that genetic test results be incorporated into the criteria for LQTS, CPVT, and SQTS.[23] These recommendations were endorsed by the American College of Cardiology Foundation (ACCF, 2013), American Heart Association (AHA, 2013), Pediatric and Congenital Electrophysiology Society (PACES, 2013), and Association for European Paediatric and Congenital Cardiology (AEPC, 2013). No specific recommendations were made for when to perform genetic testing, since this topic was addressed elsewhere.

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Introduction

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Charcot-Marie-Tooth Neuropathy Genetic Testing

MOL.TS.148.A
v2.0.2024

Introduction

Genetic testing for Charcot-Marie-Tooth (CMT) disease is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
CMT Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
CMT Known Familial Mutation Analysis	81403
Hereditary Peripheral Neuropathies (eg, Charcot-Marie-Tooth, spastic paraplegia), Genomic Sequence Analysis Panel, must include sequencing of at least 5 peripheral neuropathy-related genes (eg, BSCL2, GJB1, MFN2, MPZ, REEP1, SPAST, SPG11, SPTLC1)	81448
PMP22 Deletion/Duplication Analysis	81324
PMP22 Known Familial Mutation Analysis	81326
PMP22 Sequencing	81325

Criteria

Introduction

Requests for Charcot-Marie-Tooth (CMT) genetic testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, and
  - Pathogenic CMT-related mutation in a 1st or 2nd degree biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Distal muscle weakness and atrophy, or
  - Weak ankle dorsiflexion (e.g. foot drop), or
  - Distal sensory loss, or
  - Depressed or absent tendon reflexes, or
  - Foot deformity (e.g. high arches, hammer toes, pes cavus), or
  - Electrodiagnostic studies consistent with a peripheral neuropathy, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Age 18 years or older

PMP22 Deletion/Duplication Analysis

- Previous Genetic Testing:
  - No previous PMP22 deletion/duplication analysis, and
  - No known CMT-related mutation in the member of the member's family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Distal muscle weakness and atrophy, or
  - Weak ankle dorsiflexion (e.g. foot drop), or
  - Distal sensory loss, or
  - Depressed or absent tendon reflexes, or
  - Foot deformity (e.g. high arches, hammer toes, pes cavus), AND
- The member does not have an underlying non-genetic cause for their neuropathy (e.g. diabetic neuropathy, vitamin B12 deficiency, chronic inflammatory

demyelinating polyneuropathy), or clinical suspicion for a gene mutation remains high even in the presence of a non-genetic cause, AND

- Member's electrodiagnostic studies are consistent with a primary demyelinating neuropathy

CMT Neuropathy Multigene Panel

Multi-gene panels will be considered medically necessary when the following criteria are met:

- Previous Genetic Testing:
  - No previous CMT neuropathy multi-gene panel testing, and
  - No known CMT-related mutation in the member or the member's family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Distal muscle weakness and atrophy, or
  - Weak ankle dorsiflexion (e.g. foot drop), or
  - Distal sensory loss, or
  - Depressed or absent tendon reflexes, or
  - Foot deformity (e.g. high arches, hammer toes, pes cavus), AND
- The member does not have an underlying non-genetic cause for their neuropathy (e.g. diabetic neuropathy, vitamin B12 deficiency, chronic inflammatory demyelinating polyneuropathy), or clinical suspicion for a gene mutation remains high even in the presence of a non-genetic cause, AND
- The panel includes the genes with the highest diagnostic yield for the member's suspected CMT neuropathy subtype, AND
- Member's electrodiagnostic studies are consistent with an axonal neuropathy or combined axonal and demyelinating neuropathy (e.g., CMT1 is NOT the most likely diagnosis), OR
- Member's electrodiagnostic studies are consistent with a primary demyelinating neuropathy (e.g., CMT1 is the most likely diagnosis) and PMP22 deletion/duplication analysis was previously performed and was negative

Other Considerations

Broad CMT neuropathy panels are not medically necessary when a narrower panel is available and more appropriate based on the clinical findings.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81448*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
    - When the test is billed with multiple stacked codes, only sequencing of the following genes may be considered for reimbursement, based on electrodiagnostic findings and the family history:
        - Primary demyelinating neuropathy with negative PMP22 deletion/duplication analysis (CMT1 suspected): MPZ, PMP22, LITAF (SIMPLE) and EGR2.
        - Primary axonal neuropathy (CMT2 suspected): MFN2, MPZ and HSPB1 (HSP27). If there is no evidence of male-to-male transmission in the family, GJB1 (for CMTX) is also reimbursable.
        - Combined axonal and demyelinating neuropathy (intermediate CMT suspected): DNM2, YARS, MPZ, and GNB4.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is Charcot-Marie-Tooth Hereditary Neuropathy?

Definition

Charcot-Marie-Tooth Hereditary Neuropathy (CMT) is a group of inherited genetic conditions characterized by chronic motor and sensory polyneuropathy.[1]

Prevalence

CMT is the most common inherited neurological disorder. The prevalence of all CMT types is 1 in 2,500.[1,2]

Symptoms

The key finding in CMT is symmetric, slowly progressive distal motor neuropathy of the arms and legs, usually beginning in the first to third decade and resulting in weakness and atrophy of the muscles in the feet and/or hands. This is expressed as distal muscle weakness and atrophy, weak ankle dorsiflexion, depressed tendon reflexes, and pes cavus foot deformity (e.g. high arched feet).[1]

Cause

The most common cause of CMT is a large chromosome 17 duplication involving the PMP22 gene (CMT1A), but more than 80 different genes have been associated with CMT.[1]

As more genes causing CMT were identified and as the overlap of neuropathy phenotypes and modes of inheritance became apparent, the previous alphanumeric classification system proved unwieldy and inadequate. In 2018, Magy et al proposed a gene-based classification of inherited neuropathies, which includes a comprehensive list of CMT-associated genes and correlation with the alphanumeric classification.[3] An additional advantage of this classification system is that a patient's findings can be described in terms of mode of inheritance, neuropathy type, and gene.

Establishing a specific genetic cause of CMT hereditary neuropathy can aid in discussions of prognosis and risk to family members.[1]

Inheritance

CMT can be inherited in an autosomal dominant, autosomal recessive, or an X-linked manner.[1] De novo cases are reported, but the proportion ranges widely depending on the gene involved.[1]

Diagnosis

The clinical diagnosis of CMT in a symptomatic person is based on characteristic findings of peripheral neuropathy on medical history and physical examination.[1] CMT needs to be distinguished from the following entities: systemic disorders with neuropathy, other types of hereditary neuropathy, distal myopathies, hereditary sensory neuropathies (HSN), and acquired disorders.[1]

Molecular genetic testing can be used to establish a specific diagnosis, which aids in understanding the prognosis and risk assessment for family members.[1]

A 1.5Mb duplication at 17p11.2 that includes the PMP22 gene is the most common cause of CMT, accounting for up to 50% of cases.[1] Therefore, PMP22 deletion/duplication analysis is recommended as a first tier diagnostic test.[1] If negative, a multi-gene testing panel may be indicated.

Management

Management of CMT is based on the symptoms present, and is often accomplished through a multidisciplinary team.[1] Treatment addresses neurological deficits and mobility issues, often including physical and occupational therapies and orthoses to aid in walking.[1]

Survival

Life span is normal in many forms of CMT, but quality of life is often impacted by the degree of physical disability experienced.[1]

Test information

Introduction

Testing for CMT may include known familial mutation analysis, deletion/duplication analysis, and/or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to

conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

CMT multi-gene testing panels include a wide variety of genes associated with CMT neuropathy. The following are points to consider regarding multi-gene testing panels for CMT:

- Multi-gene testing panels may include genes without clear management recommendations. A comprehensive panel with simultaneous testing of most known genes for CMT may not be cost-effective or necessary.[1,4]
- Multi-gene testing panels may vary in technical specifications (e.g. depth of coverage, large deletion/duplication analysis, etc).
- Given differences in testing methods and sensitivity, single-gene testing after a negative multi-gene testing panel may be warranted if there is a high clinical suspicion for a particular syndrome.
- The genes included on a multi-gene testing panel may vary. The medical record should document the performing laboratory and genes tested.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to CMT genetic testing.

American Academy of Neurology

Evidence-based guidelines from the American Academy of Neurology (AAN, 2022) recommended testing for CMT, but with a tiered approach:[5]

- “Genetic testing should be conducted for the accurate diagnosis and classification of hereditary neuropathies.”
    - This is considered a level A recommendation which is defined as “established as effective, ineffective or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population.”
- “Genetic testing may be considered in patients with cryptogenic polyneuropathy who exhibit a hereditary neuropathy phenotype. Initial genetic testing should be guided by the clinical phenotype, inheritance pattern, and electrodiagnostic features and should focus on the most common abnormalities which are CMT1A duplication/HNPP deletion, Cx32 (GJB1), and MFN2 mutation screening.”

- This is considered a level C recommendation which is defined as “possibly effective, ineffective or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population.”

- “There is insufficient evidence to determine the usefulness of routine genetic testing in patients with cryptogenic polyneuropathy who do not exhibit a hereditary neuropathy phenotype.”

  - This is considered a level U recommendation which is defined as “data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.”

Selected Relevant Publications

DiVincenzo et al. [2014] described their experience testing more than 17,000 patients for CMT using a commercially available comprehensive panel of 14 genes.[6] Overall, they identified a mutation in 18.5% of patients. Notably they state that “Among patients with a positive genetic finding in a CMT-related gene, 94.9% were positive in one of four genes (PMP22, GJB1, MPZ, or MFN2). The results of our study in a population in over 17,000 individuals support the initial genetic testing of four genes (PMP22, GJB1, MPZ, and MFN2) followed by an evaluation of rarer genetic causes in the diagnostic evaluation of CMT.” [6]

Dohrn et al. [2017] examined over 600 patients with either a CMT phenotype, hereditary sensory neuropathy, familial amyloid neuropathy, or small fiber neuropathy using an NGS multigene panel.[2] At least one putative pathogenic mutation was identified in 121 cases (19.8%); the most frequently affected genes were PMP22, GJB1, MPZ, SH3TC2, and MFN2. Likely or known pathogenic variants in HINT1, HSPB1, NEFL, PRX, IGHMBP2, NDRG1, TTR, EGR2, FIG4, GDAP1, LMNA, LRSAM1, POLG, TRPV4, AARS, BIC2, DHTKD1, FGD4, HK1, INF2, KIF5A, PDK3, REEP1, SBF1, SBF2, SCN9A, and SPTLC2 were detected with a declining frequency. One pathogenic variant in MPZ was identified after being previously missed by Sanger sequencing. The authors conclude that panel-based NGS “is a useful, time and cost effective approach to assist clinicians in identifying the correct diagnosis and enable causative treatment considerations”.[2]

Bacquet et al [2018] compared the diagnostic yield of targeted NGS with their previous step-wise Sanger sequencing strategy.[7] A cohort of 123 unrelated patients affected with diverse forms of inherited peripheral neuropathies including CMT (23% CMT1, 52% CMT2), distal hereditary motor neuropathy (9%), hereditary sensory and autonomic neuropathy (7%), and intermediate CMT (6.5%) were evaluated using an 81-gene NGS panel. Pathogenic variants were identified in 49 of 123 patients (~40%). In this cohort, the most frequently mutated genes were: MFN2, SH3TC2, GDAP1, NEFL, GAN, KIF5A and AARS, respectively. “Panel-based NGS was more efficient in familial cases than in sporadic cases (diagnostic yield 49% vs 19%, respectively). NGS-based search for copy number variations, allowed the identification of three duplications in three patients and raised the diagnostic yield to 41%. This yield is two times higher than the one obtained previously by gene Sanger sequencing screening. The impact of panel-based NGS screening is particularly important for demyelinating CMT (CMT1)

subtypes, for which the success rate reached 87% (36% only for axonal CMT2)." [7] While NGS panels were able to identify causal variants in a shorter and more cost-effective time, the authors caution that this approach, "leads to the identification of numerous variants of unknown significance, which interpretation requires interdisciplinary collaborations between molecular geneticists, clinicians and (neuro) pathologists".[7]

Gemelli et al [2022] examined a cohort of 585 patients (447 index cases), 64.9% of whom had a demyelinating neuropathy and 35.1% of whom had an axonal neuropathy. Combining a gene-by-gene approach or targeted gene panels based on clinical presentation, a genetic diagnosis was achieved in 66% of all patients, with the following distribution: CMT1A (48%), HNPP (14%), CMT1X (13%), CMT2A (5%), and P0-related neuropathies (7%), accounting all together for 87% of all the molecularly defined neuropathies.[8]

In a 2023 expert-authored review, the following step-wise genetic testing strategy was recommended:[1]

- Step 1: "Single-gene testing for PMP22 duplication/deletion is recommended as the first test in all probands with CMT. PMP22 duplication (a 1.5-Mb duplication at 17p11.2 that includes PMP22) accounts for as much as 50% of all CMT and, thus, PMP22 deletion/duplication analysis is recommended as the first test for all probands with CMT."
- Step 2: "A multigene panel that includes the eight most commonly involved genes (i.e.,GDAP1,GJB1,HINT1,MFN2,MPZ,PMP22, SH3CT2, and SORD) as well as some or all of the other genes listed in Table 4 is most likely to identify the genetic cause of the neuropathy while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype."
- Step 3: "Comprehensive genomic testing - which does not require the clinician to determine which gene(s) are likely involved – may be considered if a genetic cause has not been identified in Step 1 and Step 2. Exome sequencing is most commonly used; genome sequencing is also possible."
- "Given the complexity of interpreting genetic test results and their implications for genetic counseling, health care providers should consider referral to a neurogenetics center or a genetic counselor specializing in neurogenetics..."
- "For asymptomatic minors at risk for adult-onset conditions for which early treatment would have no beneficial effect on disease morbidity and mortality, predictive genetic testing is considered inappropriate, primarily because it negates the autonomy of the child with no compelling benefit. Further, concern exists regarding the potential unhealthy adverse effects that such information may have on family dynamics, the risk of discrimination and stigmatization in the future, and the anxiety that such information may cause."

References

Introduction

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CHARGE Syndrome and CHD7 Disorder Genetic Testing

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Introduction

CHARGE Syndrome and CHD7 disorder genetic testing are addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
CHD7 Deletion/Duplication Analysis	81479
CHD7 Known Familial Mutation Analysis	81403
CHD7 Sequencing	81407

Criteria

Introduction

Requests for CHD7 genetic testing are reviewed using the following criteria.

CHD7 Known Familial Mutation Analysis

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing of CHD7 that would detect the familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals
  - Known family mutation in CHD7 in 1st degree biological relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CHD7 Sequencing

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous CHD7 sequencing, and
  - No known CHD7 mutation in the family, and
  - Chromosomal microarray, if performed, was negative, AND
- Diagnostic Testing for Symptomatic Individuals
  - The member is suspected to have CHARGE syndrome, but the diagnosis is in question because member meets ONLY ONE of the following using the Blake or Verloes criteria (see Table: *Clinical Diagnostic Criteria for Typical CHARGE Syndrome*, below)
    - 2 major criteria and 1 minor criterion, or
    - 2 major criteria and 0 minor criteria, or
    - 1 major criterion and 3 minor criteria, AND
- Molecular test results will impact medical management, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CHD7 Deletion/Duplication Analysis

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous CHD7 deletion/duplication testing, and
  - Previous CHD7 sequencing was performed and was negative, and
  - No known CHD7 mutation in the family, and
- Diagnostic Testing for Symptomatic Individuals
  - The member meets the above criteria for CHD7 sequencing, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is CHARGE Syndrome/CHD7 disorder?

Definition

CHARGE syndrome is a clinically variable syndrome involving multiple congenital anomalies of diverse organ systems.[1] The phenotype has been expanded to CHD7 disorder, which encompasses the full spectrum of clinical findings in individuals with pathogenic CHD7 mutations. This guideline focuses on CHARGE syndrome, as the majority of individuals found to have CHD7 mutations have clinical findings typical of CHARGE syndrome.[2]

Incidence

CHARGE syndrome occurs in approximately 1/10,000 newborns with an estimated range of 1/8,500 – 1/15,000.[1-3] The disorder is pan-ethnic.[3]

Symptoms

CHARGE was the acronym initially used to describe an association of eye colobomas, heart defects, choanal atresia, growth retardation, genital anomalies, and ear malformations.[1] Following the discovery that heterozygous CHD7 variants cause CHARGE syndrome, molecular genetic testing of family members of probands with CHARGE syndrome expanded the phenotypic spectrum to include phenotypes that do not fulfill the previously proposed CHARGE syndrome clinical diagnostic criteria.[1,2] Additional symptoms associated with CHD7-related disorder phenotype include cleft lip and/or palate, developmental delay, hearing loss, cranial nerve anomalies, vestibular defects, hypothyroidism, hypogonadotropic hypogonadism, tracheoesophageal anomalies, brain anomalies, seizures, renal anomalies, and characteristic dysmorphic facial features.[1,2] Thus, CHD7 disorder exhibits a high degree of clinical variability even among individuals in the same family and among individuals from different families with the same pathogenic variant.[1,2] Given this variability, the presence of a CHD7 mutation is "not equivalent to a diagnosis of CHARGE syndrome."[2]

Cause

CHARGE syndrome and CHD7 disorder are caused by mutations in the CHD7 gene. This gene plays a role in guidance of neural crest cell migration.[4] Sequencing the CHD7 gene will find a causative mutation in 98% of affected individuals.[2] Approximately 2% of mutations identified in CHD7 are whole or partial gene deletions.[2]

Inheritance

CHARGE syndrome and CHD7 disorder are considered autosomal dominant disorders. Although some cases of parent to child transmission of CHARGE syndrome have been reported, most cases are simplex (the only case in the family) and CHD7 mutations, if identified, are typically de novo.[1,2]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

If neither parent is affected, there is a 1-2% risk of recurrence, most likely due to germline mosaicism.[2]

Diagnosis

Two common sets of clinical diagnostic criteria for CHARGE syndrome have been described.[1] The Blake criteria (first published in 1998 and updated in 2009) set out major and minor diagnostic criteria to be used in diagnosing typical CHARGE syndrome.[5,6] The Verloes criteria provide a means of diagnosing typical CHARGE syndrome (see Table), as well as minor presentations termed partial CHARGE (criteria: 2 major and 1 minor) and atypical CHARGE (criteria: 2 major and 0 minor or 1 major and 3 minor).[7] There are no clinical diagnostic criteria for the phenotypic spectrum associated with CHD7 disorder.

Clinical Diagnostic Criteria for Typical CHARGE Syndrome (Adapted from Bergman et al 2011)[1]

Criteria Set	Major Criteria	Minor Criteria
Blake[5,6] (4 Major or 3 Major and 3 Minor)	Coloboma or microphthalmia Choanal atresia or stenosis External ear anomaly/ middle ear malformation/ mixed sensorineural deafness Cranial nerve dysfunction	Cardiac defect Tracheo-esophageal defects Genital hypoplasia or delayed puberty Cleft lip and/or palate Developmental delay Growth retardation Characteristic facial features

Criteria Set	Major Criteria	Minor Criteria
Verloes[7] (3 Major or 2 Major and 2 Minor)	Ocular coloboma Choanal atresia Hypoplastic semicircular canals of the inner ear	Cardiac or esophageal malformation Malformation of the middle or external ear Rhombencephalic dysfunction including sensorineural deafness Hypothalamo-hypophyseal dysfunction (gonadotropin or growth hormone deficiency) Intellectual disability

Management

Management of CHARGE syndrome and CHD7 disorder is based on the variable clinical manifestations. Airway management and cardiac assessment are essential in the newborn period, as is addressing feeding and growth difficulties.[2] Other recommended evaluations and surveillance include the following:[2]

- Ophthalmologic assessment
- Audiologic assessment
- ENT assessment, including imaging to assess middle and inner ear defects
- Genitourinary assessment, including renal ultrasound
- Endocrine evaluation if puberty is delayed or if there is presence of genital anomalies
- Cranial nerve assessment / swallowing studies
- Gastrointestinal assessment for esophageal atresia or trachea-esophageal fistula
- Developmental assessment

Survival

“Life expectancy highly depends on the severity of manifestations; mortality can be high in the first few years when severe birth defects (particularly complex heart defects) are present and often complicated by airway and feeding issues. In childhood, adolescence, and adulthood, decreased life expectancy is likely related to a combination of residual heart defects, infections, aspiration or choking, respiratory issues including obstructive and central apnea, and possibly seizures. Despite these complications, the life expectancy for many individuals can be normal.”[2]

Test Information

Introduction

Testing for CHARGE syndrome and CHD7 disorder may include known familial mutation analysis, next generation sequencing, or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and Evidences

Introduction

The following section includes relevant guidelines and evidence pertaining to CHARGE syndrome and CHD7 disorder genetic testing.

Selected Relevant Publications

van Ravenswaaij-Arts et al., 2022

An expert authored review updated in 2022 stated:[2]

- “With the current widespread use of multigene panels and comprehensive genomic testing, it has become apparent that the phenotypic spectrum of

heterozygous CHD7 pathogenic variants has broadened to encompass CHARGE syndrome as well as subsets of features that comprise the CHARGE syndrome phenotype ."

- “CHD7 disorder, refers to the entire phenotypic spectrum that can be associated with heterozygous CHD7 pathogenic variants and emphasizes both the need to evaluate an individual found to have a CHD7 pathogenic variant for medically actionable manifestations in the entire phenotypic spectrum (regardless of clinical findings that prompted molecular genetic testing) and the importance of counseling families that the finding of a CHD7 pathogenic variant is not equivalent to a diagnosis of CHARGE syndrome."
- “The diagnosis of CHD7 disorder is established in a proband with suggestive clinical and imaging findings and a heterozygous pathogenic variant in or deletion of CHD7 identified by molecular genetic testing."
- "Sequence analysis of CHD7 is performed to detect small intragenic deletions/insertions and missense, nonsense, and splice site variants. Note: Depending on the sequencing method used, single-exon, multiexon, or whole-gene deletions/duplications may not be detected. If no variant is detected by the sequencing method used, the next step is to perform gene-targeted deletion/duplication analysis to detect exon and whole-gene deletions or duplications and/or chromosomal microarray (CMA) to detect whole-gene deletions."
- “Because CHD7 disorder typically includes multiple congenital anomalies, it is also reasonable to pursue chromosomal microarray testing first, unless classic features of CHD7 disorder (e.g., the CHARGE syndrome phenotype) are apparent."
- “Management of the manifestations of CHD7 disorder can be complex and require a multidisciplinary approach involving clinicians, therapists, and educators."
- “Requires routine follow up of manifestations identified in infancy/childhood, as well as ongoing monitoring of growth, development, educational progress, behavior, and possible endocrine issues."
- “Because of the increased risk of post-anesthesia airway complications, procedures requiring anesthesia should be minimized and combined whenever possible."

van Ravenswaaij-Arts and Martin, 2017

In a review of the etiology and diagnosis, van Ravenswaaij-Arts and Martin stated:[8]

- “In clinically typical individuals with CHARGE syndrome, the tests of first choice are CHD7 Sanger sequencing and chromosomal microarray to screen for deletions and/or MLPA to test for exonic-deletions.”

- "CHD7 pathogenic variants have been described in very mildly affected individuals, for example, individuals with isolated hypogonadotropic hypogonadism [HH] due to CHD7 missense variants."
- "It is recommended that individuals with HH and a CHD7 variant be clinically screened for CHARGE syndrome features such as balance problems and deafness, amongt [sic] others."
- "One to two percent of individuals who test positive have an intragenic or whole CHD7 gene deletion that can be detected by microarray analysis, although for small exonic deletions, MLPA is preferred."
- "Most individuals with CHARGE syndrome are sporadic, but recurrence has been documented. ... Parent-child transmission with a recurrence risk of 50% is predominantly seen in milder presentations of the syndrome, although intrafamilial variability is high and a mildly affected parent does not exclude a more severely affected child. If the pathogenic CHD7 variant of a proband cannot be detected in leukocyte DNA of the parents, there remains a 1-2% recurrence risk due to germline mosaicism."

Hefner and Fassi, 2017

In a review of genetic counseling issues in CHARGE syndrome, Hefner and Fassi stated:[9]

- "[Genetic counseling] is particularly important in CS [CHARGE syndrome], as it is extremely complex and variable in its presentation and in its natural history."
- "Despite the identification of pathogenic CHD7 variants in the majority of cases, the diagnosis of CS remains clinical...with genetic testing being particularly helpful in borderline clinical cases."
- "As CS can affect any organ system in the body, the features overlap with countless other syndromes. The top candidates in the differential diagnosis of CS are 22q11.2 deletion syndrome (22q) and Kabuki syndrome (KS). VACTERL association also has a good deal of overlap, but typically does not have significant dysmorphic features."
- "CMA is often performed initially for fetuses or infants with multiple anomalies. This is reasonable as 22q is far more common than CS and CMA can identify other rare microdeletions or microduplications with overlapping features."
- "If CMA is nondiagnostic, CHD7 genetic testing (sequencing and deletion/duplication analysis) is recommended in the presence of any major feature of CS with multiple anomalies. If CHD7 analysis is nondiagnostic, whole exome sequencing (WES) may be considered."
- "Every individual with CS has his or her own unique set of medical and developmental issues. Medical management of CS involves comprehensive monitoring of multiple organ systems by a multitude of specialists."

- "Appropriate therapies will involve not only traditional therapies (occupational, physical, speech, and language therapies, etc.) but require the expertise of DB [deafblind] specialists. DB specialists are professionals expert in the unique needs of children with multiple sensory impairments."
- Genetic counseling should include information on prognosis including mortality, morbidity, and sensory, motor and intellectual expectations.

Bergman et al., 2011

In addressing molecular testing for CHARGE syndrome, Bergman and colleagues suggested that CHD7 testing, including sequencing and deletion analysis, should be considered in individuals with:[1]

- 3 cardinal features
- 2 cardinal features and 1 supportive feature
- 2 cardinal features if imaging shows semicircular canal abnormalities
- 1 cardinal feature and 1 supportive feature if imaging shows semicircular canal abnormalities

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Chromosomal Microarray for Solid Tumors

MOL.TS.344.A
v2.0.2024

Introduction

Chromosomal microarray analysis of solid tumors is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure code(s)
Cytogenomic Neoplasia Microarray Analysis	81277

Criteria

Chromosomal microarray on solid tumor tissue is medically necessary in individuals who meet the following criteria:

- Member has been diagnosed with:
  - Cancer of the central nervous system, or
  - Soft tissue sarcoma, AND
- Rendering laboratory is a qualified provider of service per Health Plan policy.

What are chromosome abnormalities in cancer?

Introduction

Chromosomal aberrations are known to contribute to tumorigenesis.[1]

Chromosome Abnormalities in Cancer

A chromosome abnormality is any difference in the structure, arrangement, or amount of genetic material packaged into the chromosomes. Chromosome abnormalities have been identified in many types of cancer, including leukemias, lymphomas, and solid tumors. Chromosome abnormalities can include deletions, duplications, balanced or

unbalanced rearrangements, and gain or loss of whole or partial chromosomes. These abnormalities can play a key role in the development, diagnosis, and monitoring of cancer. The cytogenetics of a cancer can also change over time or in response to treatment. Therefore, chromosome analysis can be used to monitor disease progression and treatment response.

“[C]ancer is thought to be a consequence of genomic alteration accumulation, such as single-nucleotide variants (SNVs) and copy number variants (CNVs), and structural rearrangements, which encompass deletions, duplications, inversions, insertions, and translocations that could lead to novel fusion genes.” [2]

Some chromosome abnormalities are characteristic of certain types of malignancy, and can be used to classify a type or subtype of cancer. For example, codeletion of 1p and 19q along with IDH1/2 mutations indicate oligodendroglioma.[3]

“The presence of specific chromosomal and genetic alterations exclusively observed in malignant cells helps in cancer diagnosis and prognosis, allowing also to quantify residual disease. Several different types and sizes of chromosomal abnormalities can be found in human cancers, being the products of these dysregulated genes and cellular pathways specific targets for new drugs.” [2]

Test information

Introduction

Chromosome analysis of solid tumors can be done through traditional cytogenetic testing (karyotype), fluorescence in situ hybridization (FISH), or chromosomal microarray. This guideline addresses only chromosomal microarray on solid tumors.

Chromosomal Microarray

CMA testing generally works by fluorescently tagging DNA from an individual’s test sample with one color and combining it with a control sample tagged in a different color. The two samples are mixed and then added to the array chip, where they compete to hybridize with the DNA fragments on the chip. By comparing the test sample versus the control, computer analysis can determine where genetic material has been deleted or duplicated in the individual.

There are a growing number of CMA testing platforms, including non-chip based applications, which differ in approach and resolution. Clinical laboratories may not only differ in the arrays that they utilize but also in their reporting practices. Although testing guidelines do not endorse one CMA over another, it is typically advisable that coverage of an ordered CMA is better than that offered by a standard karyotype and that the minimum resolution of the CMA provided by the laboratory is adequate. The inclusion of analysis of subtelomeric regions and known microdeletion syndromes with CMA testing obviates the need for additional FISH analysis.

CMA testing offers advantages over conventional karyotyping with regard to resolution and yield. However, there are some limitations of CMA testing including:

- the inability to detect
  - balanced chromosomal rearrangements such as translocations or inversions
  - certain forms of polyploidy
  - sex chromosome aneuploidy dependent on the gender control used
  - low level mosaicism
  - some marker chromosomes
- the detection of CNVs of uncertain clinical significance
- the inability to differentiate free trisomies from unbalanced Robertsonian translocations.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to chromosomal microarray in solid tumors.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2019) provided technical standards and guidelines for interpretation and reporting of acquired copy number abnormalities and loss of heterozygosity in neoplastic disorders:[4]

- "Genomic testing of hematologic malignancies and solid tumors at the time of disease presentation provides information that is crucial for diagnosis and management. This evaluation may include G-banded chromosome analysis, fluorescence in situ hybridization (FISH) analysis, chromosomal microarray analysis (CMA), gene expression and fusion studies, targeted gene sequencing, as well as gene sequencing panels."
- "[A] unified approach for the clinical interpretation, classification, and reporting of all somatic variants will become a necessity."
- Tier 1 variants are those with a strong clinical significance, and several cytogenetic abnormalities in CNS cancers are classified as Tier 1. Additionally, select cytogenetic abnormalities are classified as Tier 1 in the following cancers:
  - Renal cell carcinoma
  - Pediatric embryonal cancers
  - Breast cancer
  - Bone cancer
  - Gastrointestinal stromal tumors

- Mesothelioma

- "The laboratory must ensure that the clinical report accurately describes the findings and clearly communicates their clinical significance."

The American College of Medical Genetics and Genomics (ACMG, 2016) provided technical standards and guidelines for chromosome analysis in solid tumor-acquired chromosome abnormalities:[5]

- "Genetic analysis of solid tumors and lymphomas at diagnosis provides information critical for diagnosis and patient management."
- "Analysis of tumor tissues may be accomplished by conventional chromosome analysis, fluorescence in situ hybridization (FISH) analysis, chromosomal microarray (CMA) analysis, molecular analysis, or a combination of methodologies."
- "The method(s) chosen for evaluation of a tumor at the time of biopsy or resection will depend on the differential diagnosis, clinical indications, available tissue, available methodologies, and initial histopathology of the tumor tissue."
- "CMA can provide valuable information to supplement that of chromosomal and FISH analyses. Isolated tumor DNA hybridized to whole-genome copy number and/or single nucleotide polymorphism microarrays allows detection of loss, gain, and amplification of regions of DNA, which may not otherwise be detected."
- "[T]umor materials should be studied with available methods to gain as much information as possible at the time of initial study. At a time of suspected disease recurrence or metastasis, the initial genetic data will be used to confirm recurrence or metastasis, assess clonal disease evolution, or reveal a new malignant process."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) guideline on soft tissue sarcoma stated:[6]

- "Morphologic diagnosis based on microscopic examination of histologic sections remains the gold standard for sarcoma diagnosis. However, several ancillary techniques are useful in support of morphologic diagnosis, including IHC, classical cytogenetics, electron microscopy, and molecular genetic testing. Molecular genetic testing has emerged as an ancillary testing approach since many sarcoma types harbor characteristic gene aberrations, including single base pair substitutions, deletions and amplifications, and translocations. Molecular testing utilizes multiple techniques such as fluorescence in situ hybridization (FISH) approaches or polymerase chain reaction (PCR)-based methods and next-generation sequencing (NGS)-based methods (including DNA and RNA sequencing)."

The National Comprehensive Cancer Network (NCCN, 2023) guideline on central nervous system cancers stated:[7]

- "With the use of genetic and molecular testing, histologically similar CNS neoplasms can be differentiated more accurately in terms of prognosis and, in some instances, response to different therapies."
- "Molecular characterization of primary CNS tumors has substantially impacted clinical trial eligibility and risk stratification in the past 10 years, thereby evolving the standard of care towards an integrated tumor diagnosis in neuro-oncology".
- "Molecular/genetic characterization does not replace standard histologic assessment, but serves as a complementary approach to provide additional diagnostic and prognostic information that often enhances treatment selection."
- "Some diffusely infiltrative astrocytomas lack the histologic features of glioblastoma (necrosis and/or microvascular proliferation) but have the molecular hallmarks of glioblastoma, including one or more of the following: EGFR amplification; gain of chromosome 7 and loss of chromosome 10; and TERT promoter mutation. In such cases, the tumor can now be diagnosed as "Glioblastoma, IDH-wild-type, WHO grade 4". Because these tumors have similar clinical outcomes as typical grade 4 glioblastomas, they may be treated as such.
- “Recommendation: 1p19q testing is an essential part of molecular diagnostics for oligodendroglioma.”
- While this is most often assessed by FISH or PCR, array-based testing or NGS may also be used.

World Health Organization

The World Health Organization (WHO, 2021) classification of tumors of the central nervous system stated:[3]

- "Because of the growing importance of molecular information in CNS tumor classification, diagnoses and diagnostic reports need to combine different data types in a single "integrated" diagnosis. Such integrated diagnoses are implicit in the use of WHO CNS5…Thus, to display the full range of diagnostic information available the use of layered (or tiered) diagnostic reports is strongly encouraged… Such reports feature an integrated diagnosis at the top, followed by layers that display histological, molecular, and other key types of information."
- "In the updated fourth edition CNS classification from 2016, the common diffuse gliomas of adults were divided into 15 entities, largely because different grades were assigned to different entities (eg, Anaplastic oligodendroglioma was considered a different type from Oligodendroglioma) and because NOS designations were assigned to distinct entities (eg, Diffuse astrocytoma, NOS). WHO CNS5, on the other hand, includes only 3 types: Astrocytoma, IDH-mutant; Oligodendroglioma, IDH-mutant and 1p/19q-codeleted; and Glioblastoma, IDH-wildtype."
- "...[A]ll IDH-mutant diffuse astrocytic tumors are considered a single type (Astrocytoma, IDH-mutant) and are then graded as CNS WHO grade 2, 3, or 4. Moreover, grading is no longer entirely histological, since the presence of CDKN2A/

B homozygous deletion results in a CNS WHO grade of 4, even in the absence of microvascular proliferation or necrosis."

- "For IDH-wildtype diffuse astrocytic (NB: diffuse and astrocytic) tumors in adults, a number of papers have shown that the presence of 1 or more of 3 genetic parameters (TERT promoter mutation, EGFR gene amplification, combined gain of entire chromosome 7 and loss of entire chromosome 10 [+7/−10]) appears sufficient to assign the highest WHO grade. WHO CNS5 therefore incorporates these 3 genetic parameters as criteria for a diagnosis of Glioblastoma, IDH-wildtype. As a result, Glioblastoma, IDH-wildtype should be diagnosed in the setting of an IDH-wildtype diffuse and astrocytic glioma in adults if there is microvascular proliferation or necrosis or TERT promoter mutation or EGFR gene amplification or +7/−10 chromosome copy number changes."
- "Several molecular biomarkers are also associated with classification and grading of meningiomas, including SMARCE1 (clear cell subtype), BAP1 (rhabdoid and papillary subtypes), and KLF4/TRAF7 (secretory subtype) mutations, TERT promoter mutation and/or homozygous deletion of CDKN2A/B (CNS WHO grade 3), H3K27me3 loss of nuclear expression (potentially worse prognosis), and methylome profiling (prognostic subtyping."

Selected Relevant Publication

Ribeiro and colleagues stated in an expert-authored review (2019):[2]

- “Chromosome translocations, inversions, and insertions are frequently found in solid tumors…” however, “only few biomarkers have been approved for clinical practice that could change clinical decision making, helping in the therapeutic choices and patient management, showing the complexity of cancer and the lack of a strong bridge between the laboratory and clinicians.”

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Chromosomal Microarray Testing For Developmental Disorders (Prenatal and Postnatal)

MOL.TS.150.A
v2.0.2024

Introduction

Chromosomal microarray (CMA) testing for developmental disorders in the prenatal or postnatal setting is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Chromosomal Microarray [BAC], Constitutional	81228
Chromosomal Microarray [CGH], Constitutional	S3870
Chromosomal Microarray [SNP], Constitutional	81229
Cytogenomic (Genome-wide) Analysis for Constitutional Chromosomal Abnormalities; Interrogation of Genomic Regions for Copy Number and Loss-of-heterozygosity Variants, Low-pass Sequencing Analysis	81349

Criteria

Introduction

Requests for chromosomal microarray (CMA) testing for developmental disorders in the prenatal and postnatal setting are reviewed using these criteria.

Criteria

- Genetic Counseling:

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Diagnostic Testing for Symptomatic Individuals:
  - No previous CMA testing,* and
  - Testing performed on living child or adult, and
  - Diagnosis cannot be made on clinical evaluation alone, and
  - A more appropriate targeted test is not available (e.g., chromosome analysis, fluorescence in situ hybridization [FISH], single gene sequencing, etc.), and
  - Common aneuploidy (trisomy 13, 18, 21, or sex chromosome) is not a suspected diagnosis, and
  - One of the following presentations:
    - Developmental delay/intellectual disability (DD/ID), or
    - Autism spectrum disorder, or
    - Major congenital cardiac anomaly†, or
    - Multiple congenital anomalies†, OR

- Diagnostic Testing for Intrauterine Fetal Demise or Stillbirth:
  - No previous CMA testing in the same pregnancy, and
  - A more appropriate targeted test is not available (e.g., chromosome analysis, FISH, single gene sequencing, etc.), and
  - Common aneuploidy (trisomy 13, 18, 21, or sex chromosome) is not a suspected diagnosis, and
  - One of the following presentations:
    - Major congenital cardiac anomaly†, or
    - Multiple congenital anomalies†, or
    - Fetal demise or stillbirth occurred at 20 weeks of gestation or later, OR

- Diagnostic Prenatal Testing:
  - No previous CMA testing in the same pregnancy, and
  - The member has sufficient risk of fetal copy number variant (CNV) to justify invasive prenatal diagnosis. [It is important to note that invasive diagnostic procedures such as chorionic villus sampling and amniocentesis are associated with risks; the provider and member must have determined that the associated benefits outweigh the risks.], AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy

*Microarray is considered a first-tier test in the evaluation of postnatal developmental disorders. Therefore, it often is not necessary to do chromosome analysis or FISH in conjunction with microarray. Microarray requests following such testing will require review.

†**Multiple congenital anomalies** defined as 1) two or more major anomalies affecting different organ systems or 2) one major and two or more minor anomalies affecting different organ systems. **Major structural abnormalities** are generally serious enough as to require medical treatment on their own (such as surgery) and are not minor developmental variations that may or may not suggest an underlying disorder.

Chromosomal Microarray (CMA) Exclusions and Considerations

If routine karyotype and CMA are ordered simultaneously, only the most appropriate test based on clinical history will be considered for coverage. If CMA has been performed, the following tests are often excessive and thus not considered medically necessary. Each test may require medical necessity review.

- Routine karyotype: Full karyotype in addition to CMA is typically considered excessive. However, a limited 5 cell analysis may be approved in addition to CMA if criteria for CMA are met. This approval may be subject to claims review to ensure that the appropriate procedure code for a limited 5 cell analysis is billed (CPT 88261 x1, 88230 x1, 88291 x1).
- FISH Analysis
- Telomere Analysis
- More than one type of microarray analysis (i.e. if 81228 performed, 81229 is not medically necessary)
- CMA is not considered medically necessary in cases of family history of chromosome rearrangement in phenotypically normal individuals.
- CMA is not considered medically necessary in individuals experiencing infertility, structurally normal pregnancy losses that occur at less than 20 weeks, or recurrent pregnancy loss.
- When a multigene deletion/duplication panel is being requested and billed using a microarray procedure code (typically 81228 or 81229), please refer to the *Genetic Testing by Multigene Panels* clinical use guideline; do not apply the criteria in this guideline.
- CMA for delineation of deletion, duplication, or translocation breakpoints will be reviewed on a case by case basis.
- CMA for determination of whether a translocation is balanced or unbalanced with be reviewed on a case by case basis.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- CMA is only reimbursable once per lifetime.
- When CMA is otherwise reimbursable, the following limitations apply:
    - Only one type of microarray analysis (e.g., 81228, 81229 or 81439) will be reimbursed.
    - A limited 5 cell chromosome analysis (88261x1, 88230x1, 88291x1) may be reimbursed in addition to the CMA.
    - FISH or other procedure codes that do not accurately describe the test methodology performed (e.g. 88271) are not eligible for reimbursement of CMA.
- If CMA has been performed, the following tests are not reimbursable:
    - Routine karyotype
    - FISH Analysis
    - Telomere Analysis

What are Copy Number Variants in Developmental Disorders?

Introduction

Copy number variation is when the number of copies of genetic material differs between individuals.

Copy Number Variants (CNVs)

Copy number variants (CNVs) are deletions and duplications of genetic material. CNVs account for a significant proportion of congenital anomalies and developmental disorders without a clear etiology based on clinical findings. CNVs are detected using chromosomal microarray (CMA) testing. CMA is known by several names including array-comparative genomic hybridization (aCGH) and single-nucleotide polymorphism arrays (SNP-array).

Prevalence

Intellectual disability (ID) and congenital anomalies affect approximately 3-4% of the general population.[1] The incidence of global developmental delay (GDD) is comparable to ID. Autism spectrum disorders (ASD), which now includes autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), and Asperger syndrome are also of increasing concern, with recent CDC incidence figures estimating 1 in 36 affected children.[2]

Sixty to eighty percent of major structural anomalies are identified prenatally by ultrasound evaluation.[3]

Cause

The etiology of developmental disorders and congenital anomalies is complex. Some developmental problems may be caused by non-genetic factors, such as injury, birth complications, endocrine disorders, toxic exposures, and infection. However, genetic causes also play a significant role.[4-7]

A clinical genetics evaluation can identify a cause in a portion of individuals with ID, GDD, or ASD. Identifying an underlying genetic cause in these individuals may:[4-7]

- provide diagnostic and prognostic information
- improve health screening and prevention for some conditions
- allow for testing of family members and accurate recurrence risk counseling, and
- empower the individual and family to acquire needed services and support

CMA on chorionic villi or amniocytes is indicated in any pregnancy in which diagnostic testing for chromosome abnormalities and CNVs is desired.[8-10] Identifying an underlying genetic cause in these individuals may:[5]

- provide diagnostic and prognostic information
- guide prenatal management and decision-making, and
- allow for testing of family members and accurate recurrence risk counseling.

Parental Testing

If a CNV is detected in a child, it may be helpful to test both parents to determine whether the CNV is inherited or a new (de novo) genetic change. This information along with parental findings can be used to weigh the possibilities of a benign vs. pathogenic variant. However, even with parental studies, the clinical outcome may remain unclear.[1,8] A de novo variant is more likely to represent a pathologic abnormality.[1]

Clinical Classification of CNVs

In a joint consensus recommendation, the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome resource (ClinGen) introduced updated standards to help reduce discordance in clinical classifications of CNVs, including those detected during postnatal or prenatal testing.[11] The standards include a semi-quantitative point-based scoring system metric for CNV classification, including separate scoring metrics for copy number losses and copy number gains. Evaluation of the inheritance pattern, including whether the CNV is inherited or a new (de novo) genetic change, factors into this scoring system.

Test information

Introduction

Testing for developmental disorders in the prenatal and postnatal setting may include CMA testing.

Chromosomal Microarray

CMA testing generally works by fluorescently tagging DNA from an individual's test sample with one color and combining it with a control sample tagged in a different color. The two samples are mixed and then added to the array chip, where they compete to hybridize with the DNA fragments on the chip. By comparing the test sample versus the control, computer analysis can determine where genetic material has been deleted or duplicated in the individual.

There are a growing number of CMA testing platforms, including non-chip based applications, which differ in approach and resolution. Clinical laboratories may not only differ in the arrays that they utilize but also in their reporting practices. Although testing guidelines do not endorse one CMA over another, it is typically advisable that coverage of an ordered CMA is better than that offered by a standard karyotype and that the minimum resolution of the CMA provided by the laboratory is adequate. The inclusion of analysis of subtelomeric regions and known microdeletion syndromes with CMA testing obviates the need for additional FISH analysis.

CMA testing offers advantages over conventional karyotyping with regard to resolution and yield. However, there are some limitations of CMA testing including:

- the inability to detect
    - balanced chromosomal rearrangements such as translocations or inversions
    - certain forms of polyploidy
    - sex chromosome aneuploidy dependent on the gender control used
    - low level mosaicism
    - some marker chromosomes

- the detection of CNVs of uncertain clinical significance
- the inability to differentiate free trisomies from unbalanced Robertsonian translocations.

Diagnostic Yield

Diagnostic yield for CMA differs based on clinical presentation. The results of one multicenter trial of CMA in the prenatal setting reported that CMA identified a clinically relevant deletion or duplication in 6% of prenatal cases with a structural anomaly and normal karyotype. In addition, 1.7% of prenatal cases with an indication of advanced maternal age or positive screening results and normal karyotype had a clinically relevant deletion or duplication identified by CMA.[9]

In a large series of fetuses with ultrasound anomalies and normal conventional karyotype, CMA detected chromosome abnormalities in 5% of fetuses and up to 10% in those with 3 or more anatomic abnormalities.[12] A recent cohort study utilizing amniocentesis samples reported karyotype detected abnormalities in 5.41% of fetuses and CMA detected abnormalities in 9.14% of fetuses. The detection rate of CMA combined with karyotype was 0.35% higher than CMA alone and 4.08% higher than karyotype alone.[13]

Some forms of mosaic aneuploidy will only be detected by a cultured sample, as typically required for karyotype and would not be observed using CMA on a direct sample.[14]

The diagnostic yield for CMA across various clinical settings is presented below.

- Approximately 10-19% of people with unexplained ID or developmental delay (DD) will have CNVs.[1,6,7,15]
- CMA finds a pathogenic CNV in 5% to 14% of those with ASD who are tested clinically by this method.[16]
- The diagnostic yield in individuals with ASD is higher in those with a syndromic presentation, meaning that they have additional findings.[4]
- About 13% of spontaneous pregnancy losses that occurred between 10 and 20 weeks gestation had CNVs identified in one small prospective study.[17] Another study showed an approximate 55% diagnostic yield when performing CMA in first trimester losses.[18,19]
- CMA had a diagnostic yield of "41.9% in all stillbirths, 34.5% in antepartum stillbirths, and 53.8% in stillbirths with anomalies."[20]
- CMA may also be useful in the workup of non-immune fetal hydrops.[21,22]
- For prenatal cases with a structural anomaly and normal karyotype, about 6% will have a clinically relevant deletion or duplication identified by CMA.[9]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to CMA testing for developmental disorders in the prenatal and postnatal setting.

American Academy of Pediatrics

The American Academy of Pediatrics (AAP, 2014; reaffirmed 2019) Committee on Genetics recommended genetics evaluation for all individuals following the diagnosis of GDD or ID. They stated:[23]

"[i]f diagnosis is unknown and no clinical diagnosis is strongly suspected, begin the stepwise evaluation process:

- Chromosomal microarray should be performed in all.
- Specific metabolic testing should be considered and should include serum total homocysteine, acyl-carnitine profile, amino acids; and urine organic acids, glycosaminoglycans, oligosaccharides, purines, pyrimidines, GAA/creatine metabolites.
- Fragile X genetic testing should be performed in all."

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2010; reaffirmed 2020) Professional Practice and Guidelines Committee recommended CMA as a first-tier test for the evaluation of individuals who have the following:[5,6]

- “Multiple anomalies not specific to a well-delineated genetic syndrome.”
- “Apparently non-syndromic DD [developmental delay]/ID [intellectual disability].”
- “Autism spectrum disorders”

American College of Obstetricians and Gynecologists and Society for Maternal Fetal Medicine

The American College of Obstetricians and Gynecologists and Society for Maternal Fetal Medicine (ACOG/SMFM, 2016; reaffirmed 2023) joint committee opinion on chromosomal microarray stated:[24]

- “Chromosomal microarray analysis of fetal tissue (i.e. amniotic fluid, placenta, or products of conception) is recommended in the evaluation of intrauterine death or stillbirth when further cytogenetic analysis is desired because of the test's increased likelihood of obtaining results and improved detection of causative abnormalities.”
- “Additional information is needed regarding the clinical use and cost-effectiveness in cases of recurrent miscarriage and structurally normal pregnancy losses at less than 20 weeks of gestation.”

- “The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published.”
- "Prenatal chromosomal microarray analysis is recommended for a patient with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. This test typically can replace the need for fetal karyotype."
- "In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing, either fetal karyotyping or chromosomal microarray analysis can be performed."

The American College of Obstetricians and Gynecologists and Society for Maternal Fetal Medicine (ACOG/SMFM, 2016) practice bulletin on prenatal diagnostic testing stated:[25]

- CMA is recommended "as the primary test (replacing conventional karyotype) for patients undergoing prenatal diagnosis for the indication of a fetal structural abnormality detected by ultrasound examination."
- "It is recommended that chromosomal microarray analysis be made available to any patient choosing to undergoing invasive diagnostic testing."

European Heart Rhythm Association, Heart Rhythm Society, Asia Pacific Heart Rhythm Society, and Latin American Heart Rhythm Society

The European Heart Rhythm Association, Heart Rhythm Society, Asia Pacific Heart Rhythm Society, and Latin American Heart Rhythm Society (EHRA/HRS/APHRS/LAHRS, 2022) issued consensus statements regarding genetic testing for cardiac conditions.[26] The consensus statements were categorized as follows:

- Supported by strong observational evidence and author's consensus
- Some evidence and general agreement favor the usefulness/ efficacy of a test
- There is evidence or general agreement not to recommend a test

The following recommendations were made for chromosomal microarray:[26]

- Regarding antenatal testing: "When foetal congenital heart disease (CHD) is identified on antenatal ultrasound examinations, a chromosomal microarray (CMA) or CNV sequencing (CNV seq) of foetal tissue [amniocentesis or chorionic villous sample (CVS)] should be offered." [Supported by strong observational evidence and author's consensus]
- Regarding neonates and infants requiring investigation or procedures for complex CHD: "CMA or CNV seq is indicated in infants with CHD to identify pathogenic CNVs." [Supported by strong observational evidence and author's consensus]

- Regarding individuals with CHD and extracardiac anomalies: "CMA or CNV seq is indicated in patients with CHD and extracardiac anomalies to identify pathogenic CNVs." [Supported by strong observational evidence and author's consensus]
- Regarding sporadic non-syndromic CHD (excluding neonates or infants): "CMA or CNV seq for pathogenic CNVs may be performed in older individuals with sporadic non-syndromic CHD." [Some evidence and general agreement favor the usefulness/ efficacy of a test]

International Standard Cytogenomic Array Consortium

The International Standard Cytogenomic Array Consortium (ISCA, 2010) recommended offering CMA as a first-tier genetic test, in place of karyotype, for individuals with unexplained developmental delay/intellectual disability, autism spectrum disorders, or birth defects.[1]

Society for Maternal Fetal Medicine

The Society for Maternal Fetal Medicine (SMFM, 2016) published a consult series that stated:[27]

- We recommend that CMA "be offered when genetic analysis is performed in cases with fetal structural anomalies and/or stillbirth and replaces the need for fetal karyotype in these cases." (GRADE 1A).

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Cystic Fibrosis Genetic Testing

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Introduction

Cystic fibrosis testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
CFTR Targeted Mutation Analysis	81220
CFTR Known Familial Mutation Analysis	81221
CFTR Full Gene Sequencing	81223
CFTR Deletion/Duplication Analysis	81222
CFTR Poly T Tract (5T) Genotyping	81224

Criteria

Introduction

Requests for cystic fibrosis (CF) genetic testing are reviewed using these criteria.

CFTR Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing that would identify the familial mutation(s), AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individuals who have a suspected diagnosis of cystic fibrosis and the familial mutations to be tested were identified in 1st degree biologic relative(s), OR
- Mutation Identification to Guide Pharmacologic Therapy Selection

  - Individuals who meet diagnostic criteria for CF and are eligible for FDA-approved CFTR mutation-specific therapies, OR
- Carrier Screening:
  - Be of reproductive age and have potential and intention to reproduce, and
  - Familial CFTR mutation(s) in known biologic relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CFTR Targeted Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for CFTR mutation(s), AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individuals with an intermediate range/equivocal sweat chloride test (30-59mmol/L), or
  - Individuals with a negative sweat chloride test when symptoms of CF are present, or
  - Infants with meconium ileus or other symptoms indicative of CF and are too young to produce adequate volumes of sweat for sweat chloride test, or
  - Infants with an elevated IRT value on newborn screening, or
  - Fetus with finding of echogenic bowel on ultrasound, or
  - Males with oligospermia/azoospermia/congenital absence of vas deferens (CAVD), OR
- Mutation Identification to Guide Pharmacologic Therapy Selection
  - Individuals who meet diagnostic criteria for CF and are eligible for FDA-approved CFTR mutation-specific therapies, OR
- Carrier Screening:
  - Individuals of reproductive age and have potential and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CFTR Sequencing

- Genetic Counseling:

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Diagnostic Testing for Symptomatic Individuals:
  - Previous CFTR standard panel, if performed, was non-diagnostic (fewer than 2 pathogenic mutations detected), and
  - Individuals with a negative or equivocal sweat chloride test, and unexplained chronic obstructive pulmonary disease (COPD) or bronchiectasis with unexplained chronic or recurrent sinusitis and abnormal pulmonary function tests (PFTs), or
  - Infants with meconium ileus or other symptoms indicative of CF and are too young to produce adequate volumes of sweat for sweat chloride test, or
  - Infants with an elevated immunoreactive trypsinogen (IRT) value on newborn screening and fewer than 2 pathogenic mutations identified on standard panel testing, OR

- Mutation Identification to Guide Pharmacologic Therapy Selection
  - Individuals who meet diagnostic criteria for CF and are eligible for CFTR FDA-approved genotype-based therapies, OR

- Carrier Screening:
  - General Population Screening (e.g., no family history of CF):
    - No previous CFTR testing, and
    - Be of reproductive age and have potential and intention to reproduce, OR
  - High-risk Screening (e.g., family history of CF):
    - Previous CFTR standard panel, if performed, was negative, and
    - An individual with a family history of CF with an unknown mutation, or
    - An individual whose reproductive partner is a known CF carrier, has a diagnosis of CF, or has a diagnosis of CFTR-related CAVD, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CFTR Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous CFTR deletion/duplication testing, and

- Previous CFTR gene sequencing was non-diagnostic (fewer than 2 pathogenic mutations detected), and
- No known familial mutation, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CFTR Intron 9 Poly T Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous CFTR intron 9 poly T testing, AND
- Diagnostic Testing:
  - CFTR mutation analysis performed and R117H mutation detected, or
  - Diagnosis of male infertility (congenital absence of vas deferens [CAVD], obstructive azoospermia), or
  - Diagnosis of non-classic CF, OR
- Carrier Testing:
  - CFTR mutation analysis performed and R117H mutation detected, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Note For information regarding CFTR testing for individuals with pancreatitis, please refer to the guideline *Hereditary Pancreatitis Genetic Testing*, as this testing is not addressed here.

What is cystic fibrosis?

Definition

Classic cystic fibrosis (CF) is a genetic disorder that causes chronic lung disease, pancreatic insufficiency, and male infertility.[1,2] It is caused by mutations in the CFTR gene.[1]

Prevalence

CF affects at least 100,000 individuals worldwide.[1] While CF is most common in individuals with northern European ancestry, it can occur in any ethnic group.[2]

Symptoms

Symptoms associated with CF may include:[1]

- Frequent respiratory infections
- Bronchiectasis
- Pancreatic exocrine insufficiency
- Elevated sweat chloride levels
- Meconium ileus in newborns
- Congenital absence of the vas deferens (CAVD; can be unilateral or bilateral).

Pulmonary disease is the major cause of morbidity and mortality in individuals with CF.[1]

CFTR-Related Disorders

Several other conditions that share some clinical similarities to CF, are also caused by mutations in the CFTR gene, but do not meet the diagnostic criteria for CF. These are called "CFTR-related disorders" and include congenital bilateral absence of vas deferens (CBAVD/CAVD), acute recurrent or chronic pancreatitis, and some respiratory tract conditions such as bronchiectasis, sinusitis, and nasal polyps.[1,3]

CAVD is frequently identified after semen analysis shows absent sperm (azoospermia). CAVD is often caused by one severe CFTR mutation and one non-CF causing mutation or 2 non-CF causing mutations.[1]

CFTR-Related Metabolic Syndrome / CF Screen Positive, Inconclusive Diagnosis

CFTR-related metabolic syndrome/CF screen-positive, inconclusive diagnosis (CRMS/CF-SPID) is defined as "[a]n asymptomatic infant with a positive NBS result for CF and either a sweat chloride value <30 mmol/L and two CFTR variants at least one of which has unclear phenotypic consequences OR an intermediate sweat chloride value (30–59 mmol/L) and one or zero CF causing variants".[4] The majority of infants with CRMS/CF-SPID remain healthy. Some will convert to a CF diagnosis, and there is potential for developing a CFTR-Related Disorder (CFTR-RD) later in life.[4]

Cause

CF is caused by mutations in the CFTR gene.

Inheritance

CF is an autosomal recessive condition.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

The diagnosis of CF can be made based on clinical symptoms and evidence of CFTR dysfunction, which may include elevated sweat chloride or nasal potential difference, or the identification of 2 CFTR mutations.[1] In newborns, the diagnosis is made based on elevated trypsinogen on newborn screening and the presence of 2 CFTR mutations.[1]

Most signs of CF cannot be identified on prenatal ultrasound examination. However, pregnancies in which fetal echogenic bowel is identified on ultrasound are at an increased risk to be affected with CF.[1]

Prenatal diagnosis for CF can be performed on a sample from chorionic villus sampling (CVS) or amniocentesis:[1]

- If both parents are known carriers, a mutation panel that includes both parental mutations is typically the test of choice.
- If only one parent is a carrier, or if testing is indicated because of echogenic bowel, testing with a large mutation panel or sequencing and deletion/duplication analysis offers greater sensitivity.

Newborn screening (NBS) programs include screening for CF, though the screening protocol may vary by state.[5]

The American College of Medical Genetics has defined a panel of 23 common, pan-ethnic mutations that occur at a frequency of at least 0.1% in patients with cystic fibrosis.[6,7] While this panel was created for carrier screening purposes, the CF diagnostic guidelines also endorse its use in that setting for most patients.[2] Laboratories performing mutation panel testing routinely include all of these mutations. Many laboratories expand their panels with more mutations intended to increase the detection rate, particularly in non-Caucasian populations. Expanded mutation panels generally test for 70 or more CFTR mutations. The detection rates of expanded panels vary by laboratory and depend on the mutations included and the patient's ethnicity.[1]

CFTR-sequencing detects more than 97% of mutations.[1] The frequency of deletions and duplications is estimated to be less than 5% of all detected CFTR variants, but this may be an underestimate.[7]

Management

Management of CF addresses respiratory and digestive issues through inhaled medications and replacement of pancreatic enzymes.

There are several FDA-approved mutation-specific therapies.[8]

Survival

CF Foundation Patient registry data from 2021 indicate that the median predicted survival for people with CF is about 53 years.[9]

Test information

Introduction

Testing for cystic fibrosis tests may include known familial mutation analysis, targeted mutation analysis, NGS sequencing, deletion/duplication analysis, and intron 9 poly-T and TG analysis (previously called intron 8 or IVS8 poly-T analysis).

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Intron 9 poly-T and TG analysis

Intron 9 (formerly intron 8 or IVS 8) poly-T analysis identifies the number of thymidine bases in intron 9 of the CFTR gene. The three common variants are 5T, 7T, and 9T, with 7T and 9T being considered normal variants.[1]

"The 5T allele by itself is associated with variable penetrance for CF and CAVD based on the status of an adjacent poly TG tract, which usually contains 11, 12, or 13 repeats (c.1210–34TG[11], c.1210–34TG[12], c.1210–34TG [13]). When paired with a known CF-causing variant, 5T and 11TG variants in cis rarely confer an increased risk for CAVD in males while 5T in cis with 12TG or 13TG confers risk for CAVD and rarely for nonclassic CF. Given the commonness of the 5T allele (one in ten individuals carry a 5T variant), interpretation of its disease liability should ideally be performed in the context of the number of associated TG repeats." [7]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to cystic fibrosis testing.

The American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2020) technical standard for CFTR variant testing stated:[7]

- "As a way to ensure that CFTR variant testing for carrier screening and diagnostic testing purposes remains comprehensive, pan-ethnic, and up-to-date, the ACMG recommends either a classification-based reporting approach or a classification-based (targeted) testing approach (which has historically been used for CFTR carrier screening."
- "For those laboratories who wish to continue using a targeted testing approach, the ACMG-23 variant panel remains as the minimum list of CFTR variants that should be included. Laboratories may want to consider adding additional variants to their panel depending on the ethnic composition of their expected test population. However, the minimum list of CFTR variants recommended for pan-ethnic carrier screening has not been increased at this time."

- "Targeted and comprehensive approaches are both acceptable for the testing of individuals regardless of race, ethnicity, or test indication."
- "The ACMG recommends that laboratories performing initial CFTR variant testing on an individual can use either targeted or comprehensive methods to evaluate the gene…If pathogenic or likely pathogenic CFTR variants have been confirmed in both biological parents, or an affected full sibling, only targeted methods should be used."
- "For all prenatal, postnatal, and adult diagnostic testing indications for CFTR, the ACMG recommends the reporting of R117H status as well as the results from at least the associated polyT tract. For all adult carrier screening indications for CFTR, polyT status should be reported when the R117H variant is detected; laboratories may also want to consider reporting the results from the associated polyT tract in the partner of an individual who had a pathogenic or likely pathogenic variant detected during screening."

ACMG (2023) issued a position statement updating the minimum set of CFTR variants to be included for CF carrier screening that stated:[10]

- "The new set of 100 variants represents an updated minimum CFTR carrier screening variant set, but it does not represent a limit on the total number of variants that a laboratory can choose to assess, and it is likely that laboratories may already have many (but likely not all) of these variants included as a part of their tests."
- "The workgroup is also aware that there are not likely any existing targeted CF tests available that contain all of the newly recommended variants. However, some laboratories may have previously chosen to offer CF carrier screening using either Sanger or NGS of CFTR, and these methods should encompass all of the genomic regions containing the recommended variants."

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2017; Reaffirmed 2023) issued a committee opinion on carrier screening for genetic conditions that stated:[11]

- "Cystic fibrosis carrier screening should be offered to all women who are considering pregnancy or are currently pregnant."
- "Complete analysis of the CFTR gene by DNA sequencing is not appropriate for routine carrier screening."
- "For couples in which both partners are unaffected but one or both has a family history of cystic fibrosis, genetic counseling and medical record review should be performed to determine if CFTR mutation analysis in the affected family member is available."
- "If a woman's reproductive partner has cystic fibrosis or apparently isolated congenital bilateral absence of the vas deferens, the couple should be provided

follow-up genetic counseling by an obstetrician–gynecologist or other health care provider with expertise in genetics for mutation analysis and consultation."

- "If both partners are found to be carriers of a genetic condition, genetic counseling should be offered. Prenatal diagnosis and advanced reproductive technologies to decrease the risk of an affected offspring should be discussed."
- "Carrier screening for a particular condition generally should be performed only once in a person's lifetime, and the results should be documented in the patient's health record. Because of the rapid evolution of genetic testing, additional mutations may be included in newer screening panels. The decision to rescreen a patient should be undertaken only with the guidance of a genetics professional who can best assess the incremental benefit of repeat testing for additional mutations."

American Urological Association in partnership with the American Society for Reproductive Medicine

The American Urological Association in partnership with the American Society for Reproductive Medicine (2021) published guidelines on the diagnosis and treatment of infertility in males that stated:[12]

- "Clinicians should recommend Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutation carrier testing (including assessment of the 5T allele) in men with vasal agenesis or idiopathic obstructive azoospermia. (Expert Opinion)"
- "For men who harbor a CFTR mutation, genetic evaluation of the female partner should be recommended. (Expert Opinion)"
- "Specifically, studies suggest that mutations in the CFTR gene are present in up to 80% of men with congenital bilateral absence of the vas deferens (CBAVD), 20% of men with CUAVD and 21% of men with idiopathic epididymal obstruction."
- "As the goal of genetic testing is to help identify the etiology as well as provide counseling on potential offspring transmission, expanded carrier screening or gene sequencing should be considered. In addition to classic mutations, the 5-thymidine (5T) variant of the polythymidine tract in the splice site of intron 8 (which regulates exon 9 splicing efficiency) is also commonly found in men with obstructive azoospermia due to CFTR abnormalities."

Cystic Fibrosis Foundation

Consensus-based guidelines from the Cystic Fibrosis Foundation (2017) outline the ways in which a CF diagnosis can be established (see below). Characteristic features of CF include chronic sinopulmonary disease (such as persistent infection with characteristic CF pathogens, chronic productive cough, bronchiectasis, airway obstruction, nasal polyps, and digital clubbing), gastrointestinal/nutritional abnormalities (including meconium ileus, pancreatic insufficiency, chronic pancreatitis, liver disease, and failure to thrive), salt loss syndromes, and obstructive azoospermia in males (due to CAVD).[2]

When at least one characteristic feature is present, a diagnosis of CF can be established by:

- Two abnormal sweat chloride values; or
- Identification of two CF-causing CFTR gene mutations; or
- Characteristic transepithelial nasal potential difference (NPD)

In the absence of symptoms, a CF diagnosis can be established in:

- A newborn with two CF-causing CFTR gene mutations identified via newborn screening

"Individuals who are screen-positive and meet sweat chloride criteria for CF diagnosis should undergo CFTR genetic testing if the CFTR genotype was not available through the screening process or is incomplete." "Even in the presence of a positive sweat test, the identification of 2 CF-causing mutations should be confirmed in a clinical genetics laboratory capable of performing in-depth genetic analysis when required to further define CF risk (eg, the length of polyT tracts with the c.350G>A [legacy:R117H] CFTR mutation). Confirmation of genetic testing results with an FDA-approved companion diagnostic test also has additional value in therapy selection and access."[2]

These guidelines further state that, “Individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range (30- 59 mmol/L) on 2 separate occasions may have CF. They should be considered for extended CFTR gene analysis and/ or CFTR functional analysis."[2]

Society for Maternal-Fetal Medicine

The Society for Maternal Fetal Medicine (SMFM, 2021) statement on the evaluation of soft ultrasound markers such as fetal echogenic bowel identified during ultrasound stated:[13]

- “...for fetuses with isolated echogenic bowel, we recommend an evaluation for cystic fibrosis and fetal cytomegalovirus infection and a third-trimester ultrasound examination for reassessment and evaluation of growth (GRADE 1C)”.

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Decipher Prostate Cancer Classifier

MOL.TS.294.A
v2.0.2024

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Decipher Prostate Cancer Classifier	81542

Criteria

Decipher Prostate RP

- No previous gene expression profile testing performed for this diagnosis of cancer, AND
- Member is post-radical prostatectomy, AND
- Post-surgical PSA is undetectable (below 0.2mg/dl), AND
- No evidence of lymph node metastasis identified, AND
- One or more of the following adverse features identified in the surgical specimen:
  - positive surgical margin(s), or
  - extracapsular extension, or
  - seminal vesicle invasion, AND
- Test is being requested to inform adjuvant treatment decisions.

Decipher Prostate Biopsy

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.

- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What are gene expression profiling tests for prostate cancer?

Definition

Prostate cancer (PC) is the most common cancer in men, and metastatic prostate cancer is a leading cause of cancer-related deaths worldwide. It is considered a heterogeneous disease with highly variable prognosis.[1]

- At the time of diagnosis of localized PC, patients typically undergo a prognostic risk assessment with routine clinical and pathological tests to assess the probability of subsequent progression or metastasis. These prognostic assessments help to identify lower risk patients with indolent disease who may opt for active surveillance (AS), or higher risk patients with more aggressive disease who may benefit from a treatment intervention.
- High-risk prostate cancer (PC) patients treated with radical prostatectomy (RP) also undergo risk assessment to assess future disease prognosis and determine optimal treatment strategies. Post-RP pathology findings, such as disease stage, baseline Gleason score, time of biochemical recurrence (BCR) after RP, and PSA doubling-time, are considered strong predictors of disease-associated metastasis and mortality. Following RP, up to 50% of patients have pathology or clinical features that are considered at high risk of recurrence and these patients usually undergo post-RP treatments, including adjuvant or salvage therapy or radiation therapy, which can have serious risks and complications. According to clinical practice guideline recommendations, high risk patients should undergo 6 to 8 weeks of radiation therapy (RT) following RP. However, approximately 90% of high-risk patients do not develop metastases or die of prostate cancer, and instead may be appropriate candidates for alternative treatment approaches, including AS. As such, many patients may be subjected to unnecessary follow-up procedures and their associated complications, highlighting the need for improved methods of prognostic risk assessment.[2,3]
- Several genomic biomarkers have been commercially developed to augment the prognostic ability of currently available routine clinical and pathological tests and identify those patients either at the time of diagnosis of localized PC or after radical prostatectomy (RP) most and least likely to benefit from a specific treatment strategy. Prognostic genomic tests, including gene expression profiling tests, may help to avoid overtreatment by reclassifying those men originally identified as high risk, but who are unlikely to develop metastatic disease. Genomic biomarkers may also play a role in assisting clinicians to tailor personalized and more appropriate treatments for subgroups of PC patients, and improve overall health outcomes.[2,3]

Decipher

Test information

- Gene expression profiles (GEPs) evaluate the expression of several genes using one sample. Gene expression is determined through RNA analysis, using either reverse transcriptase (RT) polymerase chain reaction (PCR) or DNA microarrays.[4]
- According to the manufacturer, “Decipher® uses an oligonucleotide microarray to measure the expression of up to 1.4 million RNAs (e.g., mRNA, lncRNA) extracted from formalin-fixed, paraffin-embedded (FFPE) prostate specimens. Decipher testing on tumor specimens provides the probability of high-grade disease at radical prostatectomy (biopsy specimens only), 5-year probability of clinical metastasis, and 10-year prostate cancer specific mortality. A gene expression signature is used to generate the Decipher score, which ranges from 0 to 1.0."[5]
- Decipher Prostate Biopsy
    - Decipher Prostate Biopsy results are “intended for use as an adjunct to conventional clinical risk factors for determining metastatic potential and prognosis of patients diagnosed with localized prostate cancer.”[6]
    - “Decipher Prostate Biopsy predicts a patient’s risk for metastasis or prostate cancer mortality, as well as adverse pathology at RP, using the gene expression profile of FFPE prostate cancer tissue samples collected at biopsy. Decipher Prostate Biopsy classifies as low risk those who may be safely followed with active surveillance, or as high risk those who would potentially benefit from immediate treatment.”[5]
- Decipher Prostate Radical Prostatectomy (RP)
    - Decipher Prostate RP results are intended as “an adjunct to conventional clinical variables and models currently used for determining prognosis and treatment of prostate cancer patients after radical prostatectomy.”[7] Clinical validity studies have evaluated patients designated as very low-, low-, favorable intermediate-, unfavorable intermediate, high, and very high risk per the National Comprehensive Cancer Network (NCCN) risk groups for prostate cancer.
    - Decipher Prostate RP “predicts a patient’s risk for metastasis or prostate cancer mortality for men with adverse pathology or PSA persistence / recurrence following RP using the gene expression profile of FFPE prostate cancer tissue samples collected at RP. Decipher Prostate RP classifies as low risk those who may be safely observed, or as high risk those who would potentially benefit from treatment or treatment intensification.” [5]

Guidelines and evidence

American Association of Clinical Urologists

The American Association of Clinical Urologists (AACU) has issued a position statement on genomic testing in prostate cancer that states the following:[8]

- "The AACU supports the use of tissue-based molecular testing as a component of risk stratification in prostate cancer treatment decision making."

American Society of Clinical Oncology

The American Society of Clinical Oncology (ASCO, 2020) issued a guideline on molecular biomarkers in prostate cancer. This guideline states:[9]

- "Are there molecular biomarkers to diagnose clinically significant prostate cancer?"
    - "Recommendation 2.1. Commercially available molecular biomarkers (ie, Oncotype Dx Prostate [now Genomic Prostate Score], Prolaris, Decipher, and ProMark) may be offered in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. Routine ordering of molecular biomarkers is not recommended (Type: Evidence based; Evidence quality: Intermediate; Recommendation: Moderate)."
    - "Recommendation 2.2. Any additional molecular biomarkers evaluated do not have sufficient data to be clinically actionable or are not commercially available and thus should not be offered (Type: Evidence based; Evidence quality: Insufficient; Strength of recommendation: Moderate)."
- "Are there molecular biomarkers to guide the decision of postprostatectomy adjuvant versus salvage radiation?"
    - "Recommendation 3.1. The Expert Panel recommends consideration of a commercially available molecular biomarker (eg, Decipher Genomic Classifier) in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. In the absence of prospective clinical trial data, routine use of genomic biomarkers in the postprostatectomy setting to determine adjuvant versus salvage radiation or to initiate systemic therapies should not be offered (Type: Evidence based; Evidence quality: Intermediate; Strength of recommendation: Moderate)."
    - "Recommendation 3.2. Any additional molecular biomarkers evaluated do not have sufficient data to be clinically actionable or are not commercially available and thus should not be offered (Type: Evidence based; Evidence quality: Insufficient; Strength of recommendation: Moderate)."

American Urological Association and American Society of Radiation Oncology

The American Urological Association and American Society for Radiation Oncology (AUA/ASTRO, 2022) published an evidence-based guideline on localized prostate cancer endorsed by the Society of Urologic Oncology (SGO) that stated:[10]

- "Clinicians may selectively use tissue-based genomic biomarkers when added risk stratification may alter clinical decision-making. (Expert Opinion)"

- "Clinicians should not routinely use tissue-based genomic biomarkers for risk stratification or clinical decision-making. (Moderate Recommendation; Evidence Level: Grade B)"
- "Regarding tissue-based genomic biomarkers, several currently available commercial tests, including Prolaris, Oncotype Dx [now Genomic Prostate Score], and Decipher, variously offer prediction of adverse pathology as well as the risks of biochemical recurrence, metastasis, and prostate cancer death. However, most of the reported studies to date that evaluated the prognostic ability of these genomic tests did not meet inclusion criteria for the systematic review as the studies used surgical (ie, prostatectomy) rather than biopsy specimens."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) Clinical Practice Guidelines on Prostate Cancer stated the following regarding molecular assays:[11]

- "Patients with NCCN low, favorable intermediate, unfavorable intermediate, or high-risk disease and life expectancy ≥10 y may consider the use of the following tumor-based molecular assays: Decipher, Oncotype DX Prostate [now Genomic Prostate Score], and Prolaris.."
- "Retrospective case cohort studies have shown that these assays provide prognostic information independent of NCCN or CAPRA risk groups, which include likelihood of death with conservative management, likelihood of biochemical recurrence after radical prostatectomy or EBRT [external beam radiation therapy], likelihood of adverse pathologic features after radical prostatectomy, and likelihood of developing metastasis after operation, definitive EBRT, or post-recurrence EBRT."
- "These molecular biomarker tests have been developed with extensive industry support, guidance, and involvement, and have been marketed under the less rigorous FDA regulatory pathways for biomarkers. Although full assessment of their clinical utility requires prospective randomized clinical trials, which are unlikely to be done, the panel believes that men with low or favorable intermediate disease and life expectancy greater than or equal to 10 years may consider the use of Decipher, Oncotype DX Prostate [now Genomic Prostate Score], or Prolaris during initial risk stratification. Patients with unfavorable intermediate- and high-risk disease and life expectancy greater than or equal to 10 years may consider the use of Decipher or Prolaris."

With regard to the use of Decipher post-radical prostatectomy (RP), NCCN stated:[11]

- "The panel recommends use of nomograms and consideration of age and comorbidities, clinical and pathologic information, PSA levels, PSADT, and Decipher molecular assays to individualize treatment discussion."
- "Decipher molecular assay should be considered if not previously performed to inform adjuvant treatment if adverse features are found post-RP." (category 2B)

- "... Decipher may be considered to inform adjuvant treatment if adverse features are found after radical prostatectomy and during workup for radical prostatectomy PSA persistence or recurrence (category 2B for the latter setting)."
- "Adverse laboratory/pathologic features include: positive margin(s); seminal vesicle invasion; extracapsular extension; or detectable PSA."

Selected Relevant Publications

The majority of the evidence for Decipher retrospectively evaluates the association between the Decipher score and adverse pathology, biochemical recurrence, or metastasis in men post-RP.[12-35] Low quality evidence suggests Decipher results are associated with metastasis and adverse pathology at initial biopsy. However, these findings are weakened by several limitations, including: overlapping patient populations, retrospective study designs, small sample sizes, and reporting of surrogate outcomes. Several decision impact studies suggest Decipher results may influence clinical decision-making; however, it remains unclear if Decipher-based decision-making ultimately leads to improvements in patient health outcomes. Future trials should prospectively evaluate the impact of Decipher testing on clinical decision-making in large independent cohorts of men and include sufficient follow-up to capture patient-relevant outcomes (e.g., mortality, recurrence, and metastasis).

Clinical trials may be ongoing. Additional information can be found at https://clinicaltrials.gov.

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Decipher

DecisionDx Uveal Melanoma

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v2.0.2024

Introduction

DecisionDX testing for uveal melanoma is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
DecisionDx-PRAME	81401
DecisionDx-UMSeq	81479
DecisionDx Uveal Melanoma Gene Expression Profile	81552

Criteria

Introduction

Requests for DecisionDX testing for uveal melanoma are reviewed using these criteria.

DecisionDX-UM

DecisionDx-UM testing is medically necessary when the following criteria are met:

- No previous DecisionDx-UM testing performed after current diagnosis when a result was successfully obtained, AND
- Member has primary, localized uveal melanoma, AND
- No evidence of metastatic disease, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

DecisionDx-PRAME

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the

outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.

- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

DecisionDx-UMSeq

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What is Uveal Melanoma?

Definition

Uveal melanoma (UM) is a rare cancer of the eye, arising in the choroid, ciliary body or iris of the eye, with about 1500 new cases per year in the US. It accounts for about 5% of all melanomas in the US.[1]

- The diagnosis is usually established by clinical assessment combined with ancillary diagnostic testing, using fluorescein angiography and ultrasonography.[2]
- Despite relatively high cure rates of the primary tumor following treatment,[3] metastatic disease to the liver has been reported to occur in 20 to 50% of individuals with UM. Median survival after metastasis detection has been reported to be approximately 9 months.[4]
- As a result, accurate prognostic assessment for metastatic risk is considered crucial for an individual's survival. Conventional prognostic evaluation of UM involves clinical and pathologic criteria, such as age, tumor diameter, tumor thickness, ciliary body involvement, tumor cell morphology, extracellular matrix patterning, and extraocular tumor extension.[4,5]
- Some experts have questioned the accuracy of these methods to predict metastasis.[6,7] As such, new molecular techniques examining the genetic

composition of tumor cells have been introduced to improve prognostic evaluations potentially allowing for more targeted surveillance and treatment options for UM. Additionally, it may also facilitate referral of high-risk individuals to clinical trials.[7,8]

Test information

Introduction

DecisionDx-UM is a 15 gene panel that measures gene expression of 12 genes present in ocular melanoma (CDH1, ECM1, EIF1B, FXR1, HTR2B, ID2, LMCD1, LTA4H, MTUS1, RAB31, ROBO1, and SATB1) and 3 control genes (MRPS21, RBM23, and SAP130). This test is designed to assess the risk of metastasis within 5 years.[9]

- DecisionDx-UM test results are reported as follows:[9,10]
  - Class 1A – very low risk (2%) of metastasis within 5 years
  - Class 1B – intermediate risk (21%) of metastasis within 5 years
  - Class 2 – high risk (72%) of metastasis within 5 years
- DecisionDx-PRAME is a test that can be added on to the DecisionDx-UM assay for additional information regarding prognosis. According to Castle Biosciences,"PRAME [preferentially expressed antigen in melanoma] is usually not expressed in normal adult tissues, but in some cancers, PRAME expression is elevated. Studies have suggested that elevated PRAME expression ("PRAME positive") in a Class 1 uveal melanoma tumor may be associated with an increased risk of metastasis compared to a Class 1 tumor that does not express PRAME ("PRAME negative")." [11]
- The manufacturer also offers the DecisionDX-UMSeq test, which is gene sequencing panel including 7 genes (GNAQ, GNA11, CYSLTR2, PLCB4, EIF1AX, SF3B1, and BAP1).[12,13] "This genomic information can be used to help guide your care, and may also become useful in the future as UM scientific research and therapeutics evolve." [12]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to DecisionDx testing for uveal melanoma.

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) stated the following regarding gene expression for uveal melanoma.[8]

- "Biopsy of the primary tumor may provide prognostic information that can help inform frequency of follow-up and may be needed for eligibility for clinical trials. Biopsy is typically performed before the tumor is irradiated and can often be performed at the time of primary treatment depending on the procedure modality. If biopsy is performed, molecular/chromosomal testing for prognostication is preferred over cytology alone. The risk/benefits of biopsy for prognostic analysis should be carefully considered and discussed."
- "For patients who had a biopsy of their primary tumor, certain molecular features have been shown to be prognostic for risk of distant spread and should be used for risk stratification."
- Gene expression profiling as described by Onken et al[14] was recommended as part of the stratification in determining the class of the tumor [Class 1A (low risk), Class 1B (medium risk), or Class 2 (high risk)] to inform frequency of follow-up.
    - "It has been shown that class 2 was associated with a 5-fold to 20-fold higher risk of metastasis than class 1."
- "PRAME expression, present in about a third of uveal melanomas has also been associated with increased risk of metastasis… [and can be] an indicator of high risk to be used to inform frequency of follow-up."

Selected Relevant Publications

Based on the review of the available peer-reviewed published literature, the DecisionDx-UM 15-gene assay has sufficient evidence for use as a prognostic test in individuals diagnosed with primary, localized uveal melanoma to assist clinicians with predicting disease severity and improving disease management strategies.[3,14-25,30,31]

DecisionDX-PRAME and DecisionDX-UMSeq

There is currently insufficient evidence regarding use of DecisionDX-PRAME.[26-29] Clinical validity and clinical utility studies are lacking. Additional studies are needed to determine whether DecisionDX-PRAME improves clinical outcomes more than DecisionDX-UM alone. There is minimal evidence evaluating use of DecisionDX-UMSeq.[32] As a result, no conclusions can be drawn regarding the value and usefulness of these two tests.

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DermTech Pigmented Lesion Assay

MOL.TS.282.A
v2.0.2024

Introduction

DermTech Pigmented Lesion Assay (PLA) is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure code
DermTech Pigmented Lesion Assay	0089U

Criteria

Introduction

Requests for DermTech PLA are reviewed using the following criteria.

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What is melanoma?

Definition

According to the American Academy of Dermatology (AAD), the incidence of primary cutaneous melanoma has been increasing substantially for several decades. The incidence of melanoma has been reported to be increasing at a rate of 3% to 7%

annually among fair-skinned Caucasian populations, which is faster than other major cancers.[1]

Melanoma accounts for the majority of skin cancer related deaths, but treatment is nearly always curative with early detection of disease. Minimal depth (thin) melanomas have a cure rate of nearly 100%, while tumors with a Breslow depth of greater than 4mm have a 10-year survival rate of less than 50%.[1]

Standard of care for the assessment of clinically suspicious pigmented skin lesions is surgical biopsy with pathologic evaluation. However, histopathology is believed to have inherent limitations. Some lesions that are likely to be true melanomas based on clinical behavior do not meet the complete set of histologic criteria to establish a melanoma diagnosis.[1] There is also considerable interrater variability with visual image and pattern recognition of skin lesions.[2] In an effort to improve patient survival, a number of novel noninvasive techniques have been developed to classify pigmented skin lesions at an earlier stage.[3]

Test information

Introduction

The Pigmented Lesion Assay (PLA) is a non-invasive method for the biopsy of clinically atypical pigmented lesions or moles using an adhesive patch to obtain mRNA from the surface of the suspicious lesion.

According to the manufacturer, the PLA assesses gene expression consistent with melanoma and is intended as a decision making aid for the clinician to determine whether or not to biopsy a pigmented skin lesion, clinically suspicious for melanoma.[4] The test is intended for use on pigmented lesions suspicious for melanoma that meet at least one of the A (asymmetry) B (border) C (color) D (diameter) E (evolving) criteria for which the clinician would like additional information prior to surgical biopsy. Uses of the PLA include the following: lesions being followed for change; lesions in cosmetically sensitive areas of the body; lesions on individuals with possible risks for complications during surgical biopsy; or lesions among individuals who refuse biopsy.[4]

The PLA is a non-invasive method for the assessment of clinically atypical pigmented lesions or moles using an adhesive patch to obtain mRNA from the surface of the suspicious lesion. The method of adhesive tape stripping has been used to obtain RNA from the stratum corneum for gene expression of other disorders, such as allergic and irritant skin reactions and psoriasis.[5] The PLA detects the expression of 2 specific genes, PRAME and LINC00518, both of which are believed to play key roles in oncogenesis and both of which have been shown to be elevated in melanoma. If sufficient maternal is available, a DNA-based TERT Add-On Assay can be performed to detect TERT promoter mutations as well. If one or more of these biomarkers is detected, the test is considered positive. The positive lesions generally undergo surgical biopsy to definitively establish a melanoma diagnosis.[4] The test manufacturer notes that this assay cannot be used on mucous membranes, palms of the hands, or soles of the feet.[2]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to DermTech PLA.

American Academy of Dermatology

The American Academy of Dermatology (AAD) acknowledged that the clinical and prognostic significance of the use of biomarkers and mutational analysis is still unclear and there are gaps regarding their clinical usefulness that have yet to be addressed.[3] The 2019 guideline stated:

- “Ancillary diagnostic molecular techniques (eg, CGH, FISH, GEP) may be used for equivocal melanocytic neoplasms.”
- "Routine molecular testing, including GEP [gene expression profiling], for prognostication is discouraged until better use criteria are defined. The application of molecular information for clinical management (eg, sentinel lymph node eligibility, follow-up, and/or therapeutic choice) is not recommended outside of a clinical study or trial."
- “Once a lesion has been identified as clinically concerning, dermoscopy can improve diagnostic accuracy and/or help direct optimal and adequate tissue sampling in the case of very large lesions or those in cosmetically or functionally sensitive areas. Newer noninvasive techniques (eg, reflectance confocal microscopy [RCM], as well as electrical impedance spectroscopy, gene expression analysis, optical coherence tomography, and others can also be considered as these become more readily available.”
- “Lingering questions remain regarding the degree to which the selected gene sets represent genes associated with tumor progression, how they compare with current well-characterized prognostic factors and AJCC eighth edition survival data, and whether they improve prognostic models enough to affect patient management and outcomes. As such, the WG discourages routine baseline GEP for prognostication.”
- “There is insufficient evidence to recommend routine molecular profiling assessment for baseline prognostication. Evidence is lacking that molecular classification should be used to alter patient management outside of current guidelines (eg, NCCN and AAD). The criteria for and the utility of prognostic molecular testing, including GEP, in aiding clinical decision making (eg, SLNB eligibility, surveillance intensity, and/or therapeutic choice) needs to be evaluated in the context of clinical study or trial.”
- “Noninvasive genomic methods (eg, adhesive patch “biopsy”) are being investigated to further classify melanocytic lesions as either benign or malignant to guide the need for further biopsy. The uptake of 1 or more of these technologies will eventually depend on cumulative evidence regarding their effectiveness, clinical utility, cost versus benefit, and competing strategies.”

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) made no recommendation to consider or use the DermTech PLA test in the evaluation of skin lesions suspicious for melanoma.[6]

With regard to GEP, NCCN offered the following guidance:[6]

- "Currently, there is insufficient evidence to support incorporation of current GEP tests into melanoma care. The use of gene expression profiling GEP [gene expression profiling] tests according to specific AJCC-8 melanoma stage (before or after SNLB [sentinel lymph node biopsy]) requires further prospective investigation in large, contemporary data sets of unselected patients. Prognostic GEP tests to differentiate melanomas at low versus high risk for metastasis should not replace pathologic staging procedures and are not recommended outside of the context of a clinical study or trials. Moreover, since there is a low probability of metastasis in stage I melanoma and a higher proportion of false-positive results using these tests, GEP testing should not guide clinical decision-making in this subgroup.On an individual basis, the likelihood of a positive SLNB may be informed by the use of multivariable nomograms/ risk calculators. Ongoing prospective investigation will further inform the use of GEP tests for SLNB risk prediction."
- “Pre-diagnostic clinical modalities (ie, total-body photography and sequential digital dermoscopy), noninvasive imaging and other technologies (eg, reflectance confocal microscopy, electrical impedance spectroscopy) may aid in surveillance for new primary melanoma, particularly in patients with high mole count and/or presence of clinically atypical nevi. For melanocytic neoplasms that are clinically/dermoscopically suspicious for melanoma, pre-diagnostic noninvasive patch testing may also be helpful to guide biopsy decisions”

With regard to diagnostic testing, NCCN stated:[6]

- "Melanocytic neoplasms of uncertain biological potential present a unique challenge to pathologists and treating clinicians. Ancillary tests to differentiate benign from malignant melanocytic neoplasms include immunohistochemistry (IHC), and molecular testing via comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), gene expression profiling (GEP), single nucleotide polymorphism (SNP) array, and next-generation sequencing (NGS). These tests may facilitate a more definitive diagnosis and guide therapy in cases that are diagnostically uncertain or controversial by histopathology. Ancillary tests should be used as adjuncts to clinical and expert dermatopathologic examination and therefore be interpreted within the context of these findings."

With regards to prognostic testing, NCCN stated:[6]

- "Despite commercially available GEP tests being marketed to risk stratify cutaneous melanomas, current GEP platforms do not provide clinically actionable prognostic information when combined or compared with known clinicopathologic factors (eg, sex, age, primary tumor location, thickness, ulceration, mitotic rate, lymphovascular

invasion, microsatellites, and/or SLNB status) or multivariable nomograms/risk calculators. Furthermore, the clinical utility of these tests to inform treatment recommendations and predict patient outcomes has not been established."

- "Various studies of prognostic GEP tests suggest their role as an independent predictor of worse outcome. However, GEP is not superior to Breslow thickness, ulceration, or SLN status and it remains unclear whether available GEP tests are reliably predictive of outcome across the risk spectrum of cutaneous melanoma. Validation studies on prospectively collected, independent cohorts … are necessary to define the clinical utility of molecular prognostic GEP testing as an adjunct to AJCC staging or as part of the multidisciplinary decision-making process to guide surveillance imaging, SLNB, and adjuvant therapy."
- "Gene expression profiling for melanoma could be an enormously valuable contribution to understanding the biology of the disease. However, the difficulty of embracing gene expression profiling as an independent predictor of outcome is illustrated by the inconsistency of results across studies aimed at defining the most predictive gene sets for melanoma. Comparison of the gene signatures identified in these studies show minimal overlap in specific genes thought to be predictive of outcome. The identification and validation of a prognostic gene expression profile is a complicated multi-step and often multi-study process, and there are many ways in which specifics of study design and methodology can impact the end result. The lack of overlap in gene signatures identified as prognostic for melanoma is likely due to substantial differences in study design and methodology. Efforts to develop gene expression profiling prognostic assays for other types of cancer have also resulted in limited or partial overlap in the "gene signature" identified by different studies."
- "While there is interest in newer prognostic molecular techniques such as gene expression profiling to help differentiate benign from malignant neoplasms, or to help distinguish melanomas at low- versus high-risk for metastasis, routine (baseline) genetic testing of primary cutaneous melanomas (before or following SLN biopsy [SLNB]) is not recommended outside of a clinical study."

Selected Relevant Publications

There is insufficient evidence to support the use of DermTech PLA to accurately discriminate between early melanoma and non-melanoma in individuals with clinically suspicious lesions.[1,2,7-20] A recurring limitation within the evidence base is the assumption that non-biopsied PLA negative results are true negatives without follow up assessment for confirmation. Additional limitations include retrospective study designs, small individual study populations, overlapping patient populations, varying follow up times, and a lack of reported health outcomes.

Based on the current evidence, PLA testing may have a high negative predictive value and influence clinical management decisions regarding biopsy but it remains unclear if these PLA-based decisions result in clinically meaningful patient outcomes. Well-designed studies that report the impact of PLA testing on clinical management

decisions together with the health outcomes that result from those decisions are needed to confirm the utility of the DermTech PLA test.

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Early Onset Familial Alzheimer Disease Genetic Testing

MOL.TS.162.A
v2.0.2024

Introduction

Early onset familial Alzheimer disease genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
APP Deletion/Duplication	81479
APP Known Familial Mutation	81403
APP Sequencing	81406
EOFAD Multigene panel	81479
PSEN1 Deletion/Duplication	81479
PSEN1 Known Familial Mutation	81403
PSEN1 Sequencing	81405
PSEN2 Known Familial Mutation	81403
PSEN2 Sequencing	81406

Criteria

Introduction

Requests for early onset familial Alzheimer disease (EOFAD) testing are reviewed using these criteria.

PSEN1, PSEN2, or APP Known Familial Mutation Testing

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, and
  - PSEN1, PSEN2, or APP mutation identified in a 1st or 2nd degree biological relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Dementia diagnosed ≤65 years of age, OR
- Predictive Testing:
  - Age 18 years or older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

PSEN1 Full Sequence and Deletion/Duplication Analysis

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous PSEN1 sequencing or deletion/duplication analysis, and
  - No known PSEN1, PSEN2, or APP mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Dementia diagnosed ≤65 years of age, and
  - Family history of dementia in 1st or 2nd degree relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

APP Full Sequence and Deletion/Duplication Analysis

- Criteria for PSEN1 analysis are met, AND
- No previous APP sequencing or deletion/duplication analysis, AND
- PSEN1 sequencing and deletion/duplication analysis were performed, and no mutations were detected, AND
- No mutations detected in PSEN2 sequencing, if performed.

PSEN2 Full Sequence Analysis

- Criteria for PSEN1 analysis are met, AND
- No previous PSEN2 sequencing analysis, AND

EOFAD

- PSEN1 sequencing and deletion/duplication analysis were performed, and no mutations were detected, AND
- No mutations detected in APP sequencing, if performed.

Multigene Panel (PSEN1, APP, and PSEN2 ONLY)

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous testing for EOFAD, and
  - No known PSEN1, PSEN2, or APP mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Dementia diagnosed less than or equal to 65 years of age, and
  - Family history of dementia in 1st of 2nd degree relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

When otherwise reimbursable, the following limitations apply:

- When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81479*).
- When use of a panel code is not possible, each billed component procedure will be assessed independently.
- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.

- When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement in a tiered fashion:
    - PSEN1
    - APP
    - PSEN2

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is early onset familial Alzheimer disease?

Definition

Alzheimer disease (AD) is characterized by adult onset, progressive dementia with cerebral cortical atrophy, beta amyloid plaque formation, and intraneuronal neurofibrillary tangles.[1]

Prevalence

The general population lifetime risk of AD is about 10%.

Familial AD

Familial AD (3 or more affected individuals in a family) accounts for about 25% of all AD, including late and early onset.[1]

Most familial AD is late-onset, but in less than 2% of cases, symptoms start at an unusually young age (called “early onset familial Alzheimer disease” or EOFAD).[1]

Symptoms

Common findings include memory loss, confusion, speech issues, hallucinations, and personality and behavioral changes such as poor judgment, agitation, and withdrawal.[1,2] Symptoms of AD usually start after 60-65 years old; however, symptoms of EOFAD begin at 65 years or younger.[1]

EOFAD is suspected when:[1]

- More than one family member has AD; and
- Symptoms occur before the age of 65.

EOFAD

Cause

Table 1 below summarizes three subtypes of EOFAD.[1]

- While not clinically distinguishable, the underlying genetic cause differs. Among families with EOFAD, 60-80% will have a detectable mutation in the APP, PSEN1, or PSEN2 gene.[1] Therefore, some families with EOFAD will not have an identifiable mutation by current testing. There may be other disease causing genes that have not been identified to date.
- Most people with EOFAD have an affected parent. In cases where there appears to be no parent affected, most people have a second degree relative with the condition. De novo (new) mutations are possible. However, they have not been reported in EOFAD.[1,2]
- Reduced penetrance of EOFAD-associated mutations has been described.[1]

Table 1

Gene	Proportion of EOFAD cases	Average age of onset
APP	10-15%	40s to 50s (occasionally 60s)
PSEN1	20-70%	40s to early 50s
PSEN2	~5%	40 to 75

Inheritance

EOFAD is an autosomal dominant disorder.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Diagnosis

The diagnosis of AD relies on clinical assessment, which may include mental status testing, neurological examinations, diagnostic tests, and brain imaging.[3] Genetic testing of APP, PSEN1, or PSEN2 is another tool to establish the diagnosis in individuals with early onset AD and a positive family history. In asymptomatic individuals with a mutation in one of these genes, there is an increased likelihood they will develop EOFAD however reduced penetrance has been documented.[1]

Because of the implications of predictive testing, pretest genetic counseling should include limitations of predictive testing and potential consequences with regard to health, life, and disability insurance coverage; employment and educational discrimination; and changes in social and family dynamics.[1] Predictive testing is considered inappropriate for asymptomatic minors who are at risk for adult-onset conditions if there is not an early treatment option expected to have a beneficial effect on the disease morbidity and mortality.[1]

Management

There is no cure for AD however some medications may help with symptoms such as memory loss and confusion. “Key elements of a strategy to maximize dementia outcomes include regular monitoring of patient's health and cognition, education and support to patients and their families, initiation of pharmacologic and non-pharmacologic treatments as appropriate, and evaluation of patient/family motivation to volunteer for a clinical trial."[3]

Survival

The survival for individuals with EOFAD is unknown due to the rarity of the condition and a paucity of longitudinal studies. In individuals with late-onset AD diagnosed at age 65 or older, the average survival is four to eight years after the diagnosis is made but can be as long as 20 years.[3] EOFAD is believed to have a more aggressive disease course that late-onset AD with faster progression.[1]

Test information

Introduction

Testing for EOFAD may include known familial mutation analysis, next generation sequencing, deletion/duplication analysis, and/or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and

insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Given the significant overlap in clinical manifestations and age of onset in AD, single-gene testing is typically not recommended.[1] A multigene panel that includes PSEN 1/2 and APP is most likely to identify the genetic cause but also limit identification of variants of uncertain significance.[1]

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to EOFAD testing.

American College of Medical Genetics and Genomics and National Society of Genetic Counselors

The American College of Medical Genetics and Genomics (ACMG, 2011) and The National Society of Genetic Counselors NSGC, 2011) stated:[4]

- "Testing for genes associated with early-onset autosomal dominant AD should be offered in the following situations:"
    - "A symptomatic individual with EOAD in the setting of a family history of dementia or in the setting of an unknown family history (e.g., adoption)".
    - "Autosomal dominant family history of dementia with one or more cases of EOAD."
    - "A relative with a mutation consistent with EOAD (currently PSEN 1/2 or APP)."

Amyloid Imaging Taskforce, Society of Nuclear Medicine and Molecular Imaging, and Alzheimer's Association

The Amyloid Imaging Taskforce (AIT, 2013), Society of Nuclear Medicine and Molecular Imaging (SNMMI, 2013), and the Alzheimer's Association referenced genetic testing in their recommendations:[5]

- "The use of amyloid PET in lieu of genotyping for suspected autosomal dominant mutation carriers is considered inappropriate. The optimal clinical evaluation in these cases is careful collection of a family history, followed (if appropriate) by genetic counseling prior to and after genetic testing for known mutations. Future use of amyloid PET in autosomal dominant mutation carriers could include determination of whether the amyloid deposition phase of their illness has begun. In the setting of a complete clinical evaluation, including serial neuropsychological testing, this information may be useful in identifying one disease-related milestone that, along with the genetic information, aids decision making."

European Federation of Neurological Societies

The European Federation of Neurological Societies (EFNS, 2010) Alzheimer's diagnosis and management guidelines addressed genetic testing:[6]

- "Screening for known pathogenic mutations can be undertaken in patients with appropriate phenotype or a family history of an autosomal dominant dementia." (No evidence level assigned.) They add, "Testing of patients with familial dementia and of unaffected at-risk-relatives should be accompanied by neurogenetic counseling and undertaken only after full consent and by specialist centers. Pre-symptomatic testing may be performed in at risk member of family-carrying mutation. It is recommended that the Huntington's disease protocol is followed for pre-symptomatic testing."

Selected Relevant Publications

A 2018 expert-authored review stated:[1]

- "Establishing a specific genetic cause of Alzheimer disease (AD): Can aid in discussions of prognosis (which are beyond the scope of this GeneReview) and genetic counseling (Section 4); Usually involves a medical history, physical examination, and laboratory testing to exclude disorders included in the differential diagnosis (see Section 1), family history, and genomic/genetic testing."
- "Because familial AD and nonfamilial AD appear to have the same clinical and pathologic phenotypes, they can only be distinguished by family history and/or by molecular genetic testing."
- "Because of the significant overlap in clinical manifestations and age of onset in AD, single-gene testing (i.e., sequence analysis, followed by gene-targeted deletion/duplication analysis) is rarely useful and typically NOT recommended."

EOFAD

- "Predictive testing for asymptomatic adults at risk for APP-, PSEN1-, or PSEN2-related EOFAD is possible if the pathogenic variant has been identified in an affected family member."

References

Introduction

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Ehlers-Danlos Syndrome Genetic Testing

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Introduction

Ehlers-Danlos syndrome (EDS) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
EDS Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
EDS Known Familial Mutation Analysis	81403

Criteria

Introduction

Requests for Ehlers-Danlos syndrome (EDS) genetic testing are reviewed using these criteria.

EDS Known Familial Mutation Analysis

- Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy) , AND

- Previous Genetic Testing:
  - No previous testing that would detect the familial mutation, AND
- Diagnostic Testing for an Autosomal Dominant EDS:
  - Known mutation identified in 1st degree biological relative. (Note: 2nd or 3rd degree relatives may be considered when 1st degree relatives are unavailable or unwilling to be tested), OR
- Diagnostic Testing and Carrier Screening for an Autosomal Recessive EDS:
  - Known mutation(s) identified in 1st, 2nd, or 3rd degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - Family history of an autosomal dominant type of EDS with a known mutation identified in a previous child or either parent, or
  - Both parents carry a known mutation for an autosomal recessive type of EDS, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

EDS Single Gene Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous sequencing of the requested gene, AND
- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND
- The member does not have a family history of a known EDS gene mutation that would explain their clinical symptoms, AND
- The member meets the below 2017 minimal criteria suggestive for an EDS type associated with the requested gene test:
  - For COL5A1 and/or COL5A2 analysis: criteria for classical EDS met, or
  - For TNXB analysis: criteria for classical-like EDS met, or
  - For COL1A1* analysis: criteria met for one of the following EDS types:
    - Classical EDS, or
    - Vascular EDS, or

- Arthrochalasia EDS, or
- Member displays one or more of the following:
  - Arterial rupture at a young age, or
  - Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology, or
  - Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears, or
  - Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma, or
  - Member has one minor criterion for vEDS and a family history of arterial rupture, colonic rupture, uterine rupture, or carotid-cavernous sinus fistula (CCSF), OR

- For COL1A2* analysis: criteria met for one of the following EDS types:
  - Cardiac valvular EDS, or
  - Arthrochalasia EDS, or

- For COL3A1* analysis: criteria for vascular EDS met, or
  - Member displays one or more of the following:
    - Arterial rupture at a young age, or
    - Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology, or
    - Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears, or
    - Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma, or
    - Member has one minor criterion for vEDS and a family history of arterial rupture, colonic rupture, uterine rupture, or carotid-cavernous sinus fistula (CCSF), OR
- For ADAMTS2 analysis: criteria for dermatosparaxis EDS met, or
- For PLOD1 and/or FKBP14 analysis: criteria for kyphoscoliotic EDS met, or
- For ZNF469 and/or PRDM5 analysis: criteria for brittle cornea syndrome met, or
- For B3GALT6, B4GALT7, and/or SLC39A13 analysis: criteria for spondylodysplastic EDS met, or

- For CHST14 and/or DSE analysis: criteria for musculocontractural EDS met, or
- For COL12A1 analysis: criteria for myopathic EDS met, or
- For C1R and/or C1S analysis: criteria for periodontal EDS met, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

* For non-EDS indications, refer to any available disorder-specific guidelines or general guidelines, *Hereditary Connective Tissue Disorder Genetic Testing*, or *Genetic Testing to Diagnose Non-Cancer Conditions*, as appropriate. COL1A1 and COL1A2 are also associated with osteogenesis imperfecta, Caffey disease, and skeletal dysplasias. COL3A1 is also associated with familial thoracic aortic aneurysm and dissection (TAAD).

Exceptions and Other Considerations

For information on multigene panel testing, please refer to the guideline *Hereditary Connective Tissue Disorder Genetic Testing*, as this testing is not addressed here.

The following are not medically necessary indications for EDS gene sequencing and deletion/duplication analysis:

- Member's personal and/or family history are consistent with hypermobile EDS or the related clinical entity, “joint hypermobility syndrome”
- Isolated nonsyndromic joint hypermobility, including both asymptomatic and symptomatic forms (e.g., “hypermobility spectrum disorders”)

What is Ehlers-Danlos Syndrome?

Definition

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders. Although all types of EDS affect the joints and skin, additional features vary by type.[1]

Prevalence

The combined prevalence of all types of EDS appears to be at least 1 in 5,000 individuals worldwide, with the most common being the hypermobile type.[1]

Symptoms

An unusually large range of joint movement (hypermobility) occurs with most forms of EDS, and is especially prominent in the hypermobile type.[1]

- Generalized joint hypermobility is typically assessed using a 9-point scale called the Beighton criteria. Adults 50 or younger with a Beighton score of ≥5, adults older

than 50 with a Beighton score ≥4, and pre-pubertal children and adolescents with a Beighton score ≥6, are considered to have generalized joint hypermobility.[2-4] In people with a Beighton score 1 point below the age-specific cut-off, a positive 5-point questionnaire result (2 or more positive answers) can be taken as evidence of generalized joint hypermobility.[4]

- Generalized joint hypermobility is relatively common, occurring in 2-57% of different populations.[2]
- Joint hypermobility can be a feature of other connective tissue disorders (e.g. Marfan syndrome, skeletal dysplasias, and other disorders), myopathic disorders, and other chromosomal and molecular disorders. Joint hypermobility may also occur as an isolated, nonsyndromic finding.[3]
- Joint hypermobility may be asymptomatic, or associated with musculoskeletal complications such as chronic pain and disturbed proprioception. Individuals with symptomatic joint hypermobility who do not have hypermobile EDS or another identifiable cause are considered to have "hypermobility spectrum disorders (HSDs)." [3]
- Six types of EDS were originally delineated in 1997.[5] In 2017, clinical criteria were updated and revised to include thirteen EDS types:[4]
    - Classical EDS
    - Classical-like EDS
    - Cardiac-valvular EDS
    - Vascular EDS
    - Hypermobile EDS
    - Arthrochalasia EDS
    - Dermatosparaxis EDS
    - Kyphoscoliotic EDS
    - Brittle cornea syndrome
    - Spondylodysplastic EDS
    - Musculocontractural EDS
    - Myopathic EDS
    - Periodontal EDS

Cause and Inheritance

Ehlers-Danlos syndrome may be an autosomal recessive or autosomal dominant disorder, depending on the type.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

The genetic basis and inheritance of the various types of EDS are summarized in the table below:[4]

EDS Type	Inheritance	Genetic basis	Protein
Classical EDS	Autosomal dominant	Major: COL5A1, COL5A2 Rare: COL1A1 c.934C>T	Type V collagen Type I collagen
Classical-like EDS	Autosomal recessive	TNXB	Tenascin XB
Cardiac valvular EDS	Autosomal recessive	COL1A2 (biallelic mutations that lead to COL1A2 NMD & absence of pro α2(I) collagen chains)	Type I collagen
Vascular EDS	Autosomal dominant	COL3A1	Type III collagen
Hypermobile EDS	Autosomal dominant	Unknown	Unknown
Arthrochalasia EDS	Autosomal dominant	COL1A1 COL1A2	Type I collagen
Dermatosparaxis EDS	Autosomal recessive	ADAMTS2	ADAMTS-2
Kyphoscoliotic EDS	Autosomal recessive	PLOD1 FKBP14	LH1 FKBP22
Brittle cornea syndrome	Autosomal recessive	ZNF469 PRDM5	ZNF469 PRDM5

EDS Type	Inheritance	Genetic basis	Protein
Spondylodysplastic EDS	Autosomal recessive	B4GALT7 B3GALT6 SLC39A13	β4GalT7 β3GalT6 ZIP13
Musculocontractural EDS	Autosomal recessive	CHST14 DSE	D4ST1 DSE
Myopathic EDS	Autosomal recessive or dominant	COL12A1	Type XII collagen
Periodontal type	Autosomal dominant	C1R C1S	C1r C1s

Diagnosis

A diagnosis of EDS can be established with the identification of a pathogenic mutation or mutations in a causative gene. Furthermore, as outlined in the guidelines and evidence section, international clinical criteria have been published.[4]

Clinical genetic testing is available for most types of EDS (see table above), and is used to confirm the final diagnosis when it is clinically suspected.[4]

- >90% of individuals with classical EDS have a mutation in COL5A1 or COL5A2.[4,6]
- >95% of individuals with vascular EDS have a mutation in COL3A1.[7]
- Mutation detection rates for the rarer EDS types are mostly unknown.

Hypermobile EDS (hEDS) continues to require a clinical diagnosis, since the genetic etiology of this type is not yet known.[4,8]

Management

There is no cure for EDS. Management is focused on prevention and treatment of symptoms. This may consist of medication for pain, physical therapy, protection of joints, monitoring for and treating common complications, and psychosocial support.[9]

Survival

The prognosis will depend on the type of EDS and associated symptoms. Most types of EDS do not affect life expectancy. Given the rarity of some types (such as dermatosparaxis and musculocontractural), the natural history and prognosis may not be firmly established. The severe forms of EDS (vascular and cardiac-valvular) usually affect lifespan. The kyphoscoliotic form may also affect lifespan if there are vascular symptoms and/or restrictive lung disease.[10]

Test information

Introduction

Testing for EDS may include known familial mutation analysis, single gene analysis, and/or multigene panel testing. Known familial mutation analysis and single gene analysis are addressed by this guideline.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Multigene Panel Testing

With the availability of NGS technology, EDS genetic testing is increasingly performed as a panel test that includes multiple EDS genes. In addition, these panels often include other hereditary connective tissue disorders with overlapping phenotypes. For information on multigene panel testing, please refer to the guideline *Hereditary Connective Tissue Disorder Genetic Testing*, as this testing is not addressed here.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to EDS genetic testing.

International Consortium on the Ehlers-Danlos Syndromes

According to the International Consortium on the Ehlers-Danlos Syndromes (2017):[4]

- “In view of the vast genetic heterogeneity and phenotypic variability of the EDS subtypes, and the clinical overlap between many of these subtypes, but also with

other hereditary connective tissue disorders, the definite diagnosis relies for all subtypes, except hEDS, on molecular confirmation with identification of (a) causative variant(s) in the respective gene."

- "Molecular diagnostic strategies should rely on NGS technologies, which offer the potential for parallel sequencing of multiple genes. Targeted resequencing of a panel of genes…is a time- and cost-effective approach for the molecular diagnosis of the genetically heterogeneous EDS. When no mutation (or in case of an autosomal recessive condition only one mutation) is identified, this approach should be complemented with a copy number variant (CNV) detection strategy to identify large deletions or duplications, for example Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR, or targeted array analysis."
- "The diagnosis of hEDS remains clinical as there is yet no reliable or appreciable genetic etiology to test for in the vast majority of patients."

As defined in the sections below, the International Consortium developed clinical criteria for the Ehlers-Danlos syndromes.[4]

2017 International Criteria for Classical EDS

Minimal criteria suggestive for Classical EDS (cEDS):

- Major criterion 1, PLUS either:
  - Major criterion 2, and/or
  - At least three minor criteria.

Major criteria for cEDS	Minor criteria for cEDS
1. Skin hyperextensibility and atrophic scarring 2. Generalized joint hypermobility	1. Easy bruising 2. Soft, doughy skin 3. Skin fragility (or traumatic splitting) 4. Molluscoid pseudotumors 5. Subcutaneous spheroids 6. Hernia (or history thereof) 7. Epicanthal folds 8. Complications of joint hypermobility (e.g., sprains, luxation/subluxation, pain, flexible flatfoot) 9. Family history of a first-degree relative who meets clinical criteria

2017 International Criteria for Classical-like EDS

Minimal criteria suggestive for Classical-like EDS (clEDS):

- All three major criteria, AND
- A family history compatible with autosomal recessive transmission.

Major criteria for clEDS	Minor criteria for clEDS
1. Skin hyperextensibility, with velvety skin texture and absence of atrophic scarring 2. Generalized joint hypermobility with or without recurrent dislocations (most commonly shoulder and ankle) 3. Easy bruisable skin/spontaneous ecchymoses	1. Foot deformities: broad/plump forefoot, brachydactyly with excessive skin; pes planus; hallux valgus; piezogenic papules 2. Edema in the legs in absence of cardiac failure 3. Mild proximal and distal muscle weakness 4. Axonal polyneuropathy 5. Atrophy of muscles in hands and feet 6. Acrogeric hands, mallet finger(s), clinodactyly, brachydactyly 7. Vaginal/uterus/rectal prolapse

2017 International Criteria for Cardiac-Valvular EDS

Minimal criteria suggestive for Cardiac-Valvular EDS (cvEDS)

- Major criterion 1, AND
- A family history compatible with autosomal recessive inheritance, PLUS either:
    - One other major criterion, and/or
    - At least two minor criteria.

Major criteria for cvEDS	Minor criteria for cvEDS
1. Severe progressive cardiac-valvular problems (aortic valve, mitral valve) 2. Skin involvement: skin hyperextensibility, atrophic scars, thin skin, easy bruising 3. Joint hypermobility (generalized or restricted to small joints)	1. Inguinal hernia 2. Pectus deformity (especially pectus excavatum) 3. Joint dislocations 4. Foot deformities: pes planus, pes planovalgus, hallux valgus

2017 International Criteria for Vascular EDS

Minimal criteria suggestive for Vascular EDS (vEDS):

- A family history of the disorder, and/or
- Arterial rupture or dissection in individuals less than 40 years of age, and/or
- Unexplained sigmoid colon rupture, and/or
- Spontaneous pneumothorax in the presence of other features consistent with vEDS, and/or
- A combination of the other minor clinical features listed below.

Major criteria for vEDS	Minor criteria for vEDS
1. Family history of vEDS with documented causative variant in COL3A1 2. Arterial rupture at a young age 3. Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology 4. Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears 5. Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma	1. Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back 2. Thin, translucent skin with increased venous visibility 3. Characteristic facial appearance 4. Spontaneous pneumothorax 5. Acrogeria 6. Talipes equinovarus 7. Congenital hip dislocation 8. Hypermobility of small joints 9. Tendon and muscle rupture 10. Keratoconus 11. Gingival recession and gingival fragility 12. Early onset varicose veins (under 30 and nulliparous if female)

2017 International Criteria for Hypermobile EDS

Diagnosis of Hypermobile EDS (hEDS) requires the simultaneous presence of criteria 1 AND 2 AND 3:

- Criteria 1: Generalized joint hypermobility

- Criterion 2: Two or more among the features (A-C) listed in the table below must be present (for example: A and B; A and C; B and C; A and B and C).
- Criterion 3: All of the following prerequisites must be met:
    - Absence of unusual skin fragility, and
    - Exclusion of other heritable and acquired connective tissue disorders, including autoimmune rheumatologic conditions, and
    - Exclusion of alternative diagnoses that may also include joint hypermobility by means of hypotonia and/or connective tissue laxity.

Feature A	Feature B	Feature C
A total of 5 must be present: 1. Unusually soft or velvety skin 2. Mild skin hyperextensibility 3. Unexplained striae 4. Bilateral piezogenic papules of the heel 5. Recurrent or multiple abdominal hernia(s) 6. Atrophic scarring involving at least two sites 7. Pelvic floor, rectal, and/or uterine prolapes in children, men or nulliparous women without a history of morbid obesity or other known predisposing medical condition 8. Dental crowding and high or narrow palate 9. Arachnodactyly 10. Arm span-to-height ≥ 1.05 11. Mitral valve prolapse (MVP) 12. Aortic root dilatation with Z-score > +2	Positive family history, with one or more first degree relatives independently meeting the current diagnostic criteria for hEDS.	Must have at least one 1. Musculoskeletal pain in two or more limbs, recurring daily for at least 3 months. 2. Chronic, widespread pain for ≥ 3 months 3. Recurrent joint dislocations or frank joint instability, in the absence of trauma: a. Three or more atraumatic dislocations in the same joint or two or more atraumatic dislocations in two different joints occurring at different times, or b. Medical confirmation of joint instability at two or more sites not related to trauma

2017 International Criteria for Arthrochalasia EDS

Minimal criteria suggestive for Arthrochalasia EDS (aEDS):

- Major criterion 1, PLUS either:
  - Major criterion 3, and/or
  - Major criterion 2 and at least two other minor criteria.

Major criteria for aEDS	Minor criteria for aEDS
1. Congenital bilateral hip dislocation 2. Severe generalized joint hypermobility, with multiple dislocations/subluxations 3. Skin hyperextensibility	1. Muscle hypotonia 2. Kyphoscoliosis 3. Radiologically mild osteopenia 4. Tissue fragility, including atrophic scars 5. Easy bruisable skin

2017 International Criteria for Dermatosparaxis EDS

Minimal criteria suggestive for Dermatosparaxis EDS (dEDS):

- Major criterion 1, AND
- Major criterion 2, PLUS either:
  - One other major criterion, and/or
  - Three minor criteria.

Major criteria for dEDS	Minor criteria for dEDS
1. Extreme skin fragility with congenital or postnatal skin tears 2. Characteristic craniofacial features, which are evident at birth or early infancy, or evolve later in childhood 3. Redundant, almost lax skin, with excessive skin folds at the wrist and ankles 4. Increased palmar wrinkling 5. Severe bruisability with a risk of subcutaneous hematomas and hemorrhage 6. Umbilical hernia 7. Postnatal growth retardation 8. Short limbs, hands and feet 9. Perinatal complications due to connective tissue fragility	1. Soft and doughy skin texture 2. Skin hyperextensibility 3. Atrophic scars 4. Generalized joint hypermobility 5. Complications of visceral fragility (e.g., bladder rupture, diaphragmatic rupture, rectal prolapse) 6. Delayed motor development 7. Osteopenia 8. Hirsutism 9. Tooth abnormalities 10. Refractive errors (myopia, astigmatism) 11. Strabismus

2017 International Criteria for Kyphoscoliotic EDS

Minimal criteria suggestive for Kyphoscoliotic EDS (kEDS):

- Major criterion 1, AND
- Major criterion 2, PLUS either:
    - Major criterion 3, and/or
    - Three minor criteria (either general or gene-specific criteria).

Major criteria for kEDS	Minor criteria for kEDS	Gene-specific minor criteria for kEDS
1. Congenital muscle hypotonia 2. Congenital or early onset kyphoscoliosis (progressive or non-progressive) 3. Generalized joint hypermobility with dislocations/subluxations (shoulders, hips, and knees in particular)	1. Skin hyperextensibility 2. Easy bruisable skin 3. Rupture/aneurysm of a medium-sized artery 4. Osteopenia/ osteoporosis 5. Blue sclerae 6. Hernia (umbilical or inguinal) 7. Pectus deformity 8. Marfanoid habitus 9. Talipes equinovarus 10. Refractive errors (myopia, hypermetropia)	PLOD1 1. Skin fragility (easy bruising, friable skin, poor wound healing), widened atrophic scarring 2. Scleral and ocular fragility/rupture 3. Microcornea 4. Facial dysmorphology FKBP14 1. Congenital hearing impairment (any type) 2. Follicular hyperkeratosis 3. Muscle atrophy 4. Bladder diverticula

2017 International Criteria for Brittle Cornea Syndrome

Minimal criteria suggestive for Brittle Cornea Syndrome (BCS):

- Major criterion 1, PLUS either:
  - At least one other major criterion, and/or
  - Three minor criteria.

Major criteria for BCS	Minor criteria for BCS
1. Thin cornea, with or without rupture (central corneal thickness often <400 µm) 2. Early onset progressive keratoconus 3. Early onset progressive keratoglobus 4. Blue sclerae	1. Enucleation or corneal scarring as a result of previous rupture 2. Progressive loss of corneal stromal depth, especially in central cornea 3. High myopia, with normal or moderately increased axial length 4. Retinal detachment 5. Deafness (often mixed, progressive, higher frequencies often more severely affected) 6. Hypercompliant tympanic membranes 7. Developmental dysplasia of the hip 8. Hypotonia in infancy, usually mild if present 9. Scoliosis 10. Arachnodactyly 11. Hypermobility of distal joints 12. Pes planus, hallux valgus 13. Mild contractures of fingers (especially fifth) 14. Soft, velvety skin, translucent skin

2017 International Criteria for Spondylodysplastic EDS

Minimal criteria suggestive for Spondylodysplastic EDS (spEDS):

- Major criterion 1, AND
- Major criterion 2, PLUS
- Characteristic radiographic findings and at least 3 other minor criteria (general or type-specific).

Major criteria for spEDS	Minor criteria for spEDS	Gene-specific minor criteria for spEDS
1. Short stature (progressive in childhood) 2. Muscle hypotonia (ranging from severe congenital, to mild later-onset) 3. Bowing of limbs	1. Skin hyperextensibility, soft, doughy skin, thin translucent skin 2. Pes planus 3. Delayed motor development 4. Osteopenia 5. Delayed cognitive development	B4GALT7 1. Radioulnar synostosis 2. Bilateral elbow contractures or limited elbow movement 3. Generalized joint hypermobility 4. Single transverse palmar curve 5. Characteristic craniofacial features 6. Characteristic radiographic findings 7. Severe hypermetropia 8. Clouded cornea
		SLC39A13 1. Protuberant eyes with bluish sclerae 2. Hands with finely wrinkled palms 3. Atrophy of the thenar muscles, tapering fingers 4. Hypermobility of distal joints 5. Characteristic radiologic findings

Major criteria for spEDS	Minor criteria for spEDS	Gene-specific minor criteria for spEDS
		B3GALT6 1. Kyphoscoliosis (congenital or early onset, progressive) 2. Joint hypermobility, generalized or restricted to distal joints, with joint dislocations 3. Joint contractures (congenital or progressive) (especially hands) 4. Peculiar fingers (slender, tapered, arachnodactyly, spatulate, with broad distal phalanges) 5. Talipes equinovarus 6. Characteristic craniofacial features 7. Tooth discoloration, dysplastic teeth 8. Characteristic radiographic findings 9. Osteoporosis with multiple spontaneous fractures Ascending aortic aneurysm 10. Lung hypoplasia, restrictive lung disease

2017 International Criteria for Musculocontractural EDS

Minimal criteria suggestive for Musculocontractural EDS (mcEDS):

- At birth or in early childhood:

- Major criterion 1, AND
- Major criterion 2

- In adolescence and in adulthood:
  - Major criterion 1, AND
  - Major criterion 3.

Major criteria for mcEDS	Minor criteria for mcEDS
1. Congenital multiple contractures, characteristically adduction-flexion contractures, and/or talipes equinovarus (clubfoot) 2. Characteristic craniofacial features, which are evident at birth or in early infancy 3. Characteristic cutaneous features including skin hyperextensibility, easy bruisability, skin fragility with atrophic scars, increased palmar wrinkling	1. Recurrent/chronic dislocations 2. Pectus deformities (flat, excavated) 3. Spinal deformities (scoliosis, kyphoscoliosis) 4. Peculiar fingers (tapering, slender, cylindrical) 5. Progressive talipes deformities (valgus, planus, cavum) 6. Large subcutaneous hematomas 7. Chronic constipation 8. Colonic diverticula 9. Pneumothorax/pneumohemothorax 10. Nephrolithiasis/cystolithiasis 11. Hydronephrosis 12. Cryptorchidism in males 13. Strabismus 14. Refractive errors (myopia, astigmatism) 15. Glaucoma/elevated intraocular pressure

2017 International Criteria for Myopathic EDS

Minimal criteria suggestive for Myopathic EDS (mEDS):

- Major criterion 1, PLUS either:
  - One other major criterion and/or
  - Three minor criteria

Major criteria for mEDS	Minor criteria for mEDS
1. Congenital muscle hypotonia, and/or muscle atrophy, that improves with age 2. Proximal joint contractures (knee, hip, and elbow) 3. Hypermobility of distal joints	1. Soft, doughy skin 2. Atrophic scarring 3. Motor developmental delay 4. Myopathy on muscle biopsy

2017 International Criteria for Periodontal EDS

Minimal criteria suggestive for Periodontal EDS (pEDS):

- Major criterion 1, OR major criterion 2, PLUS
  - At least two other major criteria and one minor criterion.

Major criteria for pEDS	Minor criteria for pEDS
1. Severe and intractable periodontitis of early onset (childhood or adolescence) 2. Lack of attached gingiva 3. Pretibial plaques 4. Family history of a first-degree relative who meets clinical criteria	1. Easy bruising 2. Joint hypermobility, mostly distal joints 3. Skin hyperextensibility and fragility, abnormal scarring (wide or atrophic) 4. Increased rate of infections 5. Hernias 6. Marfanoid facial features 7. Acrogeria 8. Prominent vasculature

Selected Relevant Publication

An expert-authored review in 2018 stated the following regarding hEDS:[8]

"If an individual's personal or family history is suggestive of one of the other types of EDS or another hereditary disorder of connective tissue or arterial fragility syndrome,

analysis of an associated gene or multi-gene connective tissue disease panel may be appropriate. Failure to identify a pathogenic variant with such multiple gene testing reduces the likelihood of an arterial fragility syndrome, but does not completely rule it out, especially in the setting of a positive personal or family history of arterial fragility. Negative testing for an arterial fragility syndrome also does not confirm a diagnosis of hEDS. Therefore, such testing is not recommended in the absence of specific suggestive signs, symptoms, or family history."

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Expanded Carrier Screening Panels

MOL.TS.165.A
v2.0.2024

Introduction

Expanded carrier screening panels are addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Ashkenazi Jewish Genetic Disorders Sequencing	81412
ASPA Targeted Mutation Analysis	81200
BCKDHB Targeted Mutation Analysis	81205
BLM Targeted Mutation Analysis	81209
Carrier Screening Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
CFTR Targeted Mutation Analysis	81220
CFTR Deletion/Duplication Analysis	81222
CFTR Sequencing	81223
DMD Deletion/Duplication Analysis	81161
FANCC Targeted Mutation Analysis	81242
FMR1 Expansion Analysis	81243

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Procedures addressed by this guideline	Procedure codes
FMR1 Methylation Analysis	81244
G6PC Targeted Mutation Analysis	81250
GBA Targeted Mutation Analysis	81251
Genesys Carrier Panel	0400U
Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders, genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH)	81443
GJB2 Sequencing	81252
GJB6 Targeted Mutation Analysis	81254
HBA1/HBA2 Targeted Mutation Analysis	81257
HBA1/HBA2 Sequencing	81259
HBA1/HBA2 Deletion/Duplication Analysis	81269
HBB Targeted Mutation Analysis	81361
HBB Deletion/Duplication Analysis	81363
HBB Sequencing	81364
Hemoglobin Electrophoresis	83020
HEXA Targeted Mutation Analysis	81255
IKBKAP Targeted Mutation Analysis	81260
MCOLN1 Targeted Mutation Analysis	81290
SERPINA1 Targeted Mutation Analysis	81332
SMN1 Gene Analysis; Dosage/Deletion Analysis (eg, carrier testing), includes SMN2 Analysis, if performed	81329
SMPD1 Targeted Mutation Analysis	81330
UNITY Carrier Screen	0449U

Criteria

Introduction

Requests for expanded carrier screening panels are reviewed using these criteria.

Individually billed gene tests

Individual gene tests included in expanded carrier screening panels that will be separately billed should be evaluated based on the medical necessity criteria for each gene test. See Coverage Guidance below.

Single panel code billed

Medical necessity must be established for full gene **sequencing** of at least two conditions included in the panel. This does not include:

- targeted mutation testing (i.e. known familial mutation testing), or
- molecular methodologies other than sequencing (i.e. fragile X or other gene methylation analysis; SMN1 dosage/deletion analysis [e.g., carrier testing]; deletion/duplication analysis of any gene by MLPA or similar platform), or
- non-molecular methodologies (i.e. hemoglobin electrophoresis for hemoglobinopathies)

Coverage guidance

This table describes coverage guidance around the most commonly performed carrier screening tests. It also includes the test types addressed by population-based carrier screening guidelines. When the test is not addressed in this table, refer to the general guideline: *Genetic Testing for Carrier Status*. For these additional tests to be medically necessary, there will generally need to be a specific known increased risk for that condition such as a known family history or a reproductive partner who is known to be a carrier of or affected with the condition.

Coverage Guidance for Genes Included in Expanded Carrier Screening Multi-Gene Panels

Condition groups	Condition	Gene	CPT Code	Required Claim Code	Coverage
Pan-Ethnic Conditions	Cystic fibrosis	CFTR	81220	NONE	MOL.TS.158
			81222	NONE	MOL.TS.158
			81223	NONE	MOL.TS.158
	Spinal muscular atrophy	SMN1/ SMN2	81329	SMN1SMN2	MOL.TS.225
	Fragile X	FMR1	81243	NONE	MOL.TS.172

Condition groups	Condition	Gene	CPT Code	Required Claim Code	Coverage
	syndrome		81244	NONE	MOL.TS.172
	Ashkenazi Jewish genetic disorders **				MOL.TS.129
	Bloom syndrome	BLM	81209	NONE	MOL.TS.132
	Canavan disease	ASPA	81200	NONE	MOL.TS.145
	Dihydrolipoamide dehydrogenase deficiency	DLD	81479	DLD	MOL.CU.110
	Familial dysautonomia	IKBKAP	81260	NONE	MOL.CU.110
	Familial hyperinsulinism	ABCC8	81401	ABCC8	MOL.CU.110
	Fanconi anemia, type C	FANCC	81242	NONE	MOL.CU.110
	Gaucher disease, type 1	GBA	81251	NONE	MOL.TS.173
	Glycogen storage disease, type 1A	G6PC	81250	NONE	MOL.CU.110
	Joubert syndrome, type 2	TMEM216	81479	TMEM216	MOL.CU.110
	Maple syrup disease, type 1B	BCKDHB	81205	NONE	MOL.CU.110
	Mucolipidosis, type IV	MCOLN1	81290	NONE	MOL.CU.110

Condition groups	Condition	Gene	CPT Code	Required Claim Code	Coverage
	Nemaline myopathy, type 2	NEB	81400	NEB	MOL.CU.110
	Niemann-Pick disease, type A	SMPD1	81330	NONE	MOL.TS.207
	Tay-Sachs disease	HEXA	81255	NONE	MOL.TS.226
	Usher syndrome, type 1F	PCDH15	81400	PCDH15	MOL.CU.110
	Usher syndrome, type 3	CLRN1	81400	CLRN1	MOL.CU.110
Hemoglobinopathy screening	Hemoglobinopathies	NONE	83020	NONE	Cover without review
	Sickle cell anemia, Beta Thalassemia	HBB	81361	HBB	MOL.TS.308
			81363	HBB	MOL.TS.308
			81364	HBB	MOL.TS.308
	Alpha thalassemia	HBA1/HBA2	81257	NONE	MOL.TS.308
			81269	HBA1HBA2	MOL.TS.308
			81259	HBA1HBA2	MOL.TS.308

Note **The single Ashkenazi Jewish Carrier Screening guideline should be sufficient to assess the appropriateness of all tests in this category in most circumstances. The available individual gene test policies are provided should additional information be useful.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on

allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Panel will be billed with a single procedure code (e.g., 81443), to represent all genes being sequenced.
    - No single gene components of the panel have been performed and reimbursed previously, or billed separately on the same date of service,
- Individual gene tests that are separately billed and do not meet medical necessity criteria are not a reimbursable service. It will be at the laboratory, provider, and member's discretion to determine if a multi-gene panel remains the preferred testing option, recognizing that only a portion of the panel may be reimbursed by insurance.
- The following should not be billed as part of 81443 and should not count toward the requirement of two conditions meeting medical necessity requirements:
    - Spinal muscular atrophy carrier testing should be billed separately using 81329.
    - Fragile X carrier testing should be billed separately using 81243.
    - Carrier testing performed due to the sole indication of Ashkenazi Jewish ancestry should be billed with 81412.
- When otherwise reimbursable, the following limitations apply:
    - Any individual gene or multi-gene panel is only reimbursable once per lifetime.

What are expanded carrier screening panels?

Definition

Expanded carrier screening panels, also known as multiplex carrier screening panels or universal carrier screening, are designed to identify carrier status or predict risk for multiple genetic diseases in a single test. It is typically offered to individuals planning a pregnancy or currently pregnant.

Prevalence

The genetic diseases that are tested for range in severity from lethal in infancy to so mild an affected individual may never develop symptoms. Some conditions are quite common, especially in certain ethnic groups, while others are rare.

It is generally believed that all people carry several recessive gene mutations. An estimated 1 in 580 births has an autosomal recessive condition and 1 in 2000 has an X-linked condition.[1]

Inheritance

Expanded carrier screening panels may include autosomal recessive and X-linked conditions.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

X-Linked Inheritance

In X-linked inheritance, the mutation is carried on the X chromosome. Females have two X chromosomes, and males have one. Males typically have more severe symptoms than females. A female with a mutation has a 50% chance to pass that mutation to her children. A male with a mutation cannot pass the mutation to any sons, but will pass it to all daughters. A process called X-inactivation in females results in random inactivation of expression of one X-chromosome in each cell of the body. For females with one mutation, the percentage and distribution of cells with expression of the X chromosome carrying the mutation can influence the degree of severity.

Common uses

Expanded carrier screening is most commonly done for reproductive planning, to identify couples at risk for having a child with a recessive inherited disorder. In most cases, couples who have a child with a recessive inherited disorder have no family history of that disorder or any other risk factors.

Carrier screening for a specific disorder may be indicated when there is a positive family history, when a reproductive partner is a carrier of or is affected with a recessive disorder, or when there is a known increased risk based on ethnicity or other factors.

Test information

Introduction

Expanded carrier screening panels determine carrier status for numerous genetic conditions simultaneously for the purposes of reproductive planning.

Expanded carrier screening panels

Several expanded carrier screening panels are available. Each test has a unique set of diseases included in novel and proprietary genetic testing platforms. The number of mutations tested varies considerably by condition, ranging from a single mutation for rare conditions to over 100 mutations for cystic fibrosis. Many panels consist of full gene sequencing. Complete testing information, including a list of all conditions screened and the technology used, can be found at a laboratory's website.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to expanded carrier screening.

American College of Obstetrics and Gynecology

The American College of Obstetrics and Gynecology (ACOG, 2017; Reaffirmed 2023) published a committee opinion that stated the following regarding Expanded Carrier Screening:[2]

- “Ethnic-specific, panethnic, and expanded carrier screening are acceptable strategies for prepregnancy and prenatal carrier screening. Each obstetrician–gynecologist or other health care provider or practice should establish a standard approach that is consistently offered to and discussed with each patient, ideally before pregnancy. After counseling, a patient may decline any or all carrier screening.” “Given the multitude of conditions that can be included in expanded carrier screening panels, the disorders selected for inclusion should meet several of the following consensus-determined criteria: have a carrier frequency of 1 in 100 or greater, have a well-defined phenotype, have a detrimental effect on quality of life, cause cognitive or physical impairment, require surgical or medical intervention, or have an onset early in life. Additionally, screened conditions should be able to be diagnosed prenatally and may afford opportunities for antenatal intervention to improve perinatal outcomes, changes to delivery management to optimize newborn and infant outcomes, and education of the parents about special care needs after birth.”
- “Carrier screening panels should not include conditions primarily associated with a disease of adult onset.”

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2021) released an educational practice resource on carrier screening.[3] This consensus statement asserted that general population carrier screening should be ethnicity and family history agnostic. To accomplish this, screening all individuals in the

prenatal/preconception period for autosomal recessive and X-linked conditions with a carrier frequency of >1/200 was suggested. ACMG generated a list of 113 genes meeting these criteria.

Concerns with large panels

Although the number of large panels being offered by laboratories is increasing, most of the included tests are not indicated for each person being tested.

Issues with expanded carrier screening include:

- Many included tests have not been recommended for population-based carrier screening and should therefore only be performed when there is a specific known increased risk, such as a family history of the condition.
- Some conditions included in expanded carrier screens are exceedingly rare except in certain ethnicities, or the carrier frequency in the general population may not be known. Therefore, the residual risk for an individual after a negative expanded carrier screen may not be provided by the laboratory.[3]
- Mutation analysis may not be the preferred initial screening test for some conditions. For example, a CBC with RBC indices is the initial screening test for beta-thalassemia followed by hemoglobin analysis for individuals with microcytic anemia.[4,5] Measuring hexosaminidase A activity may be preferable to mutation analysis for Tay-Sachs carrier screening, especially in non-Jewish populations.[5]
- Some expanded carrier screens include testing for conditions that are relatively mild, treatable, or have onset in adulthood.
- Depending on ethnicity, current expanded carrier screening panels are expected to identify up to 40% of people tested as carriers of a recessive gene mutation. Therefore, if this screening is routinely offered, many patients will require counseling for a positive result, and partner testing must be offered. The most complete partner testing is often by full gene sequencing. Availability of partner testing, cost, turnaround time, and the possibility of identifying a variant of unknown significance by sequencing make this a complex clinical scenario to manage in the routine reproductive setting.

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Introduction

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Facioscapulohumeral Muscular Dystrophy Genetic Testing

MOL.TS.290.A
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Introduction

Facioscapulohumeral muscular dystrophy genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
D4Z4 region (FSHMD1A) deletion analysis	81404
D4Z4 region (FSHMD1A) methylation analysis	81479
FSHMD1 characterization of 4qA/4qB haplotypes	81404
SMCHD1 sequencing	81479
SMCHD1 deletion/duplication analysis	81479

Criteria

Introduction

Requests for facioscapulohumeral muscular dystrophy (FSHD) testing are reviewed using the following criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND

- Diagnostic Testing for Symptomatic Individuals:
  - D4Z4 deletion and permissive 4A haplotype in a 1st, 2nd, or 3rd degree biologic relative with a clinical diagnosis of FSHD, or
  - Abnormal D4Z4 methylation or disease-causing SMCHD1 mutation and permissive 4A haplotype in a 1st, 2nd, or 3rd degree biologic relative with a clinical diagnosis of FSHD, OR
- Presymptomatic Testing for Asymptomatic Individuals:
  - Member is 18 years of age or older, AND
  - One of the following has been identified in a 1st, 2nd, or 3rd degree biologic relative:
    - D4Z4 deletion and permissive 4A haplotype in a 1st, 2nd, or 3rd degree biologic relative with a clinical diagnosis of FSHD, or
    - Abnormal D4Z4 methylation or disease-causing SMCHD1 mutation and permissive 4A haplotype in a 1st, 2nd, or 3rd degree biologic relative with a clinical diagnosis of FSHD, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

D4Z4 Targeted Analysis and Haplotyping

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No redundant previous FSHD related testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - The member has a probable clinical diagnosis of FSHD based on the following:
    - Weakness of facial muscles, or
    - Either weakness of scapular stabilizers or foot dorsiflexors, and
    - Member has the following:
      - No involvement of the extrinsic ocular muscles (responsible for eyeball movement), and
      - Muscle biopsy, if available, is not consistent with another diagnosis, and
      - EMG, if available, does not show myotonia or neurogenic changes, and
      - Creatine kinase, if performed, is less than 1500 IU/L, AND

- The member does not have a known underlying cause for their symptoms, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

D4Z4 Methylation Analysis

- Previous Genetic Testing:
  - No redundant previous FSHD related testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - The member meets the above criteria for D4Z4 deletion and haplotype analysis, and
  - The member has previously had negative D4Z4 deletion testing, and
  - The member has a permissive 4A haplotype, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

SMCHD1 Analysis

- Previous Genetic Testing:
  - No redundant previous FSHD related testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - The member meets the above criteria for D4Z4 methylation analysis, and
  - The member has low D4Z4 methylation analysis results (less than 25%), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Facioscapulohumeral Muscular Dystrophy?

Definition

Facioscapulohumeral muscular dystrophy (FSHD) is both a genetic & epigenetic condition characterized by progressive muscle weakness involving facial, scapular, and humeral muscle groups early, and pelvic and peroneal muscle groups later.[1,2] There are two types of FSHD (FSHD1 and FSHD2) that are clinically identical, but distinguished by their different genetic causes.

Prevalence

Prevalence is estimated between 4-10 per 100,000.[3] Approximately 95% of FSHD cases are FSHD1; the remaining cases are FSHD2.[2]

Symptoms

Signs and symptoms can begin anytime between childhood and adulthood. More than 50% of individuals with FSHD demonstrate findings by age 20 years, but some individuals remain asymptomatic throughout their lives.[3] There is a severe infantile form of FSHD in which muscle weakness is present from birth.[3]

Symptoms of FSHD include:[1-4]

- Progressive facial muscle weakness (seen by difficulty closing the eyes, raising the eyebrows, whistling, frowning, puffing the cheeks, or showing teeth)
- Progressive shoulder girdle muscle weakness and atrophy
- Upper arm weakness and atrophy ("Popeye arms"), often asymmetric
- Pelvic muscle weakness and atrophy develop later
- Gait weakness, foot drop, calf hypertrophy
- Scapular winging
- Exercise intolerance
- Pain
- Extra-muscular manifestations include hearing loss (common) and vision deterioration (rare)

Severity ranges from almost asymptomatic weakness to severe restrictions of activities of daily living with approximately 20% of individuals requiring a wheelchair.

Cause

FSHD is caused by inappropriate expression of the DUX4 gene in muscle cells. The DUX4 gene is located within a microsatellite region called D4Z4, and relaxation of the chromatin in this region is believed to cause the aberrant expression.[3]

In FSHD1, the chromatin relaxation is caused by a deletion or contraction of a repeated stretch of DNA (called the D4Z4 repeat). Symptoms arise when this deletion occurs in the context of a permissive nearby haplotype (called 4A). Inheritance with another haplotype results in non-penetrance of the deletion, and FSHD1 is not likely.

In FSHD2, the chromatin relaxation is caused by the loss of methylation at D4Z4. This is commonly caused by a mutation in the SMCHD1 gene or, very rarely, the DNMT3B gene.[2,3]

Inheritance

The pattern of inheritance differs between FSHD1 and FSHD2.

FSHD1 is inherited in an autosomal dominant pattern, with symptoms only occurring when the D4Z4 deletion occurs in the presence of the permissive haplotype. Without

the presence of a specific chromosome 4A haplotype, a D4Z4 region deletion will not lead to the FSHD1 disorder.

FSHD2 inheritance is digenic, with symptoms only occurring when a mutation in SMCHD1 or DNMT3B occurs with the permissive 4A haplotype. The inheritance is not simply autosomal dominant, as SMCHD1 and DNMT3B sort independently from the permissive 4A haplotype locus: they are not always inherited together or from the same parent, as is the case with FSHD1.

Between 10 and 30% of individuals diagnosed with FSHD have no family history. In these putative non-familial cases the genetic change occurred either de novo or the parents may be mosaic for the causative genetic change.

Diagnosis

Diagnosis of FSHD is suggested by clinical phenotype and inheritance pattern, and confirmed by molecular testing. Because of the complex inheritance, careful correlation between clinical presentation and molecular result is essential.

- Diagnostic features should include a facial, scapular, humeral, and/or peroneal distribution of weakness and atrophy. Presence of a clinical phenotype more consistent with FSHD than other myopathies is an important diagnostic consideration. Note, myotonic dystrophy type 1 and 2 are very similar to FSHD and may only be distinguished by molecular testing.
- Biochemical abnormalities are nonspecific but point in the direction of muscle damage. Creatine kinase (CK) is normal to elevated, but it is not typically greater than 1500 IU/L.[3]
- EMG shows mild myopathic changes.
- Muscle biopsy is usually reserved for cases in which molecular testing is inconclusive. If a muscle biopsy is performed, results typically show nonspecific, chronic myopathic changes and dystrophy. Occasionally there can be inflammatory changes present significant enough to suggest an inflammatory myopathy.

The University of Rochester's National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy defines definite FSHD diagnosis as:[5]

- Weakness of facial muscles, and
- Either of the following
    - Scapular weakness, or
    - Foot dorsiflexor weakness, AND
- Absence of eye involvement (ptosis or extraocular muscle weakness), and
- Absence of an alternative diagnosis on muscle biopsy, and
- EMG results that do not demonstrate myotonia or neurogenic changes

Probable FSHD diagnosis is defined as either:[4]

- Weakness of facial muscles, or
- Either of the following
  - Scapular weakness, or
  - Foot dorsiflexor weakness, and
- Absence of eye involvement (ptosis or extraocular muscle weakness), and
- Absence of an alternative diagnosis on muscle biopsy, and
- EMG results that do not demonstrate myotonia or neurogenic change

OR

- Weakness of facial muscles, and
- Either of the following
  - Scapular weakness, or
  - Foot dorsiflexor weakness, and muscle biopsy and/or EMG results are not available

Treatment

There are no disease-modifying treatments currently available for FSHD. Management is symptom driven and primarily consists of support needed to address loss of strength. Hearing loss and rarer sequelae such as vision impairment or decreased lung function should be assessed and addressed as needed.

Standard of care and management guidelines for confirmed FSHD diagnosis include:[6]

- Evaluation by physical therapy to address functional limitations
- Help determining standard follow-up schedules to monitor for complications (such as pulmonary function testing and ophthalmologic screenings), and the need for assistive devices
- Assessments for hearing and vision loss and other orthopedic interventions
- Pain management to avoid compounding existing mechanical limitations

Survival

FSHD is not typically life shortening, but does lead to increased morbidity.

Test information

Introduction

Testing for FSHD may include known familial mutation analysis, targeted analysis with haplotyping, methylation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

FSHD1 Testing: Targeted Analysis and Haplotyping

Molecular testing for FSHD starts with assessment for the more common FSHD1. This testing consists of detecting contractions of the D4Z4 locus (reported as a number of D4Z4 repeats) and determination of the associated haplotype, using Southern blot analysis and optical genome mapping.[7]

- The normal range is defined as 12-100 repeat units.
- The FSHD-associated repeat range is defined as 1-10; however, to be pathogenic, the contraction needs to occur in the context of the permissive 4A haplotype.
- Borderline repeat lengths of 10 or 11 require clinical phenotype to interpret, as they may or may not be associated with FSHD in a given individual, even in the presence of the 4A haplotype. These are considered reduced penetrance alleles.

This analysis will detect causative variants in 95% of clinically affected individuals.[3]

FSHD2 Testing: Methylation Analysis and SMCHD1 Sequencing

Molecular testing for FSHD2 consists of determining the methylation status of the D4Z4 region.

- D4Z4 methylation (methylation-sensitive restriction enzyme and Southern blot): methylation levels below 25% are consistent with an FSHD2 diagnosis. Again, to be pathogenic, the contraction needs to occur in the context of the permissive 4A haplotype.
- If hypomethylation is identified, SMCHD1 next generation sequencing may be performed to determine the causative mutation.
- SMCHD1 deletion/duplication analysis will find gene rearrangements that are too large to be detected by sequencing. Large deletions in SMCHD1 are infrequently

reported; therefore, deletion/duplication analysis is done as second tier testing in FSHD2.

- DNMT3B gene sequencing may detect rare causative mutations.

This analysis will detect causative variants in less than 5% of clinically affected individuals.[3]

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to FSHD testing.

American Academy of Neurology

The American Academy of Neurology Evidenced-based Guideline for Clinicians (2015) considered the following to be Level B practice recommendations:[6]

- "Clinicians should obtain genetic confirmation of FSHD1 in patients with atypical presentations and no first-degree relatives with genetic confirmation of the disease."
- "Large D4Z4 deletion sizes (contracted D4Z4 allele of 10-20 kb) should alert the clinician that the patient is more likely to develop more significant disability and at an earlier age. Patients with large deletions are also more likely to develop symptomatic extramuscular manifestations."

European Neuromuscular Center

According to the 171st European Neuromuscular Center International Workshop: Standards of Care and Management of FSHD (2010): if a physician suspects FSHD clinically, genetic testing is the preferred diagnostic test.[8,9]

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FSHMD

Familial Hypercholesterolemia Genetic Testing

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Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
APOB Common Variants	81401
APOB Sequence Analysis	81407
FH Known Familial Mutation Analysis	81403
FH Multigene Panel	81479
LDLR Sequence Analysis	81406
LDLR Deletion/Duplication Analysis	81405
PCSK9 Sequence Analysis	81406

Criteria

Introduction

Requests for familial hypercholesterolemia (FH) genetic testing are reviewed using these criteria.

Known Familial Mutation Testing for Familial Hypercholesterolemia

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of LDLR, APOB, or PCSK9 that would detect the familial mutation, and
  - LDLR, APOB, or PCSK9 mutation identified in 1st, 2nd or 3rd degree biological relative, AND

- Diagnostic Testing:
  - LDL cholesterol of >120 mg/dL in the absence of treatment, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

LDLR Full Sequence and Deletion/Duplication Analysis

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous LDLR sequencing or deletion/duplication testing, and
  - No known LDLR, APOB, or PCSK9 mutation in the family, AND
- Diagnostic Testing:
  - Member meets either the Dutch criteria or the Simon Broome criteria for possible or probable FH, and
  - Genetic testing is necessary because there is uncertainty in the clinical diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

APOB Targeted Mutation Analysis or Full Sequence Analysis

- Criteria for LDLR sequencing and deletion/duplication analysis is met, AND
- No previous full sequence analysis of APOB, AND
- No mutations detected in full sequencing or deletion/duplication testing of LDLR or PCSK9 sequencing, if previously performed, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

PCSK9 Full Sequence Analysis

- Criteria for LDLR sequencing and deletion/duplication analysis is met, AND
- No previous genetic testing for PCSK9, AND
- No mutations detected in full sequencing or deletion/duplication analysis of LDLR or APOB sequencing, if previously performed, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

LDLR, APOB, PCSK9 Multigene Panels

FH multi-gene panels, limited to testing for LDLR, APOB, and PCSK9, will be reimbursed when the following criteria are met:

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous LDLR, APOB, or PCSK9 sequencing or deletion/duplication testing, and
  - No known LDLR, APOB, or PCSK9 mutation in the family, AND
- Diagnostic Testing:
  - Member meets the Dutch criteria or the Simon Broome criteria for possible or probable FH, and
  - Genetic testing is necessary because there is uncertainty in the clinical diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Exclusions

Genetic testing for the sole purpose of treatment decisions (i.e. PCSK9 inhibitors) in the absence of a clinical suspicion supported by either the Dutch or Simon Broome criteria is not medically necessary.

What is familial hypercholesterolemia?

Definition

Familial hypercholesterolemia (FH) is a genetic disorder characterized by very high levels of low-density lipoprotein (LDL) cholesterol.

Prevalence

About 1 in 200-250 individuals worldwide have heterozygous FH (they have 1 FH-causing mutation), but may be higher in certain ethnicities.[1]

Approximately one in 1 million individuals have homozygous FH (they have 2 FH-causing mutations). This is much more severe than heterozygous FH.[2] Individuals with this type of FH typically have severe coronary heart disease by their mid-20s; the rate of death or the need for surgical treatment of heart problems by the teenage years is high.[1]

Symptoms

FH is a genetic disorder characterized by very high levels of low-density lipoprotein (LDL) cholesterol: usually >190 mg/dL in untreated adults and >130 mg/dl in untreated children/adolescents.[1] This leads to an increased risk for coronary heart disease (CHD), including heart attacks, at an early age.[1,3,4]

- Men with untreated FH have a 50% risk for a coronary event by age 50.[1,2]
- Women with untreated FH have a 30% risk for a coronary event by age 60.[1,2]

Individuals with untreated FH have about a 20 fold increase for coronary heart disease.[1]

Cause

Most cases of FH are caused by mutations in one of three genes: LDLR, APOB, PCSK9.[1] However, mutations in these genes only account for approximately 60%-80% of FH.[1]

There are likely other genes that are not known at the present time that make up the remaining 20%-40% of cases of FH; therefore, a negative genetic test does not rule out a diagnosis of FH.[1]

Inheritance

FH is an autosomal dominant disorder.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Although not included in this guideline, it is important to note that there is an autosomal recessive form of hypercholesterolemia which is caused by mutations in the LDLRAP1 gene. There is also a milder autosomal dominant form, Familial Combined Hyperlipidemia, which is usually caused by mutations in the LPL gene.[1]

Diagnosis

A clinical diagnosis of FH is suspected based on some combination of personal and family history of very high cholesterol, premature CHD, and cholesterol deposits, such as tendon xanthomas and corneal arcus.[4] At least three organizations have attempted to define clinical diagnostic criteria for FH, but all criteria have recognized limitations.[5,6]

Genetic testing for FH can confirm a diagnosis of FH, particularly in borderline clinical cases.[7-9]

MEDPED criteria[6]

Table gives required cholesterol levels and family history for diagnosing FH.

Total Cholesterol (LDL), mg/dL

Patient's age	Patient has 1st degree relative with FH	Patient has 2nd degree relative with FH	Patient has 3rd degree relative with FH	General population
<20	220 (155)	230 (165)	240 (170)	270 (200)
20-29	240 (170)	250 (180)	260 (185)	290 (220)
30-39	270 (190)	280 (200)	290 (210)	340 (240)
40 or older	290 (205)	300 (215)	310 (225)	360 (260)

Dutch criteria[6]

Definitive FH: Greater than 8 points; Probable FH: 6-8 points; Possible FH: 3-5 points; Unlikely FH <3 points

Points	Description
1 point	First-degree relative with premature* cardiovascular disease or LDL >95th percentile, or personal history of premature peripheral or cerebrovascular disease or LDL 155-189 mg/dL**
2 points	First-degree relative with tendinous xanthoma and/or corneal arcus, or first-degree relative age <18 with LDL >95th percentile, or personal history of coronary artery disease
3 points	LDL 190-249 mg/dL**
4 points	Corneal arcus in patient age <45 years
5 points	LDL 250-329 mg/dL**
6 points	Tendon xanthoma
8 points	LDL ≥330 mg/dL**

Note * Premature: less than 55 years in men; less than 60 years in women

** Please note that these are LDL level cut offs for untreated individuals.

Simon Broome criteria[5]

Definitive FH

- Total cholesterol (LDL cholesterol): 290 (190) mg/dL or higher in adults or 260 (155) mg/dL or higher in pediatric patients and tendon xanthoma in patient or in first-or second-degree relative, or
- DNA mutation

Probable FH

- Total cholesterol (LDL): 290 (190) mg/dL in adults or 260 (155) mg/dL in pediatric patients, and
- Family history of myocardial infarction (MI) at age <50 in second-degree relative or at age <60 in first-degree relative or family history of total cholesterol >290 mg/dL in first- or second-degree relative

Management

Early and aggressive LDL-lowering with high doses of potent statins or statin combination therapy significantly lowers CHD morbidity and mortality for individuals with FH.[10,11] Statins are contraindicated during pregnancy due to concerns for teratogenicity and should be discontinued prior to conception.[1] Due to considerable overlap between the LDL levels of those with FH and common multifactorial hypercholesterolemia, FH often goes undiagnosed until middle age, when much of the preventive value of cholesterol-lowering therapy is lost.[12]

The US Food and Drug Administration (FDA) has approved several medications for FH homozygous and heterozygous mutation carriers.[13] However, there have been no guidelines recommending that genetic testing should be performed for the sole purpose of treatment decisions in the absence of a clinical suspicion of FH.

Less than 10% of individuals with FH are adequately treated.[7]

Once a mutation is found in an affected person, single-site testing should be offered to at-risk family members to allow for appropriately early intervention.[14-17]

Survival

Individuals with untreated FH have a much higher risk of dying from a coronary event than those in the general population.[2]

Adequate treatment with statins and other medications significantly decreases morbidity and mortality.[1] In one study, survival to age 39 in those treated since childhood was 100%, while in their affected parents, the survival rate was 93%.[18]

Test information

Introduction

Testing for FH may include known familial mutation analysis, targeted or full single gene sequence analysis, deletion/duplication analysis, and/or multigene panels.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Over 1000 LDLR mutations have been characterized so sequence analysis is required. Major gene deletions and rearrangements account for an estimated 9% of mutations and require specialized deletion testing to detect them.[19]

APOB mutations are primarily found in a limited region of the gene, with the R3500Q mutation being most common.[19] Laboratory testing may be done by targeted mutation analysis for a limited number of APOB mutations or sequencing of the gene region where these mutations are generally found.[1] Deletions and duplications of APOB are not commonly reported in individuals with FH.[1]

Mutations in PCSK9 are the least common genetic cause of FH with less than 5% of cases being attributed.[20] No deletions or duplications have been reported to cause FH.[1]

The proportion of FH attributed to each gene and recommended testing differs. See the Table: Molecular Genetic Testing for FH.

Molecular Genetic Testing for FH

Gene	Proportion of FH Attributed to Mutations in Gene[1]	Test Method[1]
LDLR	60%-80%	Sequence Analysis Deletion/Duplication
APOB	1%-5%	Targeted Analysis Sequencing Analysis Deletion/Duplication
PCSK9	0%-3%	Targeted Analysis Sequencing Analysis
Unknown	20%-40%	NA

Guidelines and evidence

Canadian Cardiovascular Society

The Canadian Cardiovascular Society (CCS, 2018) published an updated position statement that stated the following:[21]

- "We recommend that genetic testing be offered, when available, to complement a diagnosis of FH and enable cascade screening (Strong Recommendation, High-Quality Evidence)."
- "The decision to request genetic screening should be made by the treating physician after discussion with the patient."
- "We suggest that if available, genetic testing should be used to stratify the ASCVD risk in patients with FH (Weak Recommendation, Moderate-Quality Evidence)."
- "We recommend that patients with HoFH be referred to a specialized lipid clinic and undergo complete evaluation for genetic analysis, presence of ASCVD, and

aggressive lipid-lowering therapies, including consideration for extracorporeal LDL-C removal, lomitapide, and PCSK9 inhibitors (Strong Recommendation, Moderate-Quality Evidence)."

Cardiac Society of Australia and New Zealand

Consensus-based guidelines from The Cardiac Society of Australia and New Zealand (CSANZ, 2016) stated:[8]

- "Although the clinical picture of FH will be clear-cut in many instances, the diagnostic criteria suggest that genetic testing can provide certainty of diagnosis in some cases where confounding factors such as borderline cholesterol levels, inconclusive family histories or tendon injuries have resulted in a diagnostic dilemma."

European Atherosclerosis Society

The European Atherosclerosis Society Consensus Panel (2015) stated the following: [22]

- "Given the proven atherogenicity of LDL-C in experimental models and in humans with FH, with evidence that exposure to even moderate hypercholesterolaemia increases the long-term risk of a new CHD event, and given the lifelong benefit of genetically determined low LDL-C concentrations, there is an urgent need to identify and treat FH early to maximize therapeutic benefit…. Detection of a pathogenic mutation, usually in the LDLR gene, is the gold standard for diagnosis of FH."

National Institute for Health and Care Excellence

Evidence-based guidelines by the National Institute for Health and Care Excellence (NICE, 2019) supported genetic testing for FH as follows:[15]

- "Use the Simon Broome or Dutch Lipid Clinic Network (DLCL) criteria to make a clinical diagnosis of FH in primary care settings. This should be done by a healthcare professional competent in using the criteria."
- "Refer the person to an FH specialist service for DNA testing if they meet the Simon Broome criteria for possible or definite FH, or they have a DLCN score greater than 5."
- "Healthcare professionals should offer all people with FH a referral to a specialist with expertise in FH for confirmation of diagnosis and initiation of cascade testing."
- "Inform all people who have an identified mutation diagnostic of FH that they have an unequivocal diagnosis of FH even if their LDL-C concentration does not meet the diagnostic criteria ..."
- "In a family where a DNA mutation is identified, not all family members may have inherited the mutation. When DNA testing has excluded FH in a member of a family,

healthcare professionals should manage the person's coronary heart disease risk as in the general population."

- "In children aged 0–10 years at risk of FH because of 1 affected parent, offer a DNA test at the earliest opportunity. If testing of a child at risk has not been undertaken by the age of 10 years, offer an additional opportunity for a DNA test."

National Lipid Association

The National Lipid Association expert panel on Familial Hypercholesterolemia (NLA, 2011) made the following recommendations regarding genetic testing:[20]

- "Genetic screening for FH is generally not needed for diagnosis or clinical management but may be useful when the diagnosis is uncertain."
- "Identification of a causal mutation may provide additional motivation for some patients to implement appropriate treatment."
- "Importantly, a negative genetic test does not exclude FH, since approximately 20% of clinically definite FH patients will not be found to have a mutation despite an exhaustive search using current methods."

In a statement on genetic testing in dyslipidemia (2020), the NLA stated:[23]

- "Patients with severe primary hypercholesterolemia, and suspected to have FH, are at high risk of ASCVD; the precise genotype is not predictive in an individual patient."
- "Intensity of treatment should be guided by LDL-C elevation rather than the underlying genotype."
- "Prospective studies are needed to determine whether genetic testing for FH in addition to routine lipid profile testing will alter cardiovascular outcomes by identifying the appropriate LDL-C–lowering therapy based on a patient's gene mutations."

Selected Relevant Publication

A Journal of the American College of Cardiology Scientific Expert Panel (2018) statement on clinical genetic testing for FH stated:[16]

- "Because FH is common yet underdiagnosed, it is expected that genetic testing will facilitate the diagnosis of FH, the initiation and intensity of recommended lipid-lowering therapy (LLT), and the identification of affected relatives, thus reducing the burden of cardiovascular disease in families with FH."

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Familial Malignant Melanoma Genetic Testing

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Introduction

Familial malignant melanoma (FMM) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
CDKN2A Deletion/Duplication Analysis	81479
CDKN2A Known Familial Mutation Analysis	81403
CDKN2A Sequencing	81404
CDK4 Known Familial Mutation Analysis	81403
CDK4 Sequencing	81479

Criteria

Introduction

Requests for familial malignant melanoma (FMM) genetic testing are reviewed using these criteria.

Single Gene Sequencing and Deletion/Duplication Analysis

Due to the low diagnostic yield of single gene sequencing and deletion/duplication analysis, testing of a single gene is not considered medically necessary.

Other Considerations

FMM testing may be performed as part of a multigene, multisyndrome panel. For information on multigene, multisyndrome panel testing, please refer to the guideline *Hereditary Cancer Syndrome Multigene Panels*, as this testing is not addressed here.

What is familial malignant melanoma?

Definition

Familial malignant melanoma (FMM) is a strongly inherited form of melanoma.

Prevalence

The lifetime risk for a cutaneous melanoma for someone born in the U.S is 1 in 34 women and 1 in 53 men. [1] The incidence continues to rise dramatically.[1] Most melanoma is sporadic. It is usually the result of a combination of genetic susceptibility (probably from several relatively low risk gene variants such as those involved with pigment) and environmental risk factors such as sun exposure.[1-4]

About 4-8% of people with melanoma have a family history of at least one first-degree relative (parent, child, sibling) with melanoma.[3,5] Less than 1% to 2% have multiple affected relatives, which suggests a stronger genetic susceptibility.[2,5]

Symptoms

People who inherit an FMM mutation do not always develop melanoma. Data for CDKN2A mutations suggest that in the United States the melanoma risk is 50% by age 50 and 76% by age 80.[4] The likelihood may vary with geographic location and sun exposure.[5]

Carriers of the CDKN2A p16-Leiden mutation have been found to have between 17% to 25% risk for pancreatic cancer. Estimates from studies using population-based identification of subjects have shown a 7.4 relative-risk (95% CI 2.3 to 18.7) for pancreatic cancers in families with other CDKN2A (p16) mutations.[6]

Cause

Several genes have been linked to a higher risk of melanoma in families. CDNK2A gene mutations account for most of the currently identifiable FMM mutations, followed by CDK4 mutations.[7]

Inheritance

FMM is an autosomal dominant disorder.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Diagnosis

FMM is most likely in a family when there are three or more close relatives diagnosed with melanoma.[2] Other factors that may also suggest FMM include:[2,4,5]

- Melanoma diagnosed younger than usual (average diagnosis age 30s versus 50s in people without FMM)
- More than one melanoma primary in the same individual
- Melanoma and pancreatic cancer in the same family
- Multiple, atypical moles, called dysplastic nevi that are often larger than 5mm in diameter with irregular borders. Melanoma with multiple nevi has also been called familial atypical mole-malignant melanoma syndrome. However, the presence or absence of such moles is no longer viewed as a reliable predictor of FMM in a family.

CDKN2A next generation sequencing identifies the majority of FMM-causing mutations and, in the absence of a known familial mutation, is usually the first step in testing. The likelihood that genetic testing will identify an FMM mutation varies with the personal and family history. The chance of finding a CDKN2A mutation is:

- 20-40% of people with melanoma from a family with at least 3 affected first-degree relatives.[2,7]
- Less than 5% of those with only 2 affected first-degree relatives[2]
- 15% in someone with multiple melanoma primaries and no known family history[2]
- 25-40% in people diagnosed with familial atypical mole-malignant melanoma syndrome - a subset of FMM characterized by >50 atypical nevi with characteristic microscopy features[8]
- 74% of families with FMM and pancreatic cancer[7]

CDK4 next generation sequencing, sometimes of only exon 2, is also available, but mutations are uncommon, accounting for only 2-3% of FMM cases.[7]

Management

For all individuals with a pathogenic mutation in CDKN2A, "consider pancreatic cancer screening beginning at age 40 years (or 10 years younger than the earliest exocrine pancreatic cancer diagnosis in the family, whichever is earlier)".[9] NCCN does not comment on pancreatic cancer screening for individuals with CDK4 mutations.

For individuals with a mutation in a hereditary melanoma gene such as CDKN2A or CDK4, "[t]hese individuals should be instructed on photoprotection and monthly self-skin screening examinations and should receive a regular skin screening examination by a medical professional. The frequency of examination by a health care provider should be tailored to account for the melanoma status and the difficulty of the examination, with higher-risk individuals receiving more frequent examinations ranging

from every 3 to 12 months. If the individual has a personal history of melanoma, examinations should be in accordance with NCCN guidelines."[10]

Survival

The increased risk for malignant tumors is the largest factor impacting survival.

Special Considerations

Familial melanoma is also associated with other inherited cancer syndromes such as Li Fraumeni syndrome, hereditary breast and ovarian cancer syndrome, PTEN hamartoma tumor syndrome, inherited retinoblastoma, BAP1 tumor predisposition syndrome, and xeroderma pigmentosum.[2,11,12]

Test information

Introduction

FMM testing may include known familial mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to FMM testing.

American Cancer Society

The American Cancer Society (ACS, 2019) stated:[13]

- "Some families with high rates of melanoma have mutations in genes such as CDKN2A (also known as p16). Tests for some of these gene changes are now available, although doctors aren't sure how useful they are at this time. In part, this is because people with any of the factors above are already known to have a higher risk of melanoma regardless of whether they carry a mutated gene, so it's not always clear how helpful the genetic testing results would be."

Melanoma Genetics Consortium

The Melanoma Genetics Consortium (GenoMEL,1999), an international research collaborative group, published a consensus statement which stated:[2]

- "DNA testing for mutations in known melanoma susceptibility genes should only rarely be performed outside of defined research programs. With this general proviso, two distinct clinical situations need further consideration: families in which a CDKN2A mutation has been identified in a proband as part of a research study and families for which no prior testing of affected individuals has been conducted."
- "Individuals who choose to undergo genetic testing [in a research setting] should have a second independent diagnostic (as distinct from research) DNA test performed in an accredited genetic testing laboratory."
- For at-risk relatives with a known familial mutation, test sensitivity is virtually 100%. However, the likelihood of developing melanoma in mutation-positive individuals is largely unknown and there is "lack of proved efficacy of prevention and surveillance strategies based on DNA testing, even for mutation carriers." They do acknowledge potential benefits could include enhanced motivation to adhere to prevention and screening guidelines, earlier melanoma diagnosis if the biopsy threshold is lower, and lower anxiety for those who learn they are negative for a known family mutation.

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) evidence and consensus based guidelines stated:[1]

- "Consider genetic counseling referral for p16/CDKN2A mutation testing in the presence of 3 or more invasive cutaneous melanomas, or a mix of invasive melanoma, pancreatic cancer, and/or astrocytoma diagnosis in an individual or family."

- "Multigene panel testing that includes CDKN2A is recommended for patients with invasive cutaneous melanoma who have a first degree relative diagnosed with pancreatic cancer."
- "Testing for other genes that can harbor melanoma-predisposing mutations may be warranted."

Special Considerations

- FMM genetic testing outside of the research setting is not currently recommended for several reasons, including:
  - Currently available testing does not detect a mutation in a significant number of people who appear to have FMM. Therefore, a negative result cannot rule out FMM and should not change the prevention and screening plan for at-risk people.[2]
  - Individuals with FMM mutations need essentially the same prevention and screening as anyone at high risk for melanoma (family history, pigmentation, multiple moles, history of blistering sunburn).[2] Therefore, identifying an FMM-causing mutation is also not expected to change screening or treatment for melanoma.[5,14,15]
  - When a family FMM mutation has been found, other relatives who test negative for that mutation at best only return to the background risk for melanoma (which may be as high as 1 in 25) and still need regular skin screening.[2]
  - A significant percentage of people with recognized FMM mutations do not develop melanoma, which is especially true when sun exposure is limited by geography or prevention.[4]

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Friedreich Ataxia Genetic Testing

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Introduction

Friedreich ataxia genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
FXN gene analysis; evaluation to detect abnormal (expanded) alleles	81284
FXN gene analysis; characterization of alleles (eg, expanded size)	81285
FXN gene analysis; full gene sequence	81286
FXN gene analysis; known familial variant(s)	81289
FXN gene analysis, deletion/duplication	81479
Genomic Unity FXN Analysis	0233U

Criteria

Introduction

Requests for Friedreich ataxia (FRDA) testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No previous FXN gene analysis performed that would have identified the known familial mutation, AND
- Known disease-causing mutation in FXN gene identified in 1st degree relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

GAA Trinucleotide Repeat Analysis

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous GAA repeat analysis of FXN performed, and
  - Member does not have a known mutation in both copies of the FXN gene, AND
- Individual has been diagnosed with cerebellar ataxia, regardless of age of onset, AND
- Family history is consistent with autosomal recessive inheritance (including simplex cases), AND
- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Sequence Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member does not have a known mutation in both copies of the FXN gene, and
  - No previous sequencing analysis of the FXN gene, and
  - Previous GAA trinucleotide repeat analysis was performed and revealed a GAA expansion on only one allele, and
  - Meets criteria for GAA trinucleotide repeat analysis, and
  - Testing is needed to confirm the diagnosis of Friedreich Ataxia, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Deletion/duplication Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Genetic Testing:
  - Member does not have a known mutation in both copies of the FXN gene, and
  - Previous GAA trinucleotide repeat analysis was performed and revealed a GAA expansion on only one allele, and
  - Previous GAA sequencing was performed and did not identify a mutation on either FXN allele, and
  - Meets criteria for GAA trinucleotide repeat analysis, and
  - Testing is needed to help confirm the diagnosis of Friedreich Ataxia, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Exclusions and Other Considerations

For information on multigene panels, please refer to the guideline *Hereditary Ataxia Multigene Panel Genetic Testing,* Friedreich ataxia as this testing is not addressed here.

What is Friedreich Ataxia?

Definition

Friedreich ataxia (FRDA) is an inherited neuromuscular condition.

Prevalence

FRDA is the most common inherited ataxia in European, Middle Eastern, south Asian (Indian subcontinent), and North African populations.[1] The prevalence is 2:100,000-4:100,000.[1] The carrier frequency is 1:60-1:100.[1]

Symptoms

FRDA is characterized by progressive ataxia (lack of coordination of muscle movements) of the limbs and gait, dysarthria (difficulty articulating speech), absent lower limb reflexes, sensory loss, and muscle weakness.[1-3] About two-thirds of individuals with FRDA also have cardiomyopathy (weakening of the heart muscle).[1] Approximately 30% of individuals with FRDA have diabetes mellitus.[1] Other features include pes cavus, sensorineural hearing loss, and optic atrophy.[3]

Symptoms typically present before 25 years of age, with the mean age of symptom onset between 10 and 15 years.[1,2] However, about 25% of affected individuals have an atypical form with later onset and/or retained reflexes.[1] Shorter GAA repeat expansions tend to be associated with later onset of symptoms.[1,3]

Cause

FRDA is caused by mutations in the FXN gene. Most mutations in the FXN gene cause a section of DNA, called a GAA triplet repeat, to expand.[1] The GAA expansion results in reduced levels of the protein, frataxin.[3] A minority (less than 5%) of affected people have a different type of mutation in the FXN gene.

Inheritance

FRDA is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

The diagnosis of FRDA is confirmed when disease-causing mutations are found in both copies of the FXN gene (biallelic mutations).[1] Approximately 96% of individuals with FRDA have disease-causing GAA triplet repeat expansions in both FXN genes.[1] About 4% have a single disease-causing GAA triplet repeat expansion and a second FXN gene mutation not in the GAA repeat region.[1] In this case, different genetic testing, such a next generation sequencing, is required to identify the second mutation.

The main result categories are based on the number of GAA triplet repeats:[1]

- 5 to 33 GAA repeats: normal range
- 34 to 65 repeats: described as normal, but possibly unstable with regard to reproductive risk; have rarely been reported in individuals presenting with atypical FRDA
- 44 to 66 repeats: borderline; the "shortest repeat length associated with disease has not been clearly determined."[1]
- 66 or more repeats: disease-causing; usually people with typical FRDA have 600 to 1200 repeats.[1] "The age of onset, presence of leg muscle weakness/wasting, duration until wheelchair use, and prevalence of cardiomyopathy, pes cavus, and scoliosis have all shown statistically significant inverse correlations with the size of the expanded GAA repeat." [1]

Single or multi-exon deletions or duplication of FXN are rare but have been reported.[1]

Very few people who have been clinically diagnosed with FRDA have no GAA expansion in the FXN gene, though some are reported with only one mutation

identified.[1] These people may have mutations in another gene, although another disease causing gene has not yet been identified.[1,4]

Management

Management of FRDA is largely supportive, and includes the use of walking aids and wheelchairs for ambulation, physical therapy, speech therapy, occupational therapy, and other assistive devices.[1]

Survival

The survival range for FRDA varies. The mean age of death is 36.5 years, with a median age of 30 years.[1] Some individuals have been documented to live into their 60s and 70s. Cardiac issues, particularly progressive heart failure, arrhythmias, and cardioembolic stroke attributable to atrial fibrillation, are the most common cause of death among individuals with FRDA.[3] Potential therapeutic targets focused on two general principles, increasing frataxin expression and reducing oxidative stress, are currently under investigation.[3]

Test Information

Introduction

Testing for FRDA may include known familial mutation analysis and trinucleotide repeat testing. If needed for affected individuals with only one expended repeat identified, next generation sequencing and/or deletion/duplication analysis can be subsequently performed.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Analysis for known familial mutations is typically performed by trinucleotide repeat expansion analysis. Some mutations may require sequencing or deletion/duplication analysis.

Trinucleotide Repeat Testing

Repeat expansion genetic testing allows for the determination of the size of a repeated DNA sequence. This testing may involve more than one test methodology. Smaller repeat expansions are typically identified using certain types of polymerase chain reaction (PCR), while larger expansions may require Southern blot. More comprehensive repeat expansion testing that utilizes next generation sequencing and exome sequencing methods is under development.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and Evidence

Introduction

This section includes relevant guidelines and evidence pertaining to genetic testing for FRDA.

American College of Medical Genetics and Genomics

An overview published by the American College of Medical Genetics (ACMG, 2013) stated the following regarding testing for hereditary ataxias:[5]

- “Establishing the diagnosis of hereditary ataxia requires:
    - Detection on neurological examination of typical clinical signs including poorly coordinated gait and finger/hand movements, dysarthria (incoordination of speech), and eye movement abnormalities such as nystagmus, abnormal saccade movements, and ophthalmoplegia.
    - Exclusion of nongenetic causes of ataxia (see Differential Diagnosis below).

  - Documentation of the hereditary nature of the disease by finding a positive family history of ataxia, identifying an ataxia-causing mutation, or recognizing a clinical phenotype characteristic of a genetic form of ataxia."

- "Differential diagnosis of hereditary ataxia includes acquired, nongenetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, and paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung, and the idiopathic degenerative disease multiple system atrophy (spinal muscular atrophy). The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available."
- "Testing strategy when the family history suggests autosomal recessive inheritance
  - A family history in which only sibs are affected and/or when the parents are consanguineous suggests autosomal recessive inheritance. Because of their frequency and/or treatment potential, FRDA, A-T, AOA1, AOA2, AVED, and metabolic or lipid storage disorders such as Refsum disease and mitochondrial diseases should be considered."
- "Testing simplex cases. A simplex case is a single occurrence of a disorder in a family, sometimes incorrectly referred to as a "sporadic" case.
  - If no acquired cause of the ataxia is identified, the probability is ~13% that the affected individual has SCA1, SCA2, SCA3, SCA6, SCA8, SCA17, or FRDA, and mutations in rare ataxia genes are even less common.
  - Other possibilities to consider are a de novo mutation in a different autosomal dominant ataxia, decreased penetrance, alternative paternity, or a single occurrence of an autosomal recessive or X-linked disorder in a family such as fragile X-associated tremor/ataxia syndrome.
  - Although the probability of a positive result from molecular genetic testing is low in an individual with ataxia who has no family history of ataxia, such testing is usually justified to establish a specific diagnosis for the individual's medical evaluation and for genetic counseling.
  - Always consider a possible nongenetic cause such as multiple system atrophy, cerebellar type in simplex cases."

European Federation of Neurological Sciences

- The European Federation of Neurological Sciences (EFNS, 2014) stated the following regarding testing for ataxia:[4]
  - For symptomatic individuals with a family history consistent with autosomal recessive cerebellar ataxia, the first step in the suggested diagnostic approach included analysis for Friedreich ataxia.

        - "Step 1: mutation analysis of the FRDA gene for Friedreich's ataxia (although one can refrain from this in the case of severe cerebellar atrophy), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, creatine kinase (CK) and a-fetoprotein. Also consider doing nerve conduction studies/EMG (presence versus absence of peripheral neuropathy, axonal versus demyelinating) and referral to an ophthalmologist (retinitis pigmentosa, cataract, cherry red spot etc.) (Table S2) (good practice point)."
    - "In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA6, and DRPLA (in Asian patients) (level B), the step 1 panel of the recessive ataxia workup, i.e mutation analysis of the FRDA gene (level B), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, CK, and α-fetoprotein."
- For the diagnosis of Friedreich ataxia, guidelines from the European Federation of Neurological Societies (EFNS, 2010) created by consensus of expert members following literature review recommended: "In cases presenting with early onset ataxia, peripheral sensory neuropathy, and absence of marked cerebellar atrophy at MRI, genetic test for FRDA mutation is recommended (Class B)."[2]

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Gaucher Disease Genetic Testing

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Introduction

Gaucher disease genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
GBA Gene Analysis, Common Mutations	81251
GBA Known Familial Mutation Analysis	81403
GBA Sequencing	81479

Criteria

Introduction

Requests for Gaucher disease testing are reviewed using these criteria.

Carrier Testing

GBA Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Carrier Screening:
  - GBA mutation(s) identified in 1st, 2nd, or 3rd degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - GBA mutation(s) identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

GBA Common Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous GBA genetic testing, including Ashkenazi Jewish screening panels containing targeted mutation analysis for Gaucher disease, AND
- Carrier Screening:
  - Ashkenazi Jewish descent, regardless of disease status and results of glucocerebrosidase assay, and
  - Intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Diagnostic and Expanded Carrier Testing

GBA Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous GBA full sequencing analysis, and
  - If performed, testing for 4 common mutations is negative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Glucocerebrosidase enzyme activity in peripheral blood leukocytes is 0-15% of normal activity, and
  - Characteristic bone changes including osteopenia, focal lytic or sclerotic bone lesions or osteonecrosis, or
  - Hepatosplenomegaly and hematologic changes including anemia or thrombocytopenia, or
  - Primary neurologic disease which could include one or more of the following: cognitive impairment, bulbar signs, pyramidal signs, oculomotor apraxia, or seizures (progressive myoclonic epilepsy), OR
- Diagnostic Testing for Asymptomatic Carriers:
  - One mutation detected by targeted mutation analysis, and

  - Glucocerebrosidase enzyme activity in peripheral blood leukocytes is 0-15% of normal activity, OR

- Testing for Individuals with Family History or Partners of Carriers:
  - 1st, 2nd, or 3rd degree biologic relative with clinical diagnosis of Gaucher disease, familial mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for GBA mutation, and has the potential and intention to reproduce with this partner, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Gaucher Disease?

Definition

Gaucher disease is a genetic disorder of lipid metabolism that affects multiple organs and tissues.[1]

Incidence

Gaucher disease is relatively common in Ashkenazi Jewish populations, with Gaucher disease type 1 affecting about 1 in 855 people.[2] Gaucher disease is much less common in the general population, affecting about 1 in 57,000 to 1 in 100,000 people.[2] Other populations with an enrichment for this disease include Spanish, Portuguese, Swedish, Jenin Arab, Greek, and Albanian. "The prevalence of neuropathic GD [Gaucher disease] varies across ethnic groups but appears to be higher among those who are not of European origin."[2]

Symptoms

There are several types of Gaucher disease, each with varying signs and symptoms:[1,2]

- Type 1: This is the most common type of Gaucher Disease. Unlike other types, type 1 does not affect the central nervous system (CNS). Symptoms include enlargement of the liver and spleen (hepatosplenomegaly), cytopenias, coagulation abnormalities, lung disease, immunologic abnormalities, and bone abnormalities.
- Type 2/Type 3: These types are more rare, usually more severe, and affect the brain and CNS. Common symptoms include seizures, hyperextension of the spine, and lockjaw, in addition to the symptoms listed above for type 1.
- Perinatal lethal: The most severe form of Gaucher disease has symptoms that begin during pregnancy or in early infancy. Prenatal symptoms include non-immune hydrops fetalis. Early infantile symptoms include swelling, dry/scaly skin (ichthyosis), and serious neurological problems.
- Cardiovascular: This type has heart manifestations. Symptoms include the hardening of heart valves as well as eye abnormalities, bone disease, and enlarged

spleen. This form has only been reported in individuals who are homozygous for a specific variant (c.1342G>C, p.Asp448His).

- Some general genotype/phenotype correlations have been observed. However, "[s]ignificant overlap in clinical manifestations found between individuals with various genotypes precludes specific counseling about prognosis in individual cases. … Discordance in phenotype has been reported even among monozygotic twins."[2] Subtypes of Gaucher disease are identified through clinical symptoms and, with the exception of the cardiovascular type, do not correlate well with the various mutations that cause Gaucher disease.[2]

Cause

Gaucher disease is caused by mutations in the GBA gene.[1-3] The GBA gene produces the enzyme beta-glucosylceramidase, also called acid beta-glucocerebrosidase. This enzyme helps break down fatty substances in cells. Mutations in GBA lead to a buildup of these fatty substances to toxic levels. This buildup damages tissues and organs, leading to symptoms of Gaucher disease.[1-3]

Inheritance

Gaucher disease is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

A diagnosis of Gaucher disease requires 0-15% normal glucocerebrosidase enzyme activity, or detection of biallelic pathogenic variants in the GBA gene.[2] Clinical findings alone are insufficient for a definitive diagnosis of Gaucher disease.[2]

If Gaucher disease is suspected in a symptomatic person, glucocerebrosidase enzyme testing should be performed first. People affected with Gaucher disease have 0-15% the normal level of glucocerebrosidase compared to healthy individuals. Measuring glucocerebrosidase levels is a reliable way to confirm a suspected case of Gaucher disease.[2,4] Individuals with type 1 Gaucher disease typically will have 10-15% enzyme level function while individuals with Type 2 or Type 3 will have much lower levels. However, the types cannot be reliably distinguished from one another.[2] Enzyme levels within the normal range rule out Gaucher disease.[2] Enzyme testing is not appropriate to identify unaffected carriers.[2]

Genetic testing can be used to identify the disease-causing mutations in an affected person diagnosed by enzyme analysis.[1,2] Identifying the causative GBA mutations can confirm a diagnosis and impact recurrence risks and family planning. Some mutations can give prognostic information, such as whether or not CNS involvement is expected. High variability exists among phenotypes, even within families.[2]

- Clinically-available testing panels assess four or more of most common mutations in the GBA gene.
    - Four mutations (N370S, L444P, 84GG, IVS2+1) account for approximately 90% of mutations in the Ashkenazi Jewish population and approximately 50%-60% of mutations in the non-Ashkenazi Jewish population.[1,2]
    - Some laboratories include other mutations in their panels.
    - GBA common mutation analysis is widely available as part of carrier screening panels. These panels are often ethnicity based, but can also be pan-ethnic screens, including a variety of conditions affecting multiple ethnic groups. GBA common mutation testing is offered as part of an “Ashkenazi Jewish Panel” that includes several other genetic diseases that are more common in this population.[2,5-7]
        - For information on Ashkenazi Jewish carrier screening, please refer to the guideline *Ashkenazi Jewish Carrier Screening,* as this testing is not addressed here.
- Next generation sequencing of the entire coding region of the GBA gene will detect mutations that the GBA mutation panel would not.[1,2]
    - The detection rate of sequencing is approximately 99%.[2]
    - This test is indicated in people with Gaucher disease who have one or no mutations identified by mutation panel testing.
    - This test is also indicated for reproductive partners of individuals who have 1 or more GBA mutations.

Management

There is no cure for Gaucher disease. The main therapeutic options are enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). "Individuals with type 1 GD report improved health-related quality of life after 24-48 months of ERT. … Individuals with type 2 GD and pyramidal tract signs are not likely to respond to ERT or SRT, perhaps because the underlying neuropathology is cell death rather than lysosomal storage of GL1."[2] ERT can be used for individuals with Type 3 disease, but will not improve any symptoms involving the CNS as treatment does not cross the blood-brain barrier.[2] "SRT used in combination with ERT for type 3 GD with progressive neurologic disease does not appear to alter ultimate prognosis."[2]

Survival

Many individuals with Type 1 disease can expect a normal lifespan. Type 2 is more severe than type 3, and affected individuals usually do not survive past childhood. Individuals with Type 3 have more slowly progressing symptoms and can survive into adulthood. Infants affected with the perinatal lethal type usually survive only a few days after birth.[1,2]

Test information

Introduction

Testing for Gaucher disease may include known familial mutation analysis, targeted mutation analysis, or next generation sequencing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Gaucher disease testing. Professional guidelines generally supported Gaucher disease carrier screening for those at increased risk.[5-7]

American College of Medical Genetics and Genomics

Consensus guidelines from the American College of Medical Genetics and Genomics (ACMG, 2008) recommended routine carrier screening for a group of disorders that includes Gaucher disease when at least one member of the couple is Ashkenazi Jewish and that couple is pregnant or planning pregnancy.[6]

ACMG (2021) released an educational practice resource on carrier screening.[7] This consensus statement asserted that general population carrier screening should be ethnicity and family history agnostic. To accomplish this, screening all individuals in the prenatal/preconception period for autosomal recessive and X-linked conditions with a carrier frequency of >1/200 was suggested. ACMG generated a list of 113 genes, which included the GBA gene, meeting the criteria.

American College of Obstetricians and Gynecologists

Consensus guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2020) stated:[5]

- "Some experts have advocated for a more comprehensive screening panel for those of Ashkenazi descent, including tests for several diseases that are less common [than the four conditions mentioned above] (carrier rates 1 in 15 to 1 in 168)." A list of autosomal recessive conditions for which screening could be considered, inclusive of Gaucher disease, was provided in this guideline.

International Working Group of Gaucher Disease

The Diagnostic Working Group of the International Working Group of Gaucher Disease (IWGGD, 2022) published guidelines for implementation and interpretation of testing for the diagnosis of Gaucher Disease type 1.[8] The guideline addressed biochemical and genetic testing. In this guideline, the GBA gene is denoted as GBA1 and BGLU refers to enzyme activity assayed by the use of an artificial substrate. They stated the following specific to genetic testing of GBA:

- The following had a level of evidence of II and IV ("retrospective cohort studies or case series with consistent results") and "Grade B (Recommendation)":
    - "Molecular analysis of the GBA1 gene should always be performed when biomarker results or phenotype are at odds with the enzymology and is highly recommended in subjects with BGLU activity below normal reference intervals in cells to further support/confirm the diagnosis of GD and provide genetic counseling. ..."

- "Genetic testing could be done as a primary test (before testing enzymatic activity). However, results should be interpreted with caution since GBA1 testing is challenging … Therefore, confirmation of diagnosis through the assessment of enzymatic activity in patient's cells is mandatory."
- "Genetic testing is the most reliable method to detect heterozygous carriers and it should be made available to family members at risk of being a carrier."
- "In all cases, molecular testing should be accompanied by a pre and post-test genetic counseling delivered by a counsellor experienced in GD to ensure informed choices."
- "Sequencing analysis of GBA1 exons and intron exon boundaries should be performed as the primary molecular test. It should be performed using specific long template amplification of the GBA gene (avoiding the amplification of the pseudogene) followed by Sanger sequencing or NGS specifically designed to avoid reads misalignments."
- "GBA1 could be included in gene panels analyzed by NGS. This technology allows the detection of point mutations, although false positive results have been reported. Therefore, point mutations detected by NGS methods should always be confirmed by Sanger sequencing. Standard workflows are not suitable for the detection of large deletions or recombinant alleles."
- "Segregation of alleles by identifying variants in parents, should be determined."
- "The presence of homozygous pathogenetic variants not confirmed in parents, as well as the absence of pathogenetic variants (in one or both allele) after sequencing should always be questioned and additional investigations should be performed. ..."
- "Variants should be classified following the ACMG criteria and in case of identification of VUS, pathogenicity should be investigated by functional analysis."

- The following had a level of evidence of V ("case reports") and "Grade D (Option)":
  - "In the absence of pathogenetic variants in the GBA1 gene in subjects with a clinical phenotype compatible with GD, increased chitotriosidase activity, increased levels of GlcSph [glucosylsphingosine] and normal or low BGLU activity in cells, a Sap C [Saposin C] deficiency should be suspected and the PSAP gene analyzed."

Selected Relevant Publications

A 2023 expert-authored review recommended the following testing strategy for diagnosis of an affected person.[2] These recommendations were supported by the ACMG Work Group on Diagnostic Confirmation of Lysosomal Storage Diseases.[9]

- "The diagnosis of GD relies on demonstration of deficient glucocerebrosidase (glucosylceramidase) enzyme activity in peripheral blood leukocytes or other nucleated cells or by the identification of biallelic pathogenic variants in GBA."[2]
- "Sequence analysis of GBA is performed first and followed by gene-targeted deletion/duplication analysis if only one or not pathogenic variant is found. Targeted analysis for pathogenic variants can be performed first, particularly in individuals of Ashkenazi Jewish ancestry. ... Molecular genetic testing can be used to identify carriers among at-risk family members once the pathogenic variants have been identified in the family."[2]

References

Introduction

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Epilepsy Genetic Testing

MOL.TS.257.A
v2.0.2024

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures covered by this guideline	Procedure codes
CACNA1A Full Gene Sequence	81185
CSTB Full Gene Sequence	81189
CSTB Gene Analysis; evaluation to detect abnormal alleles	81188
Epilepsy Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Epilepsy Gene Known Familial Mutation Analysis	81403
Epilepsy Gene Panel (must include analyses for ALDH7A1, CACNA1A, CDKL5, CHD2, GABRG2, GRIN2A, KCNQ2, MECP2, PCDH19, POLG, PRRT2, SCN1A, SCN1B, SCN2A, SCN8A, SLC2A1, SLC9A6, STXBP1, SYNGAP1, TCF4, TPP1, TSC1, TSC2, and ZEB2)	81419
Genomic Unity CACNA1A Analysis	0231U
Genomic Unity CSTB Analysis	0232U

400 Buckwalter Place Boulevard, Bluffton, SC 29910 (800) 918-8924

Criteria

This guideline applies to all epilepsy testing, including single gene analysis and multi-gene panels, which are defined as assays that simultaneously test for more than one epilepsy gene. Coverage criteria differ based on the type of testing being performed (i.e., individual epilepsy genes separately chosen versus pre-defined panels of epilepsy genes).

Epilepsy single gene tests

Epilepsy single gene tests are considered medically necessary when the following criteria are met:

- The member has a condition that will benefit from information provided by the requested epilepsy gene testing based on at least one of the following criteria:
    - The member displays clinical features of the condition for which testing is being requested and a particular treatment is being considered for the member that requires a genetic diagnosis, OR
    - A particular antiepileptic drug (AED) is being considered for the member and the AED is contraindicated for individuals with mutations in the requested gene, defined by ONE of the following criteria:
        - A neurology therapy FDA label requires results from the genetic test to effectively or safely use or avoidance of the therapy for the member's epilepsy type and the member has not previously had a trial of the therapy, or
        - An American neurological society specifically recommends the testing for the safe and effective use or avoidance of a therapy and the member has not previously had a trial of the therapy, OR
    - The member meets all criteria in a test-specific guideline, if available (see Table: *Common epilepsy genes, associated conditions and applicable guidelines*), AND
- The member does not have a known underlying cause for their seizures (e.g. tumor, head trauma, known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Epilepsy multi-gene panels

Epilepsy multigene panels are considered medically necessary when all of the following criteria are met:

- The member has a diagnosis of early infantile epileptic encephalopathy, OR
- The member has a diagnosis of infantile spasms, OR
- The member has a diagnosis of intractable, neonatal seizures, OR

- The member has a diagnosis of febrile seizures with at least one episode of status epilepticus, OR
- The member has a progressive neurological disease defined by the following:
    - Member has epilepsy with persistent loss of developmental milestones, and
    - Member's seizures are worsening in severity and/or frequency despite treatment, OR
- A particular antiepileptic drug (AED) is being considered for the member and there are 2 or more genes on the panel for which the AED is contraindicated for individuals with mutations in that gene by ONE of the following:
    - A neurology therapy FDA label requires results from the genetic test to effectively or safely use or avoidance the therapy for the member's epilepsy type and the member has not previously had a trial of the therapy, or
    - An American neurological society specifically recommends the testing for the safe and effective use or avoidance of a therapy and the member has not previously had a trial of the therapy, AND
- The member does not display clinical features of a specific condition for which testing is available (e.g. Tuberous Sclerosis, Angelman Syndrome, Rett Syndrome, etc.), AND
- The member does not have a known underlying cause for their seizures (e.g. tumor, head trauma, known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other considerations

This guideline may not apply to genetic testing for indications that are addressed in test-specific guidelines. Please see the test-specific list of guidelines for a complete list of test-specific panel guidelines.

Genetic testing for a specific gene is medically necessary only once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest. Further, given rapidly advancing knowledge regarding genetic variations in epilepsy and in normal or healthy populations, re-analysis of genetic tests may be warranted at regular intervals.

Table: Common epilepsy genes, associated conditions and applicable guidelines

This is a representative list of known epilepsy genes and is not all inclusive:

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
ALDH7A1	81406	Pyridoxine-Dependent Epilepsy	Epilepsy Genetic Testing	MOL.TS.257
ARX	81404	ARX-Related Neurodevelopmental Disorders	Epilepsy Genetic Testing	MOL.TS.257
ATP1A2	81406	Familial Hemiplegic Migraine	Epilepsy Genetic Testing	MOL.TS.257
ARGHEF9	81479	ARGHEF9-Related Epilepsy (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
CACNA1A	81185	Familial Hemiplegic Migraine, Episodic Ataxia	Epilepsy Genetic Testing	MOL.TS.257
CDKL5	81406	Infantile Spasms; Early Seizure Variant Rett Syndrome	Epilepsy Genetic Testing	MOL.TS.257
CHD2	81479	CHD2-Related Neurodevelopmental Disorders (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
CHRNA2	81479	ADNFLE	Epilepsy Genetic Testing	MOL.TS.257
CHRNA4	81405	ADNFLE	Epilepsy Genetic Testing	MOL.TS.257
CHRNB2	81405	ADNFLE	Epilepsy Genetic Testing	MOL.TS.257
CLN3	81479	Neuronal Ceroid Lipofuscinosis	Epilepsy Genetic Testing	MOL.TS.257
CLN5	81479	Neuronal Ceroid Lipofuscinosis	Epilepsy Genetic Testing	MOL.TS.257

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
CLN8	81479	Neuronal Ceroid Lipofuscinosis	Epilepsy Genetic Testing	MOL.TS.257
CNTNAP2	81406	Pitt-Hopkins-Like Syndrome	Epilepsy Genetic Testing	MOL.TS.257
CSTB*	81188 81189 81190	PME (Unverrict-Lundborg)	Epilepsy Genetic Testing	MOL.TS.257
DEPDC5	81479	DEPDC5-Related Epilepsy	Epilepsy Genetic Testing	MOL.TS.257
EFHC1	81406	Susceptibility to Juvenile Absence & Myoclonic Epilepsies	Epilepsy Genetic Testing	MOL.TS.257
EPM2A	81404	PME (Lafora Disease)	Epilepsy Genetic Testing	MOL.TS.257
FOLR1	81479	Cerebral Folate Transport Deficiency	Epilepsy Genetic Testing	MOL.TS.257
FOXG1	81404	Congenital Variant Rett Syndrome	Epilepsy Genetic Testing	MOL.TS.257
GABRA1	81479	GABRA1-Related Epilepsy (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
GABRB3	81479	GABRB3-Related Epilepsy (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
GABRG2	81405	GABRG2-Related Epilepsy (GEFS+ included)	Epilepsy Genetic Testing	MOL.TS.257

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
GAMT	81479	Creatine Deficiency Syndromes	Epilepsy Genetic Testing	MOL.TS.257
GATM	81479	Creatine Deficiency Syndromes	Epilepsy Genetic Testing	MOL.TS.257
GRIN2A	81479	GRIN2A-Related Speech Disorders & Epilepsy (Landau-Kleffner included)	Epilepsy Genetic Testing	MOL.TS.257
KCNJ10	81404	EAST/SeSAME Syndrome	Epilepsy Genetic Testing	MOL.TS.257
KCNQ2	81406	KCNQ2-Related Disorders (BFNS & EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
KCNQ3	81479	KCNQ3-Related Disorders (BFNS included)	Epilepsy Genetic Testing	MOL.TS.257
KCNT1	81479	KCNT1-Related Disorders (ADNFLE & EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
KCTD7	81479	PME With or Without Inclusions, Neuronal Ceroid Lipofuscinosis	Epilepsy Genetic Testing	MOL.TS.257
LGI1	81479	Autosomal Dominant Partial Epilepsy with Auditory Features	Epilepsy Genetic Testing	MOL.TS.257

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
MBD5	81479	MBD5 Haploinsufficiency	Epilepsy Genetic Testing	MOL.TS.257
MECP2	81302	Classic Rett Syndrome; MECP2-Related Epileptic Encephalopathy (males)	Rett Syndrome Testing	MOL.TS.224
MEF2C	81479	Intellectual disability, Stereotypic Movements, Epilepsy, and/or Cerebral Malformations	Epilepsy Genetic Testing	MOL.TS.257
NHLRC1	81403	PME (Lafora Disease)	Epilepsy Genetic Testing	MOL.TS.257
NRXN1	81479	Pitt-Hopkins-Like Syndrome	Epilepsy Genetic Testing	MOL.TS.257
PCDH19	81405	Epilepsy & Intellectual Disability Limited to Females	Epilepsy Genetic Testing	MOL.TS.257
PNKP	81479	PNKP-Related Epilepsy (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
PNPO	81479	Pyridoxamine 5'-Phosphate Oxidase Deficiency	Epilepsy Genetic Testing	MOL.TS.257
POLG	81406	POLG-Related Disorders (Alpers Syndrome included)	Epilepsy Genetic Testing	MOL.TS.257
PRICKLE1	81479	PME	Epilepsy Genetic Testing	MOL.TS.257

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
PPT1	81479	Neuronal Ceroid Lipofuscinosis	Epilepsy Genetic Testing	MOL.TS.257
PRRT2	81479	PRRT2-Related Disorders	Epilepsy Genetic Testing	MOL.TS.257
SCARB2	81479	Action Myoclonus-Renal Failure Syndrome; PME	Epilepsy Genetic Testing	MOL.TS.257
SCN1A	81407	SCN1A-Related Disorders (Dravet syndrome & GEFS+ included)	Epilepsy Genetic Testing	MOL.TS.257
SCN1B	81404	SCN1B-Related Disorders (GEFS+ & EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
SCN2A	81479	SCN2A-Related Disorders (BFIS & EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
SCN8A	81479	SCN8A-Related Disorders (BFIS & EOEE Included)	Epilepsy Genetic Testing	MOL.TS.257
SLC19A3	81479	Biotin-Thiamine-Responsive Basal Ganglia Disease	Epilepsy Genetic Testing	MOL.TS.257
SLC2A1	81405	GLUT1 Deficiency	Epilepsy Genetic Testing	MOL.TS.257
SLC25A22	81479	SLC25A22-Related Epilepsy (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257

Gene	CPT	Condition	Applicable guideline name	Applicable guideline number
SLC9A6	81406	Christianson Syndrome	Epilepsy Genetic Testing	MOL.TS.257
SPTAN1	81479	SPTAN1-Related Epilepsy (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
STXBP1	81406	STXBP1-Related Disorders (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
TBC1D24	81479	TBC1D24-Related Disorders (EOEE included)	Epilepsy Genetic Testing	MOL.TS.257
TCF4	81406	Pitt-Hopkins Syndrome	Epilepsy Genetic Testing	MOL.TS.257
TSC1	81406	Tuberous Sclerosis	Epilepsy Genetic Testing	MOL.TS.257
TSC2	81407	Tuberous Sclerosis	Epilepsy Genetic Testing	MOL.TS.257
TPP1	81479	Neuronal Ceroid Lipofuscinosis	Epilepsy Genetic Testing	MOL.TS.257
UBE3A	81406	Angelman Syndrome	Angelman Syndrome Testing	MOL.TS.126
ZEB2	81405	Mowat-Wilson Syndrome	Epilepsy Genetic Testing	MOL.TS.257

Note *90% of Unverrict-Lundborg syndrome is due to a repeat expansion in CSTB that may not be detected using next-generation sequencing and requires specific testing for repeat expansions.

ADNFLE = Autosomal Dominant Frontal Lobe Epilepsy; BFIS = Benign Familial Infantile Seizures; BFNS = Benign Familial Neonatal Seizures; EOEE = Early-Onset

Epileptic Encephalopathy; GEFS+ = Generalized Epilepsy with Febrile Seizures Plus; PME = Progressive Myoclonic Epilepsy

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81419*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is epilepsy?

Definition

Epilepsy is a neurological condition that causes seizures.

Prevalence

Epilepsy is one of the most common disorders, with an estimated prevalence of 6 in 1000 people worldwide.[1,2]

Symptoms

Epilepsy can manifest in different ways, including different types of seizures or with multiple neurodevelopmental and medical complications besides seizures. Seizure types include generalized seizures (absence seizures, tonic-clonic seizures) and focal seizures (simple focal seizures, complex focal seizures, secondary generalized seizures, among others).

Cause

Epilepsy has multiple causes. These include, but are not limited to, acquired causes such as stroke, brain tumor, head injury, and central nervous system infection.[2] There are also numerous genetic conditions associated with epilepsy. It is estimated that approximately 40% of individuals with seizures have an underlying genetic basis for their condition (see Table 1 for a list of common genetic causes).[3]

Epileptic encephalopathy is a group of disorders in which seizures are accompanied by developmental delays, cognitive impairment, or a host of other neurological issues such as feeding difficulties, sleep dysregulation, and behavioral problems.[4] Knowledge regarding the genetic basis of these disorders has increased significantly in the last decade due to the advent of high throughput Next Generation Sequencing methods, resulting in wider availability of multi-gene panel testing. The following are examples of epileptic encephalopathies:

- Ohtahara Syndrome (Early Infantile Epileptic Encephalopathy)
    - “Characterized by early onset intractable tonic spasms, suppression-burst pattern on interictal EEG, and poor prognosis.” [5]
    - “To date various genes, which have essential roles in the brain’s neuronal and interneuronal functions, have been reported to be associated with Ohtahara syndrome. For instance, syntaxin binding protein 1 (STXBP1) regulates synaptic vesicle release; aristaless-related homeobox (ARX) acts as a regulator of proliferation and differentiation of neuronal progenitors; solute carrier family 25 member 22 (SLC25A22) encodes a mitochondrial glutamate transporter13; and potassium voltage-gated channel, KQT-like subfamily, member 2 (KCNQ2) plays a key role in a cell’s ability to generate and transmit electrical signals.” [6]
- Dravet Syndrome (Severe Myoclonic Epilepsy of Infancy)
    - “Clinical cardinal features include febrile or afebrile generalized or hemiconvulsions starting in the first year of life, seizure evolution to a mixture of intractable generalized (myoclonic or atonic seizures, atypical absences) and

focal seizures, normal early development, subsequent psychomotor retardation, and normal brain imaging at onset." [5]

- "In most of the cases with Dravet syndrome, one single gene has been involved, in contrast to other epileptic encephalopathy syndromes. SCN1A mutations have been shown in at least 80% of patients with Dravet syndrome." [6]

- Infantile Spasms (West Syndrome and X-linked Infantile Spasms)
  - "West syndrome is characterized by a specific seizure type, i.e., epileptic spasms, a unique interictal EEG pattern termed hypsarrhythmia, and psychomotor retardation. Spasms start within the first year of life, mainly between 4 and 6 months of age." [5]
  - "There are multiple genetic determinants of infantile spasms, which are usually explained by mutations in distinct genes. Genetic analysis of children with unexplained infantile spasms have demonstrated mutations on the X chromosome in genes such as ARX, cyclin-dependent kinase-like 5 (CDKL5), and UDP-N-acetylglucosaminyltransferase subunit (ALG13) as well as de novo mutations in autosomal genes, including membrane-associated guanylate kinase, WW and PDZ domain containing protein 2 (MAGI2), STXBP1, sodium channel alpha 1 subunit (SCN1A), sodium channel protein type 2 subunit alpha (SCN2A), g-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3), and dynamin 1 (DNM1)." [6]

- Epilepsy and Intellectual Disability Limited to Females
  - "Epilepsy and intellectual disability limited to females (EFMR) is an underrecognized disorder with X-linked inheritance but surprisingly only affecting females while sparing transmitting males. Seizure, cognitive, and psychiatric phenotypes show heterogeneity. Seizures start from the age of 6 to 36 months and may be precipitated by fever. Seizure types include GTCS, myoclonic and tonic seizures, absences, and focal." [5]
  - "Different mutations of PCDH19 (protocadherin 19), including missense, nonsense, and frameshift mutations, have been reported as the cause of EFMR." [5]

- Whole-genome screening for CNVs identifies potentially pathogenic deletions or duplications in ~5% of patients with a range of epilepsy phenotypes, including focal epilepsy, generalized epilepsies, epileptic encephalopathies, fever-associated epilepsy syndromes, and patients with neurodevelopmental disorders and epilepsy.[7]

Inheritance

Inheritance patterns differ between various epilepsy syndromes including dominant, X-linked, recessive, and mitochondrial causes, in addition to epilepsy caused by de novo (or new) genetic mutations. Clinical heterogeneity is also seen in these conditions.

Diagnosis

An electroencephalograph (EEG) can be used to help diagnose epilepsy and possibly give information as to the seizure type. A brain magnetic resonance imaging (MRI) scan can further help define whether epilepsy is caused by a structural brain abnormality or help determine the origin of epilepsy.

Genetic testing for epilepsy is complicated by many factors. Epilepsy syndromes frequently have overlapping features, such as the types of seizures involved and/or additional clinical findings. Many (if not most) epilepsy syndromes, including epileptic encephalopathy, are genetically heterogeneous, and can be caused by mutations in a number of different genes. Sometimes, the inheritance pattern or the presence of pathognomonic features makes the underlying syndrome clear. However, in many cases, it can be difficult to reliably diagnose an epilepsy syndrome based on clinical and family history alone.

NGS-based testing has been shown to dramatically improve the diagnostic rate for children and adults with epilepsy, as well as significantly shorten the time from assessment to diagnosis.[8-10] The diagnostic yield of NGS in patients with epileptic encephalopathies ranges is estimated to be 20-30%.[11,12]

Clinical information (e.g. age of onset, seizure type, EEG results, etc.) or family history may be used in some cases to help narrow down the suspected cause. In these cases, it may be possible to identify a narrow subset of genes that may be responsible for a person's epilepsy.[5,6]

Management

Treatment for epilepsy ranges from antiepileptic drugs (AEDs) to the ketogenic diet to vagal nerve stimulation to epilepsy surgery in the most severe situations. Not all treatments will work for everyone and often, it takes multiple treatment trials to find a regimen that is successful. In a rapidly growing number of epilepsy disorders, knowing the genetic mutation that is responsible for the epilepsy has been shown to help guide management and provide more disease-specific treatment.[13,14]

Survival

Lifespan is dependent upon seizure control and the underlying cause of the individual's epilepsy.

Test information

Introduction

Genetic testing for epilepsy may consist of next-generation sequencing or multigene panels.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to genetic testing for epilepsy.

National Society of Genetic Counselors

The National Society of Genetic Counselors practice guideline on epilepsy (2022), which was endorsed by the American Epilepsy Society (AES), stated:[15]

- “We strongly recommend comprehensive, multi-gene testing, such as ES/GS [exome sequencing/genome sequencing] or MGP [multigene panels] as a first-tier test. We conditionally recommend ES/GS over MGP as the first-tier test.”
- “The MGP panel should have a minimum of 25 genes and include copy number analysis.”
- “MGPs are valuable clinical tools that can be employed in a number of clinical scenarios, for example, when an individual presents with a defined epilepsy syndrome for which a subset of genes should be interrogated more robustly than through ES when GS is unavailable. Additionally, if urgent results are required and rapid ES/GS is unavailable, a targeted MGP may be considered. MGPs may also be utilized as a first-tier test when access to ES/GS, or the additional genetic counseling required to implement such testing, may be limited.”

Selected Relevant Publications

Peer reviewed and expert authored articles are presented below.

- In 2016, a peer reviewed article on genetic testing for epileptic encephalopathy stated the following:
  - "Second line investigations: Targeted next generation sequencing panels of epileptic encephalopathy genes for individuals with epileptic encephalopathy." [4]
- In 2016, a peer reviewed article on genetic causes of early-onset epileptic encephalopathy stated the following:[6]
  - "Molecular-based studies on early-onset epileptic encephalopathies should be performed, necessitating programmed genetical algorithms. If the phenotype could be determined with clinical findings, specific gene testing would be helpful in diagnosis. However, if the phenotype could not be determined because of overlapping phenotypes of different syndromes and the spectrum of phenotypes seen in different mutations, the use of gene panels for epilepsy would increase the probability of correct diagnosis. In a recent study, the rate of diagnosis with targeted single gene sequencing has been reported as 15.4%, whereas the rate has increased to 46.2% with the utility of epilepsy gene panels."
- A Task Force for the ILAE Commission of Pediatrics (2015) published recommendations for the management of infantile seizures. These recommendations included the following on treatments:[16]
  - "for Dravet syndrome, strong evidence supports that stiripentol is effective (in combination with valproate and clobazam), whereas weak evidence supports that topiramate, zonisamide, valproate, bromide, and the ketogenic diet are possibly effective; and for Ohtahara syndrome, there is weak evidence that most antiepileptic drugs are poorly effective."
  - "Genetic evaluation for Dravet syndrome and other infantile-onset epileptic encephalopathies should be available at tertiary and quaternary levels of care (optimal intervention would permit an extended genetic evaluation) (level of evidence—weak recommendation, level C)"
  - "Early diagnosis of some mitochondrial conditions may alter long-term outcome, but whether screening at quaternary level is beneficial is unknown (level of evidence U)"
- A systematic evidence review and meta-analyses of the diagnostic yield of genetic tests commonly utilized for patients with epilepsy was conducted in 2022.[17] Studies that utilized genome sequencing, exome sequencing, multigene panel, and/or genome-wide comparative genomic hybridization/chromosomal microarray (CGH/CMA) in cohorts (n ≥ 10) ascertained for epilepsy were included.
  - Overall diagnostic yield across all test modalities was 17%, with the highest yield for GS (48%), followed by ES (24%), MGP (19%), and CGH/CMA (9%).

- Phenotypic factors that were significantly associated with increased diagnostic yield included the presence of:
  - developmental and epileptic encephalopathy, and/or
  - neurodevelopmental comorbidities.

- Multiple peer-reviewed articles have shown that epilepsy multi-gene panels have a significant diagnostic yield when seizure onset is in infancy or early childhood.[10,18-20] The diagnostic yields in adults with epilepsy tend to be lower.[21,22]

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Nonsyndromic Hearing Loss and Deafness Genetic Testing

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v2.0.2024

Introduction

Nonsyndromic hearing loss and deafness genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
GJB2 Gene Analysis	S3840
GJB2 Known Familial Mutation Analysis	81253
GJB2 Sequencing	81252
GJB6 Common Variant Analysis	81254
Hearing Loss (e.g., nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); Genomic Sequence Analysis Panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3, USH1C, USH1G, USH2A, and WFS1	81430
Hearing Loss (e.g, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); Duplication/Deletion Analysis Panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes	81431

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Procedures addressed by this guideline	Procedure codes
Hearing Loss and Deafness Gene Tests	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
MT-RNR1 Sequencing	81403
MT-RNR1 Targeted Mutation Analysis	81401
MT-TS1 Sequencing	81403
MT-TS1, MT-RNR1 Targeted Mutation Analysis	81401

Criteria

Introduction

Requests for nonsyndromic hearing loss and deafness testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Previous testing:
  - Member has not previously had testing that would detect the known familial mutation(s), AND
- Member has a 1st, 2nd, or 3rd degree biological relative with a pathogenic mutation(s) in a gene associated with nonsyndromic hereditary hearing loss or deafness, AND
- Member is at risk of inheriting the pathogenic mutation based on the family history and the inheritance pattern associated with the mutation, AND
- Diagnostic testing:

- Member has nonsyndromic hearing loss or deafness that is consistent with the mutation in the family, OR

- Carrier testing:
  - Member is of reproductive age, and
  - Member has ability and intention to reproduce, or
  - Member is currently pregnant.

GJB2 Sequencing

- Previous testing:
  - Member has not previously had GJB2 sequencing, and
  - No known pathogenic hearing loss/deafness gene variants in a biological relative, AND
- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and
  - Prelingual onset of hearing loss (prior to speech development), and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss, and
  - Member's family history is consistent with autosomal recessive inheritance (including simplex cases), OR
- Carrier screening
  - Member is of reproductive age, and
  - Has potential and intention to reproduce, and
  - Has a reproductive partner who is a carrier of a GJB2/GJB6 mutation, or
  - Has a reproductive partner with GJB2/GJB6-related deafness.

GJB6 Common Variant Analysis for 309kb and 232kb Deletions

- Previous testing:
  - Member has not previously had GJB6 common variant analysis or deletion/duplication analysis, AND
- Diagnostic Testing:

- Member meets criteria for GJB2 sequencing, and
- No mutation or only one mutation identified on GJB2 sequencing, OR

- Carrier screening
  - Member is of reproductive age, and
  - Has potential and intention to reproduce, and
  - Has a 1st, 2nd, or 3rd-degree biological relative with a GJB6 variant, or
  - Member meets criteria for GJB2 sequencing, and
  - No mutation identified on GJB2 sequencing.

MT-RNR1 Targeted Mutation Analysis for m.1555A>G Mutation

- Previous testing:
  - Member has not previously had MT-RNR1 targeted mutation analysis, and
  - No known pathogenic hearing loss/deafness gene variants in a biological relative, AND
- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss, and
  - Member has at least one of the following risk factors for MT-RNR1 related deafness:
    - History of aminoglycoside antibiotic exposure (gentamycin, tobramycin, amikacin, kanamycin, or streptomycin), or
    - Member's family history is strongly suggestive of mitochondrial inheritance (no transmission through a male).

MT-RNR1 Sequencing

- Previous testing:
  - Member has not previously had MT-RNR1 sequencing, and
  - No mutations detected in any previous MT-RNR1 testing (targeted m.1555A>G mutation analysis), and

- No known pathogenic hearing loss/deafness gene variants in a biological relative, AND

- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss, and
  - Member has at least one of the following risk factors for MT-RNR1 related deafness:
    - Aminoglycoside antibiotic exposure (gentamycin, tobramycin, amikacin, kanamycin, or streptomycin) prior to hearing loss onset, or
    - Member's family history is strongly suggestive of mitochondrial inheritance (no transmission through a male).

MT-TS1 Sequencing

- Previous testing:
  - Member has not previously had MT-TS1 analysis, and
  - No mutations detected in any previous MT-TS1 testing (targeted variant analysis), and
  - No known pathogenic hearing loss/deafness gene variants in a biological relative, AND

- Diagnostic Testing:
  - Member has a formal diagnosis of bilateral sensorineural hearing loss, and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies, or other signs of syndromic hearing loss, and
  - Member's family history is strongly suggestive of mitochondrial inheritance (no transmission through a male).

Nonsyndromic Hearing Loss and Deafness Multigene Panel Testing

Multi-gene panels will be considered medically necessary when the following criteria are met:

- Previous testing:
  - Member has not previously had a hearing loss panel, and
  - No known pathogenic hearing loss/deafness gene variants in a biological relative, AND
- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss.

Other considerations

Broad hearing loss and deafness panels may not be medically necessary when a narrower panel is available and more appropriate based on the clinical findings.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
  - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81430 to represent a sequencing panel and 81431 to represent a deletion/duplication panel)*.
  - When use of a panel code is not possible, each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.

- If appropriate first-tier tests cannot be determined on the basis of clinical and family histories, only the following genes may be considered for reimbursement: GJB2, STRC, SLC26A4, TECTA, MYO15A, MYO7A.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is nonsyndromic hearing loss and deafness?

Definition

Nonsyndromic hearing loss (NSHL) is defined as partial or total hearing loss that does not occur with other medical conditions or symptoms.[1]

Prevalence

It is estimated that up to 3/1000 children are born with hearing loss in one or both ears.[1] About 15% of adults in America have some level of hearing loss.[2]

Symptoms

Approximately 70-80% of genetic hearing loss is nonsyndromic, with no related systemic findings.[3,4] Some syndromic forms of hearing loss and deafness may masquerade as nonsyndromic in infancy and early childhood, before additional symptoms emerge. For example, goiter does not develop until puberty or adulthood in Pendred syndrome; retinitis pigmentosa emerges in adolescence in Usher syndrome; and males with Deafness-Dystonia-Optic Neuronopathy (Mohr-Tranebjaerg) Syndrome begin having progressive neurological symptoms in their teens.[3,5]

Cause

Approximately 35% of cases of prelingual hearing loss are attributed to environmental causes, including viral (cytomegalovirus) or bacterial (meningitis) infection, trauma, prenatal exposure to certain drugs, and other environmental factors.[3] The remaining 65% of cases are thought to be genetic, either as part of a recognized genetic syndrome, or as isolated, nonsyndromic hearing loss (NSHL).[3]

GJB2-related autosomal recessive hearing loss is the most common cause of congenital severe-to-profound non-progressive sensorineural hearing loss.[6] Carrier frequency for GJB2-related hearing loss is dependent on population. Based on current available data, the highest carrier rate (~8%) is reported in the East Asian population.[6]

Inheritance

NSHL can exhibit autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance patterns.[3,6,7] Autosomal recessive inheritance accounts for 80% of NSHL, while 15-19% is autosomal dominant, and ~1% is mitochondrial or X-linked.

Diagnosis

In the United States, >98% of newborns have hearing screening which can identify congenital hearing loss.[3] Diagnosis of hearing loss may involve physiologic testing (including auditory brainstem response or ABR/BAER) and/or audiometry.[3]

Management

Management of congenital hearing loss or deafness may include hearing aids, cochlear implants, and appropriate educational interventions[1]. Uncovering the genetic etiology of the hearing loss may also identify (or allay concerns about) comorbidities that may require referral for specialty care.[3,4]

Survival

NSHL is not associated with decreased survival.

Test information

Introduction

Testing for NSHL may include known familial mutation analysis, targeted mutation analysis, multigene panel testing, or single gene analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Single Gene Analysis

Under certain circumstances, technologies used in multigene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion remains for a particular syndrome after negative multigene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted.

NSHL and deafness multigene panels include a wide variety of genes associated with nonsyndromic hearing loss and deafness. Multigene nonsyndromic hearing loss and deafness panels may also include genes for syndromes that mimic nonsyndromic hearing loss (e.g. Usher syndrome, Pendred syndrome, Jervell and Lange-Nielsen syndrome, etc.).

A study of 440 individuals with genetic hearing loss found mutations in ~40% of cases tested with a multigene panel. The only feature with an adverse effect on test yield was unilateral hearing loss, for which the panel only identified mutations in 1% of cases.[5] In another study, the mutation detection rate was ~60% via multigene panel; multigene panel testing was noted to be more cost-effective than single gene testing.[8]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to genetic testing for nonsyndromic hearing loss and deafness.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2022) stated:[4]

- A comprehensive genetic evaluation is recommended for all cases of congenital deafness or hearing loss with onset in childhood or early adulthood. Cytomegalovirus (CMV) testing is important for cases of congenital hearing loss (HL). The testing should be completed within the first three weeks of life if possible. Ancillary testing (e.g. electrocardiogram, renal ultrasound, temporal bone imaging and ophthalmology examination) remains important, as results may support genetic testing selection or interpretation of variants. The clinical utility of these tests should

be evaluated on a case-by-case basis since genetic testing via NGS panels may soon become more cost-effective.

- Genetic testing to confirm a diagnosis of suspected syndromic hearing loss is recommended based on clinical findings. For apparently nonsyndromic hearing loss, a tiered approach was recommended: "Unless clinical and/or family history suggests a specific etiology, comprehensive HL [hearing loss] gene panel testing should be initiated. If panel testing is negative, genome-wide testing, such as ES [exome sequencing] or GS [genome sequencing], may be considered. However, issues related to genomic testing, such as the likelihood of incidental or secondary findings, will have to be addressed."
- Hearing loss panels should include those genes recommended by the ClinGen Hearing Loss Gene Curation Expert Panel.[9]
- "If genetic testing reveals variant(s) in an HL-related gene, gene-specific genetic counseling should be provided, followed by appropriate medical evaluations and referrals."
- "If genetic testing fails to identify an etiology for a patient's HL, the possibility of a genetic etiology remains. This point must be emphasized because it can be misunderstood by clinicians and by patients and their families. For interested patients and families, further genetic testing may be pursued on a research basis."

International Pediatric Otolaryngology Group

The International Pediatric Otolaryngology Group (IPOG, 2016) stated:[10]

- "In the setting of unilateral hearing loss, genetic testing has a limited role unless syndromic hearing loss is suspected."
- "After and [sic] audiogram and physical exam, comprehensive genetic testing (CGT) that relies on next generation sequencing (NGS) methodologies should guide subsequent workup in children with bilateral sensorineural hearing loss."
- "Diagnostic rates for single gene testing for GJB2/GJB6 vary significantly based on the patient's ethnicity, and do not outperform the diagnostic rates for comprehensive genetic testing. In cases where CGT is unavailable, single gene testing can be directed by the audiometric phenotype and ethnicity."
- The general consensus of the authors was that temporal bone imaging “should not be a routine part of the diagnostic algorithm for bilateral symmetric sensorineural hearing loss.”

Selected Relevant Publications

Expert-authored reviews of nonsyndromic hearing loss stated:

- "A hearing loss multigene panel that includes all genes implicated in nonsyndromic hearing loss and disorders that mimic nonsyndromic hearing loss including GJB2and other genes of interest … is most likely to identify the genetic cause of the

condition while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype."[6]

- "Analytic methods used for this panel must include the detection of deletions of GJB2, either intragenic or whole gene, and deletions that include sequences upstream of GJB2 (comprising either GJB6 and portions of CRYL1or just portions of CRYL1) that delete cis-regulatory regions of GJB2, thereby abolishing GJB2 expression."[6]
- Regarding mitochondrial NSHL, the diagnosis should be suspected in individuals with moderate-to-profound hearing loss and a family history suggestive of maternal inheritance (e.g. no transmission through a male), or onset of hearing loss after exposure to an aminoglycoside antibiotic.[7]
    - "In individuals with hearing loss following aminoglycoside exposure, molecular testing for the pathogenic variants m.1555A>G and m.1494C>T in MT-RNR1 and m.7445A>C/T/G in MT-TS1 can be done first."
    - An alternative strategy is to perform multigene panel testing that includes both MT-RNR1 and MT-TS1, plus other genes of interest.
    - If targeted mtDNA testing and/or multigene panel testing including these mtDNA genes fail to confirm a diagnosis, mitochondrial genome sequencing can be considered. Mitochondrial genome sequencing should be performed prior to multigene panel testing if there is a clear mitochondrial inheritance pattern.

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Hereditary Ataxia Multigene Panel Genetic Testing

MOL.TS.310.A
v2.0.2024

Introduction

Hereditary ataxia multigene panel testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Genomic Unity Ataxia Repeat Expansion and Sequence Analysis	0216U
Genomic Unity Comprehensive Ataxia Repeat Expansion and Sequence Analysis	0217U
Hereditary Ataxia Multigene Panel	81479
Hereditary Ataxia Multigene Panel (including sequencing of at least 15 genes)	81443

Criteria

Introduction

Requests for hereditary ataxia multigene panel testing are reviewed using these criteria.

Multigene Panel Testing

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous testing of requested genes, and

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  - No known mutation identified by previous analysis, and
  - No known familial mutation in a gene known to cause ataxia, AND

- Diagnostic Testing for Symptomatic Individuals
  - Individual has been diagnosed with cerebellar ataxia, regardless of age of onset, AND
- Documentation from ordering provider indicating how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), AND
- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation, etc), AND
- Family and medical history do not point to a specific genetic diagnosis or pattern of inheritance for which a more focused test or panel, such as a nucleotide repeat analysis panel, would be appropriate, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Other Considerations

- For information on spinocerebellar ataxia (SCA) panel testing, please refer to the guideline *Spinocerebellar Ataxia Genetic Testing*, as this testing is not addressed here.
- Gene panels that are specific to hereditary ataxias will be considered for medical necessity according to the criteria outlined in this guideline. Test methodology should be appropriate to the disease-causing mutations that are commonly reported for the disorder in question (e.g., sequencing-only panels will not detect triplet repeat or large deletion/duplication mutations).

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any multi-gene panel is only reimbursable once per lifetime.

- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81443, 81479, 0216U, or 0217U)*.
    - Analysis of individual genes will not be reimbursed separately (i.e. multiple stacked codes are not eligible for reimbursement).

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are hereditary ataxias?

Definition

The hereditary ataxias are a group of genetic disorders. They are characterized by slowly progressive uncoordinated, unsteady movement and gait, and often poor coordination of hands, abnormal eye movements, and slurred speech. Cerebellar atrophy is also frequently seen via brain imaging.[1]

Prevalence

Prevalence estimates vary. The prevalence is approximately 2.7/100,000 and 3.3/100,000 for autosomal dominant and autosomal recessive hereditary ataxias, respectively.[2] One study in Norway estimated the prevalence of hereditary ataxia at 6.5 per 100,000 people.[3]

Symptoms

Although hereditary ataxias are made up of multiple different conditions, they are characterized by slowly progressive uncoordinated, unsteady movement and gait, and often poor coordination of hands, abnormal eye movements, and slurred speech. Cerebellar atrophy is also frequently seen via brain imaging.[1]

Cause

Hereditary ataxias are caused by pathogenic mutations in one of numerous genes.[1] The following genes are associated with hereditary ataxia; however, this list is not intended to be all inclusive: ATN1, ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, TBP, FXN, and FMR1. Several of the ataxias are caused by nucleotide repeat expansions.

Testing for these conditions is performed by expansion analysis to identify the number of repeats. Expansion analysis can be performed for diagnostic testing, presymptomatic testing, as well as prenatal testing.

Inheritance

Most hereditary ataxias, including the spinocerebellar ataxias (SCA), dentatorubral-pallidoluysian atrophy (DRPLA), and episodic ataxia (EA) types 1 and 2, are inherited in an autosomal dominant manner. A few of the hereditary ataxias, including Friedreich ataxia and ataxia telangiectasia, are inherited in an autosomal recessive manner. Fragile X tremor/ataxia syndrome is an X-linked ataxia.[1]

Diagnosis

The diagnosis of hereditary ataxia is suspected based on clinical and family history, neurological exam, and neuroimaging studies.[1] Acquired causes of ataxia — including alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, and tumors — should be ruled out.[1]

Molecular genetic testing can be used to establish a specific diagnosis. In the absence of a family history, it can be difficult to differentiate the type or subtype of hereditary ataxia based on clinical features.[1] One study found that in approximately 13% of apparently sporadic ataxias, a causative genetic change was identified.[4]

Management

Treatment of ataxia is largely supportive, and includes the use of canes and walkers for ambulation, speech therapy, and other assistive devices.[1]

Survival

The survival range of the hereditary ataxias varies across the multiple conditions included in this group.

Test Information

Introduction

Testing for hereditary ataxias may include known familial mutation analysis, single gene testing, nucleotide repeat expansion analysis, or multigene panel testing. This guideline only addresses multigene panel testing.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical

overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and Evidence

Introduction

This section includes relevant guidelines and evidence pertaining to hereditary ataxia testing.

European Federation of Neurological Sciences

The European Federation of Neurological Sciences (EFNS, 2014) stated the following regarding testing for hereditary ataxias:[5]

- "In the case of a family history that is compatible with an autosomal dominant cerebellar ataxia, screening for SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 is recommended (Level B). In Asian patients, DRPLA should also be tested for."
- "If mutation analysis is negative, we recommend contact with or referral to a specialized clinic for reviewing the phenotype and further genetic testing (good practice point)."
- "In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA6, and DRPLA (in Asian patients) (level B), the step one panel of the recessive ataxia workup, i.e. mutation analysis of the FRDA gene (level B), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, CK, and alpha-fetoprotein."

Selected Relevant Publications

De silva R, Greenfield J, Cook A, et al. (2019) recommended referral to clinical genetics services and/or genetic testing as part of the diagnostic work-up for adults with progressive ataxia. They recommended the following as secondary [first line] care:[6]

- "Genetic tests for FRDA, SCA 1, 2, 3, 6, 7 (12,17) and FXTAS"

Hadjivassiliou M, Martindale J, Shanmugarajah P, et al (2017) stated the following with regard to testing for hereditary ataxias:[4]

- "We have shown that patients with early onset idiopathic ataxia (irrespective of family history) are much more likely to have a genetic aetiology (81%) than those with late onset idiopathic ataxia (55%). One possible selection criterion for genetic testing is early onset ataxia. Additional selection criteria may include the presence of other clinical features, for example, 1% of patients with histologically

suspected/genetically confirmed mitochondrial disease had ataxia with other clinical features (eg, deafness, diabetes, myoclonus, etc) and only 9% pure ataxia."

- "Furthermore, the presence of severe cerebellar atrophy without any clinical correlation and with well-preserved spectroscopy of the cerebellum often suggests that the ataxia is long standing (maybe even early onset) and slowly progressive. Patients should therefore be offered genetic testing. The pattern of cerebellar involvement on MR spectroscopy may also direct to a particular diagnosis. Most genetic ataxias involve both the hemispheres and the vermis while the majority of immune-mediated acquired ataxias (eg, gluten ataxia, anti-GAD ataxia and primary autoimmune cerebellar ataxia) have a predilection for the vermis."

Jayadev S and Bird T (2013) stated the following: [7]

The "differential diagnosis of hereditary ataxia includes acquired, nongenetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, and paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung, and the idiopathic degenerative disease multiple system atrophy (spinal muscular atrophy). The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available."

- Regarding establishing the diagnosis of hereditary ataxias:
  - "Detection on neurological examination of typical clinical signs including poorly coordinated gait and finger/hand movements, dysarthria (incoordination of speech), and eye movement abnormalities such as nystagmus, abnormal saccade movements, and ophthalmoplegia."
  - "Exclusion of nongenetic causes of ataxia."
  - "Documentation of the hereditary nature of the disease by finding a positive family history of ataxia, identifying an ataxia-causing mutation, or recognizing a clinical phenotype characteristic of a genetic form of ataxia."
- Regarding testing when the family history suggests autosomal dominant inheritance:
  - "An estimated 50–60% of the dominant hereditary ataxias can be identified with highly accurate and specific molecular genetic testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, and DRPLA; all have nucleotide repeat expansions in the pertinent genes."
  - "Because of broad clinical overlap, most laboratories that test for the hereditary ataxias have a battery of tests including testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA14, and SCA17. Many laboratories offer them as two groups in stepwise fashion based on population frequency, testing first for the more common ataxias, SCA1, SCA2, SCA3, SCA6, and SCA7. Although pursuing multiple genes simultaneously may seem less optimal than serial genetic testing, it is important to recognize that the cost of the battery of ataxia tests often is equivalent to that of an MRI. Positive results from the molecular

genetic testing are more specific than MRI findings in the hereditary ataxias. Guidelines for genetic testing of hereditary ataxia have been published."

- "Testing for the less common hereditary ataxias should be individualized and may depend on factors such as ethnic background (SCA3 in the Portuguese, SCA10 in the Native American population with some exceptions [Fujigasaki et al., 2002]); seizures (SCA10); presence of tremor (SCA12, fragile X-associated tremor/ataxia syndrome); presence of psychiatric disease or chorea (SCA17); or uncomplicated ataxia with long duration (SCA6, SCA8, and SCA14). Dysphonia and palatal myoclonus are associated with calcification of the dentate nucleus of cerebellum (SCA20)."
- "If a strong clinical indication of a specific diagnosis exists based on the affected individual's examination (e.g., the presence of retinopathy, which suggests SCA7) or if family history is positive for a known type, testing can be performed for a single disease."

- Regarding testing when the family history suggests autosomal recessive inheritance:
  - "A family history in which only sibs are affected and/or when the parents are consanguineous suggests autosomal recessive inheritance. Because of their frequency and/or treatment potential, FRDA, A-T, AOA1, AOA2, AVED, and metabolic or lipid storage disorders such as Refsum disease and mitochondrial diseases should be considered."
- Regarding testing for a simplex case:
  - "If no acquired cause of the ataxia is identified, the probability is ~13% that the affected individual has SCA1, SCA2, SCA3, SCA6, SCA8, SCA17, or FRDA, and mutations in rare ataxia genes are even less common."
  - "Other possibilities to consider are a de novo mutation in a different autosomal dominant ataxia, decreased penetrance, alternative paternity, or a single occurrence of an autosomal recessive or X-linked disorder in a family such as fragile X-associated tremor/ataxia syndrome."
  - "Although the probability of a positive result from molecular genetic testing is low in an individual with ataxia who has no family history of ataxia, such testing is usually justified to establish a specific diagnosis for the individual's medical evaluation and for genetic counseling."
  - "Always consider a possible nongenetic cause such as multiple system atrophy, cerebellar type in simplex cases."

References

Introduction

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Hereditary Cancer Syndrome Multigene Panels

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Introduction

Hereditary cancer syndrome multigene panel testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
BRCAplus	0129U
BreastNext	0102U
Chromosomal Microarray [BAC], Constitutional	81228
Chromosomal Microarray [SNP], Constitutional	81229
Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and loss-of-heterozygosity variants, low-pass sequencing analysis	81349
ColoNext	0101U
CustomNext + RNA: APC	0157U
CustomNext + RNA: MLH1	0158U
CustomNext + RNA: MSH2	0159U
CustomNext + RNA: MSH6	0160U
CustomNext + RNA: PMS2	0161U
CustomNext + RNA: Lynch (MLH1, MSH2, MSH6, PMS2)	0162U
GeneticsNow Comprehensive Germline Panel	0474U

Procedures addressed by this guideline	Procedure codes
Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including BRCA1, BRCA2,CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53	81432
Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11	81433
Hereditary cancer syndrome multigene gene panel	81479
Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11	81435
Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11	81436
Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); genomic sequence analysis panel, must include sequencing of at least 6 genes, including MAX, SDHB, SDHC, SDHD, TMEM127, and VHL	81437

Procedures addressed by this guideline	Procedure codes
Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); duplication/deletion analysis panel, must include analyses for SDHB, SDHC, SDHD, and VHL	81438
OvaNext	0103U
ProstateNow Prostate Germline Panel	0475U
+RNAinsight for ATM	0136U
+RNAinsight for BRCA1/2	0138U
+RNAinsight for BreastNext	0131U
+RNAinsight for CancerNext	0134U
+RNAinsight for ColoNext	0130U
+RNAinsight for GYNPlus	0135U
+RNAinsight for OvaNext	0132U
+RNAinsight for PALB2	0137U
+RNAinsight for ProstateNext	0133U

Criteria

Introduction

Requests for hereditary cancer syndrome panel testing are reviewed using these criteria.

Hereditary Cancer Multi-Syndrome Panels

This guideline applies only to testing performed as a multi-syndrome panel for hereditary cancer. For information on single gene or single syndrome requests, please refer to a test-specific policy, if available, as this testing is not addressed here. If none is available, please refer to the clinical use guideline *Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes*.

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No known cancer-predisposing mutation in the family, AND
- No previous hereditary cancer syndrome multi-gene panel testing, AND
- No previous hereditary cancer syndrome testing for any gene on the panel, AND

- One of the following is met:
  - Member has a personal diagnosis of cancer consistent with the hereditary cancer syndrome that is suspected in the family, or
  - Member is not affected with cancer but is the most informative person in the family to test and an affected family member cannot proceed with testing. If the member is not the most informative person to test, documentation must be provided by the ordering physician's office clearly documenting that it is impossible to test the most informative family member and describing the reason the unaffected member is being tested at this time, AND
- One of the following is met:
  - Member has a personal history of invasive cutaneous melanoma and a first degree biological relative diagnosed with pancreatic cancer (multi-syndrome panel must include CDKN2A), or
  - Member meets criteria for BRCA Analysis based on current eviCore guideline *BRCA Analysis*, or
  - Member meets criteria for Lynch Syndrome Genetic Testing based on current eviCore guideline *Lynch Syndrome Genetic Testing*, or
  - Member meets criteria for Familial Adenomatous Polyposis Syndrome Genetic Testing based on current eviCore guideline *Familial Adenomatous Polyposis Syndrome Genetic Testing*, or
  - Member meets criteria for MUTYH Associated Polyposis Genetic Testing based on current eviCore guideline *MUTYH Associated Polyposis Genetic Testing*, or
  - Member meets criteria for two other separate hereditary cancer syndromes based on eviCore guidelines that are included on the panel, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Deletion/Duplication Analysis

- Member meets criteria for sequencing above, AND
- Previous sequencing panel, if applicable, was performed and no mutations identified.

RNA Testing for Hereditary Cancer Syndromes

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves

patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.

- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

Hereditary cancer testing reflex or update panels (e.g. MyRisk Update) will be reimbursed when the following criteria are met:

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No known cancer-causing mutation in the family, AND
- No previous hereditary cancer syndrome multi-gene panel testing, AND
- Testing for one condition, for which the member meets eviCore criteria, was performed and billed separately. A multi-gene panel is now being considered and will be billed at a rate comparable to single syndrome pricing, AND
- Member meets medical necessity criteria for at least one additional condition included in the panel that was not already tested (e.g., hereditary breast and ovarian cancer was already performed, but Lynch syndrome criteria are also met). Please refer to test-specific guidelines for details.
    - Although not a complete list, the following are considered separate conditions:
        - Hereditary breast cancer - this includes both BRCA1/2 and PALB2 (Note that if BRCA1/2 testing was already performed and PALB2 criteria are now met, PALB2 testing alone would be medically necessary and not an update panel.)
        - Lynch syndrome
        - Li-Fraumeni syndrome
        - Familial adenomatous polyposis
        - Cowden syndrome
        - Peutz-Jeghers syndrome
        - MUTYH-associated polyposis

Other considerations

- Genetic testing is only medically necessary once per lifetime. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
    - A single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously.
    - If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- RNA testing is not reimbursable.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81432 or other DNA-based panel code in the table at the beginning of this policy)*.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are hereditary cancer syndromes?

Definition

When a mutation in a single gene causes a significantly increased risk for certain cancers, it is called a hereditary cancer syndrome. Hereditary cancer syndromes are usually characterized by a pattern of specific cancer types occurring together in the same family, younger cancer diagnosis ages than usual, and/or other co-existing non-cancer conditions.

Prevalence

Most cancer is sporadic and believed to be caused by a mix of behavioral or lifestyle, environmental, and inherited risk factors. However, about 5-10% of cancers are believed to have a major inherited component.[1]

Hereditary cancer syndromes

There are more than 50 hereditary cancer syndromes.[1] Some of the most common are listed below with associated cancers.[2]

- Hereditary breast and ovarian cancer syndrome (HBOC): breast, ovarian/fallopian tube/primary peritoneal cancer, pancreatic, prostate cancers
- Lynch syndrome: colorectal, endometrial, small bowel, stomach, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, brain, sebaceous adenoma, and keratoacanthoma tumors
- Familial adenomatous polyposis: colorectal and other gastrointestinal cancers, gastrointestinal tract polyps (adenomas, fundic gland), osteomas, desmoids, thyroid cancer and hepatoblastoma
- MUTYH-associated polyposis: colorectal and other gastrointestinal cancers, adenomas, hyperplastic polyps
- Cowden syndrome: benign and malignant tumors of the breast, endometrium, and thyroid; cancer and polyps (hamartomas) in the colon and rectum
- Li-Fraumeni syndrome: soft tissue sarcoma, osteosarcoma, leukemia, melanoma, and cancer of the breast, pancreas, colon, adrenal cortex, stomach, esophagus and brain
- Peutz-Jeghers syndrome: polyps (hamartomas) in the stomach, small intestine and colon, and pancreas, lung, breast, uterine and non-epithelial ovarian cancer

Many hereditary cancer syndromes can include the same types of cancer and therefore have overlapping clinical findings. For example, breast cancer is a feature of HBOC, Li-Fraumeni syndrome, Cowden syndrome, and other hereditary cancer syndromes. The pattern of cancers in the family or pathognomonic features may help determine the underlying syndrome. However, in many cases it can be difficult to reliably diagnose hereditary cancer syndromes based on clinical and family history alone.

Genes associated with hereditary cancer syndromes

The National Comprehensive Cancer Network (NCCN) suggested specific genes that may contribute to hereditary cancers.[3-5] They are provided in the table below.

Hereditary cancer type	Associated genes
Breast cancer	ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, STK11, TP53
Colon cancer / polyposis	APC, AXIN2, BMPR1A, CHEK2, EPCAM, GREM1, MLH1, MLH3, MSH2, MSH3, MSH6, MUTYH, NTHL1, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TP53
Ovarian cancer	BRCA1, BRCA2, BRIP1, MLH1, MSH2, MSH6, PALB2, PMS2, EPCAM, RAD51C, RAD51D, and STK11
Pancreatic cancer	ATM, BRCA1, BRCA2, CDKN2A, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2, STK11, TP53
Prostate cancer	ATM, BRCA1, BRCA2, CHEK2, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2

Test information

Introduction

Testing for hereditary cancer syndromes may include multigene panel testing.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to hereditary cancer syndrome multigene panel testing.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG has published several statements or standards that offer general guidance on the clinical application of large-scale sequencing, including recommendations regarding counseling around unexpected results, variants of unknown significance, and minimum requirements for reporting apply to many NGS applications.[6-8]

ACMG (2021) published a technical standard for use of NGS in the clinical laboratories which stated:[7]

- "Choosing an appropriate NGS-based test is the responsibility of the ordering health-care provider. Given the large number of tests (https://www.ncbi.nlm.nih.gov/gtr/) available to the clinician, the clinical laboratory often provides critical advice in test selection. Ordering providers must weigh considerations of sensitivity, specificity, cost, and turnaround time for each clinical situation."
- "Test development must consider the variant types that will be detected in the genes or regions of the genome interrogated."

In a 2020 technical standard on gene sequencing panels, ACMG stated:[8]

- "Gene sequencing panels are a powerful diagnostic tool for many clinical presentations associated with genetic disorders. Advances in DNA sequencing technology have made gene panels more economical, flexible, and efficient."
- "Due to differences in decision-making processes in the absence of clear professional standards, genes included on similar disease-focused panels vary between laboratories. With the ability to sequence multiple genes simultaneously, it is imperative to evaluate critically the validity of gene–disease associations prior to test design."
- "Transparency is imperative when performing a gene sequencing panel so that ordering providers know what the test includes and what it does not."
- Gene panels should:
    - "Maximize clinical specificity by limiting or excluding GUSs [genes of uncertain significance], thereby minimizing detection of VUS [variants of uncertain significance]"
    - "Employ auxiliary assays for genes/regions that cannot be interrogated with current sequencing technology to maximize the clinical utility."

In a 2020 statement on whether all individuals with breast cancer should receive BRCA1/2 testing, ACMG stated:[9]

- "With the advances in sequencing technologies and increasing access to and expanding indications for genetic testing, it remains critical to ensure that implementation of testing is based on evidence. Currently, there is insufficient evidence to recommend genetic testing for BRCA1/2 alone or in combination with multi-gene panels for all breast cancer patients."

American College of Obstetricians and Gynecologists

In a Committee Opinion, the American College of Obstetricians and Gynecologists (ACOG, 2019) stated:[10]

- "If a hereditary cancer risk assessment suggests an increased risk of a hereditary cancer syndrome, referral to a specialist in cancer genetics or a health care provider with expertise in genetics is recommended for expanded gathering of family history information, risk assessment, education, and counseling, which may lead to genetic testing and tailored cancer screening or risk reduction measures, or both."
- "Genetic testing may be performed using a panel of multiple genes through next-generation sequencing technology. This multigene testing process increases the likelihood of finding variants of unknown significance, and it also allows for testing for pathogenic and likely pathogenic variants in multiple genes that may be associated with a specific cancer syndrome or family cancer phenotype (or multiple phenotypes)."

American Society of Breast Surgeons

The American Society of Breast Surgeons (2019) published a consensus guideline on genetic testing for hereditary breast cancer. They stated the following:[11]

- "Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling and make recommendations to their patients regarding genetic testing and arrange testing. When the patient's history and/or test results are complex, referral to a certified genetic counselor or genetics professional may be useful. Genetic testing is increasingly provided through multi-gene panels. There are a wide variety of panels available, with different genes on different panels. There is a lack of consensus among experts regarding which genes should be tested in different clinical scenarios. There is also variation in the degree of consensus regarding the understanding of risk and appropriate clinical management of mutations in some genes."
- "Genetic testing should be made available to all patients with a personal history of breast cancer. Recent data support that genetic testing should be offered to each patient with breast cancer (newly diagnosed or with a personal history). If genetic testing is performed, such testing should include BRCA1/BRCA2 and PALB2, with other genes as appropriate for the clinical scenario and family history. For patients with newly diagnosed breast cancer, identification of a mutation may impact local treatment recommendations (surgery and potentially radiation) and systemic therapy. Additionally, family members may subsequently be offered testing and tailored risk reduction strategies."
- "Genetic testing should be made available to all patients with a personal history of breast cancer. Every patient being seen by a breast surgeon, who had genetic testing in the past and no pathogenic variant was identified, should be re-evaluated

and updated testing considered. In particular, a patient who had negative germline BRCA1 and 2 testing, who is from a family with no pathogenic variants, should be considered for additional testing. Genetic testing performed prior to 2014 most likely would not have had PALB2 or other potentially relevant genes included and may not have included testing for large genomic rearrangements in BRCA1 or BRCA2."

- "Genetic testing should be made available to patients without a history of breast cancer who meet NCCN guidelines. Unaffected patients should be informed that testing an affected relative first, whenever possible, is more informative than undergoing testing themselves. When it is not feasible to test the affected relative first, then the unaffected family member should be considered for testing if they are interested, with careful pre-test counseling to explain the limited value of "uninformative negative" results. It is also reasonable to order a multi-gene panel if the family history is incomplete (i.e., a case of adoption, patient is uncertain of exact type of cancer affecting family members, among others) or other cancers are found in the family history, as described above."

American Society of Clinical Oncology

The American Society of Clinical Oncology (ASCO, 2020) published the following recommendations after a consensus conference on germline testing in prostate cancer:[12]

- "For men with metastatic PCA, broader panel testing may be appropriate, particularly if considering treatment or clinical trial options."
    - Recommended priority genes for individuals with metastatic prostate cancer include BRCA1/2 and mismatch repair genes.
    - Recommended priority gene for individuals with nonmetastatic prostate cancer is BRCA2.
    - Additional genes can be considered in either group depending upon personal or family history.
- "Reflex testing may be considered for all patients, but especially for men with nonmetastatic disease considering AS or men without PCA for early detection, which allows for initial testing of genes that inform management."

National Comprehensive Cancer Network (NCCN)

The National Comprehensive Cancer Network (NCCN) made the following general recommendations for using multi-gene panels in evaluating risk for breast and ovarian cancer and now includes this option in some management algorithms:[3-5]

- "Multi-gene testing is a new and rapidly growing field, but there is currently a lack of evidence regarding proper procedure and risk management strategies that should follow testing, especially when P/LP [pathogenic or likely pathogenic] variants are found for moderate-penetrance genes and when a VUS is found. For this reason,

the NCCN panel recommends that multi-gene testing be offered in the context of professional genetic expertise, with pre- and post-test counseling being offered." [4]

- "An individual's personal and/or family history may be explained by more than one inherited cancer syndrome; thus, phenotype-directed testing based on personal and family history through a tailored multi-gene panel test is often more efficient and cost-effective and increases the yield of detecting a P/LP variant in a gene that will impact medical management for the individual or their at-risk family members."[3]
- "There may also be a role for multi-gene testing in individuals who have tested negative for a single syndrome, but whose personal or family history remains suggestive of an inherited susceptibility."[3]
- "Because commercially available tests differ in the specific genes analyzed, variant classification, and other factors (eg methods of DNA/RNA analysis or option to reflex from a narrow to a larger panel; provision of financial assistance for cascade testing of relatives), it is important to consider the indication for testing and expertise of the laboratory when choosing the specific laboratory and test panel." [3]
- "Multi-gene testing can include "intermediate" penetrant (moderate-risk) genes. For many of these genes, there are limited data on the degree of cancer risk, and there may currently be no clear guidelines on risk management for carriers of P/LP variants. Not all genes included on available multi-gene tests will change risk management compared to that based on other risk factors such as family history." [3]
- "P/LP variants in many breast, ovarian, pancreatic, and prostate cancer susceptibility genes involved in DNA repair may be associated with rare autosomal recessive conditions, thus posing risks to offspring to offspring if the partner is also a carrier." [3]
- "As more genes are tested, there is an increased likelihood of finding variants of unknown significance (VUS), mosaicism, and clonal hematopoiesis of indeterminate potential (CHIP)." [3]
- "Multi-gene panel testing increases the likelihood of finding P/LP variants, however, some genes do not have clear clinical actionability or have a clear impact on change in medical management." [3]
- "It may be possible to refine risks associated with both moderate and high-penetrance genes, taking into account the influence of gene/gene or gene/environment interactions. In addition, certain P/LP variants in a gene may pose higher or lower risk than other P/LP variants in that same gene. This information should be taken into consideration when assigning risks and management recommendations for individuals and their at-risk relatives."[3]

NCCN Practice Guidelines for Genetic/Familial High-Risk Assessment: Colorectal (2023) stated the following regarding genetic testing:[4]

- "The introduction of multi-gene testing for hereditary forms of cancer has rapidly altered the clinical approach to testing affected patients and their families. Based on NGS technology, these tests simultaneously analyze a set of genes that are associated with a specific family cancer phenotype or multiple phenotypes."

- "When more than one gene can explain an inherited cancer syndrome, multigene testing is more efficient than single-gene testing, or sequential single syndrome testing."
- "Chances of finding a VUS or pathogenic variant with uncertain clinical management increase as the number of genes included in the multigene panel increase."
- “There is also a role for multi-gene testing in individuals who have tested negative (indeterminate) for a single syndrome, but whose personal or family history remains strongly suggestive of an inherited susceptibility.”
- “As is the case with high-risk genes, it is possible that the risks associated with moderate-risk genes may not be entirely due to that gene alone, but may be influenced by gene/gene or gene/environment interactions. In addition, certain pathogenic variants in a gene may pose higher or lower risk than other pathogenic variants in that same gene. Therefore, it may be difficult to use a known pathogenic variant alone to assign risk for relatives.”
- "In many cases, diagnosing mutations in moderate-penetrance genes does not change management compared to management based on family history alone."
- "It is for these and other reasons that multigene testing is ideally offered in the context of professional genetic expertise for pre- and post-test counseling. Individuals with the recommended expertise include certified genetic counselors, as well as clinicians who have had extensive training and/or experience in identification and management of hereditary syndromes."
- "Multi-gene testing may be preferred, particularly for patients with a strong family history or if the age of CRC diagnosis is less than 50 years."
- Germline multigene testing that “includes all polyposis and colorectal cancer genes” is preferred for the following individuals when there is no known pathogenic variants in any polyposis gene in the family:
    - “Personal history of 20 or more cumulative adenomas”
    - “Multifocal/bilateral congenital hypertrophy of retinal pigment epithelium (CHRPE)”
    - “Consider testing if a personal history of between 10-19 cumulative adenomas, desmoid tumor, hepatoblastoma, cribriform-morular variant of papillary thyroid cancer, unilateral CHRPE,or if individual meets criteria for SPS [Serrated Polyposis Syndrome] with at least some adenomas.”

NCCN Practice Guidelines for Prostate Cancer (2023) stated the following regarding genetic testing:[5]

- "If criteria are met, germline multigene testing that includes at least BRCA1, BRCA2, ATM, PALB2, CHEK2, HOXB13, MLH1, MSH2, MSH6, and PMS2 is recommended."

- "Additional genes may be appropriate depending on clinical context. For example, HOXB13 is a prostate cancer risk gene that does not have therapeutic implications in advanced disease, but testing may have utility for family counseling."
- Germline genetic testing is recommended for all men with high-risk, very-high-risk, regional (node positive), or metastatic prostate cancer.

NCCN Practice Guidelines for Cutaneous Melanoma (2023) stated the following regarding genetic testing:[13]

- “Multigene panel testing that includes CDKN2A is recommended for patients with invasive cutaneous melanoma who have a first degree relative diagnosed with pancreatic cancer.”
- “Testing other genes that can harbor melanoma-predisposing mutations may be warranted.”

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Hereditary Connective Tissue Disorder Genetic Testing

MOL.TS.268.A
v2.0.2024

Introduction

Hereditary connective tissue disorder genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Aortic Dysfunction or Dilation Duplication/ Deletion Analysis Panel	81411
Aortic Dysfunction or Dilation Genomic Sequencing Analysis Panel	81410
Hereditary Connective Tissue Disorder Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Hereditary Connective Tissue Disorder Known Familial Mutation Analysis	81403

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Criteria

Introduction

Requests for hereditary connective tissue disorder genetic testing are reviewed using these criteria.

Hereditary connective tissue disorder testing includes single genes as well as multi-gene panels, which are defined as assays that simultaneously test for more than one hereditary connective tissue disorder gene. Medical necessity determination generally relies on criteria established for testing individual genes.

Medical necessity criteria differ based on the type of testing being performed (i.e., individual hereditary connective tissue disorder genes separately chosen versus pre-defined panels of genes).

Hereditary Connective Tissue Disorder single gene tests are considered medically necessary when the following criteria are met:

- The member has or is suspected to have a condition that will benefit from information provided by the requested hereditary connective tissue disorder gene testing based on at least one of the following:
    - The member displays clinical features of the condition for which testing is being requested and a genetic diagnosis would result in changes to the member's medical management, OR
    - The member meets all criteria in a test-specific guideline, if available (see *table: Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines*), AND
- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Hereditary Connective Tissue Disorder multi-gene panels are considered medically necessary when the following criteria are met:

- Clinical documentation is provided stating that the member has, or is suspected to have, at least TWO conditions included in the panel, and medical necessity is established for these conditions based on the following:
    - The member displays clinical features of the condition for which testing is being requested, and a genetic diagnosis would result in changes to the member's medical management, OR
    - The member meets all criteria in a test-specific guideline, if available, (see table: *Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines*), AND
- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND

- Clinical features are not sufficiently specific to suggest a single causative gene, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other considerations

This guideline may not apply to genetic testing for indications that are addressed in test-specific guidelines. Please see the test-specific list of guidelines for a complete list of test-specific panel guidelines.

Broad connective tissue disorder panels may not be medically necessary when a narrower panel is available and more appropriate based on the clinical findings.

Genetic testing is only medically necessary once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be medically necessary if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

The following are not medically necessary indications for Hereditary Connective Tissue Disorder testing:

- Member's personal and/or family history are consistent with hypermobile EDS or the related clinical entity, "joint hypermobility syndrome"
- Isolated joint hypermobility, including both asymptomatic and symptomatic forms (e.g., “hypermobility spectrum disorders”)

Table: Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines

Condition, Gene, CPT, Applicable guideline

Condition	Gene	CPT	Applicable guideline
Arterial tortuosity syndrome	SLC2A10	81479	MOL.TS.268
Congenital contractural arachnodactyly	FBN2	81479	MOL.TS.268
Cutis laxa	ALDH18A1	81479	MOL.TS.268
	ATP6V0A2	81479	MOL.TS.268
	EFEMP2	81479	MOL.TS.268
	ELN	81479	MOL.TS.268

Condition	Gene	CPT	Applicable guideline
	FBLN5	81479	MOL.TS.268
	LTBP4	81479	MOL.TS.268
	PYCR1	81479	MOL.TS.268
Ehlers-Danlos syndrome (EDS)	ADAMTS2	81479	MOL.TS.267
	B3GALT6	81479	MOL.TS.267
	B4GALT7	81479	MOL.TS.267
	C1R	81479	MOL.TS.267
	C1S	81479	MOL.TS.267
	CHST14	81479	MOL.TS.267
	COL1A1	81408	MOL.TS.267
	COL1A2	81408	MOL.TS.267
	COL12A1	81479	MOL.TS.267
	COL3A1	81479	MOL.TS.267
	COL5A1	81479	MOL.TS.267
	COL5A2	81479	MOL.TS.267
	DSE	81479	MOL.TS.267
	FKBP14	81479	MOL.TS.267
	PLOD1	81479	MOL.TS.267
	PRDM5	81479	MOL.TS.267
	SLC39A13	81479	MOL.TS.267

Condition	Gene	CPT	Applicable guideline
	TNXB	81479	MOL.TS.267
	ZNF469	81479	MOL.TS.267
FLNA deficiency (periventricular nodular heterotopia)	FLNA	81479	MOL.TS.268
Homocystinuria (cystathionine beta-synthase deficiency)	CBS	81401 81406	MOL.TS.268
Juvenile polyposis/hereditary hemorrhagic telangiectasia	SMAD4	81406	MOL.TS.268
	SMAD4	81405	MOL.TS.268
Loeys-Dietz syndrome	SMAD3	81479	MOL.TS.268
	SMAD2	81479	MOL.TS.268
	TGFB2	81479	MOL.TS.268
	TGFB3	81479	MOL.TS.268
	TGFBR1	81405	MOL.TS.268
	TGFBR2	81405	MOL.TS.268
MED12-related disorders	MED12	81401 81479	MOL.TS.268
Marfan syndrome	FBN1	81408	MOL.TS.202
	TGFBR1	81405	MOL.TS.202
	TGFBR2	81405	MOL.TS.202
NOTCH1-related aortic valve disease/ Adams-Oliver syndrome	NOTCH1	81407	MOL.TS.268

Condition	Gene	CPT	Applicable guideline
Occipital horn syndrome/Menkes	ATP7A	81479	MOL.TS.268
Osteogenesis imperfecta	COL1A1	81408	MOL.TS.268
	COL1A2	81408	MOL.TS.268
Pseudoxanthoma elasticum	ABCC6	81479	MOL.TS.268
Shprintzen-Goldberg syndrome	SKI	81479	MOL.TS.268
Stickler syndrome	COL11A1	81479	MOL.TS.268
	COL11A2	81479	MOL.TS.268
	COL2A1	81479	MOL.TS.268
	COL9A1	81479	MOL.TS.268
	COL9A2	81479	MOL.TS.268
	COL9A3	81479	MOL.TS.268
Thoracic aortic aneurysm and dissection (TAAD)	ACTA2	81405	MOL.TS.227
	BGN	81479	MOL.TS.227
	COL3A1	81479	MOL.TS.227
	FBN1	81408	MOL.TS.227
	LOX	81479	MOL.TS.227
	MAT2A	81479	MOL.TS.227
	MFAP5	81479	MOL.TS.227
	MYH11	81408	MOL.TS.227

Condition	Gene	CPT	Applicable guideline
	MYLK	81479	MOL.TS.227
	PRKG1	81479	MOL.TS.227
	SMAD3	81479	MOL.TS.227
	TGFB2	81479	MOL.TS.227
	TGFB3	81479	MOL.TS.227
	TGFBR1	81405	MOL.TS.227
	TGFBR2	81405	MOL.TS.227

Note Several genes in this table are associated with multiple genetic disorders, including some not listed above. The test should be reviewed for the appropriate condition/indication.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- Broad connective tissue disorder panels are not reimbursable when a narrower panel is available and more appropriate based on the clinical findings.
- When otherwise reimbursable, the following limitations apply:
    - Both the sequencing and deletion/duplication components of genetic testing for clinically indicated gene(s) will be reimbursed.

- When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81410 or 81479*).
- When use of a panel code is not possible, each billed component procedure will be assessed independently.
- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
- Procedure codes representing multiple methods for deletion/duplication testing will not be reimbursable for the same panel. When deletion/duplication testing is not part of a single panel CPT code being billed, deletion/duplication testing is reimbursable in only one of the following ways:
  - A single CPT code specific to the performed deletion/duplication analysis panel (e.g. 81411, 81479), or
  - A single microarray procedure (e.g. 81228 or 81229)

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are hereditary connective tissue disorders?

Definition

Hereditary connective tissue disorders (HCTDs) are a group of disorders that affect the connective tissues that support the skin, bones, joints, heart, blood vessels, eyes, and other organs.[1]

- While specific features vary by type, an unusually large range of joint movement (hypermobility) and cardiovascular disease (such as thoracic aortic aneurysms and dissections, or TAAD) are features that are present in many HCTDs. Medical management may differ based on the underlying genetic etiology.
- In many cases, a careful clinical examination by a specialist familiar with clinical features of these conditions can help to point toward one condition or group of conditions. In these cases, testing for gene(s) associated with a single condition or group of conditions would be most appropriate. However, in some cases, it can be difficult to reliably diagnose an HCTD based on clinical and family history alone.
- Although connective tissue disorders as a whole are common, individual hereditary connective tissue disorders are relatively uncommon.[1]

- There are more than 200 connective tissue disorders.[2] Some of the most common types are summarized below:

  - **Arterial tortuosity syndrome (ATS)** — An autosomal recessive disorder associated with severe and widespread tortuosity of the aorta and middle-sized arteries, with an increased risk of aneurysms and dissections. Other features include stenosis of the aorta and/or pulmonary arteries, characteristic facies with high palate and dental crowding, and soft/doughy skin. Additional connective tissue disorder features that may be present include skeletal findings (scoliosis, pectus anomalies, joint laxity), hernias, hypotonia, and ocular involvement (myopia, keratoconus). SLC2A10 is the only gene known to be associated with ATS. Sequence variants are the most common; exon deletions have been reported in a couple cases.[3]
  - **Congenital contractural arachnodactyly (CCA) (Beals syndrome)** — An autosomal dominant disorder characterized by a Marfan-like appearance (tall, slender habitus in which arm span exceeds height) and long, slender fingers and toes (arachnodactyly). Most affected individuals have a “crumpled” appearance to their ears and most have contractures of major joints (knees and ankles) at birth. Hip contractures, adducted thumbs, and club foot may occur. The majority of affected individuals have muscular hypoplasia. Kyphosis/scoliosis is present in about half of all affected individuals. Dilatation of the aorta is occasionally present. FBN2 is the only gene in which pathogenic variants are known to cause congenital contractural arachnodactyly, "however, locus heterogeneity is likely given that only 25%-75% of individuals with clinically diagnosed CCA have an identifiable FBN2 pathogenic variant."[4]
  - **Cutis laxa** — A group of disorders characterized by lax, sagging skin that often hangs in loose folds, causing the face and other parts of the body to have a droopy appearance. Extremely wrinkled skin may be particularly noticeable on the neck and in the armpits and groin. Other features may include arterial aneurysm and dissection, emphysema, and inguinal or umbilical hernia. There are autosomal dominant, autosomal recessive, and X-linked forms. Causative autosomal genes include ELN, FBLN5, ATP6V0A2, EFEMP2, ALDH18A1, PYCR1, and LTBP4.[5,6] The X-linked form is due to mutations in ATP7A (see also Occipital Horn Syndrome).[5]
  - **Ehlers Danlos syndromes (EDS)** — A heterogeneous group of disorders, the majority of which share the features of joint hypermobility and skin involvement. There are 13 types: classical, classical-like, cardiac-valvular, vascular, hypermobile (includes “joint hypermobility syndrome”), arthrochalasia, dermatosparaxis, kyphoscoliotic, spondylodysplastic, musculocontractural, myopathic, periodontal, and brittle cornea syndrome. Some types have autosomal dominant inheritance, while others are autosomal recessive. Hypermobile type is the most common, but its genetic etiology is currently unknown. Genetic testing is available for the other EDS types (see Table: *Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines* for a list of genes).[7,8]

- **Homocystinuria due to cystathionine beta-synthase deficiency** — An autosomal recessive metabolic disorder in which affected individuals have markedly elevated plasma total homocysteine and methionine. Clinical features include involvement of the eye (ectopia lentis and/or severe myopia), skeletal system (excessive height, long limbs, scoliosis, and pectus excavatum), and vascular system (thromboembolism). Many have developmental delay/intellectual disability. Treatment involves maintenance of normal or near-normal plasma homocysteine concentrations using a specialized diet and vitamin supplementation. The diagnosis can be substantiated by detection of biallelic pathogenic mutations in the CBS gene. Sequence analysis detects 95-98% of mutations, while deletion/duplication analysis detects <5%.[9]
- **Loeys-Dietz syndrome (LDS)** — LDS is an autosomal dominant disorder that affects many parts of the body.[10] LDS is caused by mutations in six genes: TGFBR2 (55-60%), TGFBR1 (20-25%), SMAD3 (5-10%), TGFB2 (5-10%), TGFB3 (1-5%), or SMAD2 (1-5%). Major manifestations of this condition include "vascular findings (cerebral, thoracic, and abdominal arterial aneurysms and/or dissections), skeletal manifestations (pectus excavatum or pectus carinatum, scoliosis, joint laxity, arachnodactyly, talipes equinovarus, cervical spine malformation and/or instability), craniofacial features (widely spaced eyes, strabismus, bifid uvula / cleft palate, and craniosynostosis that can involve any sutures), and cutaneous findings (velvety and translucent skin, easy bruising, and dystrophic scars)."[10] Given that there is no clinical diagnostic criteria established for LDS, genetic testing, either through serial single-gene testing or use of a multigene panel, can establish the diagnosis.[10]
- **Marfan syndrome (MFS)** — MFS is an autosomal dominant disorder that affects connective tissue in many parts of the body.[11] MFS is caused by mutations in the FBN1 gene. Up to 93% of people meeting diagnostic criteria for MFS will have a mutation in this gene. Diagnostic criteria, called the Ghent criteria, exist for MFS. Major manifestations of the disease include aortic enlargement and ectopia lentis. Other features include, but are not limited to, bone overgrowth and joint laxity, long arms and legs, scoliosis, sternum deformity (pectus excavatum or carinatum), long thin fingers and toes, dural ectasia (stretching of the dural sac), hernias, stretch marks on the skin, and lung bullae. Symptoms can present in males or females at any age. Symptoms typically worsen over time. Infants who present with symptoms typically have the most severe disease course.[11]
- **NOTCH1-related aortic valve disease** — NOTCH1 variants can be associated with autosomal dominant congenital heart defects affecting the left ventricular outflow tract (LVOT), most commonly bicuspid aortic valve (BAV). Adult-onset aortic valve calcification is a frequent feature. NOTCH1 variants have also been identified in 4.2% of individuals with sporadic BAV and much less frequently with other LVOT malformations. Mutations in this gene are also associated with Adams-Oliver syndrome, which is characterized by aplasia cutis congenita of the scalp and malformations of the limbs, brain, and cardiovascular system.[12]

- **Osteogenesis imperfecta (OI)** — A group of disorders associated with a propensity to fractures with little or no trauma. Additional features may include skeletal anomalies, short stature, hearing loss, and blue/gray sclera. The severity is highly variable, ranging from a mild form with few fractures and normal life expectancy, to severe forms with neonatal lethality. OI types I-IV account for the majority of cases, and are caused by heterozygous mutations in the COL1A1 and COL1A2 genes. Inheritance is autosomal dominant. Autosomal recessive forms of OI are rare, and can be associated with mutations in a number of different genes.[13]
- **FLNA Deficiency** — FLNA deficiency is associated with a phenotypic spectrum that includes FLNA-related periventricular nodular heterotopia (PVNH). FLNA deficiency is an X-linked condition that is prenatally or neonatally lethal in most males. Therefore, most affected individuals are female. In addition to PVNH, some individuals have connective tissue anomalies such as joint hypermobility, aortic dilation, and other vascular anomalies. 90% of individuals with FLNA-related PVNH have a sequence variant; about 10% of probands have a variant detected by deletion/duplication analysis.[14]
- **Stickler syndrome** — A disorder characterized by ocular findings (myopia, cataract and retinal detachment), hearing loss, craniofacial findings (midfacial underdevelopment and cleft palate), mild spondyloepiphyseal dysplasia and/or early-onset arthritis. Clinical diagnostic criteria are available. Greater than 90% of cases are due to mutations in COL2A1 or COL11A1. Mutations in these genes are inherited in an autosomal dominant pattern. Mutations in COL9A1, COL9A2, and COL9A3 are rare, and inherited in an autosomal recessive pattern.[15]
- **Thoracic Aortic Aneurysm and Dissection (TAAD)** — Familial TAAD is defined as dilatation and/or dissection of the thoracic aorta, absence of clinical features of MFS, LDS or vascular EDS, and a positive family history of TAAD. Approximately 30% of families with heritable thoracic aortic disease (HTAD) who do not have a clinical diagnosis of MFS or another syndrome have a causative mutation in one of 15 known HTAD-related genes (see the Table: Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines).[16]

Test information

Introduction

Testing for hereditary connective tissue disorders may include next-generation sequencing or multigene panels.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene

sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Clinical genetic testing is available for many HCTDs. However, hypermobile EDS (hEDS), joint hypermobility syndrome, and isolated joint hypermobility, including "hypermobility spectrum disorders", continue to require a clinical diagnosis, since the genetic etiology of these disorders is not yet known.[8]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to hereditary connective tissue disorder genetic testing.

- No current U.S guidelines address the use of multi-gene panels in HCTDs.
- An expert-authored review (updated in 2018)[17] stated the following regarding hEDS: "If an individual's personal or family history is suggestive of one of the other types of EDS or another hereditary disorder of connective tissue or arterial fragility syndrome..., analysis of an associated gene or multigene connective tissue disease panel may be appropriate. Failure to identify a pathogenic variant with such multiple gene testing reduces the likelihood of an arterial fragility syndrome, but does not completely rule it out, especially in the setting of a positive personal or family history of arterial fragility. Negative testing for an arterial fragility syndrome also does not confirm a diagnosis of hEDS. Therefore, such testing is not recommended in the absence of specific suggestive signs, symptoms, or family history."
- According to the International Consortium on the Ehlers-Danlos Syndromes (2017):[8]
    - "In view of the vast genetic heterogeneity and phenotypic variability of the EDS subtypes, and the clinical overlap between many of these subtypes, but also with other HCTDs, the definite diagnosis relies for all subtypes, except hEDS, on

molecular confirmation with identification of (a) causative variant(s) in the respective gene."

- "Molecular diagnostic strategies should rely on NGS technologies, which offer the potential for parallel sequencing of multiple genes. Targeted resequencing of a panel of genes…is a time- and cost-effective approach for the molecular diagnosis of the genetically heterogeneous EDS. When no mutation (or in case of an autosomal recessive condition only one mutation) is identified, this approach should be complemented with a copy number variant (CNV) detection strategy to identify large deletions or duplications, for example Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR, or targeted array analysis."
- "The diagnosis of hEDS remains clinical as there is yet no reliable or appreciable genetic etiology to test for in the vast majority of patients."

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HFE Hemochromatosis Genetic Testing

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Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
HFE Targeted Mutation Analysis (common variants)	81256
HFE Sequence Analysis	81479
HFE Deletion/Duplication Analysis	81479

Criteria

HFE known familial mutation testing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of the HFE gene that would detect the known familial mutation, AND
- Presymptomatic/Asymptomatic Genetic Testing:
  - HFE mutation(s) identified in 1st degree biological relative, OR
- Diagnostic Testing:
  - HFE mutation(s) identified in 1st degree biological relative, and
  - Serologic evidence of iron overload (e.g., a transferrin saturation greater than or equal to 45% and/or elevated ferritin), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

HFE targeted mutation testing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of the HFE gene, AND
- Presymptomatic/Asymptomatic Genetic Testing:
  - Documented family history of first-degree relative with HFE hemochromatosis, OR
- Diagnostic Testing:
  - Serologic evidence of iron overload (e.g., transferrin saturation greater than or equal to 45% and/or elevated ferritin), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

HFE gene sequence analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous sequencing of the HFE gene, and
  - Previous targeted HFE genetic testing performed and zero or one mutation identified, AND
- Diagnostic Testing:
  - Serologic evidence of iron overload (e.g., transferrin saturation greater than or equal to 45% and/or elevated ferritin), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

HFE deletion/duplication analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous deletion/duplication analysis of the HFE gene, and

- Previous HFE sequencing performed and zero or one mutation identified, AND

- Diagnostic Testing:
  - Serologic evidence of iron overload (e.g., transferrin saturation greater than or equal to 45% and/or elevated ferritin), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is HFE hemochromatosis?

Definition

HFE hemochromatosis is a disorder marked by high absorption of iron by the mucosa of the small intestine.[1]

Prevalence

About 1 in 200 to 1 in 400 non-Hispanic whites in North America are affected with HFE hemochromatosis.[2] The disorder is less common among African Americans, Hispanics, and Asians.[1]

Symptoms

There is a phenotypic spectrum of HFE hemochromatosis.[1]

- Clinical HFE hemochromatosis: individuals manifest end-organ damage secondary to iron overload.
- Biochemical HFE hemochromatosis: individuals have increased transferrin-iron saturation, but “the only evidence of iron overload is increased serum ferritin concentration.” [1]
- Non-expressing C282Y homozygotes: individuals with two copies of the HFE mutation C248Y have neither clinical manifestations of disease nor iron overload.

Individuals who are untreated may experience the following symptoms: abdominal pain, weakness, lethargy, weight loss, arthralgias, diabetes mellitus, and increased risk of cirrhosis when the serum ferritin is higher than 1,000 ng/mL.[1] Other findings may include progressive increase in skin pigmentation, congestive heart failure, and/or arrhythmias, arthritis, and hypogonadism.[1] Clinical HFE hemochromatosis is more common in men than women.[1]

HFE hemochromatosis is typically an adult-onset condition.[1] Juvenile forms of hereditary hemochromatosis exist, but are caused by other genes, and testing for these forms of hemochromatosis is not addressed by this guideline.

Cause

HFE hemochromatosis is caused by pathogenic mutations in the HFE gene that lead to excess iron absorption and storage in the liver, heart, pancreas, and other organs.[1]

Inheritance

HFE hemochromatosis is inherited in an autosomal recessive manner.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

When HFE hemochromatosis is suspected, serum iron studies, including transferrin saturation (TS), serum ferritin (SF), and unsaturated iron-binding capacity (UIBC), are the first step in establishing a diagnosis. HFE genetic testing is recommended if TS is greater than or equal to 45%.[3-5]

Current guidelines support HFE genetic testing in individuals with: [2,4-7]

- Serologic evidence of iron overload, considered to be a transferrin saturation greater than or equal to 45% and elevated ferritin
- A known family history of hemochromatosis
- A known family mutation in the HFE gene in a first degree relative

Common changes in the HFE gene associated with HFE hemochromatosis are C282Y, H63D, and S65C.[1]

C282Y and H63D are the most common and account for 87% of hereditary hemochromatosis in European populations.[1] The next most common cause are individually rare mutations.[8] Many labs do not test for S65C because it accounts for <1% of HFE hemochromatosis.[1] There is controversy over whether the H63D variant causes clinical disease.[2,9] The combination of these mutations determines both the chances of symptoms occurring and their severity.

Management

HFE hemochromatosis can be effectively treated in most people. Phlebotomy therapy can alleviate almost all symptoms of iron overload if initiated before organ damage occurs.[10]

Survival

Untreated HFE hemochromatosis may result in reduced lifespan due to congestive heart failure and other cardiac manifestations or end-stage liver disease.[1]

Test information

Introduction

Testing for HFE hemochromatosis may include known familial mutation analysis, targeted mutation analysis, next-generation sequencing, or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

HFE sequencing and deletion/duplication analysis may be necessary for individuals who do not have northern European ancestry.[1]

Guidelines and evidence

American Association for the Study of Liver Disease

The American Association for the Study of Liver Diseases (AASLD, 2011) Practice Guidelines stated:[9]

- "In a patient with suggestive symptoms, physical findings, or family history, a combination of transferrin saturation (TS) and ferritin should be obtained rather than relying on a single test. (1B) If either is abnormal (TS ≥45% or ferritin above the upper limit of normal), then HFE mutation analysis should be performed. (1B)"
- "The guideline developers recommend screening (iron studies and HFE mutation analysis) of first-degree relatives of patients with HFE-related HH to detect early disease and prevent complications."

American College of Gastroenterology

The American College of Gastroenterology (ACG, 2019) Clinical Guideline on Hereditary Hemochromatosis (called HH in this document) stated:[5]

- "We recommend that family members, particularly first-degree relatives, of patients diagnosed with HH should be screened for HH (strong recommendation, moderate quality of evidence)."
- "We recommend that individuals with the H63D or S65C mutation in the absence of C282Y mutation should be counseled that they are not at increased risk of iron overload (conditional recommendation, very low quality of evidence)."
- "We suggest against further genetic testing among patients with iron overload who tested negative for the C282Y and H63D alleles (conditional recommendation, very low quality of evidence)."
- "[G]enotyping for HFE mutations (C282Y) is now a standard part of the evaluation of patients in whom HH is suspected on clinical grounds or based on the finding of elevated iron studies."

American College of Physicians

The American College of Physicians (ACP, 2005) clinical practice guideline stated:[10]

- "Physicians should discuss the risks, benefits, and limitations of genetic testing in patients with a positive family history of hereditary hemochromatosis or those with elevated serum ferritin level or transferrin saturation. Before genetic testing, individuals should be made aware of the benefits and risks of genetic testing. This should include discussing available treatment and its efficacy; costs involved; and

social issues, such as impact of disease labeling, insurability and psychological well-being, and the possibility of as-yet-unknown genotypes associated with hereditary hemochromatosis."

European Association for the Study of the Liver

The European Association for the Study of the Liver (EASL, 2022) Clinical Practice Guidelines on haemochromatosis stated:[11]

- "Individuals with clinical and biochemical signs of haemochromatosis, elevated transferrin saturation and high serum ferritin concentration, or otherwise unexplained persistently elevated transferrin saturation should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained (level of evidence 2, strong recommendation, strong consensus)."
- "Adult individuals with a positive family history of first-degree relatives with haemochromatosis should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained (level of evidence 4, string recommendation, strong consensus)."

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Hereditary Pancreatitis Genetic Testing

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Introduction

Genetic testing for hereditary pancreatitis is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
CFTR Deletion/Duplication Analysis	81222
CFTR Known Familial Mutation Analysis	81221
CFTR Sequencing	81223
Hereditary Pancreatitis Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Hereditary Pancreatitis Multigene Panel	81479

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Criteria

Introduction

Requests for genetic testing for hereditary pancreatitis are reviewed using the following criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, and
  - Pathogenic pancreatitis-associated mutation(s) in a 1st degree biologic relative, AND
- Diagnostic Testing in Symptomatic Individuals:
  - Member is symptomatic (at least one documented episode of acute pancreatitis or a diagnosis of recurrent acute or chronic pancreatitis), OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Age 16 years or older

PRSS1 Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous PRSS1 analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - An unexplained, documented episode of acute pancreatitis in an individual less than 18 years of age, or
  - Recurrent acute pancreatitis (2 or more documented episodes) or chronic pancreatitis, and
    - Symptom onset prior to age 25 years, and/or

        - A first degree biologic relative with recurrent acute pancreatitis, idiopathic chronic pancreatitis, or childhood pancreatitis (less than 18 years of age) without a known cause, and
    - No known etiology for the member's pancreatitis (e.g. alcoholism, gallstones, known genetic disorder), and
    - Absence of extra-pancreatic features suggestive of a complex genetic syndrome or cystic fibrosis (e.g. chronic sinopulmonary disease, failure-to-thrive, obstructive azoospermia due to congenital absence of the vas deferens, etc.), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Pancreatitis Multi-Gene Panel

When a multi-gene panel is being requested and will be billed with the appropriate CPT panel code, the panel will be considered medically necessary when the following criteria are met:

- Genetic Counseling:
    - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
    - No previous multi-gene analysis, and
    - PRSS1 analysis, if previously performed, was negative, AND
- Diagnostic Testing for Symptomatic Individuals:
    - An unexplained, documented episode of acute pancreatitis in an individual less than 18 years of age, or
    - Recurrent acute pancreatitis (2 or more documented episodes) or chronic pancreatitis, and
        - Symptom onset prior to age 25 years, or
        - A first degree biologic relative with recurrent acute pancreatitis, idiopathic chronic pancreatitis, or childhood pancreatitis (less than 18 years of age) without a known cause, and
    - No known etiology for the member's pancreatitis (e.g., alcoholism, gallstones, known genetic disorder), and
    - Absence of extra-pancreatic features suggestive of a complex genetic syndrome or cystic fibrosis (e.g., chronic sinopulmonary disease, failure-to-thrive, obstructive azoospermia due to congenital absence of the vas deferens, etc.), AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CLDN2, PNLIP, and CEL Analysis

Individual testing of these genes for the purpose of diagnosing hereditary pancreatitis is not medically necessary.

Other considerations

Broad gastrointestinal disease panels may not be medically necessary when a narrower panel is available and more appropriate based on the clinical findings.

This guideline addresses testing for non-syndromic hereditary pancreatitis. For information on testing for syndromes that may include pancreatitis as part of a more complex phenotype (e.g. Schwachman-Diamond syndrome, CEL-related MODY, mitochondrial disorders, Johanson-Blizzard syndrome) please refer to appropriate guidelines (e.g. *Maturity-Onset Diabetes of the Young (MODY) Genetic Testing* or *Mitochondrial Disorders Genetic Testing*) or applicable clinical use guidelines, if available. For information on CFTR analysis for individuals suspected of having Cystic Fibrosis please refer to the guideline *Cystic Fibrosis Testing*, as this is not addressed here.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- CLDN2, PNLIP, and CEL Analysis are not separately reimbursable.
- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81479*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.

- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
- When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement in a tiered fashion:
  - PRSS1
  - SPINK1
  - CFTR
  - CTRC

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is pancreatitis?

Definition

Pancreatitis is inflammation of the pancreas that may be acute, recurrent acute, or chronic.[1]

Prevalence

PRSS1 mutations are identified in 5-7% of individuals with chronic pancreatitis.[2] In one US study of children, PRSS1 mutations were identified in 46% of those diagnosed with chronic pancreatitis and 17% of those with recurrent acute pancreatitis.[2]

Symptoms

Acute pancreatitis is defined as two of the three following findings:[2]

- Abdominal pain
- Elevated serum amylase or lipase (greater than 3x the upper limit of normal)
- Findings consistent with pancreatic inflammation on abdominal imaging.

Recurrent acute pancreatitis is defined as multiple (2 or more), discrete episodes of acute pancreatitis without any evidence of chronic pancreatitis.[2] There must be complete resolution of clinical and laboratory findings between episodes.

Chronic pancreatitis (CP) is a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress.[3] Common features of established and advanced CP include:

- Pancreatic exocrine dysfunction
- Pancreatic endocrine dysfunction and dysplasia.

Up to 5% of patients with chronic pancreatitis may develop pancreatic cancer.[4] The efficacy of pancreatic cancer screening has not been proven, and this screening is typically recommended to take place in a research setting.[4]

Cause

Idiopathic sporadic pancreatitis occurs when a single individual in a family is affected, and the etiology is unknown despite comprehensive investigations.

Familial pancreatitis is pancreatitis of any cause (genetic or non-genetic) that occurs in a family with a greater incidence than would be expected by chance alone.[1]

Hereditary pancreatitis (HP) is a rare cause of acute, recurrent acute, and chronic pancreatitis. It is defined as a personal history of pancreatitis and pancreatitis diagnosed in two first-degree relatives or in three second degree relatives spanning at least two generations. Beginning with the first report of a PRSS1 mutation in a family with HP, it has been shown that multiple genetic risk factors are associated with this disease.[5]

Mutations in the following genes contribute to the development of recurrent acute and chronic pancreatitis:[1]

- PRSS1 mutations are the most common cause of hereditary pancreatitis.[1,2] Mutations in this gene follow autosomal dominant inheritance and have a penetrance of approximately 40-93%, depending on the variant.[1,2]
    - The mutation detection rate for PRSS1 sequencing is approximately 94%, and deletion/duplication analysis is at least 6%.[2] N29I (p.Asn29Ile) and R122H (p.Arg122His) variants account for approximately 90% of cases of pathogenic variants observed in PRSS1-related HP.[2] Test results particularly for the PRSS1 gene, may offer prognostic information since the risk of pancreatic cancer in those with chronic pancreatitis is significantly increased.
- SPINK1 mutations may increase the severity of recurrent acute and chronic pancreatitis due to mutations in PRSS1, CFTR, CASR, or CTRC.[1,6] The majority of SPINK1 mutations are sequence variants, with deletions having been reported in a very small number of cases.[1]
- CFTR mutations are risk factors for pancreatitis. Individuals with biallelic CFTR mutations may have atypical cystic fibrosis (CF), putting them at risk for additional manifestations such as lung disease, male infertility, and chronic sinusitis.[1] The

frequency of CFTR deletions in HP has not been investigated; however they occur rarely in cystic fibrosis (approximately 1%).[1]

- CTRC mutations have been identified in individuals with recurrent acute and chronic pancreatitis. These variants were initially thought to be modifier genes, however, they have been shown to be sufficient to cause disease without other identifiable genetic or environmental risk factors.[7]
- CASR mutations may be a predisposing genetic factor for pancreatitis either in isolation or as modifying risk when other genetic causes are present.[8]
- CLDN2, CPA1, and GGT1 variants have been implicated as risk factors or modifiers for chronic pancreatitis, but less is known about the utility of screening for these mutations compared to the others mentioned above.[1,8]
- TRPV6 mutations have been reported in patients with early-onset CP not associated with alcohol consumption.[1] In a recent study, 20% patients with functionally defective TRPV6 variants also had the SPINK1 p.N34S variant.[9]
- CEL and PNLIP variants may result in an increased risk of developing pancreatitis as mutations in these genes are enriched in chronic pancreatitis patient populations. However, current data remains limited and the clinical utility of screening for these genetic variants is uncertain.[1,9]
- Rare disorders that include pancreatitis/pancreatic insufficiency as part of a more complex syndrome include Schwachman-Diamond syndrome (SBDS), mitochondrial DNA deletions, CEL-associated maturity-onset diabetes of the young (MODY), and Johanson-Blizzard syndrome (UBR1).[1]

Genes included on hereditary pancreatitis multi-gene panels may not be causative or associated with high risk for pancreatitis (e.g.: CLDN2).[1]

Inheritance

While single pathogenic variants in SPINK1, CFTR, and CTRC are associated with an increased risk of recurrent acute or chronic pancreatitis, additional unidentified modifying factors may contribute to the disease.[1] These include alcohol use, smoking, chronic kidney disease, autoimmune factors, and anatomic issues.[1] Individuals with multiple risk factors (including multiple gene mutations) have a higher risk for pancreatitis.[1]

Biallelic variants of SPINK1 have been reported to result in early onset pancreatitis, suggesting an autosomal recessive pattern of inheritance with reduced penetrance.[1]

The rare disorders of which pancreatitis is a feature have varying patterns of inheritance.

Diagnosis

Pancreatitis is diagnosed by one of the following:[1-3]

- Abdominal imaging

- Functional studies (e.g. pancreatic exocrine insufficiency or pancreatic endocrine insufficiency with diabetes mellitus)
- Histology

Genetic testing results provide important early information about the etiology of pancreatitis-related disorders.[3] Determining the etiology of a pancreatitis-related disorder may not lead to immediate treatment in some cases, but it often ends exhaustive, invasive, and expensive diagnostic testing for advanced disease. Understanding the genetic etiology also informs decisions about therapy for persistent or severe disease, such as total pancreatectomy with islet autotransplantation.[3] However, genetic testing cannot predict the age of onset or disease severity.[1,4]

Management

Treatment of HP focuses on longitudinal monitoring of endocrine and exocrine pancreatic function, enzyme and nutritional supplementation, pain management and monitoring for complications (such as decreased bone mineral density and fat-soluble vitamin deficiencies). Endoscopic and surgical therapies may be necessary in some cases. Affected individuals are discouraged from smoking and drinking alcohol.[1]

Survival

In a relatively small study of PRSS1 mutation carriers, overall survival did not differ significantly from that of the general US Caucasian population.[10] Pancreatic cancer rates were higher and contributed to mortality.

Test information

Introduction

Testing for hereditary pancreatitis may include known familial mutation analysis, next generation sequencing, and/or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis

detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to genetic testing for hereditary pancreatitis.

American College of Gastroenterology

The American College of Gastroenterology (ACG, 2013) guideline on management of acute pancreatitis stated: “Genetic testing may be considered in young patients (<30 years old) if no cause is evident and a family history of pancreatic disease is present (conditional recommendation, low quality of evidence).” [11]

The ACG (2015) guidelines on genetic testing for hereditary gastrointestinal cancer syndromes stated that having a history of hereditary pancreatitis is a risk factor for familial pancreatic adenocarcinoma, and genetic testing for pancreatitis-associated genes should be considered for pancreatic cancer patients with “a personal history of at least 2 acute attacks of acute pancreatitis of unknown etiology, a family history of pancreatitis, or early-age onset chronic pancreatitis.”[12]

The ACG (2020) guideline on chronic pancreatitis recommended genetic testing in patients with clinical evidence of a pancreatitis-associated disorder or possible CP in which the etiology is unclear, especially in younger patients.[2]

American Pancreatic Association

American Pancreatic Association (APA, 2014) guidelines stated: “Several genetic variations have been associated with pancreatitis including PRSS1, PRSS2, SPINK1, CTRC, CASR, and CFTR. The role of these gene mutations in CP is becoming increasingly recognized and better understood.” It is also noted that “knowledge of gene, gene-environment interactions may translate into new diagnostic and treatment paradigms” (Strong recommendation, level of evidence – moderate).[13]

Fourth International Symposium of Inherited Diseases of the Pancreas

The Fourth International Symposium of Inherited Diseases of the Pancreas (2007) recommended symptomatic patients be referred for genetic counseling to consider PRSS1 testing when at least one of the following conditions are met, in order to determine if they may be candidates for pancreatic cancer surveillance:[14]

- "≥2 attacks of acute pancreatitis of unknown aetiology"
- "Idiopathic chronic pancreatitis, particularly if disease onset occurs <25 years of age"
- "One first-degree or second-degree relative with pancreatitis"
- "Unexplained documented episode of childhood pancreatitis that required hospitalization and where there is concern that HP should be excluded."
- "Asymptomatic people should be referred for genetic counseling to consider testing for a PRSS1 mutation when the patient has one first-degree relative with a defined HP gene mutation."

United European Gastroenterology

United European Gastroenterology (UEG, 2018) guidelines on chronic pancreatitis stated:[15]

- "A diagnosis of cystic fibrosis needs to be ruled out in all patients with CP onset before the age of 20 years as well as in patients with so-called 'idiopathic' CP (regardless of the age of onset). (GRADE 1B, strong agreement)"
- "All patients with a family history or early onset disease (less than 20 years) should be offered genetic testing for associated variants. (GRADE 2C, strong agreement)"
- "Genetic testing was recommended to include PRSS1, SPINK1, CPA1, CTRC, CEL, and "may include screening for variants in CFTR. (GRADE 2C, strong agreement)"

Select Relevant Publications

2007 Expert Authored Review

A 2007 expert-authored guideline on non-syndromic pancreatitis stated genetic testing should be considered when an affected patient fulfills at least one of the following criteria:[16]

- "A family history of recurrent acute pancreatitis, idiopathic chronic pancreatitis, or childhood pancreatitis without a known cause"
- "Relatives known to carry mutations associated with pancreatitis"
- "A series of recurrent acute attacks of pancreatitis for which there is no other explanation"

- "An unexplained documented episode of pancreatitis as a child"
- "Idiopathic chronic pancreatitis (especially when onset of pancreatitis precedes age 25)"
- "Patients eligible for approved research protocols"
- "[...] symptomatic family members at risk of inheriting a PRSS1 mutation may wish to be tested after a mutation has been identified in the family…Testing asymptomatic individuals for CFTR and SPINK1 mutations is not recommended because a large fraction of those who carry mutations in these genes never develop pancreatitis. CFTR carrier testing should be offered to unaffected relatives of a CFTR mutation that is capable of causing classic CF."

2007 Expert Authored Review

A 2007 expert-authored review on hereditary pancreatitis recommended PRSS1 and SPINK1 mutation testing in symptomatic patients with one of the following:[17]

- "recurrent unexplained attacks of acute pancreatitis and positive family history"
- "unexplained chronic pancreatitis and a positive family history"
- "unexplained chronic pancreatitis without a positive family history after exclusion of other causes"
- "unexplained pancreatitis episode in children"

2010 Expert Authored Review

A 2010 expert-authored review on genetic testing in pancreatitis stated:[18]

- "Because of the high penetrance (80%) of the more common PRSS1 mutations, especially R112H and N29I, testing is generally accepted for diagnostic purposes in symptomatic individuals. The confirmation of a genetic etiology of pancreatitis provides a valid explanation for both symptoms and/or disease, and may be helpful to predict a lack of efficacy with various endoscopic or operative procedures."
- "[T]here is currently no clinical indication for the routine use of SPINK1 mutation testing for either diagnostic or screening purposes and has no implications in altering the management of patients with pancreatitis."
- "[T]he CTRC gene that is the most recently identified pancreatitis susceptibility gene, should be approached in a similar fashion to SPINK1 as it is also associated with a very low penetrance."
- Regarding testing for CFTR mutations, "In subjects presenting with pancreatitis, the overwhelming rationale for further testing is to exclude or confirm the diagnosis of CF [cystic fibrosis]. The traditional sweat test remains the primary diagnostic test for CF disease in the genomic age. In any symptomatic individual, diagnostic testing should include sweat testing as the primary test

and referral to a CF clinic made if sweat chloride concentration is borderline (40-59 mmol/L) or abnormal (>60 mmol/L). CFTR mutation analysis in isolation, as the first-line clinical diagnostic test, is unlikely to change management but may instead give false reassurance of the absence of CF if CFTR genotyping fails to identify mutations or alternatively be inappropriately thought to be diagnostic of CF... [T]here is currently no rationale for CFTR mutation screening for risk of pancreatitis alone."

2016 Expert Authored Review

A 2016 expert-authored review on hereditary pancreatitis stated:[19]

- "[…] targeted genetic testing of members of an established HP family may be considered in cases of unexplained [recurrent acute pancreatitis] and/or [chronic pancreatitis], an affected individual with a first or second-degree relative with pancreatitis, unexplained pancreatitis in a child requiring hospitalization and/or when there is a known mutation in the family."
- "[…] next generation sequencing approaches such as whole exome sequencing or whole genome sequencing should not be used for PRSS1 testing because of challenges in sequence alignment. If a mutation is not identified from sequencing or targeted mutation analysis, deletion/duplication analysis can be considered."
- "In families where a deleterious variant has been identified, predictive genetic testing may be considered in close family members…Genetic testing of asymptomatic family members in a family without an identifiable mutation is uninformative."
- "Genetic testing may be indicated in a child with diagnosed or suspected pancreatitis…Predictive genetic testing for asymptomatic patients less than 16 years of age is not recommended and does not have clear benefits."

2017 Expert Authored Review

A 2017 expert authored review on pediatric recurrent acute and chronic pancreatitis concluded:[20]

- "The search for a genetic cause of ARP or CP should include a sweat chloride test (even if newborn screening for cystic fibrosis (CF) is negative) and PRSS1 gene mutation testing. Genetic testing for CF should be considered if a sweat test is unable to be performed." (Strong consensus, definitely yes, 1A)
- "Mutation analysis of the genes SPINK1, CFTR and CTRC may identify risk factors for ARP or CP." (Strong consensus, definitely yes, 1B)

2020 Expert Authored Review

A 2020 expert-authored review on pancreatitis recommended molecular genetic testing in a proband with pancreatitis and at least of one of the following:[1]

- “An unexplained documented episode of acute pancreatitis in childhood”
- “Recurrent acute attacks of pancreatitis of unknown cause”
- “Chronic pancreatitis of unknown cause, particularly with onset before age 35 years without a history of heavy alcohol use (>5 drinks per day)”
- “A history of at least one relative with recurrent acute pancreatitis, chronic pancreatitis of unknown cause, or childhood pancreatitis of unknown cause”
- PRSS1 sequencing is recommended, followed by deletion/duplication analysis if sequencing is negative. Alternatively, a multi-gene panel that includes PRSS1, SPINK1, CFTR, and CTRC may be appropriate.

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Inherited Bone Marrow Failure Syndrome (IBMFS) Testing

MOL.TS.360.A
v2.0.2024

Introduction

Inherited bone marrow failure syndrome (IBMFS) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
IBMFS Multigene panel [Inherited bone marrow failure syndromes (IBMFS) (eg, Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, Shwachman-Diamond syndrome, GATA2 deficiency syndrome, congenital amegakaryocytic thrombocytopenia) sequence analysis panel, must include sequencing of at least 30 genes, including BRCA2, BRIP1, DKC1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, GATA1, GATA2, MPL, NHP2, NOP10, PALB2, RAD51C, RPL11, RPL35A, RPL5, RPS10, RPS19, RPS24, RPS26, RPS7, SBDS, TERT, and TINF2]	81441
IBMFS Multigene panel	81479

Criteria

Introduction

This guideline applies to inherited bone marrow failure syndrome (IBMFS) multi-gene panels, which are defined as assays that simultaneously test for more than one inherited bone marrow failure gene.

400 Buckwalter Place Boulevard, Bluffton, SC 29910 (800) 918-8924

IBMFS Multigene Panel

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous testing of the requested genes, and
  - No known IBMFS pathogenic variant in the family or
  - If there is a known IBMFS pathogenic variant in the family, testing has been performed and is negative, and a diagnosis of IBMFS is still suspected, AND
- The member has or is suspected to have a condition that will benefit from information provided by the requested IBMFS gene testing based on at least one of the following:
  - The member meets all criteria in a test-specific guideline, if available, or
  - The following criteria are met:
    - The member displays clinical features of the condition for which testing is being requested:
      - unexplained chronic cytopenia with or without associated congenital physical anomalies consistent with the condition, or
      - sporadic aplastic anemia, or
      - myelodysplastic syndrome, or
      - lack of cytopenias but classic physical findings, cancer diagnosis, or family history, and
    - Acquired etiologies have been considered and ruled out when possible (e.g., immune-mediated or viral), and
    - Predicted impact on health outcomes, including immediate impact on medical management based on the molecular results, and
    - Family and medical history do not point to a specific genetic diagnosis or pattern of inheritance for which a more focused test or panel would be appropriate, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Note An alternative sample, such as DNA from a skin biopsy, may need to be considered in a patient with MDS/AML and/or when there is concern for somatic reversion events.

Other considerations

This guideline may not apply to genetic testing for indications that are addressed in test-specific guidelines. Please see the test-specific list of guidelines for a complete list of test-specific panel guidelines.

Table: Select Inherited Bone Marrow Failure Syndromes

Myelodysplastic syndrome (MDS) is a heterogeneous group of disorders characterized by dysplastic changes in the bone marrow, cytopenias, and an increased risk to develop acute myeloid leukemia (AML). MDS is primarily a sporadic disease that occurs in older individuals, but inherited forms have been described. Familial MDS disorders are typically inherited in an autosomal dominant manner but all inheritance patterns have been described.[10-12] They may only present with hematologic findings and can be caused by many of the genes listed in the table below (not an all-inclusive list).

IBMFS

Syndrome Name	Hematologic & malignancy risks	Other features	Diagnosis	Inheritance
Congenital amegakaryocytic thrombocytopenia (CAMT)[13,14]	Isolated thrombocytopenia due to ineffective megakaryocytopoiesis at birth, with elevated plasma TPO levels. Progression to pancytopenia/aplastic anemia will occur in the majority of affected individuals. Individuals are at risk to develop MDS and AML. Genotype-phenotype correlations exist and individuals with type I variants have earlier progression to bone marrow failure than those with type II.	N/A	Identification of mutations in MPL.	AR

Syndrome Name	Hematologic & malignancy risks	Other features	Diagnosis	Inheritance
Diamond-Blackfan anemia (DBA)[15,16]	Classic: characterized by profound normochromic and typically macrocytic anemia. Elevated erythrocyte adenosine deaminase (eADA) activity levels are elevated in the majority of individuals with DBA. 90% of affected individuals will experience red cell aplasia within the first year of life. Other individuals have very mild anemia, requiring no treatment. There is an increased risk to develop AML, MDS, and solid tumors such as osteosarcoma.	Congenital malformations in up to 50% of individuals with DBA including upper limb and hand malformations, craniofacial anomalies, and congenital heart disease; 30% will have growth retardation.	DBA is suspected in individuals who meet the following diagnostic criteria: • Age <1 year • Macrocytic anemia with no other significant cytopenias • Reticulocytopenia • Normal marrow cellularity with a paucity of erythroid precursors • No evidence of another acquired or inherited disorder of bone marrow function DBA is caused by a mutation in one of the following genes: GATA1, RPL5, RPL9, RPL11, RPL15, RPL18, RPL26, RPL27, RPL31, RPL35, RPL35A, RPS7, RPS10,	Usually AD GATA1- and TSR2-related DBA are XL

Syndrome Name	Hematologic & malignancy risks	Other features	Diagnosis	Inheritance
Dyskeratosis Congenita and Related Telomere Biology Disorders (DC/TBD) [9,17-20]	At increased risk for BMF, MDS, AML, and solid tumors.	Classic DC: Classic triad of nail dysplasia, lacy reticular pigmentation of the upper chest/ and or back, and oral leukoplakia. Phenotypic spectrum of TBD is broad and can also include: IUGR, cerebellar hypoplasia, immunodeficiency, retinopathy, eye abnormalities, dental abnormalities, developmental delay, short stature, microcephaly, gastrointestinal features such as liver fibrosis and genitourinary anomalies. Pulmonary fibrosis is the most common presentation of a telomere biology disorder and may be the only symptom in adults.	Identification of a mutation or mutations in one of the following genes: ACD, CTC1, DKC1, NAF1, NHP2, NOP10, PARN, POT1, RPA1, RTEL1, STN1, TERC, TERT, TINF2, WRAP53, and ZCCHC8. Approximately 70% of individuals with a clinical diagnosis are found to have a mutation in an associated gene.	AD, AR, and XL.

Syndrome Name	Hematologic & malignancy risks	Other features	Diagnosis	Inheritance
Fanconi Anemia (FA)[7,21]	At increased risk for progressive BMF with pancytopenia, usually in first decade, often initially with thrombocytopenia or leukopenia, increased risk for AML, MDS, and solid tumors (particularly of the head and neck, skin and genitourinary tract). Carriers of a subset of FA-related genes (e.g., BRCA2, PALB2, and BRIP1) have an increased risk for breast and other cancers.	Physical features are present in ~75% of individuals. These include: short stature, abnormal skin pigmentation, skeletal malformations of the upper and/or lower limbs (especially thumbs), microcephaly, ophthalmic anomalies, genitourinary tract anomalies, gastrointestinal anomalies (such as tracheoesophageal fistula), heart anomalies, otology anomalies and facial features (such as triangular face micrognathia, mid-face hypoplasia).	Increased chromosome breakage and radial forms on cytogenetic testing of lymphocytes with diepoxybutane (DEB) and mitomycin C (MMC) and/or molecular diagnosis. Fanconi Anemia is caused by a mutation or mutations in one of the following genes: BRCA1 (FANCS), BRCA2 (FANCD1), BRIP1 (FANCJ), ERCC4 (FANCQ) FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG (XRCC9), FANCI, FANCL, FANCM, PALB2 (FANCN), RAD51 (FANCR), RAD51C (FANCO), REV7 (MAD2L2/FANC	Usually AR AD (RAD51 gene) and XL (FANCB gene) cases have been reported.

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Syndrome Name	Hematologic & malignancy risks	Other features	Diagnosis	Inheritance
GATA2 deficiency[22-24]	Cytopenias, myelodysplasia. Individuals have an increased risk to develop MDS and leukemias (AML and CMML). Bone marrow is typically hypocellular with characteristic features including atypical megakaryocytes, ranging from large abnormal forms with separated nuclear lobes (osteoclast-like), to smaller forms with separated nuclear lobes, micromegakaryocytes, to small hypolobated or mononuclear megakaryocytes. The majority of cases in the pediatric population who develop MDS will have monosomy 7 on bone marrow karyotype.	Viral and bacterial infections, pulmonary alveolar proteinosis and lymphedema.	Identification of a mutation in GATA2. "GATA2 mutations have been found in up to 10% of those with congenital neutropenia and/or aplastic anemia."[22]	AD

IBMFS

Syndrome Name	Hematologic & malignancy risks	Other features	Diagnosis	Inheritance
SAMD9L ataxia-pancytopenia syndrome (ATXPC) and MIRAGE syndrome [25-27]	SAMD9L: variable hematologic cytopenias, and predisposition to marrow failure, myelodysplasia, and myeloid leukemia, sometimes associated with monosomy 7. SAMD9: Myelodysplastic syndrome and/or acute myelogenous leukemia (AML) with monosomy 7. Monosomy 7 may be transient if the clone is small, or it may persist for years before transformation to AML. These syndromes are likely underdiagnosed due to a common occurrence of genetic reversion to restore hematopoiesis.	SAMD9L: cerebellar ataxia SAMD9: MIRAGE (myelodyplasia, infection, restriction of growth, adrenal hyperplasia, genital phenotypes, and enteropathy) syndrome. Moderate-to-severe developmental delay is reported in most affected individuals. Autonomic dysfunction and renal dysfunction are also reported.	Identification of a mutation in SAMD9L or SAMD9.	AD

IBMFS

Syndrome Name	Hematologic & malignancy risks	Other features	Diagnosis	Inheritance
Severe congenital neutropenia (SCN) [1,28,29]	A "chronic state of severe neutropenia associated with a neutrophil count less than 500/uL lasting longer than 3 months, often presenting in the first year of life."[1] At increased risk of MDS and AML.	Severe/ recurrent infections, abscesses, omphalitis, oropharyngeal inflammation, cervical adenopathy, and osteopenia. With G6PC3 mutation, developmental anomalies of the cardiac and genitourinary systems are possible.	Identification of a mutation or mutations in one of the following genes: HAX1, ELANE, AK2, GFI1, CSF3R, WAS, JAGN1, G6PC3.	AD, AR, and XL.
Shwachman-diamond syndrome (SDS)[30-32]	Single or multi-lineage cytopenias. At increased risk for MDS and AML.	Exocrine pancreatic dysfunction with gastrointestinal malabsorption, malnutrition, and growth failure.	Diagnosis can be established when exocrine pancreatic dysfunction and bone marrow dysfunction are present. Identification of mutation or mutations in one of the following genes: SBDS, ELF1, DNAJC21, SRP54.	Usually AR. Some AD (SRP54 gene) cases have been reported.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81441*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are inherited bone marrow failure syndromes?

Definition

Bone marrow failure (BMF) is the inability of the bone marrow to produce a sufficient quantity of functional blood cells to meet physiologic demands.[1] BMF is typically classified into three categories, based on presumed etiology: inherited, secondary, or idiopathic.[1] Inherited bone marrow failure syndromes (IBMFSs) are a group of genetically defined disorders that are characterized by BMF. Individuals presenting with aplastic anemia (AA), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and chronic unexplained cytopenias should be evaluated for an IBMFS.[1]

Incidence

"The incidence of inherited bone marrow failures accounts for 10% to 15% of marrow aplasia and 30% of pediatric bone marrow failure disorders, with approximately 65 cases per million live births every year."[2] Seventy-five percent of children with an IBMFS have an identifiable cause.[2]

Symptoms

While specific features may vary by each type of IBMFS, features that are present in most IBMFSs include bone marrow failure with single or multi-lineage cytopenia. Many individuals have an increased risk to develop aplastic anemia (AA), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and solid malignancies.[1,3]

IBMFSs typically present with specific patterns of cytopenias, and an individual with an IBMFS may have congenital anomalies and other characteristic physical features or health issues.[1]

Phenotypic overlap between IBMFSs makes it difficult to establish a diagnosis based solely on clinical features.[3]

IBMFSs typically present within the first decade of life; however, delay in diagnosis and variability in phenotypic spectrum may lead to diagnosis into adulthood.[3]

Cause

“A wide variety of specific syndromes have been described so far with more than 80 different genes associated to IBMFSs. Based on the inheritance patterns of IBMFSs in multiplex families and the segregation of mutated alleles in known IBMFS genes of phenotypically affected family members, the disorders are considered monogenic in the vast majority of patients.” [4]

Inheritance

IBMFSs may be inherited in an autosomal dominant (AD), autosomal recessive (AR), or X-linked (XL) manner, depending on the gene involved.

Diagnosis

The diagnosis and classification of an IBMFS requires a combination of clinical, family history, physical examination, laboratory, and bone marrow findings in addition to specialized testing, such as molecular diagnostics.[5]

Timely genetic testing is essential to establish a diagnosis in the individual and to guide appropriate management, treatment, and cancer surveillance.[3] Additionally, knowing the genetic cause in the individual allows for genetic testing in family members. This information is important for their own health and a critical part of their workup if they are being considered as a possible bone marrow transplant donor.

The risk of development of cancers differs greatly between the various IBMFSs, and identification of the underlying etiology of marrow failure is imperative to assess the need for and type of cancer screening.[4]

Treatment

Treatment of IBMFSs varies depending on the specific type, but typically involves supportive care, including blood and/or specific blood cell transfusions, and in severe situations, hematopoietic stem cell transplants (HSCTs).

Survival

The survival range of IBMFSs varies across the multiple conditions included in this group. Survival is impacted by disease severity, response to initial therapy, and the age at the time of initial transplant. The overall survival for individuals with an IBMFS is also significantly impacted by the development of MDS, with disease progression occurring 4.7 months from the time of MDS diagnosis.[6]

Note For additional information on specific IBMFSs, their causes and common presentations and symptoms, see the Table: *Select Inherited Bone Marrow Failure Syndromes* at the end of this document

Test information

Introduction

The investigation and diagnosis of individuals with IBMFSs necessitates a combination of laboratory analyses (including complete blood counts with differential, telomere length studies, exocrine pancreatic function studies, bone marrow analysis, and cytogenetic studies), along with clinical assessment and genetic testing.[1] Clinical genetic testing is available for many IBMFSs, via known familial mutation analysis, single gene analysis and/or multi-gene panels.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to inherited bone marrow failure syndrome genetic testing. Although there are no current U.S. guidelines that address the use of multigene panels in IBMFSs, there are published guidelines for a subset of IBMFSs.

Fanconi Anemia

The Fanconi Anemia Research Fund Inc. (FARF, 2020) established expert guidelines for diagnosis and management of Fanconi Anemia (FA) which stated:[7]

- "The chromosome breakage test is the first test that should be performed for an individual suspected of having FA. This assay is performed in a clinical cytogenetics laboratory, often using a sample of the patient's peripheral blood. Lymphocytes isolated from the blood sample are treated with DNA cross-linking agents; the most commonly used for FA testing are diepoxybutane (DEB) and mitomycin C (MMC) and the chromosomes are examined for evidence of chromosomal breakage."
- "If the results from the chromosome breakage test are positive, genetic testing should be performed to identify the specific FA-causing variants. Genetic testing enables accurate diagnosis and improves clinical care for individuals with anticipated genotype/phenotype manifestations and for relatives who are heterozygous carriers of FA gene variants that confer increased risk for malignancy."
- Recommendations for follow-up testing are made based on the results of the chromosome breakage studies:
    - Negative: No further testing for FA unless strong clinical suspicion.
    - Positive: Targeted FA gene panel and deletion/duplication analysis.
    - Equivocal:
        - Next-generation sequencing for other chromosome instability/DNA repair syndromes
        - Skin chromosome breakage study (if not already performed)

Shwachman-Diamond Syndrome

Draft consensus guidelines for the diagnosis and treatment of Shwachman-Diamond Syndrome (SDS, 2011) stated:[8]

- “The clinical diagnosis is established by (a) documenting evidence of characteristic exocrine pancreatic dysfunction and hematological abnormalities and (b) excluding known causes of exocrine pancreatic dysfunction and bone marrow failure. Attention should be given to ruling out cystic fibrosis (the most common cause of

pancreatic insufficiency) with a sweat chloride test, Pearson disease (pancreatic insufficiency and cytopenia, marrow ring sideroblasts and vacuolated erythroid and myeloid precursors), cartilage hair hypoplasia (diarrhea and cytopenia, and metaphyseal chondrodysplasia, and more common in certain isolated populations such as the Amish), and other inherited bone marrow failure syndromes (such as dyskeratosis congenita)."

- “As the clinical diagnosis of SDS is usually difficult and patients may present at a stage when no clinical pancreatic insufficiency is evident, it is advisable to test most or all suspected cases for mutations in the SBDS gene. It is noteworthy that about 10% of patients with clinical features of SDS may be negative for mutations, and that de novo SBDS mutations have been identified in some families."

Telomere Biology Disorders

Guidelines for diagnosis and management of telomere biology disorders (TBD) were published by expert authors in consultation with a medical advisory board in 2022:[9]

- "The first step in testing for a suspected TBD is to assess the telomere length in specific subtypes of white blood cells."
- "If all or nearly all of the white blood cells' telomere lengths are determined to be very short (less than 1% length for their age), the test result is consistent with diagnosis of TBD. However, it is possible that not all individuals with a TBD will have all very short telomeres."
- "Once an individual has been identified to have clinical features and/or telomere lengths that are consistent with or suggestive of a TBD, genetic testing is recommended for TBD-associated genes to try to identify a causative gene variant."

Selected Relevant Publications

An expert-authored review (2017) stated the following regarding IBMFSs:[1]

- “Genetic testing is an indispensable tool in the diagnostic evaluation of IBMFSs that complements traditional clinical history, examination, and laboratory evaluation, especially in the setting of overlapping or adult presentations. However, clinical use of this powerful tool is currently limited by cost or access in most places."
- "In addition, even when genetic testing is available, it may fail to provide the correct diagnosis." This is because not all genes that cause IBMFS have been identified, many rare variants in known IBMFS genes cannot currently be classified as disease causing, or in the event of somatic reversion, the genetic variant(s) that cause a patient's IBMFS may not be detectable in peripheral blood cells."
- "Now and likely well into the future, the sum of all available tools is greater than any alone, and a modern IBMFS workup should include a focused history and physical examination, screening tests, and genetic evaluation whenever possible."

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IBMFS

Legius Syndrome Genetic Testing

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Introduction

Legius syndrome genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
SPRED1 Deletion/Duplication Analysis	81479
SPRED1 Known Familial Mutation Analysis	81403
SPRED1 Sequencing	81405

Criteria

Introduction

Requests for SPRED1 genetic testing are reviewed using the following clinical criteria.

SPRED1 Known Familial Mutation Analysis

Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Diagnostic Testing for Symptomatic Individuals:

- No previous genetic testing of SPRED1 by a method that would detect the familial mutation, AND
- SPRED1 mutation identified in 1st degree biological relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

SPRED1 Sequence Analysis

- No previous sequencing analysis of SPRED1, AND

- No known, pathogenic SPRED1 mutation in the member's close biologic relatives, AND
- No known, pathogenic NF1 mutation in the member or the member's close biologic relatives, AND
- Member has at least one of the following pigmentary findings suggestive of Legius syndrome:
    - Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals, with or without freckling in the axillary or inguinal regions, or
    - Six or more café-au-lait macules over 15 mm in greatest diameter in postpubertal individuals, with or without freckling in the axillary or inguinal regions, AND
- Member's personal and/or family history are not consistent with neurofibromatosis type 1 (e.g., neurofibromas, optic glioma, Lisch nodules, sphenoid dysplasia or tibial pseudoarthrosis are not present), AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the member, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

SPRED1 Deletion/Duplication Analysis

- Criteria for SPRED1 sequencing are met, AND
- No previous deletion/duplication analysis of SPRED1, AND
- No mutation detected in full sequencing of SPRED1, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

What is Legius Syndrome?

Definition

Legius syndrome is an inherited disorder characterized by multiple café-au-lait macules and axillary or inguinal freckling, without neurofibromas or other tumor symptoms of Neurofibromatosis type 1 (NF1).[1,2]

Prevalence

The prevalence of Legius syndrome is estimated at 1/46,000 to 1/75,000.[3] Studies have shown that approximately 2% of individuals meeting the diagnostic criteria for NF1 have Legius syndrome.[1]

Symptoms

Individuals with Legius syndrome have multiple café-au-lait macules and may have axillary or inguinal freckling. Other clinical features reported in some individuals with Legius syndrome include macrocephaly, Noonan-like facial features, pectus excavatum or carinatum, developmental concerns, attention deficit hyperactivity disorder (ADHD), and learning difficulties.[2]

Genetic testing may be indicated in an individual with café-au-lait macules to confirm a diagnosis and direct long-term management and surveillance. Approximately 3%-25% of individuals evaluated for NF1 who do not have an identifiable mutation in the NF1 gene are noted to have a SPRED1 pathogenic mutation.[3] Individuals with NF1 require long-term surveillance due to an increased risk of tumor development and other complications. Thus, the diagnosis of Legius syndrome may include molecular testing of the SPRED1 gene, and in some cases the NF1 gene.

Cause

Legius syndrome is caused by mutations in the SPRED1 gene. The protein product of this gene interacts with neurofibromin, the protein product of the NF1 gene.[2]

Inheritance

Legius syndrome is an autosomal dominant disorder.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Diagnosis

The diagnosis of Legius syndrome can be made in an individual without an affected parent if both of the following are present:[4]

- “Six or more café au lait macules ... bilaterally distributed and no other NF1-related diagnostic criteria except for axillary or inguinal freckling”
- “A heterozygous pathogenic variant in SPRED1 with a variant allele fraction of 50% in apparently normal tissue such as white blood cells”

“A child of a parent who meets the diagnostic criteria specified in A merits a diagnosis of Legius syndrome if one or more of the criteria [above] are present.”

SPRED1 sequence mutations, such as missense, nonsense, and splice site mutations, account for up to 89% of mutations seen in Legius syndrome.[3] Approximately 10% of the disease-causing mutations in Legius syndrome are multi-exon and whole gene deletions.[5,6]

Management

Management of Legius syndrome includes therapies for developmental delays, learning disorders, and ADHD, if present.[3]

Survival

Lifespan does not appear to be affected by Legius syndrome. Current knowledge is based on the clinical history of fewer than 300 individuals with a confirmed diagnosis of Legius syndrome.[3,5]

Test Information

Introduction

Testing for Legius syndrome may include known familial mutation analysis, sequence analysis, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to Legius syndrome genetic testing.

Selected Relevant Publication

A 2020 expert-authored review stated:[3]

- "Opinions differ on the appropriate approach when clinical information and family history cannot distinguish between NF1 and Legius syndrome. This is the case in individuals with only cafe au lait macules with or without freckling but no other signs of NF1. The assessment of pros and cons of molecular testing requires the consideration each individual's unique circumstances, including (but not limited to):
  - Clinical findings and family history
  - Age of the individual
  - Differences in recommended clinical management when the diagnosis of NF1 or Legius syndrome is established with certainty versus when the diagnosis of neither can be established with confidence
  - Psychological burden of a diagnosis or lack thereof
  - Cost of testing and surveillance
  - Odds of identifying a diagnosis of NF1 versus Legius syndrome in those with a phenotype limited to pigmentary findings."

:

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Li-Fraumeni Syndrome Genetic Testing

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Introduction

Li-Fraumeni syndrome genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
TP53 Deletion/Duplication Analysis	81479
TP53 Known Familial Mutation Analysis	81353
TP53 Sequencing	81351
TP53 Targeted Sequence Analysis	81352

Criteria

Introduction

Requests for Li-Fraumeni genetic testing are reviewed using these criteria.

TP53 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic and Predisposition Testing for Presymptomatic/Asymptomatic Individuals**:
  - Known family mutation in TP53, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

** Includes prenatal testing for at-risk pregnancies.

TP53 Sequence Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy). AND
- Previous Testing:
  - No previous sequencing of TP53, and
  - No known familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Classic Li-Fraumeni syndrome when **ALL** of the following are present:
    - Combination of an individual diagnosed less than age 45 years of age with a sarcoma, and
    - First-degree relative diagnosed less than 45 years of age with cancer, and
    - An additional first- or second-degree relative in the same lineage with cancer diagnosed less than 45 years of age, or a sarcoma at any age, OR
  - Chompret Criteria (2015) are met when **ANY** of the following are present:
    - Individual with a tumor from LFS tumor spectrum (eg, soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma) before age 46 years, and
      - at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) under the age of 56 years, or
      - at least one first- or second-degree relative with multiple primary cancers at any age, or
    - Individual with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum (eg, soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma) with the initial cancer occurring before the age of 46 years, regardless of the family history, or
    - Individual with adrenocortical carcinoma or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, at any age of onset, regardless of the family history, OR
  - Early onset breast cancer
    - Individual with breast cancer diagnosed before 31 years of age, OR

  - Individual with a tumor from LFS tumor spectrum and one or more biologic relatives (1st, 2nd, or 3rd degree) with a clinical diagnosis of LFS (relative meets classic Li-Fraumeni syndrome criteria or Chompret criteria, as listed above) and no known family mutation or no testing to date, OR
  - Individual who was diagnosed with hypodiploid acute lymphoblastic leukemia (ALL) before age 21 years, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - One or more biologic relatives (1st, 2nd, or 3rd degree) with a clinical diagnosis of LFS (relative meets classic Li-Fraumeni syndrome criteria or Chompret criteria as listed above) and no known family mutation or no testing to date, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

TP53 Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous deletion/duplication analysis of TP53, and
  - No mutation detected on full sequencing of TP53, AND
- Diagnostic or Presymptomatic Testing:
  - Meets clinical criteria for TP53 sequence analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other Considerations

LFS testing may be performed as part of a multigene, multisyndrome panel. For information on multigene, multisyndrome panel testing, please refer to the guideline *Hereditary Cancer Syndrome Multigene Panels,* as this testing is not address here.

What is Li-Fraumeni syndrome?

Definition

Li-Fraumeni syndrome (LFS) is a hereditary cancer-predisposition syndrome typically associated with soft tissue sarcoma, osteosarcoma, premenopausal breast cancer, brain tumors, and adrenocortical carcinomas. People with LFS also have an increased risk of a variety of other childhood and adult-onset cancers.[1-3]

Prevalence

In Brazil, a high prevalence of LFS is present due to a founder mutation. A specific germline TP53 mutation (c.1010G>A; p.R337H) is present in 0.3% of individuals from the South/Southeastern regions, and it is estimated that more than 300,000 Brazilian individuals have LFS.[4]

The prevalence of inherited TP53 mutations is not well established but is estimated to be 1/3,555 to 1/5,476.[1]

Symptoms

Men with LFS have a 70% or higher lifetime risk of cancer while women have a 90% or higher lifetime risk of cancer. However, penetrance may be overestimated as more individuals with non-classic personal and/or family histories of cancer are identified to have TP53 mutations.[1]

Cause

LFS is caused by mutations in the TP53 gene.

Inheritance

LFS is an autosomal dominant disorder.[1]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Diagnosis

The identification of a pathogenic mutation in the TP53 gene establishes the diagnosis.[1]

Complete TP53 gene sequencing will detect approximately 95% of known mutations.[1]

Deletion/duplication testing may be considered as a reflex test if a mutation is not found by sequencing. This method will identify gene rearrangements in an additional 1% of cases.

Management

The recommended surveillance for individuals with LFS includes whole-body MRI, ultrasound of the abdomen and pelvis, mammogram and breast MRI, clinical breast exam, brain MRI, upper endoscopy and colonoscopy, dermatologic exam, and complete physical examination.[1,4] The age for initiation of screening and the frequency at which the screenings are repeated are well-defined.[1,2]

Survival

A study followed 89 individuals who pursued or declined recommended surveillance. The five year survival rate was 88.8% and 59.6% for those in the surveillance group versus those who declined, respectively.[1]

Test information

Introduction

Testing for Li-Fraumeni may include known familial mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Li-Fraumeni genetic testing.

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) guidelines for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic outlined the following Li-Fraumeni syndrome testing criteria. These are considered a category 2A recommendation “lower level evidence with uniform NCCN consensus”:[2]

- "Individuals from a family with a known TP53 P/LP [pathogenic/likely pathogenic] variant," OR
- Classic Li-Fraumeni syndrome when ALL of the following are present:
    - "Combination of an individual diagnosed at age less than 45 years with a sarcoma AND
    - A first-degree relative diagnosed at age less than 45 years with cancer AND
    - An additional first- or second-degree relative in the same lineage with cancer diagnosed at age less than 45 years, or a sarcoma at any age," OR
- Chompret Criteria (2015 version)[5], when ANY of the following are present:
    - "Individual with a tumor from LFS tumor spectrum (eg, soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma), before 46 years of age, AND at least one first- or second-degree relative with any of the aforementioned cancer (other than breast cancer if the proband has breast cancer) before the age of 56 years, or with multiple primaries at any age OR
    - Individual with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum with the initial cancer occurring before the age of 46 years OR
    - Individual with adrenocortical carcinoma or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, at any age of onset, regardless of the family history OR
    - Breast cancer before 31 years of age".
- "Affected individuals with P/LP variant identified on tumor genomic testing that may have implications if also identified on germline testing. This should prompt a careful evaluation of personal and family history of the individual to determine the yield of germline sequencing. Somatic TP53 P/LP variants are common in many tumor types in absence of a germline P/LP variant." For information on germline testing after somatic testing, please refer to the guideline *Hereditary (Germline) Testing After Tumor (Somatic) Testing*, as this testing is not addressed here.
- Hypodiploid Pediatric Acute Lymphoblastic Leukemia (ALL)
    - The National Comprehensive Cancer Network Guidelines (NCCN, 2023) for the treatment of Pediatric Acute Lymphoblastic Leukemia stated that germline TP53 mutations are common in low hypodiploid ALL and testing should be considered.[6,7] Approximately 50% of pediatric patients (<21 years) with a

diagnosis of low hypodiploid ALL will have a germline TP53 mutation. A germline mutation has not been reported in individuals with adult-onset hypodiploid ALL.[1,7]

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Introduction

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Limb-Girdle Muscular Dystrophy Genetic Testing

MOL.TS.288.A
v2.0.2024

Introduction

Genetic testing for limb-girdle muscular dystrophy (LGMD) is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
LGMD Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
LGMD Known Familial Mutation Analysis	81403
LGMD Multigene Panel	81479 81443

Criteria

Introduction

Requests for limb-girdle muscular dystrophy (LGMD) genetic testing are reviewed using the following clinical criteria.

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LGMD Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Known family mutation(s) in LGMD subtype related gene in 1st or 2nd degree biologic relative, OR
- Presymptomatic Testing for Asymptomatic Individuals:
  - Age 18 years or older, and
  - At increased risk of developing an LGMD phenotype, and
  - Known family mutation(s) in LGMD subtype related gene in 1st or 2nd degree biologic relative, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

LGMD Single Gene Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No redundant previous LGMD related gene sequencing, and
  - No known LGMD related gene mutation in family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member displays clinical features of LGMD by the following
    - Muscle weakness and atrophy not secondary to a neurogenic cause in a limb-girdle distribution, and
    - Member does not have a congenital myopathy, and
    - EMG does not show evidence of a nerve etiology as the primary cause, OR
  - Member has had a muscle biopsy and results are consistent with the LGMD subtype for which testing is being requested, AND

- Inheritance pattern is consistent with the LGMD subtype for which testing is being requested, AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the individual, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

LGMD Multi-Gene Diagnostic Panels

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No known molecular cause of LGMD (single disease-causing mutation in dominant forms or biallelic disease-causing mutations in recessive forms) in family, and
  - No mutations or one mutation associated with recessive form of LGMD detected by single gene analysis or different mutation panel than being requested, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Muscle weakness and atrophy not secondary to a neurogenic cause in a limb-girdle distribution, and
  - Member does not have a congenital myopathy, and
  - EMG does not show evidence of a nerve etiology as the primary cause, and
  - Muscle biopsy, if available, shows dystrophic changes (degeneration / regeneration of fibers), and immunohistochemical staining may reveal aberrant or absent muscle specific proteins, AND
- Inheritance pattern not suggestive of Duchenne muscular dystrophy or other X-linked muscular dystrophies, AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the individual, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

Other considerations

Broad neuromuscular panels are not medically necessary.

If the inheritance pattern in the family is evident based on pedigree analysis, only a panel specific to the inheritance pattern is medically necessary.

If a muscle biopsy has been performed with IHC staining, only genes associated with the findings are considered medically necessary.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- For a panel code to be considered for reimbursement, it must be limited to LGMD-associated genes. Broad neuromuscular panels are not reimbursable.
- If the inheritance pattern in the family is evident based on pedigree analysis, a panel code specific to the inheritance pattern will be reimbursable; however, panels of all LGMD genes will not.
- If a muscle biopsy has been performed with IHC staining, only procedure codes for genes associated with the findings will be reimbursable.
- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81479 or 81443*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is limb-girdle muscular dystrophy?

Definition

Limb-girdle muscular dystrophy (LGMD) is a rare, inherited, heterogeneous group of over 30 myopathies with predominant involvement of the proximal musculature.[1] They

are typically progressive myopathies characterized by weakness and atrophy of muscle without primary involvement of the nervous system or neurogenic atrophy. The LGMDs are classified into groups, based on inheritance pattern. Historically, these were denoted as LGMD1 (autosomal dominant) and LGMD2 (autosomal recessive). In 2018, the European Neuromuscular Centre published new nomenclature with the types of LGMD denoted as LGMD D (autosomal dominant) and LGMD R (autosomal recessive) with the subtype denoted with a numeral to categorize the order of discovery, and inclusion of the affected protein, if known. 'LGMD unclassified' refers to individuals with symptoms consistent with LGMD but with negative genetic testing.[2]

Prevalence

Autosomal recessive LGMD is more common, with an overall prevalence of about 1/15,000.[3] Dominant forms are comparatively rare, representing 10% of LGMD cases.[3] The prevalence of specific LGMD subtypes may differ in certain populations:[1]

- LGMD R5 (previously known as LGMD2C) is more common in Roma and Tunisian populations,[1]
- LGMD R1 (previously known as LGMD2A) is more common in Southern European, Eastern European, and British populations[4], and
- LGMD R9 (previously known as LGMD2I) is more common in Northern European populations[4].

Symptoms

Signs and symptoms typically begin anytime between childhood and adulthood depending on the subtype but are generally not congenital. Symptoms can include the following:

- Upper and lower limb weakness, proximal greater than distal weakness
- Gait weakness
- Foot drop
- Cramps
- Exercise intolerance

LGMDs are most often non-syndromic and usually limited to skeletal muscle, but not always. For example, certain subtypes involve cardiac and respiratory muscles. The clinical course can range from mild, with relatively normal activity and life span, to severe with rapid onset and progression of disease.[3]

The muscle atrophy in LGMD is greatest at the shoulder girdle (scapulohumeral) and pelvic girdle (pelvifemoral), although it may progress distally. Bulbar muscles (including facial muscles and oropharyngeal muscles innervated by cranial nerves VII-XII) are relatively spared depending on the subtype of LGMD. This general pattern of girdle muscle weakness as well as onset, progression, and distribution help classify LGMD and its genetic subtypes.

Cause

There are more than 30 genes implicated in LGMD subtypes, which manifest in overlapping and variable clinical presentations.[3] The genes identified so far encode muscle proteins within the sarcomere-sarcolemma-sarcoplasm-extracellular-matrix network.[5]

Inheritance

LGMD inheritance is typically autosomal with updated LGMD subtype nomenclature reflecting autosomal dominant inheritance (LGMD D with subtypes designated by a numeral), and autosomal recessive inheritance (LGMD R with subtypes designated by a numeral). This autosomal inheritance pattern helps distinguish LGMD from the more common X-linked dystrophies (Duchenne, Becker and Emery-Dreifuss).[2,6]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

The diagnosis of muscular dystrophies is typically based on clinical phenotype and inheritance pattern.[5] Although classification schema are becoming more reliant on molecular test results, the 2014 American Academy of Neurology guidelines for LGMD still recommend genetic testing that is directed by clinical assessment.[1]

- The phenotype must be more consistent with LGMD than other myopathies
  - Muscle weakness in the proximal limbs and limb girdle (i.e., scapular winging)
  - Myopathic and not neuropathic symptoms
  - Sparing of extra-ocular muscles (although eye anomalies are seen in some severe allelic disorders)[3]
  - Onset is not congenital
  - Course is progressive

- Biochemical/histological investigation should suggest muscle damage (although findings can be non-specific)[4]
  - Creatine kinase can be elevated or normal
  - EMG typically shows myopathic rather than neuropathic changes
  - Muscle biopsy shows “dystrophic” changes” (degeneration / regeneration of fibers), and immunohistochemical staining may reveal aberrant or absent muscle specific proteins.
- Dystrophinopathy and inflammatory myopathy should be excluded
- Identification of pathogenic variants in an LGMD-associated gene can confirm a clinical diagnosis of LGMD

Given the expanding number of loci involved in LGMD subtypes, a negative molecular test result does not rule out LGMD. There are more than 50 loci implicated in LGMD subtypes.

When a specific LGMD subtype is clinically favored over another, genetic testing specific to that subgroup is supported over large panels. However, given the number of loci, and phenotypic overlap among the limb girdle muscular dystrophies, panel testing grouped by inheritance pattern is acceptable.

Large deletions in autosomal LGMD related genes are infrequently reported. Therefore, deletion/duplication analysis is done as second tier testing or first tier in some cases to help rule out X linked dystrophies if they are a part of the differential.

Management

There is no cure for LGMD. Treatment is symptom driven and includes weight control, physical therapy, surgery, use of respiratory aids, and cardiology monitoring.[1]

Survival

LGMDs have a broad range of severity. Many are life shortening and debilitating.[3]

Test information

Introduction

Testing for LGMD disease may include known familial mutation analysis, next generation sequencing, deletion/duplication analysis, and/or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if

available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to LGMD genetic testing.

American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine

The Guideline Development Subcommittee of the American Academy of Neurology (AAN, 2014) and the Practice Issues Review Panel of the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM, 2014; reaffirmed 2022) issued recommendations for the approach to genetic testing in LGMD:[1]

- Clinically directed genetic testing is recommended (See Table e-2 for reference of clinical features suggestive of LGMD subtypes).
    - Clinicians should use a clinical phenotype, inheritance pattern, and associated manifestations to guide genetic diagnosis (Level B)
    - "In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome sequencing, whole-genome sequencing, or next-generation sequencing to identify the genetic abnormality (Level C)."

Selected Relevant Publications

Studies evaluating diagnostic yield from small and large panels found both number and composition of genes sequenced have a sizeable impact. A 3-fold greater diagnostic pickup rate was seen when the LGMD panel was increased from 11 genes to a more comprehensive panel containing 41 genes (15 - 46%).[7]

Sequencing of 18 LGMD related genes in 35 individuals suspected of having a muscular dystrophy (unknown genetic diagnosis, high CK values and dystrophic changes on muscle biopsy, DMD ruled out prior to study inclusion) was reported.[8] Pathogenic variants confirmed a LGMD-related molecular etiology in 20 individuals (57.1%). The study population was ascertained through the neurology clinic at the University of Seoul, Korea. Information regarding consanguinity was not stated in the report and may not have been specifically queried in the study.[8]

While some panels are getting so large as to overlap with WES, a comprehensive panel approach has been suggested to be similar or superior to WES.[7,9,10] One study analyzed 50 families with an LGMD type distribution of muscle weakness.[9] They showed that after large LGMD panel testing as a first line diagnostic, follow-up WES did not yield further diagnosis. On the other hand, smaller panels would have missed several LGMD related genes.[9] Weaknesses of this study includes the specialized population investigated and the small sample size, albeit somewhat large for this rare disease. The population was suspected to be highly consanguineous (in Saudi Arabia) which authors suggest led in part to their 76% diagnostic yield. The authors also analyzed cost, and, despite the large panel size (759 OMIM genes), the actual cost of sequencing with batching was around $150.00 per sample. This study did not include deletion/duplication analysis. Follow-up analysis after negative large panel testing was carried out with only a small cohort of nine people. Also, the size of the large sequencing panel used approximates the size of the interpretive gene set that a bioinformatician would look at when analyzing results from WES with a myopathic proband.[9] A large gene panel may also increase the risk of incidental findings or variants of uncertain clinical significance.

A US study of 4656 individuals with clinically suspected LGMD (no prior molecular testing) underwent genetic testing via a 35-gene NGS panel (included LGMD or LGMD-like genes).[11] A molecular diagnosis was established in 27% (N=1259). There was a high prevalence of individuals with pathogenic variants in more than one LGMD

gene (N=31), raising the question of possible synergistic heterozygosity/digenic/multigenic contribution to disease presentation/progression.

A group in Australia performed exome sequencing (ES) on 60 families with LGMDs and achieved a diagnostic success rate of 45%.[12] All patients had normal dystrophin immunohistochemistry results. In 14 of the 60 families, pathogenic variants were identified in genes typically associated with other forms of inherited myopathy, highlighting the diagnostic challenge with overlapping clinical presentation among individuals with features of LGMD. An international study including 1001 undiagnosed patients from Europe and the Middle East performed exome sequencing, evaluating 429 genes involved in muscle conditions.[13] In this cohort of patients with limb-girdle weakness, they identified pathogenic or likely pathogenic variants in 87 genes, with a diagnostic yield of 52% of patients.

A US study of 55 families affected by LGMD demonstrated pathogenic variants in 22 families using exome sequencing.[5] Most of the probands had clinical muscle biopsies, and none of the muscle biopsies led to a genetic diagnosis prior to enrollment. "Among the pathogenic mutations identified in our cohort, six were found in loci not traditionally classified as being associated with LMGD (e.g., DMD, GAA, SMCHD1, VCP, FLNC, and the D4Z4 region of 4q35)", suggesting that gene panels include a broad array of muscle disease genes, beyond just LGMD, particularly given the decreasing use of muscle biopsy in clinical settings.[5]

Given the degree of phenotypic overlap among LGMD subtypes, atypical presentations of non-LGMD myopathies, and variable expressivity of LGMD, panel testing may be superior to a candidate gene approach when multiple LGMD subtypes are being considered.

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Introduction

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Liquid Biopsy Testing

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Introduction

Liquid biopsy testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
ABL1 Mutation Analysis	81170
APC Sequencing	81201
ASXL1 Full Gene Sequencing	81175
ASXL1 Mutation Analysis	81176
BRAF V600 Targeted Mutation Analysis	81210
BRCA1/2 Sequencing	81163
BRCA1 Sequencing	81165
BRCA2 Sequencing	81216
CALR Exon 9 Mutation Analysis	81219
CCND1/IGH (t(11;14)) Translocation Analysis, Major Breakpoint	81168
CEBPA Full Gene Sequencing	81218
EGFR Targeted Mutation Analysis	81235
Epic Sciences ctDNA Metastatic Breast Cancer Panel	0428U
EZH2 Common Variant(s) (e.g. codon 646)	81237
EZH2 Full Gene Sequencing	81236
FLT3 Mutation Analysis (internal tandem duplication variants)	81245
FLT3 Mutation Analysis (tyrosine kinase domain variants)	81246
FoundationOne Liquid CDx	0239U

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Procedures addressed by this guideline	Procedure codes
Guardant360 CDx	0242U
Guardant360 LDT	0326U
Guardant360 Response	0422U
Hematolymphoid Neoplasm Molecular Profiling; 5-50 Genes	81450
IDH1 Mutation Analysis	81120
IDH2 Mutation Analysis	81121
IGH@/BCL2 (t(14;18)) Translocation Analysis, Major Breakpoint Region (MBR) and Minor Cluster Region (mcr) Breakpoints	81278
InVisionFirst-Lung Liquid Biopsy, Inivata, Inc.	0388U
JAK2 Targeted Mutation Analysis (e.g exons 12 and 13)	81279
JAK2 V617F Targeted Mutation Analysis	81270
KIT D816 Targeted Mutation Analysis	81273
KIT Targeted Sequence Analysis	81272
KRAS Exon 2 Targeted Mutation Analysis	81275
KRAS Targeted Mutation Analysis, Additional Variants	81276
LiquidHALLMARK	0409U
MGMT Promoter Methylation Analysis	81287
MLH1 Sequencing	81292

Procedures addressed by this guideline	Procedure codes
Molecular Tumor Marker Test	81400 81401 81402 81403 81405 81406 81407 81408 81479
Molecular Tumor Marker Test	88271
MPL Common Variants (e.g. W515A, W515K, W515L, W515R)	81338
MPL Mutation Analysis, Exon 10	81339
MSH2 Sequencing	81295
MSH6 Sequencing	81298
NeoLAB Prostate	0011M
NPM1 Exon 12 Targeted Mutation Analysis	81310
NRAS Exon 2 and Exon 3 Analysis	81311
NTRK1 Translocation Analysis	81191
NTRK2 Translocation Analysis	81192
NTRK3 Translocation Analysis	81193
NTRK Translocation Analysis	81194
PDGFRA Targeted Sequence Analysis	81314
PMS2 Sequencing	81317
PTEN Sequencing	81321
Resolution ctDx Lung	0179U
RUNX1 Mutation Analysis	81334
SF3B1 Common Variants (e.g. A672T, E622D, L833F, R625C, R625L)	81347

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Procedures addressed by this guideline	Procedure codes
Solid Organ Neoplasm, Genomic Sequence Analysis Panel, Cell-free Nucleic Acid (eg, plasma), Interrogation for Sequence Variants; DNA Analysis or Combined DNA and RNA Analysis, Copy Number Variants and Rearrangements	81462
Solid Organ Neoplasm, Genomic Sequence Analysis Panel, Cell-free Nucleic Acid (eg, plasma), Interrogation for Sequence Variants; DNA Analysis, Copy Number Variants, and Microsatellite Instability	81463
Solid Organ Neoplasm, Genomic Sequence Analysis Panel, Cell-free Nucleic Acid (eg, plasma), Interrogation for Sequence Variants; DNA Analysis or Combined DNA and RNA Analysis, Copy Number Variants, Microsatellite Instability, Tumor Mutation Burden, and Rearrangements	81464
SRSF2 Common Variants (e.g. P95H, P95L)	81348
TERT Targeted Sequence Analysis	81345
therascreen PIK3CA RGQ PCR Kit	0177U
TP53 Sequencing	81351
TP53 Targeted Sequence Analysis	81352
U2AF1 Common Variants (e.g. S34F, S34Y, Q157R, Q157P)	81357
ZRSR2 Common Variants (e.g. E65fs, E122fs, R448fs)	81360

Criteria

Introduction

Requests for liquid biopsy testing are reviewed using the following criteria.

Companion Diagnostic (CDx) Liquid Biopsy Assay

Liquid biopsy-based companion diagnostic assays are considered medically necessary when the member meets ALL of the following criteria:

- Member has a diagnosis of cancer, AND
- Treatment with a medication for which there is a liquid biopsy-based FDA-approved companion diagnostic is being considered, AND
- FDA approval for the CDx being requested must include the member's specific cancer type as an approved indication, AND
- FDA label for the drug and indication being considered states companion diagnostic testing is necessary for member selection, AND
- Member has not had previous somatic and/or germline testing that would have identified the genetic change required to prescribe the medication under consideration, AND
- Family history:
    - Member does not have a close (1st or 2nd degree) biological relative with a known germline mutation in a gene that is a target of the requested companion diagnostic test (e.g. known familial mutation in BRCA1/2 and requested test is myChoice CDx), or
    - Member has a close (1st or 2nd degree) biological relative with a known germline mutation in a gene that is a target of the requested companion diagnostic test (e.g. known familial mutation in BRCA1/2 and requested test is myChoice CDx), and the member’s germline test was negative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Note Not all indications for medications with an FDA-approved companion diagnostic liquid biopsy test require the results of that test prior to prescribing. Testing would not be considered medically necessary when prescribed for indications that do not require the companion diagnostic.

Guardant360 LDT

When Guardant360 LDT (laboratory developed test) is being requested for indications that are outside the scope of a companion diagnostic (i.e.: non-CDx), the panel will be considered medically necessary when the following criteria are met:

- The member has a diagnosis of metastatic or recurrent non-small cell lung cancer (NSCLC), AND
- NSCLC diagnosis has been confirmed based on a histopathologic assessment of tumor tissue, AND
- No previous multi-gene panel testing has been performed for NSCLC, AND

- Insufficient tumor tissue is available for broad molecular profiling and member is unable to undergo an additional standard tissue biopsy due to documented medical reasons (i.e., invasive tissue sampling is contraindicated due to the member's clinical condition)

Liquid Biopsy Tests for Other Non-CDx Indications

These tests are considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

When otherwise reimbursable, the following will apply:

- When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81462, 81463, 81464*).

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is liquid biopsy testing?

Definition

The use of circulating tumor DNA (ctDNA) to identify genetic mutations present in a tumor is also referred to as a liquid biopsy.

- The National Cancer Institute defines a liquid biopsy as “a laboratory test done on a sample of blood, urine, or other body fluid to look for cancer cells from a tumor or small pieces of DNA, RNA, or other molecules released by tumor cells into a person's body fluids. Liquid biopsy allows multiple samples to be taken over time, which may help doctors understand what kind of genetic or molecular changes that are taking place in a tumor.” [1]
- Circulating tumor DNA is released into circulation by tumors.[2] It can be found in various substances, including blood, urine, saliva, etc.
- Analysis of ctDNA can be performed to help identify indicators of disease recurrence or disease progression. It can also help to determine if a specific treatment is indicated.
- Liquid biopsies can be used to more easily obtain serial sampling of a tumor. This is particularly useful since somatic mutations that are used in treatment decisions can change as the tumor progresses.[2] ctDNA is also thought to provide a more representative sample of the entire tumor genome as well as any metastases that may be present.[2]
- Traditional methods of performing biopsies on tumor tissue pose the following problems:[2,3]
    - Biopsies are invasive, involve risks, are typically costly, and are typically difficult to obtain.
    - Treatment decisions often rely on one single biopsy, while tumors are usually heterogeneous in nature, tumor characteristics can evolve, and information regarding metastases may not be not known.[2]
- The use of liquid biopsies can help overcome some of the above problems with traditional biopsies since they can be completed in a noninvasive manner.
- This guideline will only address the use of ctDNA as a liquid biopsy in solid tumors and hematologic malignancies. Circulating tumor cells (CTCs) can be used to help obtain information about an individual's cancer prognosis and treatment options. CTC assays are not addressed by this guideline.

Test information

Introduction

Liquid biopsy testing is an assay that utilizes ctDNA to assist with monitoring disease status and potentially determining sensitivity to certain treatments.

Liquid biopsy methodology

Testing methodology relies on the presence of ctDNA in circulation, which is typically analyzed by one of the following methods:

- Standard testing methodologies, such as polymerase chain reaction (PCR) or sequencing, are used to identify targeted mutations commonly present in tumors of a specific type.
- Methodologies such as next-generation sequencing (NGS) or array comparative genomic hybridization (aCGH) are used to identify both novel and recurrent mutations. These include whole genome sequencing or whole exome sequencing. These approaches analyze single genes, panels of genes, exomes, or genomes. Use of these approaches allows testing with no prior knowledge of genetic mutations that are present in the individual's tumor.
- Several liquid biopsy tests have been designated by the Food and Drug Administration (FDA) as companion diagnostic (CDx) assays deemed necessary for the effective use of a specific medication in the context of a specific clinical indication. Within this guideline, liquid biopsy tests that do not have the designation of companion diagnostics are referred to as non-CDx assays.

Note Tests that extract DNA from nucleated cells in the blood or bone marrow for hematologic malignancies are not considered liquid biopsies. For information on these assays, please refer to the guideline *Somatic Mutation Testing - Hematological Malignancies*, as this testing is not addressed here.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to liquid biopsy testing.

American Society of Clinical Oncology and College of American Pathologists

Based on a comprehensive systematic review of 77 scientific studies on ctDNA assays for solid tumors, an expert panel assembled by the American Society of Clinical Oncology (ASCO, 2018) and the College of American Pathologists (CAP, 2018) concluded that there is currently insufficient evidence of clinical validity and clinical utility for most ctDNA assays being used in advanced cancer.[4] There are some ctDNA assays that have demonstrated clinical validity and clinical utility with certain types of cancers, such as non-small cell lung cancer. There is no evidence for use in early stage cancer, treatment monitoring, or residual disease detection. They also stated that there is no evidence of clinical value for cancer screening outside of a clinical trial.

To establish clinical validity and clinical utility of ctDNA analyses, the expert panel recommended the following:

- “Future research studies to establish clinical validity and utility of ctDNA assays should include a patient cohort that matches the intended-use population as closely as possible and samples collected from a prospective study with defined entry criteria. Data will most frequently come from a phase II or phase III study in the patient population where it is anticipated the assay would be used in subsequent clinical practice, with the frequency of the variant under study approximately equal to that in an unselected clinical population. In prospective studies of targeted therapies, the entry criteria should allow inclusion of patients in which the variant under study is observed in the plasma, but not in the tissue analysis, to evaluate the treatment response of this population with discordant genotyping results."

European Society for Medical Oncology

The European Society for Medical Oncology (ESMO, 2022) stated the following regarding liquid biopsies (LBs) for testing in individuals with advanced cancer:[5]

- "LB assays with very high analytical and clinical specificity, and therefore positive predictive values, may be used in routine practice when the results will affect standard treatment options. The limitations of ctDNA assays, however, must be taken into account."
- "...the clinical utility of ctDNA is very much context-dependent, contingent on disease types and stages, available treatment that could effectively eradicate MRD [minimal residual disease] and intended use..."
- Tissue-based testing is the most appropriate test for the majority of individuals, while clinical scenarios exist where ctDNA assays are recommended. These include certain aggressive tumors or when tumor tissue is insufficient or not appropriate.

The guidelines also stated that insufficient evidence exists for implementing use of ctDNA assays for cancer screening, monitoring of treatment response, or detection of molecular relapse or MRD.

International Association for the Study of Lung Cancer

A consensus statement from the International Association for the Study of Lung Cancer (IASLC, 2021) on usage of liquid biopsy for advanced NSCLC made the following recommendations:[6]

- For treatment-naive individuals with advanced NSCLC, tumor genotyping with a tissue sample should be performed first if available. If the tumor tissue is of uncertain adequacy or there is concern for incomplete tumor genotyping, ctDNA genotyping can be performed concurrently or as reflex testing. When a tissue sample is unavailable for these individuals, plasma ctDNA genotyping is the recommended test.
- Usage of plasma ctDNA is recommended "[a]t the time of acquired resistance after tyrosine kinase inhibitor (TKI) therapy in an oncogene-driven NSCLC."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2024) stated the following regarding liquid biopsies for testing in individuals with non-small cell lung cancer:[7]

- “ctDNA testing should not be used in lieu of a histologic tissue diagnosis.”
- “The use of cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, most notably:”
    - "If a patient is medically unfit for invasive tissue sampling"
    - "In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA can be used; however, follow-up tissue-based analysis for all patients in which an oncogenic driver is not identified should be planned."
    - "In the initial diagnostic setting, if tissue-based testing does not completely assess all recommended biomarkers owing to tissue quantity or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing."
    - "In the initial diagnostic setting, if the feasibility of timely tissue-based testing is uncertain, concurrent cfDNA [cell-free DNA] testing may aid in biomarker evaluation for treatment selection, provided negative results are considered per above limitations."
- “…the panel feels that plasma ctDNA testing should not be used to diagnose NSCLC; tissue should be used to diagnose NSCLC. Standards and guidelines for plasma ctDNA testing for somatic variants/mutations have not been published, there is up to a 30% false-negative rate, and variants can be detected that are not related to the tumor….careful consideration is required to determine whether ctDNA findings reflect a true oncogenic driver or an unrelated finding."
- "Data suggest that plasma ctDNA testing is a useful minimally invasive test that can be used to identify ALK, BRAF, EGFR, HER2, MET exon 14 skipping, RET, ROS1, and other oncogenic biomarkers that would not otherwise be identified in patients with metastatic NSCLC.”
- “The NCCN Guidelines for NSCLC provide recommendations for individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories."

Selected Relevant Publications

Many laboratories are developing liquid biopsy assays. For many of these assays, analytical validity studies have been performed; however, data regarding the clinical validity and clinical utility of these tests is still emerging.[3,8-41]

The TRACERx study (Tracking Non-small cell lung cancer evolution through therapy (Rx)) is a large, prospective clinical trial being conducted to evaluate "the relationship between intra-tumour heterogeneity and clinical outcome following surgery and adjuvant therapy." [42] Researchers plan to analyze the individual's tumors before surgery and multiple times after surgery during their treatment regimen. Tumor tissue and ctDNA in individual's blood will be examined in approximately 840 individuals with NSCLC. This trial is expected to continue until 2026.[42]

Limited evidence suggests that liquid biopsy with Guardant360, in individuals with advanced NSCLC, may be a reasonable non-invasive alternative to tumor biopsy, particularly in individuals unable to undergo standard tissue biopsy or in cases where tumor tissues are lacking or insufficient for proper mutation analysis.[43-58]

Several systematic reviews and meta-analyses have synthesized the findings of multiple studies to evaluate the clinical validity and clinical utility of cell-free circulating tumor DNA (ctDNA) to detect a variety of advanced cancer (excluding non-small cell lung cancer and hematological malignancies).[8-12,16-41,59-63] With the exception of FDA-approved ctDNA assays, the majority of assays have limited evidence of clinical validity and very limited-to-no evidence of clinical utility for use in individuals with advanced cancer.[63] Some studies have also reported relatively high rates of discordance between ctDNA assays and tissue-based testing. There is even less evidence regarding the validity of ctDNA testing in early stage disease, during treatment monitoring, or minimal residual disease (MRD) detection.[63] Additional well-designed prospective studies are needed to establish the clinical validity and clinical utility of ctDNA assays before ctDNA assays (liquid biopsy) can be widely adopted in clinical practice.

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Lynch Syndrome Genetic Testing

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Introduction

Lynch syndrome genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
EPCAM Deletion/Duplication Analysis	81403
Genomic Unity Lynch Syndrome Analysis	0238U
Known Familial Variant Not Otherwise Specified	81403
MLH1 Deletion/Duplication Analysis	81294
MLH1 Known Familial Mutation Analysis	81293
MLH1 Sequencing	81292
MSH2 Deletion/Duplication Analysis	81297
MSH2 Known Familial Mutation Analysis	81296
MSH2 Sequencing	81295
MSH6 Deletion/Duplication Analysis	81300
MSH6 Known Familial Mutation Analysis	81299
MSH6 Sequencing	81298
PMS2 Deletion/Duplication Analysis	81319
PMS2 Known Familial Mutation Analysis	81318
PMS2 Sequencing	81317

Criteria

Introduction

Requests for Lynch syndrome genetic testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Family History:
  - Known MLH1, MSH2, MSH6, PMS2, or EPCAM mutation in a close blood relative (1st, 2nd, or 3rd degree), AND
- Age- 18 years and older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Gene Sequencing and/or Deletion/Duplication Analysis of MLH1, MSH2, MSH6, PMS2, or EPCAM

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Gene requested has not been tested previously by the same methodology (i.e., sequencing or deletion/duplication analysis), AND
- Age- 18 years or older, AND
- Familial adenomatous polyposis (FAP) has been ruled out, AND
- Diagnostic Testing for Symptomatic Individuals
  - Personal history of colorectal cancer (CRC) (or other Lynch syndrome-related tumor[***]), and
  - If colorectal cancer:
    - Colorectal cancer diagnosed before 50 years of age, or
    - Colorectal cancer diagnosed at any age with (see Figure A):
      - MSI testing of tumor tissue shows MSI-high, or
      - Immunohistochemistry (IHC) testing of tumor tissue detects absence of MLH1, MSH2, MSH6, and/or PMS2 encoded protein products, and
      - BRAF mutation analysis and/or MLH1 hypermethylation analysis performed if indicated (according to figure A) and not consistent with

sporadic CRC (sporadic CRC is likely when the tumor has MLH1 promoter hypermethylation and/or the BRAF V600E mutation.), OR

- If other Lynch syndrome-associated tumor:
  - Endometrial cancer diagnosed before age 50, or
  - Endometrial cancer diagnosed at any age with abnormal tumor testing indicative of a mutation in a mismatch repair gene (see Figure A), or
  - Presence of synchronous or metachronous Lynch syndrome-associated tumors, regardless of age, or
  - Amsterdam II criteria are met:
    - ≥ 3 close blood relatives (1st, 2nd, or 3rd degree) with Lynch syndrome-associated tumor (symptomatic member can be one of the three), and
    - One should be a first-degree relative of the other two, and
    - ≥ 2 successive generations affected, and
    - ≥ 1 diagnosed before age 50, or
  - 5% or greater risk of Lynch syndrome based on one of the following mutations prediction models (MMRPro or MMRPredict), or
  - 2.5% or greater risk of Lynch syndrome based on PREMM[5], OR

- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - IHC and/or Lynch syndrome genetic testing results from affected family member are unavailable, AND
    - Colorectal or endometrial cancer diagnosed before age 50 in a first-degree relative, or
    - Colorectal or endometrial cancer and another synchronous or metachronous Lynch syndrome-associated tumor in a first-degree relative, or
    - ≥ 3 close blood relatives (1st, 2nd, or 3rd degree) with Lynch syndrome-associated tumor, where Amsterdam II criteria are met:
      - One should be a first-degree relative of the other two, and
      - ≥ 2 successive generations affected, and
      - ≥ 1 diagnosed before age 50, OR
  - 5% or greater risk of Lynch syndrome based on one of the following mutations prediction models (MMRPro or MMRPredict), OR
  - 2.5% or greater risk of Lynch syndrome based on PREMM[5], AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy

***Lynch syndrome-associated tumors include colorectal, endometrial, small bowel, stomach, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, brain/CNS tumors (usually glioblastomas), sebaceous adenomas, and keratoacanthomas.

Other Considerations

- Lynch syndrome testing may be performed as part of a multigene, multisyndrome panel. For information on multigene, multisyndrome panel testing, please refer to the guideline *Hereditary Cancer Syndrome Multigene Panels*, as this testing is not addressed here.
- Lynch syndrome genetic testing for those with colorectal cancer is generally not indicated in the absence of abnormal microsatellite instability (MSI) and/or IHC results on the colorectal tumor. MSI and/or IHC became part of the standard NCCN recommended evaluation for all people with colorectal cancer under the age of 70 (at a minimum) in May 2013. As a result, most people affected with colorectal cancer who are appropriate candidates for Lynch syndrome testing should have access to MSI and/or IHC. Lynch syndrome genetic testing without MSI and/or IHC results will only be considered necessary in extenuating circumstances and will require medical necessity review

Billing and Reimbursement

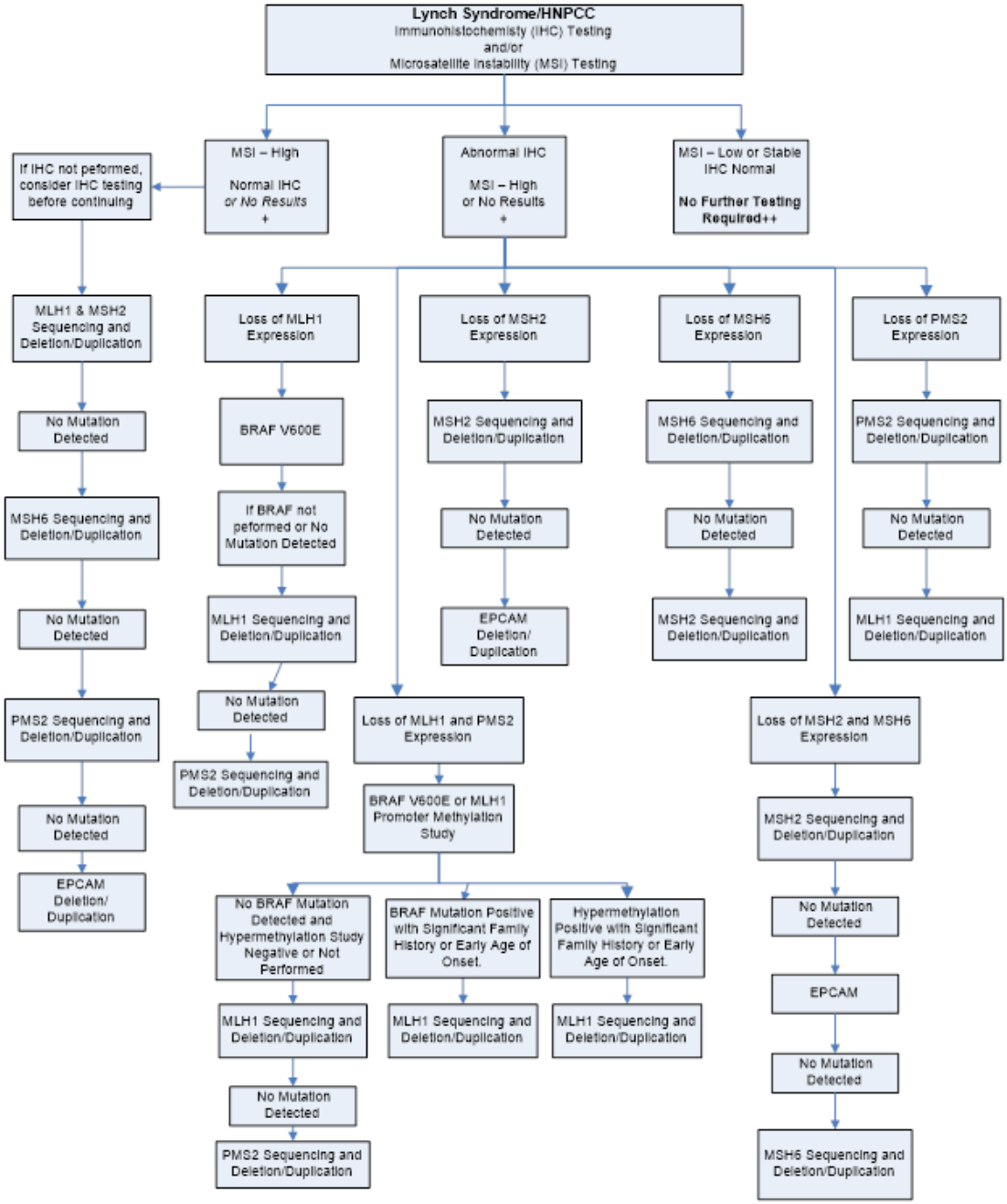
Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- For individuals that have had previous tumor testing (MSI and/or IHC), the testing algorithm as outlined in Figure A must be followed for payment of claim.

Figure A [9, 11]

Diagnostic Testing for Individuals with Previous Tumor Testing (MSI and/or IHC)



+ “Studies have shown that 45%–68% of cases with unexplained defective MMR (MSI-H and/or abnormal IHC with no evidence of MLH1 promoter hypermethylation when indicated) have biallelic somatic MMR gene inactivation (sometimes referred to as double somatic MMR mutations). Biallelic somatic MMR gene inactivation is defined by having either two pathogenic sequence variants or one pathogenic sequence variant and loss of heterozygosity [LOH] in the MMR genes. . . tumor sequencing may be

helpful for individuals with tumor testing showing dMMR and no germline pathogenic variant detected. If biallelic somatic MMR gene inactivation is identified, it is recommended that these patients and their close relatives be receive care based on their family history and NOT as if they have LS." [1]

++"If strong family history (i.e. Amsterdam criteria) or additional features of hereditary cancer syndromes (multiple colon polyps) are present, additional testing may be warranted in the proband, or consider tumor testing in another affected family member due to the possibility of a phenocopy." [12]

+++ Per NCCN guidelines, MLH1 promoter mutation analysis, not BRAF testing, is recommended for endometrial tumors when IHC testing has indicated a loss of MLH1 protein.[1]

What is Lynch syndrome?

Definition

Lynch syndrome (LS), also called hereditary non-polyposis colorectal cancer (HNPCC), is a hereditary cancer syndrome that is the most common cause of inherited colon and endometrial cancer.[1-3]

Prevalence

Lynch syndrome affects approximately 1 in 35 individuals with colorectal and endometrial cancer and around 1 in 370 individuals in the general population. Lynch syndrome accounts for 3% of all colorectal and endometrial cancer cases.[1-4]

Symptoms

Lynch syndrome is associated with up to a 61% lifetime risk for colorectal cancer and up to a 57% risk for endometrial cancer. [1] The risk is also increased for the development of the following cancers: small bowel, stomach, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, brain, bladder and prostate.[1,5] The average age of diagnosis for these cancers varies based on the gene that harbors the mutation.[1] Individuals may also develop skin lesions such as sebaceous adenomas and keratoacanthomas.[1,5]

Lynch syndrome should be suspected when the personal and family cancer history meets the *Revised Bethesda Guidelines* or the *Amsterdam II Criteria* (see below).[6,7] Risk prediction models, such as PREMM5, MMRpro, and MMRpredict, can be used to gauge the likelihood an individual has a mutation in a Lynch syndrome causative gene.[8]

Cause

Lynch syndrome is caused by mutations in any one of the following five genes: MLH1, MSH2, MSH6, PMS2, or EPCAM.[4,9]

Inheritance

Lynch syndrome is an autosomal dominant disorder.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Lynch syndrome mutations inherited in an autosomal recessive manner cause constitutional MMR deficiency syndrome (CMMR-D). Testing for CMMR-D is not addressed in this summary.[4,5]

Diagnosis

Lynch syndrome is diagnosed with the identification of a pathogenic mutation in MLH1, MSH2, MSH6, PMS2, or EPCAM.[4]

Management

Management for individuals with Lynch syndrome include more frequent cancer screenings and the option for risk reducing surgeries. The recommended management is dependent on which gene has the mutation. The recommended management guidelines include:[1,10]

- Colonoscopy: begin at 20-25 years for individuals with mutations in MLH1, MSH2, or EPCAM. Begin at 30-35 years in individuals with mutations in MSH6 or PMS2. Colonoscopy screening may begin earlier, 2-5 years earlier than the youngest diagnosis of colon cancer in the family, but not later than the aforementioned ages. Repeat colonoscopy is recommended every 1-2 years in individuals with mutations in MLH1, MSH2, or EPCAM, and every 1-3 years in individuals with mutations in MSH6 or PMS2.
- "[T]he panel suggests that aspirin may be used to reduce the future risk of CRC in patients with Lynch syndrome, but it is emphasized that the optimal dose and duration of therapy should be determined on an individual basis… Discussion of individual risks, benefits, adverse effects, and childbearing plans should also be included. The panel also recommends that providers carefully review patient-specific factors that may increase the risk of aspirin therapy, as well as factors that indicate a low future cumulative risk of CRC, as some individuals may be less likely to experience significant benefit."
- Hysterectomy and bilateral salpingo-oophorectomy (BSO) are available risk-reducing surgeries. "Timing of BSO should be individualized based on whether childbearing is complete, menopause status, comorbidities, family history, and LS gene, as risks for ovarian cancer vary by pathogenic variant." For women who decline this risk-reducing surgery, endometrial cancer screening may be an option, although a proven benefit of such screenings has not been documented. Insufficient

evidence exists in order to make a specific recommendation for prophylactic bilateral salpingo-oophorectomy for individuals with mutations in MSH6 and PMS2. Individuals with a PMS2 mutation "appear to be at no greater than average risk for ovarian cancer and may consider deferring surveillance and may reasonably elect not to have oophorectomy."

- Annual urinalysis at 30-35 years may be considered to screen for urothelial cancers. This screening may be considered in select individuals (e.g. those with a family history of urothelial cancer or in individuals with a mutation in MSH2).
- "Upper GI surveillance with EGD starting at age 30–40 years and repeat every 2–4 years, preferably performed in conjunction with colonoscopy. Age of initiation prior to 30 years and/or surveillance interval less than 2 years may be considered based on family history of upper GI cancers or high-risk endoscopic findings (such as incomplete or extensive GIM, gastric or duodenal adenomas, or Barrett esophagus with dysplasia). Random biopsy of the proximal and distal stomach should at minimum be performed on the initial procedure to assess for H. pylori (with treatment indicated if H. pylori is detected), autoimmune gastritis, and intestinal metaplasia... Individuals not undergoing upper endoscopic surveillance should have one-time noninvasive testing for H. pylori at the time of LS diagnosis, with treatment indicated if H. pylori is detected. The value of eradication for the prevention of gastric cancer in LS is unknown."
- Screening for pancreatic cancer can be considered at 50 years or 10 years younger than the earliest case of pancreatic cancer diagnosis in the family but not later than 50 years. This screening can be considered in individuals with at least one first- or second-degree relative with exocrine pancreatic cancer and on the same side of the family (or presumed same side) with the mutation in the Lynch syndrome causative gene. Notably, PMS2 mutations have not shown to increase the risk for pancreatic cancer.
- "Patients with LS should consider their risk based on the LS gene and family history of prostate cancer. The NCCN Guidelines for Prostate Cancer Early Detection recommend that it is reasonable for patients with LS to consider beginning shared decision-making about prostate cancer screening at age 40 years and to consider screening at annual intervals rather than every other year."
- "The panel recommends consideration of a skin exam every 1 to 2 years with a health care provider skilled in identifying Lynch syndrome- associated skin manifestations. The age at which to begin surveillance cannot be recommended with certainty, and therefore can be individualized."
- "Patients should be educated regarding signs and symptoms of neurologic cancer and the importance of prompt reporting of abnormal symptoms to their physicians."
- Annual physical examination starting at 20-25 years is recommended.

Special Considerations

Historically, Lynch syndrome has included the variants Muir-Torre syndrome (one or more Lynch syndrome-associated cancers and sebaceous neoplasms of the skin) and

Turcot syndrome (Lynch syndrome with glioblastoma).[4] These variant designations are considered outdated.

Test information

Introduction

Testing for Lynch syndrome may include tumor testing, known familial mutation testing, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Test Strategy

When the family Lynch syndrome mutation is known, at-risk relatives should be tested for that specific mutation only. Otherwise, genetic testing usually starts either with sequencing and deletion/duplication analysis of the gene identified from tumor IHC results. The National Comprehensive Cancer Network has outlined a comprehensive strategy for molecular testing of Lynch syndrome.[1] The first person tested should be the relative most likely to have Lynch syndrome in the family.

Testing those with a suspected Lynch syndrome-related cancer should begin with microsatellite instability or immunohistochemistry testing10325 on tumor tissue. The following table lists and describes the various testing scenarios.

When ...	Then ...
tumor tests suggest Lynch syndrome	that individual should be offered genetic testing to look for a mutation that causes Lynch syndrome. IHC studies may suggest which mismatch repair gene is likely to harbor a mutation.[1,9-11]
tumor tests are normal, and there is a young age of diagnosis or a strong family history of Lynch syndrome-associated cancers is present	genetic testing may still be warranted, or tumor testing in another family member with the most suspicious cancer history may be considered.[9]
tumor screening is not possible, and the individual meets the guideline criteria	direct genetic testing may be reasonable.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Lynch syndrome genetic testing.

Multiple Society Recommendations

The US Multi-Society Task Force (MSTF, 2014), the National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer (NSGC/CGA-ICC, jointly published, 2021), the National Comprehensive Cancer Network (NCCN, 2023), and the American College of Gastroenterology (ACG, 2015) have practice guidelines that addressed Lynch syndrome genetic testing. Generally, these recommendations agreed:[1,9,10,12]

- Test colorectal or endometrial tumors by microsatellite instability and/or immunohistochemistry first when tissue is available.
- Individuals with abnormal microsatellite instability and/or immunohistochemistry results (and no demonstrated BRAF mutation or hypermethylation of MLH1) should be offered genetic testing to identify a Lynch syndrome disease-causing mutation. Results from tumor testing should guide the genetic testing cascade. When tumor testing is not possible or results are inconclusive, genetic testing for an inherited mutation is indicated if an individual with a suspected Lynch syndrome-related cancer meets one of the first three Bethesda Guidelines or the family meets the Amsterdam Criteria (see below). If no affected family member is available for

testing, at-risk relatives can consider genetic testing if the family meets certain criteria. However, only a mutation positive result can be clearly interpreted. Mutation-negative results must be interpreted with caution; the chance of inconclusive results is high because the family mutation may not be detectable. Once a Lynch syndrome disease-causing mutation has been identified, at-risk relatives should be offered genetic testing for that specific mutation.

Manchester International Consensus Group

The Manchester International Consensus Group (2019) stated the following regarding germline testing for Lynch syndrome in women with gynecological cancer:[13]

- "The Consensus Group strongly recommends that tumor MMR or MSI status is used to identify women for germline MMR testing. There is no evidence to advocate MSI over MMR immunohistochemistry or vice versa (grade B)."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) recommended universal tumor screening for colorectal and endometrial cancers (see above). Germline multigene panel testing is recommended as an alternate or additional option for Lynch syndrome screening for the following:[1]

- "An individual with a LS-related cancer and any of the following:
  - Diagnosed <50 y
  - A synchronous or metachronous LS-related cancer regardless of age
  - 1 first-degree or second-degree relative with a LS-related cancer diagnosed <50 y
  - ≥2 first-degree or second-degree relatives with a LS-related cancer regardless of age
- Family history of any of the following:
  - ≥1 first-degree relative with a colorectal or endometrial cancer diagnosed <50 y
  - ≥1 first-degree relative with a colorectal or endometrial cancer and a synchronous or metachronous LS-related cancer regardless of age
  - ≥2 first-degree or second-degree relatives with LS-related cancers, including ≥1 diagnosed <50 y
  - ≥3 first-degree or second-degree relatives with LS-related cancers regardless of age
- Increased model-predicted risk for LS"
- "Personal history of a tumor with MMR deficiency determined by PCR, NGS, or IHC diagnosed at any age"

NCCN recommended genetic testing start with the most informative person in a family. Other close family members may consider testing if a person affected with a related cancer is not available.

Society of Gynecologic Oncology

The Society of Gynecologic Oncology (SGO, 2023) recommended that all individuals diagnosed with endometrial cancer undergo molecular testing that includes assessment of mismatch repair deficiency status.[14]

Revised Bethesda Guidelines

According to the *Revised Bethesda Guidelines*, consider Lynch syndrome tumor screening when any one of the following criteria are met:[6,15]

- colorectal cancer is diagnosed before the age of 50
- presence of synchronous or metachronous colorectal cancer, or other Lynch syndrome-associated tumor***, regardless of age
- microsatellite unstable (MSI-H) tumor pathology before the age of 60, examples include
    - tumor-infiltrating lymphocytes
    - Crohn's-like lymphocytic reaction
    - mucinous or signet-ring differentiation
    - medullary growth pattern, or
    - other reported features
- colorectal cancer diagnosed in an individual with at least one first-degree relative, including parent, sibling, or child with a Lynch syndrome-related tumor***, one of whom was diagnosed before the age of 50, or
- colorectal cancer diagnosed in an individual with at least two first- or second-degree relatives with Lynch syndrome-related tumors*** at any age.

Amsterdam II Criteria

According to *Amsterdam II Criteria*, Lynch syndrome is likely when all of the following criteria are met:[7]

- there are at least three relatives with Lynch syndrome associated tumors***
- one affected relative is a first-degree relative (parent, sibling, child) of the other two
- affected relatives are in two or more successive generations
- at least one Lynch syndrome-related tumor was diagnosed before age 50, and
- FAP has been excluded on the basis of no polyposis.

Tumors must be verified by pathology.

***Lynch syndrome-associated tumors include

- colorectal
- endometrial
- small bowel
- stomach
- ovarian
- pancreatic
- ureteral and renal pelvis
- biliary tract
- brain tumors, usually glioblastomas associated with Turcot syndrome variant
- sebaceous adenomas, and
- keratoacanthomas, associated with a Muir-Torre syndrome variant.

References

Introduction

These references are cited in this guideline.

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Marfan Syndrome Genetic Testing

MOL.TS.202.A
v2.0.2024

Introduction

Marfan syndrome genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
FBN1 Deletion/Duplication Analysis	81479
FBN1 Known Familial Mutation Analysis	81403
FBN1 Sequencing	81408
TGFBR1 Known Familial Mutation Analysis	81403
TGFBR1 Sequencing	81405
TGFBR2 Known Familial Mutation Analysis	81403
TGFBR2 Sequencing	81405

Criteria

Introduction

Requests for Marfan syndrome testing are reviewed using the following criteria.

FBN1 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of FBN1 that would detect the familial mutation, and
  - FBN1 mutation identified in 1st degree biological relative, AND

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- Rendering laboratory is a qualified provider of service per the Health Plan policy.

FBN1 Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous FBN1 sequencing, and
  - No known FBN1 mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Genetic testing is necessary because there is uncertainty in the clinical diagnosis, and
    - Aortic root enlargement (Z-score greater than or equal to 2.0) and a systemic score less than 7, without ectopia lentis, or
    - Ectopia lentis, or
  - An individual has a clinical diagnosis of Marfan syndrome based on the revised Ghent Criteria, and
    - Genetic testing is needed in order to offer testing to family members, or
    - Genetic testing is needed for prenatal diagnosis purposes, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

FBN1 Deletion/Duplication Analysis

- Criteria for FBN1 sequencing are met, AND
- No previous deletion/duplication analysis of FBN1, AND
- No mutations detected in full sequencing of FBN1, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

TGFBR1/2 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:

- No previous genetic testing of TGFBR1/2 that would detect the familial mutation, and
- TGFBR1/2 mutation identified in 1st degree biological relative, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

TGFBR2 Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous TGFBR2 testing performed, and
  - No mutations detected in full sequencing of FBN1, and
  - No mutations detected in deletion/duplication analysis of FBN1, AND
- Diagnostic Testing for Symptomatic Individuals:
  - There is a strong clinical suspicion of MFS based on the Ghent criteria (Member met testing guidelines for FBN1 sequencing), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

TGFBR1 Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous TGFBR1 testing performed, and
  - No mutations detected in full sequencing or deletion/duplication analysis of FBN1, and
  - No mutations detected in full sequencing of TGFBR2, AND
- Diagnostic Testing for Symptomatic Individuals:
  - There is a strong clinical suspicion of MFS based on the Ghent criteria (Member met testing guidelines for FBN1 sequencing), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Marfan syndrome?

Definition

Marfan syndrome is an autosomal dominant disorder that affects connective tissue in many parts of the body.

Incidence

Marfan syndrome affects 1 in 5,000 to 1 in 10,000 individuals.[1]

Symptoms

Symptoms can present in males or females at any age and typically worsen over time. Infants who present with symptoms typically have the most severe disease course.[1]

Signs and symptoms of Marfan syndrome usually include (some combination of the following):[1]

- Cardiovascular system — dilatation of the aorta, predisposition for aortic tear or rupture, mitral valve prolapse (with or without congestive heart failure), tricuspid valve prolapse, and enlargement of the proximal pulmonary artery.[1]
- Skeletal system — long bone overgrowth and joint laxity, long arms and legs, scoliosis, sternum deformity (pectus excavatum or carinatum), pes planus, long thin fingers and toes, micrognathia, retrognathia, high-arched palate, deep set eyes, malar hypoplasia, downslanting palpebral fissures, and long thin face.[1]
- Ocular system — severe myopia, dislocated lens of eye (ectopia lentis), elongation of the globe with or without flattened cornea, detached retina, glaucoma, early cataracts.[1]
- Other symptoms – dural ectasia (stretching of the dural sac), hernias, stretch marks on the skin, and lung bullae.[1]

Cause

Marfan syndrome is caused by mutations in the FBN1 gene, located on chromosome 15.[1]

- Genetic testing for Marfan syndrome typically starts with sequencing of the FBN1 gene. If negative, deletion/duplication of FBN1 should be considered.[1]
    - Sequencing of the FBN1 gene will find a causative mutation in approximately 90-93% of people with a clinical diagnosis of Marfan syndrome.[1]
    - Deletions and duplications have been described in approximately 5% of individuals with a clinical diagnosis of Marfan syndrome.[1]
- Mutations in the TGFBR1 or TGFBR2 gene have been found in some individuals with a clinical suspicion of Marfan syndrome and no identifiable FBN1 mutation.[1] Mutations in TGFBR1/2, and 4 other genes, are associated with Loeys-Dietz

syndrome (LDS). Some features of Marfan syndrome and LDS overlap. However, people with LDS typically have a greater risk of frequent aortic dissection and rupture at smaller dimensions and in early childhood.[1]

- The presence of a mutation in the FBN1 gene alone does not diagnose Marfan syndrome. FBN1 mutations may cause conditions other than Marfan syndrome. Conversely, some people who meet the clinical diagnostic criteria for Marfan syndrome do not have an identifiable FBN1 mutation.[1]

Inheritance

Marfan syndrome is inherited in an autosomal dominant fashion.[1]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Approximately 25% of cases of Marfan syndrome are the result of a new genetic change (de novo mutation) in the affected person and are not inherited from a carrier parent.[1]

Diagnosis

A clinical diagnosis of Marfan syndrome is made according to Ghent Criteria.[1-3]

- With no known family history, a Marfan syndrome diagnosis is confirmed if any ONE of the following is met:[1-3]
  - Significant aortic dilation (Z-score ≥2)/dissection + ectopia lentis**
  - Significant aortic dilation (Z-score ≥2)/dissection + FBN1 mutation
  - Aortic dilation/dissection + sufficient points from other system findings**
  - Ectopia lentis + FBN1 mutation known to be associated with aortic disease
- With a known family history, the presence of any ONE of the following is diagnostic:[1-3]
  - Ectopia lentis
  - Significant aortic root enlargement (Z-score ≥2 in those >20 years of age or ≥3 in those <20 years of age)**
  - Sufficient points (≥7) from other system findings**

** Marfan syndrome can be clinically diagnosed in these cases, provided there are not other findings that more strongly suggest Sphrintzen-Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome, which have clinical overlap. Or, these

conditions are unlikely based on genetic or collagen testing.

Systemic scoring system[1-3]

- Wrist and Thumb Sign - 3 points
- Wrist or Thumb Sign - 1 point
- Pectus Carinatum deformity - 2 points
- Pectus Excavatum or chest asymmetry -1 point
- Hindfoot deformity - 2 points
- Plan pes planus -1 point
- Pneumothorax - 2 points
- Dural Ectasia - 2 points
- Protrusio Acetabulae - 2 points
- Reduced upper seg/lower seg and inc. arm span/height ratios - 1 point
- Scoliosis or thoracolumbar kyphosis - 1 point
- Reduced elbow extension - 1 point
- 3 of 5 facial features: Dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia - 1 point
- Skin striae - 1 point
- Myopia - 1 point
- Mitral Valve Prolapse - 1 point

According to the Ghent criteria, many of the manifestations of Marfan syndrome can emerge with age. Therefore, it is not advisable to establish definitive alternative diagnosis in individuals younger than age 20 years who have some physical manifestations of Marfan syndrome but not enough for a clinical diagnosis. In this circumstance, the following is suggested:[2]

- “If the systemic score is <7 and/or borderline aortic root measurements (Z-score <3) are present (without an FBN1 pathogenic variant), use of the term ‘nonspecific connective tissue disorder’ is suggested until follow-up echocardiographic evaluation shows aortic root dilation (Z-score ≥3).” [2]
- "If an FBN1 pathogenic variant is identified in simplex or familial cases but aortic root Z-score is below 3.0, the term 'potential Marfan syndrome' should be used until the aorta reaches this threshold."[2]

Diagnostic evaluations recommended:

- Ophthalmologist evaluation with someone familiar with Marfan syndrome[1]
- Evaluation for skeletal manifestations by an orthopedist[1]
- Cardiovascular evaluations[1]

- Evaluation by a clinical geneticist and/or genetic counselor[1]

Management

The healthcare needs of individuals with Marfan syndrome are best managed by a multidisciplinary team including a clinical geneticist, cardiologist, ophthalmologist, orthopedist, and cardiothoracic surgeon. Management includes:

- Ophthalmology: annual examination with correction of refractive errors. Surgical removal of dislocated lens with artificial lens implantation.[1]
- Orthopedist: stabilization, and if needed surgical correction, of scoliosis. Repair of pectus deformity, although this is often cosmetic. Orthotics and arch supports as indicated.[1]
- Cardiology: annual echocardiography to monitor the dimensions of the ascending aorta. Medications (such as beta blockers or angiotensin receptor blockers) that reduce the stress on the aorta are usually started at diagnosis or with the notation of aortic dilatation that is significant and/or progressive.
    - Cardiothoracic surgery: "Surgical repair of the aorta is indicated either when the maximal measurement of the aortic root approaches 5.0 cm in adults or older children, when the rate of increase of the aortic root diameter approaches 0.5-1.0 cm per year, or if there is progressive and severe aortic regurgitation. For younger children, aortic root surgery should be considered once: (1) the rate of increase of the aortic root diameter approaches 0.5-1.0 cm per year, or (2) there is progressive and severe aortic regurgitation."[1] Children with Marfan syndrome may have severe and progressive mitral valve regurgitation with ventricular dysfunction requiring surgery.[1]

Avoidance of certain activities and agents are also recommended. Examples include:[1]

- Isometric exercises, contact sports, and competitive sports and activities that can exacerbate joint pain or cause injury
- Decongestants and excessive caffeine as these stimulate the cardiovascular system
- Medications that cause vasoconstriction
- Correction of refractive errors with LASIK

Survival

The greatest impact to the survival of individuals with Marfan syndrome are the manifestations in the cardiovascular system. With proper surveillance and management, the life expectancy of individuals with Marfan syndrome approximates that of individuals without Marfan syndrome.[1]

Test information

Introduction

Testing for Marfan syndrome may include known familial mutation testing, next generation sequencing, or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Additional Testing Information

Additional testing information includes the following:

TGFBR1/2 Testing

If a mutation is not found in FBN1 and there is a strong clinical suspicion of Marfan syndrome, TGFBR1/2 genetic testing may be indicated. Given the increased risk of aortic dissection and rupture at smaller dimensions and in early childhood in LDS,[1] it is important to confirm whether there is a mutation in one of these two genes.

Multigene Panel Testing

There are other conditions which can cause familial aortic aneurysm and dissections and/or have overlapping features with Marfan syndrome. Many laboratories offer panel testing for FBN1 as well as other genes that cause these

conditions.[1] Detection rates of expanded panels vary by laboratory and depend on the genes included and the methods used for testing.[1] A thorough clinical evaluation along with appropriate imaging studies will point to a specific diagnosis in many cases.[1] Testing for conditions that are clinically indicated is most appropriate.[1] Testing multiple genes, without supporting clinical features, has the potential to yield results that are difficult to interpret.[1] The chance that a variant of uncertain significance will be found increases as more genes are tested. According to the American College of Medical Genetics and Genomics, "There is no case of classic, bona fide MFS due to mutations in a gene other than FBN1." [5] Therefore, when there is a strong clinical suspicion for Marfan syndrome, genetic testing for genes other than FBN1 is typically not needed, with the exception of TGFBR1/2 testing. For information on multigene panel testing that includes Marfan Syndrome, please refer to the guideline *Hereditary Connective Tissue Disorder Genetic Testing,* as this testing is not addressed here.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Marfan syndrome.

American College of Medical Genetics and Genomics

According to the American College of Medical Genetics and Genomics (ACMG, 2012), "There is no case of classic, bona fide MFS [Marfan syndrome] due to mutations in a gene other than FBN1. However, current clinical molecular testing of FBN1 successfully detects mutations in such unequivocal patients in only about 90-95% of cases. For all of these reasons, searching for mutations in FBN1 continues to have a circumscribed role in the diagnosis of equivocal cases. Said differently, MFS remains, by and large, a clinical diagnosis." [5]

American Heart Association and American College of Cardiology

The American Heart Association and American College of Cardiology published clinical practice guidelines for the diagnosis and management of aortic disease. They stated the following regarding genetic evaluation and family screening:[6]

- Risk factors for familial thoracic aortic disease (TAD), also known as heritable thoracic aortic disease (HTAD), were outlined as:
  - "TAD and syndromic features of Marfan syndrome, Loeys-Dietz syndrome, or vascular EDS syndrome
  - TAD presenting at <60 years
  - A family history of either TAD or peripheral/intracranial aneurysms in a first- or second-degree relative

  - A history of unexplained sudden death at a relatively young age in a first- or second-degree relative"

- "In patients with aortic root/ascending aortic aneurysms or aortic dissection, obtaining a multigenerational family history of TAD, unexplained sudden deaths, and peripheral and intracranial aneurysms is recommended."
- "In patients with aortic root/ascending aortic aneurysms or aortic dissection and risk factors for HTAD, genetic testing to identify pathogenic/likely pathogenic variants (ie, mutations) is recommended."
- "In patients with an established pathogenic or likely pathogenic variant in a gene predisposing to HTAD, it is recommended that genetic counseling be provided and the patient's clinical management be informed by the specific gene and variant in the gene."
- " In patients with TAD who have a pathogenic/likely pathogenic variant, genetic testing of at-risk biological relatives (ie, cascade testing) is recommended. In family members who are found by genetic screening to have inherited the pathogenic/likely pathogenic variant, aortic imaging with TTE (if aortic root and ascending aorta are adequately visualized, otherwise with CT or MRI) is recommended."
- " In a family with aortic root/ascending aortic aneurysms or aortic dissection, if the disease-causing variant is not identified with genetic testing, screening aortic imaging of at-risk biological relatives (ie, cascade testing) is recommended."
- "In patients with aortic root/ascending aortic aneurysms or aortic dissection, in the absence of either a known family history of TAD or pathogenic/likely pathogenic variant, screening aortic imaging of first-degree relatives is recommended."
- "In patients with acute type A aortic dissection, the diameter of the aortic root and ascending aorta should be recorded in the operative note and medical record to inform the management of affected relatives."

Canadian Cardiovascular Society

The Canadian Cardiovascular Society (CCS, 2014) stated the following:[7]

- “We recommend clinical and genetic screening for suspected Marfan syndrome to clarify the nature of the disease and provide a basis for individual counseling” (Strong recommendation, High quality evidence)
- “We recommend that genetic counselling and testing be offered to first degree relatives of patients in whom a causal mutation of a TAD-associated gene is identified. We recommend that aortic imaging be offered only to mutation carriers.” (Strong recommendation, low quality evidence)

Cardiac Society of Australia and New Zealand Cardiovascular Genetic Diseases Council

The Cardiac Society of Australia and New Zealand (CSANZ, 2017) Cardiovascular Genetic Diseases Council stated the following:[8]

- "A definitive molecular genetic diagnosis can clarify an equivocal clinical picture or result in a diagnosis in an apparently phenotypically normal individual. It is unknown at this stage what proportion of patients with these different genetic mutations will develop aortic dilatation or dissection. Identification of a causal mutation allows for the provision of accurate genetic counseling, the screening of at-risk family members and offers the possibility of accurate prenatal or preimplantation genetic diagnosis."
- "Molecular confirmation of a suspected clinical diagnosis is increasingly important for guiding patient management. As an example, an individual who looks marfanoid will have more extensive arterial imaging screening if identified to have a SMAD3 mutation as opposed to an FBN1 mutation."

European Reference Network on Rare Multisystemic Cardiovascular Disease

The HTAD Rare Disease Working Group of the European Reference Network on Rare Multisystemic Cardiovascular Diseases (VASCERN, 2023) recommended a strategy for evaluation and diagnosis of individuals and families with hereditary thoracic aortic disease.[9] They recommended consideration of genetic testing, under supervision of a provider with experience in HTAD, when "there is a high suspicion of an underlying genetic aortopathy and includes:

- patients with a familial form with or without hypertension (2 first or second-degree affected relatives) of thoracic aortic dissection or aneurysm (TAA/TAD)
- sporadic TAA/TAD as defined above, at
    - any age, in the absence of arterial hypertension, or
    - <70 years of age in presence of hypertension
- patients with non-traumatic ectopia lentis compatible with MFS
- patients with a combination of TAAD and syndromic features of Marfan or LDS."

European Society of Cardiology

The European Society of Cardiology (ESC, 2014) stated the following: [10]

- “Once a familial form of TAAD is highly suspected, it is recommended to refer the patient to a geneticist for family investigation and molecular testing.” (Class I, Level C)

Joint Committee Guidelines

Joint evidence-based guidelines from the American College of Cardiology Foundation/ American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine.
(ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM, 2010) for the diagnosis and management of thoracic aortic disease include Marfan syndrome.[4] Genetic testing for Marfan syndrome is addressed in the following guidelines statements:

- "If the mutant gene (FBN1, TGFBR1, TGFBR2, COL3A1, ACTA2, MYH11) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing. Then, only the relatives with the genetic mutation should undergo aortic imaging." [Class 1, Level of Evidence C. Recommendation that procedure or treatment is useful/effective. It is based on very limited populations evaluated and only expert opinion, case studies or standard of care.]
- "The criteria for Marfan syndrome is based primarily on clinical findings in the various organ systems affected in the Marfan syndrome, along with family history and FBN1 mutations status."
- Recommend echo at baseline, repeat at 6 months to look for progression then yearly if stable (Class 1, Level of Evidence C).
- Determining genetic etiology guides prophylactic aortic surgery.

Selected Relevant Publications

An international group of Marfan syndrome experts initially proposed clinical diagnostic criteria for Marfan syndrome in 1996, called the Ghent nosology that gained wide acceptance.[11]

- The Ghent criteria were updated in 2010 and now address the role of FBN1 genetic testing in the diagnosis of Marfan syndrome.[2] They do not include guidelines about when to test for a familial mutation, but do indicate that finding a familial mutation is not sufficient evidence alone to make a definitive diagnosis, stating: "If an FBN1 mutation is identified in sporadic or familial cases but aortic root measurements are still below Z=3, we propose to use the term 'potential MFS' [Marfan syndrome] until the aorta reaches threshold" [2]

References

Introduction

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Maturity-Onset Diabetes of the Young Genetic Testing

MOL.TS.258.A
v2.0.2024

Introduction

Maturity-onset diabetes of the young (MODY) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
GCK Deletion/Duplication	81479
GCK Sequencing	81406
HNF1A Deletion/Duplication	81479
HNF1A Sequencing	81405
HNF4A Deletion/Duplication	81479
HNF4A Sequencing	81406
MODY Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
MODY Multigene Panel	81479

400 Buckwalter Place Boulevard, Bluffton, SC 29910 (800) 918-8924

Criteria

Introduction

Requests for maturity-onset diabetes of the young (MODY) genetic testing are reviewed using these criteria.

For gene testing in non-MODY contexts (e.g., neonatal diabetes, familial hyperinsulinism, etc.), refer to the general policies, *Genetic Testing to Diagnose Non-Cancer Conditions* and *Genetic Testing by Multigene Panels*, as appropriate.

HNF1A Sequencing and Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous HNF1A gene sequencing or deletion/duplication analysis, and
  - No known MODY mutation in biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has a diagnosis of diabetes prior to 35 years of age, and
  - Member has a biological parent with diabetes, and
  - Member does NOT have symptoms consistent with a specific condition or specific gene mutation, and
  - Member does NOT have any of the following features:
    - Extra-pancreatic manifestations (e.g., congenital malformations and other signs of syndromic diabetes), or
    - Pancreatic autoantibodies suggestive of type 1 diabetes, or
    - Body mass index (BMI) greater than or equal to 35 kg/m^2, or
    - Acanthosis nigricans, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

HNF4A Sequencing and Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:

- No previous HNF4A gene sequencing or deletion/duplication analysis, and
- No known MODY mutation in biologic relative, and
- Member has previous HNF1A testing with no deleterious mutation found, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Member meets criteria for HNF1A testing, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

GCK Sequencing and Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous GCK gene sequencing or deletion/duplication analysis, and
  - No known MODY mutation in biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member meets criteria for HNF1A testing and has had previous HNF1A testing with no deleterious mutation found, or
  - Member has a personal history of the following features presenting outside of pregnancy:
    - Persistent, stable elevation of fasting blood glucose (5.5-8 mmol/L), and
    - HbA1C that is no more than mildly elevated (less than or equal to 7.5%), and
    - At least one oral glucose tolerance test demonstrates a small increment (less than 4.6 mmol/L), or
  - Member has a personal history of the following features in the context of gestational diabetes:
    - Persistent elevation of fasting blood glucose (5.5-8 mmol/L) before, during, and after pregnancy, and
    - At least one oral glucose tolerance test demonstrates a small increment (less than 4.6 mmol/L) either during or after pregnancy, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Sequencing and Deletion/Duplication Analysis of ABCC8, BLK, CEL, HNF1B, INS, KCNJ11, KLF11, NEUROD1, PAX4, and PDX1

Individual testing of these genes for the purpose of diagnosing MODY is not medically necessary.

MODY Multigene Panel Testing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous MODY genetic testing, and
  - No known MODY mutation in biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has a diagnosis of diabetes prior to 35 years of age, and
  - Member has a family history of diabetes consistent with autosomal dominant inheritance, and
  - Member does NOT have symptoms consistent with a specific condition or specific gene mutation, and
  - Member does NOT have any of the following features:
    - Extra-pancreatic manifestations (e.g., congenital malformations and other signs of syndromic diabetes), or
    - Pancreatic autoantibodies suggestive of type 1 diabetes, or
    - Body mass index (BMI) greater than or equal to 35 kg/m^2, or
    - Acanthosis nigricans, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will

not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- ABCC8, BLK, CEL, HNF1B, INS, KCNJ11, KLF11, NEUROD1, PAX4, and PDX1 analysis are not separately reimbursable for the purposes of MODY testing.
- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81479*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
    - When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
        - HNF1A
        - GCK
        - HNF4A

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is MODY?

Definition

Maturity-onset diabetes of the young (MODY) is a type of monogenic diabetes characterized by non-insulin-dependent diabetes and early onset (usually before age 35 years).[1-4]

Incidence

As of 2015, approximately 30.3 million people in the United States had diabetes, or 9.4% of the population.[5] The most common types of diabetes are type 1 and type 2. The genetic basis of these types of diabetes is largely unknown. The disease is

thought to be the result of a combination of multiple genetic and environmental risk factors.[5] Monogenic forms of diabetes are rare, accounting for approximately 2% of all diabetes cases.[1-3]

Symptoms

Diabetes is a disorder that results in elevated blood glucose. Over time, the disorder can cause various health problems, including diseases of the heart, kidneys, eyes, and nervous system.

Cause

Monogenic forms of diabetes are caused by a mutation in a single gene. There are at least 14 known MODY genes. Three genes account for the majority of cases.[2-4]

- MODY3: Mutations in the hepatocyte nuclear factor-1 alpha (HNF1A) gene are the most common cause of MODY, accounting for 30-65% of all cases. This type is characterized by a progressive insulin secretory defect due to beta-cell failure. Laboratory evaluations are negative for pancreatic islet cell antibodies (ruling out type 1) and glycosuria is detectable even at low blood glucose levels (<10 mmol/l). Treatment of choice for people with this type of MODY is sulfonylureas, and a majority of individuals can be transferred from insulin to oral agents.
- MODY2: Mutations in the glucokinase gene (GCK) are the next most common cause of MODY, accounting for approximately 30-50% of cases. GCK encodes the glucokinase enzyme, which acts as the pancreatic glucose sensor. Mutations result in lifelong, stable, mild fasting hyperglycemia. HbA1C values are usually just above the high normal range. People with GCK mutations rarely require treatment. This type of MODY may be detected during pregnancy, when glucose tolerance testing is routinely performed.
- MODY1: Mutations in the hepatocyte nuclear factor-4 alpha (HNF4A) gene cause a clinical presentation similar to HNF1A. However, mutations in this gene are much less common (less than 10% of MODY). Age of onset may be later, and there is not a low renal threshold. HNF4A mutations can also cause high birth weight in newborns and transient neonatal hypoglycemia. These individuals are also more sensitive to sulfonylurea treatment.

The remaining genes are rare causes of MODY, each accounting for less than 5% of cases:[2-4]

- MODY5: Caused by heterozygous mutations in HNF1B. The vast majority of HNF1B mutations cause Renal Cysts and Diabetes Syndrome, which is associated with diabetes, renal cysts, genitourinary malformations, pancreatic atrophy, hyperuricemia, and abnormal liver function tests.
- MODY8: Caused by heterozygous mutations in CEL. Affected individuals also have pancreatic exocrine dysfunction (diabetes-pancreatic-exocrine dysfunction syndrome).

- Others include: MODY4 (PDX1/IPF-1), MODY6 (NEUROD1), MODY7 (KLF11), MODY9 (PAX4), MODY10 (INS), MODY11 (BLK), MODY12 (ABCC8), MODY13 (KCNJ11), and APPL1 (MODY14).

Other monogenic causes of pediatric diabetes include the following (not meant to be an all-inclusive list):[2,6,7]

- Permanent neonatal diabetes mellitus (PNDM), defined as persistent hyperglycemia in the first 6 months of life. It is most commonly caused by mutations in the ABCC8, KCNJ11, and INS genes. Biallelic mutations in GCK and PDX1 are less common causes.
- Transient neonatal diabetes mellitus (TNDM), which accounts for ~50% of all neonatal diabetes. Affected individuals are at risk for recurrence later in life. 70% of TNDM cases are due to 6q24 methylation defects, while ABCC8 and KCNJ11 combined account for an additional 26% of cases.
- Cystic fibrosis, caused by biallelic CFTR mutations (for more information, see test-specific guideline, *Cystic Fibrosis Genetic Testing*)
- Immune dysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX syndrome), due to mutations in FOXP3
- Maternally inherited diabetes and deafness (MIDD), caused by mutations in mitochondrial genes: MT-TL1, MT-TK, or MT-TE
- Wolcott-Rallison syndrome, due to mutations in EIF2AK3
- Wolfram syndrome, caused by mutations in WFS1 and less often CISD2
- Other genes associated with PNDM and extra-pancreatic features include GATA6, GLIS3, IER3IP1, NEUROG3, PTF1A, and RFX6.

Inheritance

MODY is typically inherited in an autosomal dominant manner.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Mutations that occur de novo in an affected individual, reduced penetrance, and variable expressivity have been reported.[4] Thus, the absence of a family history does not, by itself, rule out a diagnosis of MODY.

Diagnosis

Diabetes evaluations may include assessment of pancreatic autoantibodies, plasma glucose levels, hemoglobin A1C assessment (HbA1C), and oral glucose tolerance testing (OGTT). For young individuals in whom a diagnosis of type 1 or type 2 diabetes

is considered unlikely, genetic testing for monogenic diabetes may be considered, especially in the presence of a strong family history.[5]

Management

Like other forms of diabetes, monogenic diabetes is treated with diet, oral antidiabetic agents, and/or insulin, as required for blood sugar regulation.[4] Most individuals with MODY are not insulin-dependent. Knowledge of the specific genetic cause of MODY may help guide management.

Survival

Survival of affected individuals was reduced when compared with unaffected relatives, specifically with regard to cardiovascular-related causes of death. [8]

Test information

Introduction

Testing for MODY may include single gene sequence analysis, single gene deletion/duplication analysis, or multigene panels of various sizes.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes.

Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

MODY multigene panels include a wide variety of genes associated with MODY and monogenic diabetes in general. Some panels may also include genes associated with other types of monogenic diabetes and glycemic disorders, such as neonatal diabetes, syndromic diabetes, and familial hyperinsulinism.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to MODY genetic testing.

American Diabetes Association

The American Diabetes Association (ADA, 2023) stated:[9]

- "Children and young adults who do not have typical characteristics of type 1 or type 2 diabetes and who often have a family history of diabetes in successive generations (suggestive of an autosomal dominant pattern of inheritance) should have genetic testing for maturity-onset diabetes of the young." (A)
- "The diagnosis of monogenic diabetes should be considered in children and adults diagnosed with diabetes in early adulthood with the following findings:"
    - "Diabetes without typical features of type 1 or type 2 diabetes (negative diabetes-associated autoantibodies, no obesity, lacking other metabolic features, especially with strong family of diabetes)
    - Stable, mild fasting hyperglycemia (100-500 mg/dL [5.5-8.5 mmol/L]), stable A1C between 5.6% and 7.6% (between 38 and 60 mmol/mol), especially if no obesity"

European Molecular Genetics Quality Network

The European Molecular Genetics Quality Network (EMQN, 2008) made the following recommendations for testing:[3]

- Testing for GCK mutations (presentation outside of pregnancy):
    - Persistent, stable elevation of fasting blood glucose (5.5-8 mmol/l)
    - HbA1c just above the upper limit of normal (rarely exceeds 7.5%)
    - Oral glucose tolerance testing demonstrates a small increment (4.6 mmol/l is often used to prioritize testing)

  - May have a family history consistent with autosomal dominant inheritance

- Testing for GCK mutations (for evaluation of gestational diabetes):
  - Persistent elevation of fasting blood glucose (5.5-8 mmol/l) before, during and after pregnancy
  - At least one oral glucose tolerance test with an increment of <4.6 mmol/l (either during or after pregnancy)

- Testing for HNF1A mutations:
  - Young-onset diabetes (<25 years old)
  - Non-insulin-dependent diabetes
  - Family history of diabetes (at least two generations)
  - Absence of pancreatic islet autoantibodies
  - Glycosuria at blood glucose levels <10 mmol/l
  - Marked sensitivity to sulfonylureas
  - Features suggestive of monogenic diabetes (lack of obesity or evidence of insulin resistance, absence of acanthosis nigricans, etc)

- Testing for HNF4A mutations:
  - Should be considered when HNF1A analysis is normal but the clinical features are strongly suggestive of HNF1A
  - “When diabetic family members have marked macrosomia (>4.4 kg at term) or if diazoxide-responsive neonatal hyperinsulinism has been diagnosed in the context of familial diabetes.”
  - “Macrosomic babies with diazoxide-responsive hyperinsulinism and a strong family history of diabetes should be considered for HNF4A mutation screening.”

- Syndromic forms of diabetes, including HNF1B and CEL mutations, “are not included in these guidelines since testing is guided by the non-endocrine pancreatic or extra-pancreatic clinical features.”

International Society for Pediatric and Adolescent Diabetes

The International Society for Pediatric and Adolescent Diabetes (ISPAD, 2022) made the following recommendations:[2]

- "Testing for GCK-MODY, which is the commonest cause of persistent, incidental hyperglycemia in the pediatric population, is recommended for mild stable fasting hyperglycemia that does not progress." (B)

- "In familial autosomal dominant symptomatic diabetes, mutations in the HNF1A gene (HNF1A-MODY) should be considered as the first diagnostic possibility" (B)
- "Specific features can suggest subtypes of MODY, such as renal developmental disease or renal cysts (HNF1B-MODY), macrosomia and/or neonatal hypoglycemia (HNF4A-MODY), exocrine pancreatic dysfunction or pancreatic cysts (CEL-MODY), or hearing impairment and maternal inheritance of diabetes (mitochondrial diabetes)" (C)
- "Obesity alone should not preclude genetic testing in young persons, especially if:
    - Family history is strongly suggestive of autosomal dominant inheritance of diabetes
    - If some affected family members are NOT obese
    - And/or, there are no other features of metabolic syndrome." (C)
- "Features that suggest monogenic diabetes in children initially thought to have T1D [Type 1 diabetes] are listed below. . . none of these are pathognomonic and should be considered together rather than in isolation:"
    - "Diabetes presenting before 6 months of age (as T1D is extremely rare in this age group), or consider NDM [neonatal diabetes mellitus] if the diagnosis is between 6 and 12 months and there is no evidence of autoimmunity or if the person with diabetes has other features such as congenital defects, or an unusual family history."
    - "Family history of diabetes in one parent and other first-degree relatives of that affected parent."
    - "Absence of islet autoantibodies, especially if checked at diagnosis."
    - "Preserved β-cell function, with low insulin requirements and detectable C-peptide (either in blood or urine) over an extended partial remission phase (at least 5 years after diagnosis)."
- "In young people, T2D [Type 2 diabetes] often presents around puberty and the majority are obese. As there is no diagnostic test for T2D and because obesity has become so common in children, children and adolescents with monogenic diabetes may also be obese and can be very difficult to distinguish from T2D. One recent study found that 3% of obese youth with presumed T2D in fact carried pathogenic monogenic diabetes variants. Features that suggest monogenic diabetes in young people with suspected T2D are listed below:"
    - "Lack of consistent severe obesity among affected family members."
    - "Lack of consistent acanthosis nigricans and/or other markers of metabolic syndrome (hypertension, low HDL-cholesterol, etc.) among affected family members."

  - "Family history of diabetes in one parent and other first-degree relatives of that affected parent, especially if any affected family member lacks obesity and other markers of metabolic syndrome."
  - "Unusual distribution of fat, such as central fat with thin or muscular extremities."

- "From a clinical perspective, specific clinical scenarios when a diagnosis of monogenic diabetes should be considered include:"
  - "Diabetes presenting before 6 months of age, which is known as NDM."
  - "Autosomal dominant familial mild hyperglycemia or diabetes."
  - "Diabetes associated with extra-pancreatic features (such as, for example, congenital heart or gastrointestinal defects, brain malformations, severe diarrhea, or other autoimmune conditions in a very young child)."
  - "Monogenic IR [insulin resistance] syndromes (see below: characterized by high insulin levels or high insulin requirements; abnormal distribution of fat with a lack of subcutaneous fat, especially in extremities; dyslipidemia, especially high triglycerides; and/or significant acanthosis nigricans)."
- “Three genes are responsible for the majority of MODY cases (GCK, HNF1A, and HNF4A) … At least 14 different genes have been reported to cause diabetes with a MODY-like phenotype, and some panels will include all these genes, or possibly also many other genes associated with exceedingly rare recessive causes. It is reasonable to consider including syndromic causes such as mitochondrial diabetes, as diabetes can often be the first presenting feature and a molecular diagnosis can thereby guide monitoring and treatment of other associated features. In the modern era of expanded testing by many different laboratories, caution must be used when interpreting test results, as often there is very little information available to support the causality of rare variants in uncommon subtypes.”

National Academy of Clinical Biochemistry

The National Academy of Clinical Biochemistry (NACB, 2011) stated:[10]

- "Routine measurement of genetic markers is not of value at this time for the diagnosis or management of patients with type 1 diabetes. For selected diabetic syndromes, including neonatal diabetes, valuable information can be obtained with definition of diabetes-associated mutations. A (moderate)."
- "There is no role for routine genetic testing in patients with type 2 diabetes. These studies should be confined to the research setting and evaluation of specific syndromes. A (moderate)."

Selected Relevant Publications

A 2018 expert-authored review stated that MODY has an onset in adolescence or young adulthood, typically less than 35 years.[4]

- "Molecular genetic testing approaches to determine the associated MODY gene can include a combination of gene-targeted testing (serial singe-gene or multigene panel) and comprehensive genomic testing (chromosomal microarray analysis or exome sequencing), depending on the phenotype."
- "Serial single-gene testing. Sequence analysis of the most likely genes is performed first. If no pathogenic variant is found, gene-targeted deletion/duplication analysis to detect exon-sized deletions could be considered, especially for those genes (CEL, GCK, HNF1A, HNF1B, and HNF4A) in which whole-gene or multiexon deletions have been identified."
- "A MODY multigene panel that includes the 14 known MODY-related genes and other genes of interest is most likely to identify the genetic cause of MODY at the most reasonable cost while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype."

  a) "The genes included in the panel and the diagnostic sensitivity of the testing used for each gene vary by laboratory and are likely to change over time."

  b) "Some custom laboratory-designed multigene panels may include genes not associated with MODY but possibly associated with other types of monogenic diabetes; other custom laboratory-designed panels may not include the genes that rarely cause MODY."

  c) "In some laboratories, panel options may include a custom laboratory-designed panel and/or custom phenotype-focused exome analysis that include genes specified by the clinician."

  d) "Methods used in a panel may include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests. Note: Given that whole-gene and/or multiexon deletions have been identified in GCK, HNF1A, HNF1B, and HNF4A, a multigene panel that also includes deletion/duplication analysis is recommended."

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Introduction

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Mitochondrial Disorders Genetic Testing

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Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Genomic Unity Comprehensive Mitochondrial Disorders Analysis	0417U
Mitochondrial Disorder Known Familial Mutation Analysis	81403
MT-ATP6 Targeted Mutation Analysis	81401
MT-ND4, MT-ND6 Targeted Mutation Analysis	81401
MT-ND5 Targeted Mutation Analysis	81401
MT-TK Targeted Mutation Analysis	81401
MT-TL1 Targeted Mutation Analysis	81401
Nuclear Encoded Mitochondrial Gene Sequencing Panel	81440
TYMP Sequencing	81405
Whole Mitochondrial Genome Sequencing	81460
Whole Mitochondrial Genome Deletion/Duplication Analysis	81465

Criteria

Known Familial Mutation Testing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:

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- No previous genetic testing inclusive of the known familial mutation, and
- Disease causing mutation(s) identified in 1st degree biological relative, and
- Member is at risk to have the familial mutation based on inheritance pattern of the disorder in question, AND

- Predictive Testing for Asymptomatic Individuals:
  - 18 years of age or older, or
  - Under the age of 18 years, and
    - Test results are needed for treatment or medical screening, OR
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical examination and/or biochemical results are suggestive, but not confirmatory, of the familial diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Targeted Mutation Analysis or Single Gene Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for the mitochondrial disorder to be targeted, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical examination and/or biochemical results are suggestive, but not confirmatory, of the targeted disorder (see table titled *Select Mitochondrial Disorders)*, and
  - Inheritance pattern is consistent with the targeted mitochondrial disorder, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Whole Mitochondrial DNA (mtDNA) Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Member has not had previous whole mtDNA sequencing performed, and

- Targeted mitochondrial testing, if performed, was negative, and
- Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Member has multiple organ system involvement defined as altered function in two or more organ systems, suggestive of a mitochondrial disorder, and
  - Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, brain magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, and
  - Targeted mutation analysis is not feasible because of one of the following:
    - Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (see table titled *Select Mitochondrial Disorders*), or
    - Member's clinical presentation fits a well-described syndrome and applicable single-gene or targeted mutation analysis was negative, and
  - Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), and
  - Family history strongly suggests mitochondrial inheritance (e.g., no evidence of paternal transmission), AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Whole Mitochondrial DNA (mtDNA) Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Member has not had previous whole mtDNA deletion/duplication analysis performed, and
  - Targeted mitochondrial deletion testing, if performed, was negative , and
  - Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
- Diagnostic Testing for Symptomatic Individuals:

  - Member has multiple organ system involvement defined as altered function in two or more organ systems, suggestive of a mitochondrial disorder, and
  - Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, brain magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, and
  - Targeted mutation analysis is not feasible because of one of the following:
    - Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (see table titled *Select Mitochondrial Disorders*), or
    - Member's clinical presentation fits a well-described syndrome and applicable single-gene or targeted mutation analysis was negative, and
  - Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), and
  - Family history strongly suggests mitochondrial inheritance (e.g., no evidence of paternal transmission), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Nuclear Encoded Mitochondrial Gene Sequencing Panel

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Member has not had a previous nuclear encoded mitochondrial gene sequencing panel testing performed, and
  - Targeted nuclear-encoded mitochondrial gene testing (e.g., TYMP or POLG analysis), if performed, was negative, and
  - Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has multiple organ system involvement defined as altered function in two or more organ systems, suggestive of a mitochondrial disorder, and
  - Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes,

ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, brain magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, and

- Targeted mutation analysis is not feasible because of one of the following:
  - Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (see table titled *Select Mitochondrial Disorders*), or
  - Member's clinical presentation fits a well-described syndrome and applicable single-gene or targeted mutation analysis was negative, and
- Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), and
- Family history does not strongly suggest mitochondrial inheritance (e.g., paternal transmission is observed, autosomal inheritance is likely), AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other considerations

- For information on POLG-related disorders, please refer to the guideline *Polymerase Gamma (POLG) Related Disorders Genetic Testing*, as this testing is not addressed here.
- Testing addressed in this guideline applies to individuals in whom a mitochondrial disorder is suspected based on a constellation of findings commonly seen in these conditions. Mitochondrial genetic testing is not considered medically necessary in the following cases:
  - The individual's findings could be explained nonspecifically by a mitochondrial disorder or other neurological or myopathic condition not related to mitochondrion for which a different genetic test may be considered; or
  - Individuals who have no increased risk above the general population risk to have inherited a mitochondrial disease and have just one of the following findings in isolation: fatigue; muscle weakness; developmental delay; autism; migraines; abnormal biochemical test results (e.g., elevated lactate); psychiatric symptoms.

Table: Select Mitochondrial Disorders

Disorder, genes, CPT code, symptoms

Mitochondrial Disorder	Associated Genes / Mitochondrial DNA Mutations	CPT Code(s)	Symptoms
Leber Hereditary Optic Neuropathy (LHON)	MT-ND4, MT-ND6	81401	Bilateral painless subacute vision loss that begins in the second and third decades of life, central or cecocentral scotomas, impaired color vision
Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS)	MT-TL1, MT-ND5	81401	Stroke-like episodes, encephalopathy with seizures, and/or dementia, muscle weakness and exercise intolerance, recurrent headaches, recurrent vomiting, hearing impairment, peripheral neuropathy, learning disability, and short stature
Mitochondrial Epilepsy with Ragged Red Fibers (MERRF)	MT-TK	81401	Myoclonus, generalized epilepsy, ataxia, weakness, dementia, ragged red fibers on muscle biopsy

Mitochondrial Disorder	Associated Genes / Mitochondrial DNA Mutations	CPT Code(s)	Symptoms
Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE)	TYMP	81405	Progressive gastrointestinal dysmotility (possibly presenting as nausea, dysphagia, reflux, early satiety, vomiting after a meal, episodic abdominal pain, bloating, and/or diarrhea), cachexia, ptosis, ophthalmoplegia, leukoencephalopathy, and peripheral neuropathy
Neurogenic Muscle Weakness, Ataxia, and Retinitis Pigmentosa (NARP)	MT-ATP6	81401	Proximal neurogenic muscle weakness with sensory neuropathy, ataxia, learning difficulties, and pigmentary retinopathy

Mitochondrial Disorder	Associated Genes / Mitochondrial DNA Mutations	CPT Code(s)	Symptoms
POLG-Related Disorders (Alpers-Huttenlocher syndrome (AHS), Childhood Myocerebrohepatopathy Spectrum (MCHS), Myoclonic Epilepsy Myopathy Sensory Ataxia (MEMSA), Ataxia Neuropathy Spectrum (ANS), Autosomal Dominant or Autosomal Recessive Progressive External Ophthalmoplegia (adPEO/arPEO))	POLG	81406	Please refer to the guideline *Polymerase Gamma (POLG) Related Disorders Genetic Testing*
mtDNA Deletion Syndromes (Kearns-Sayre Syndrome (KSS), Pearson syndrome, Progressive External Ophthalmoplegia (PEO))	Full mtDNA Deletion Analysis	81465	KSS: childhood onset of pigmentary retinopathy and progressive external ophthalmoplegia Pearson syndrome: sideroblastic anemia and exocrine pancreas dysfunction PEO: ptosis

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever

appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g., ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- When otherwise reimbursable, the following limitations apply:
  - Any individual gene or multi-gene panel is only reimbursable once per lifetime.
  - When a whole mtDNA analysis or a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81460 for Whole mtDNA Sequencing, 81465 for Whole mtDNA Deletion/Duplication, and 81440 for Nuclear Encoded Mitochondrial Gene Sequencing Panels)*.
  - When use of a panel code is not possible, each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
  - If more than one test or procedure code is requested at one time, the member meets criteria for all tests requested, and mtDNA and nuclear DNA mutations (or causes) are equally likely based on personal history, clinical findings, and family history, the testing will be tiered in the following order: 81460, 81465, 81440.
  - If a single panel code is requested that includes testing of both mtDNA and nuclear DNA (e.g., 0417U), the member meets criteria for all tests described by the requested code, and mtDNA and nuclear DNA mutations (or causes) are equally likely based on personal history, clinical findings, and family history, the code will be reimbursable.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are mitochondrial disorders?

Definition

Mitochondrial disorders are conditions resulting from mutations in the nuclear (nDNA)

or mitochondrial (mtDNA) genes that are involved in the production, function, maintenance, or transmission of mitochondria.

Incidence

Mitochondrial disorders have an estimated minimum incidence of 1 in 5,000.[1]

Symptoms

Mitochondrial disorders are a clinically diverse group of diseases that may present at any age and affect a single organ or present as a multi-system condition in which neurologic and myopathic features predominate. Extensive clinical variability and phenotypic overlap exists among the many discrete mitochondrial disorders.[2,3]

Mitochondrial disease is suspected in individuals with a combination of clinical features which can include any of the following:

- Muscle: proximal myopathy or cardiomyopathy
- Nervous system: encephalopathy, seizures, dementia, stroke-like episodes, ataxia and spasticity and migraine
- Eye: ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy
- Gastrointestinal: recurrent vomiting, anorexia
- Sensorineural hearing loss
- Diabetes mellitus
- Growth: failure to thrive, short stature
- Mid- or late-pregnancy loss

Several mitochondrial disorders, due to mutations in the mtDNA, are characterized by a cluster of clinical features or syndromic presentation. These disorders are described in the table titled *Select Mitochondrial Disorders.*

Cause

Mitochondrial disorders result from dysfunction of the mitochondrial respiratory chain due to abnormality of the production, function, maintenance, or transmission of mitochondria.[2] They can be caused by mutations in either mitochondrial or nuclear DNA.

Underlying nDNA and mtDNA causes are frequently indistinguishable based on this symptomology. Diagnosis of the majority of mitochondrial conditions is based on a combination of clinical findings and genetic testing.[4,5]

For all mtDNA mutations, clinical expressivity depends on the three following factors:[2]

- The ratio of mutant mtDNA to normal mtDNA (mutational load or heteroplasmy)

- The organs and tissues in which the mutant mtDNA is found (tissue distribution), and
- The vulnerability of each tissue to impaired oxidative metabolism (threshold effect).

Inheritance

Mitochondrial conditions due to mutations in the mtDNA are maternally inherited or may be de novo. Mitochondrial conditions caused by mutations in the nuclear DNA can be maternally or paternally inherited and may follow autosomal dominant, autosomal recessive, or X-linked inheritance.

Mitochondrial Inheritance

MtDNA mutations may be de novo (not inherited) or follow maternal inheritance. This means that a female who carries the mtDNA mutation at a high mutation load will typically pass it on to all of her children. However, due to the meiotic bottleneck, the heteroplasmy level may vary significantly between generations. A male who carries the mtDNA mutation cannot pass it on to his children. Clinical expressivity of mtDNA mutations depends on the degree of heteroplasmy and the organs and tissues most affected by the mutation.

A female who carries a mtDNA mutation at high mutation load will typically pass it on to all of her children. However, due to the meiotic bottleneck, the heteroplasmy level may vary significantly between generations. A male who carries the mtDNA mutation will not pass it on to his children.[4,6] mtDNA deletions are rarely transmitted (less than 1% empiric risk).[2] If the mother is symptomatic, then the recurrence risk is approximately 4%. A male who carries the mtDNA mutation will not pass it on to his children.[4,6,7]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

X-Linked Inheritance

In X-linked inheritance, the mutation is carried on the X chromosome. Females have two X chromosomes, and males have one. Males typically have more severe symptoms than females. A female with a mutation has a 50% chance to pass that mutation to her children. A male with a mutation cannot pass the mutation to any sons, but will pass it to all daughters. A process called X-inactivation in females results in random inactivation of expression of one X-chromosome in each cell of the body. For females with one mutation, the percentage and distribution of cells with expression of the X chromosome carrying the mutation can influence the degree of severity.

Identification of a mutation in a proband may allow for informative testing of relatives at risk for diabetes, seizures, hearing loss, optic atrophy, and other findings in the corresponding phenotypic range.

Diagnosis

Clinical findings may point to a specific, well-described mitochondrial disorder, and the clinical diagnosis is often confirmed with molecular testing.[8]

The investigation and diagnosis of individuals with mitochondrial disease often necessitate a combination of techniques including clinical assessment and biochemical assessment, neuroimaging, molecular genetic studies, and sometimes muscle biopsy.

Biochemical assessment includes measurement of plasma or CSF lactate and pyruvate, glucose, creatine kinase (CK), transaminases (AST, ALT), ketone bodies, plasma acylcarnitines, and urinary organic acids. Normal plasma or CSF lactic acid concentration does not exclude the presence of a mitochondrial disorder.[2,6]

Brain magnetic resonance imaging (MRI) is recommended if CNS symptoms are present. Brain magnetic resonance spectroscopy (MRS) for elevated lactate is also useful. Neuroimaging results are not confirmatory, but may aid in the diagnosis of a mitochondrial disorder if other clinical features are present.

Molecular genetic testing for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations.[2]

If a specific disorder is not evident, analysis of an individual's family history may provide information regarding most likely inheritance patterns for a suspected mitochondrial condition. This may guide decisions to perform mtDNA sequencing, mtDNA deletion/duplication testing, nuclear encoded DNA sequencing, and/or nuclear encoded DNA deletion/duplication testing.

Management

Mitochondrial disease is not curable. However, in some cases, specific treatment recommendations can be made based on a person's definitive diagnosis. Consensus based recommendations have been published by the Mitochondrial Medicine Society for the routine care and management of individuals with mitochondrial disease.[1] Individuals at-risk for mitochondrial conditions may also benefit from clinical

assessment to initiate baseline evaluations (neurology, cardiology, ophthalmology, and audiology) and potential intervention prior to exhibiting clinical manifestations.[1,4,9]

Survival

Mitochondrial disorders are clinically heterogeneous with a wide range of severity and age of onset, depending upon the specific disorder.[1] While genetic test results alone cannot predict the exact course or phenotype of the disease, severity does correlate with mutation load for mtDNA mutations.[6,10]

Test information

Introduction

Testing for mitochondrial diseases may include known familial mutation analysis, targeted mutation analysis, mitochondrial genome sequencing, deletion/duplication analysis, and NGS panels.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

If an individual's clinical findings clearly correlate with a specific mitochondrial condition, then testing can be focused on the most appropriate approach for that condition. "False negative rates vary by genomic region; therefore, genomic testing may not be as accurate as targeted single gene testing or multigene molecular genetic testing panels." [2]

Whole Mitochondrial Genome Sequencing

Full sequencing of the entire mitochondrial genome by next generation sequencing (NGS) is capable of simultaneously detecting point mutations, deletions, and point mutation heteroplasmies in the assessment of a number of overlapping mitochondrial syndromes. Since the mitochondrial genome is highly polymorphic, this is not routinely offered unless clinical suspicion is high and there is no evidence of paternal

transmission. DNA testing can be performed on a blood specimen. Muscle biopsy is generally not necessary, but some labs accept blood, saliva, and muscle samples.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

A number of large panels are available that sequence numerous nuclear-encoded mitochondrial genes for a broad approach to testing. Multi-gene panel tests, even for similar clinical scenarios, vary considerably laboratory by laboratory in the genes that are included and in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

NGS testing is capable of simultaneously detecting point mutations, deletions, and point mutation heteroplasmies. Typically, Sanger sequence analysis will miss heteroplasmy below 20%. With suitable depth of coverage, NGS can detect heteroplasmy down to ~1%.[11,12]

Test Strategy

Due to overlap of clinical findings of mitochondrial conditions and non-mitochondrial conditions, affected individuals are more likely to have multiple tests performed before a molecular genetic cause is identified.

“In many individuals in whom molecular genetic testing does not yield or confirm a diagnosis, further investigation of suspected mitochondrial disease can involve a range of different clinical tests, including muscle biopsy for respiratory chain function.” [2]

Testing of alternative tissues by biochemical and/or molecular analysis may be required, especially if blood testing is negative and the phenotype is highly suggestive of the presence of a mutation associated with a specific gene or set of genes, or when there is a need to assess reproductive risk.

Guidelines and evidence

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2013) states the following regarding testing individuals with isolated autism for mitochondrial disorders:[13]

- "As with metabolic disorders, testing for mitochondrial disorders in persons with ASDs is recommended only if supporting symptoms or laboratory abnormalities are present."

European Federation of Neurological Sciences

The European Federation of Neurological Sciences (EFNS, 2009)[5] provided molecular diagnostic consensus-based guidelines based on literature reviews: "If the phenotype suggests syndromic MID [mitochondrial disease] due to mtDNA point mutations (MELAS, MERRF, NARP, LHON) DNA-microarrays using allele-specific oligonucleotide hybridisation, real-time-PCR or single-gene sequencing are indicated."

International Consensus Statement on Leber Hereditary Optic Neuropathy

An international consensus conference (2017) with a panel of experts from Europe and North America made the following statements regarding the clinical and therapeutic management of LHON.[14]

- "LHON primarily is a clinical diagnosis…. A definitive diagnosis of LHON is rapidly obtained by the molecular identification of one of the 3 common mtDNA mutations (m.11778G>A/MT-ND4, m.3460G>A/MT-ND1, m.14484T>C/MTND6), accounting for about 90% of cases. If this primary screen is negative and there is a high index of clinical suspicion supported by a maternal mode of inheritance in a patient with a family history, sequencing the entire mtDNA is advisable to identify other, but rare, mtDNA mutations."
- "The diagnosis of LHON should be based on a careful history, evaluation of key structural and functional visual parameters, and on a molecular confirmation of a pathogenic mtDNA mutation. The management of LHON includes genetic counseling, informing the patient about potentially preventable lifestyle risk factors and, for subacute and dynamic cases, the use of idebenone at the currently approved dose. Idebenone should be discontinued in nonresponder patients and is currently not recommended in patients in the chronic stages of the disease. These guidelines and recommendations are based on a consensus developed on the current state of the literature. Further investigations and clinical trials are needed to lead to better disease-modifying treatments and to improve the management of patients with LHON."

Mitochondrial Medicine Society

The Mitochondrial Medicine Society (MMS, 2015) developed consensus recommendations using the Delphi method.[15]

- Recommendations for DNA Testing
  - "Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations."
  - "Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling."
  - "When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered."
- Recommendations for pathology testing
  - Biopsy should only be considered when the diagnosis cannot be confirmed with DNA testing of other more accessible tissues. Muscle (and/or liver) biopsies are often not necessary and should be avoided when possible due to their invasive nature, unless other types of analyses such as pathology, enzymology, or mtDNA copy number analyses are required for diagnosis.

United Kingdom Best Practices Guideline

A working group of Clinical Scientists from the NHS Highly Specialised Service, in collaboration with national laboratory consultation, published (2023) best practice guidelines for genetic testing for mitochondrial disease.[16] The guidelines summarize current recommended technologies and methodologies for analysis of mtDNA and nuclear-encoded genes in patients with suspected mitochondrial disease, as well as genetic testing strategies for diagnosis. The guidelines outline two main alternative strategies:[16]

- "Targeted testing of 'common' mtDNA variants and/or targeted nuclear testing, followed by more comprehensive testing if required and if resources allow."
- "NGS of the mitochondrial genome and/or nuclear genes, e.g., by whole exome sequencing (WES) or whole genome sequencing (WGS)."

Targeted testing can be appropriate for routine referrals where there is not an urgent clinical need to obtain a diagnosis, for clinical presentations which are highly suggestive of a particular variant or gene, and/or where resources are limited.

Comprehensive NGS-based testing can be used for all referral indications but is particularly appropriate for more complex phenotypes and/or for urgent referrals. Simultaneous testing of both mtDNA and nDNA is recommended, as both account for a significant proportion of childhood-onset and adult-onset mitochondrial disorders.

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Multi-Cancer Early Detection Screening

MOL.TS.396.A
v2.0.2024

Introduction

Multi-cancer early detection screening tests are addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Multi-Cancer Early Detection (MCED) Screening Tests	81599 81479

Criteria

Introduction

Requests for multi-cancer early detection screening tests are reviewed using these criteria. This guideline only addresses liquid biopsy screening tests for early cancer detection. Liquid biopsy testing for other purposes, including monitoring disease status and treatment selection in solid tumors and hematologic malignancies, is not addressed by this guideline. For information on liquid biopsy testing for other purposes, please refer to the guideline *Liquid Biopsy Testing*, as this testing is not addressed here.

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What is Multi-Cancer Early Detection Screening?

Definition

Multi-cancer early detection (MCED) screening tests analyze biomarkers within blood and urine to predict the presence of cancers. Liquid biopsy to detect circulating free tumor DNA (cfDNA), circulating free tumor proteins, DNA methylation patterns, circulating free immune cell DNA and DNA fragment size is often utilized.[1,2] Multiple biomarkers, including genomic profiles, protein levels, and other analytes, may be combined to provide a comprehensive assessment of individual cancer risk. MCED tests screen for multiple cancer types simultaneously and aim to increase detection rates, particularly at earlier stages when a cancer may be more amenable to treatment.

Incidence

Each year, more than 1.5 million new cancer cases are diagnosed in the United States and over half a million individuals are expected to die from the disease.[3]

Screening and Diagnosis

Population-level screening programs are endorsed for only four cancer types in the United States: breast, cervical, colorectal, and lung cancers.[4] Other cancer types may have individualized screening recommendations, or lack any recognized screening protocols.[4] MCED screening tests are intended to complement existing screening programs and potentially increase rates of cancer detection because patients may be more willing to perform blood-based screening than currently recommended screening methodologies such as mammogram and colonoscopy. Screening for multiple cancer types at once could also allow identification of cancer types that do not have any current screening recommendations, such as pancreatic and stomach cancer.[5]

At this time, the number and type of cancers screened, and the ability to distinguish between cancer type, varies between individual tests. This is partly due to the fact that current MCED screening tests use different biomarkers to identify the presence of cancer.

Positive screening results typically prompt further investigations in an effort to confirm whether cancer is present. Investigations may include gathering of a personal and family medical history, a physical exam, laboratory tests, imaging, and biopsy as needed.[6]

Test information

Introduction

MCED screening tests utilize a variety of techniques for the measuring of biological substances from blood and other body fluids.

Multi-Cancer Early Detection Testing Methodology

MCED test methodology relies on the presence of individual or a combination of biomarkers in circulation, including cfDNA—which may be analyzed using polymerase chain reaction (PCR), methylation analysis, or next-generation sequencing (NGS). These approaches analyze single genes, panels of genes, exomes, or genomes.

Other biomarkers assessed by MCED screening tests may include routine blood and urine analysis, and levels of certain antibodies, proteins, electrolytes, and other analytes.[5,7] Genomic profiles and the combination of multiple biomarkers are used to distinguish between cancer and non-cancer signals.[7,8]

Guidelines and Evidence

Introduction

While there are no specific guidelines relating to multi-cancer early detection screening tests, the following section includes relevant guidelines and evidence that discuss the use of liquid biopsy for cancer screening.

European Society for Medical Oncology

The European Society of Medical Oncology (ESMO, 2022) provided recommendations on the use of ctDNA assays for cancer.[9] The guidelines stated that insufficient evidence exists for implementing use of ctDNA assays for cancer screening, monitoring of treatment response, or detection of molecular relapse or minimal residual disease.

United States Preventive Services Task Force

The United States Preventive Services Task Force (USPSTF, 2021) stated the following regarding liquid biopsies for cancer screening:[10,11]

- "more research is needed on the accuracy and effectiveness of emerging screening technologies such as serum- and urine-based colorectal cancer screening tests"[10]
- For lung cancer, "potential screening modalities that are not recommended because they have not been found to be beneficial include sputum cytology, chest radiography, and measurement of biomarker levels"[11]

Selected Relevant Publications

Current studies have shown variable sensitivity depending on the test product, cancer type, and cancer stage.[12-15] Clinical validation data has also not yet supported the ability of these tests to detect cancers in earlier stages. The sensitivity for identifying stage I cancers was reported in two studies to be 16.8% and 10.2%.[15,16]

Consistency in detecting early-stage cancers, identifying tissue of origin, and differentiating cancer-related variants from random and age-related variants has not

been demonstrated across MCED screening platforms, leading to practical concerns for usage of these tests in standard clinical practice. More well-designed clinical studies are needed to better define the capabilities of individual tests and document changes to clinical outcomes.

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MCED

MUTYH-Associated Polyposis Genetic Testing

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Introduction

MUTYH-associated polyposis genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
MUTYH Deletion/Duplication Analysis	81479
MUTYH Known Familial Mutation Analysis	81403
MUTYH Sequencing	81406
MUTYH Targeted Mutation Analysis	81401

Criteria

Introduction

Requests for MUTYH-associated polyposis (MAP) testing are reviewed using these criteria.

MUTYH Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing that would detect the familial mutation(s), AND
- Diagnostic or Predisposition Testing:
  - Two known MUTYH mutations in a sibling, or
  - Both parents with one or two known MUTYH mutations, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

MUTYH Targeted Mutation Analysis for Y179C and G396D Mutations

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous MUTYH testing, and
  - No mutation detected on APC gene testing, if performed, AND
- Individual is of possible Northern European descent, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical findings:
    - At least 10 cumulative adenomas, or
    - At least two adenomas, AND
      - At least 5 serrated polyps proximal to the sigmoid colon (2 or more of >10mm), or
      - > 20 serrated polyps of any size, but distributed throughout the colon, AND
  - Recessive pattern of inheritance (e.g. family history positive for only an affected sibling), OR
- Testing for Presymptomatic/Asymptomatic Individuals:
  - Reproductive partner of a person with MAP (to determine if children at risk), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

MUTYH Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous MUTYH full sequencing, and
  - Two mutations NOT identified through MUTYH targeted mutation analysis (Y179C and G396D) if performed, and

- No mutation detected on APC gene testing, if performed, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Clinical findings:
    - At least 10 cumulative adenomas, or
    - At least two adenomas, AND
      - At least 5 serrated polyps proximal to the sigmoid colon (2 or more of >10mm), or
      - > 20 serrated polyps of any size, but distributed throughout the colon, AND
  - Recessive pattern of inheritance (e.g. family history positive for only an affected sibling), OR
- Testing for Presymptomatic/Asymptomatic Individuals:
  - Reproductive partner of a person with MAP (to determine if children at risk), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

MUTYH Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - MUTYH full sequencing performed, and
  - No mutations or only one mutation detected in MUTYH through any previous testing (founder mutation panel or full gene sequencing), and
  - Meets criteria for MUTYH full sequencing, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other Considerations

MUTYH testing may be performed as part of a multigene, multisyndrome panel. For information on multigene, multisyndrome panel testing, please refer to the guideline *Hereditary Cancer Syndrome Multigene Panels*, as this testing is not addressed here.

What is MUTYH-associated polyposis?

Definition

MUTYH-associated polyposis (MAP) is an inherited colorectal cancer syndrome characterized by the development of multiple colon polyps.[1] Individuals also have an increased chance to develop duodenal adenomas which may cause duodenal cancer.[1-3] Some studies have documented an increased risk for ovarian cancer and bladder cancer.[1] Additionally, there is "some evidence of an increased risk for breast and endometrial cancer. Additional reported features include thyroid nodules, benign adrenal lesions, jawbone cysts, and congenital hypertrophy of the retinal pigment epithelium."[1] At this time, management guidelines are available for colonic and duodenal manifestations.

Prevalence

MAP is estimated to account for 0.7% of all colorectal cancer, and the prevalence of MAP is estimated to be 1/20,000 to 1/60,000.[1] It is estimated that 1-2% of individuals in Northern Europe, Australia, and the United States have a single MUTYH mutation.[1]

MUTYH mutations "account for 10%-20% of classical FAP [Familial Adenomatous Polyposis] cases without an APC mutation and for 30% of AFAP [Attenuated Familial Adenomatous Polyposis] cases.[4]

Symptoms

MAP clinical findings overlap those of FAP and AFAP. Affected individuals most often have fewer than 100 adenomas, but cases of hundreds and occasionally over 1000 polyps have been reported.[1,2] Hyperplastic and sessile serrated, and traditional serrated adenomatous polyps have also been seen individuals with MAP, although adenomas remain the most common polyp type in MAP.[1,3] Duodenal adenomas occur in 17-34% of individuals with MAP and gastric polyps have been reported in about 11%.[1,3,5] Additionally, approximately one third of individuals with MAP have been described with colorectal cancer and no polyposis.[1]

Adenomas and colorectal cancer tend to present later than FAP. MAP is "characterized by a greatly increased lifetime risk of colorectal cancer (CRC)". . . with a CRC risk of "43%-63% by age 60 yrs" and an "80%-90% lifetime risk."[1] There is also an estimated 4-5% lifetime risk for duodenal cancer.[1-3]

Cause

MAP is caused by mutations in the MUTYH gene (also called MYH).[1]

Inheritance

MAP is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

As MAP is not clinically distinguishable from FAP or AFAP, the identification of two MUTYH mutations is required to make a diagnosis of MAP.[1,6]

Two MUTYH mutations (p.Y165C and p.G382D) are particularly common and account for over 80% of MUTYH mutations in Caucasians of Northern European descent.[7] Some laboratories test for only these two mutations or offer reflex options that begin with these two mutations and proceed to full gene sequencing if two mutations are not found.

If sequencing does not find two mutations, large gene deletion/duplication analysis can be performed. It remains unknown what percentage of MAP is due to large deletions/duplications/rearrangements in the gene and thus are detectable only with this technology. However, large deletions have been reported.[1,8,9]

Surveillance

For individuals with MAP, colonoscopy screening should begin at 25-30 years (earlier colonoscopy may be indicated based on family history). If the colonoscopy is negative, repeat colonoscopy should occur every 1-2 years.[2] For positive colonoscopy findings, the treatment and surveillance is dependent on polyp burden.[2] Additional recommended screening includes upper endoscopy with complete visualization of the ampulla of Vater beginning at 30-35 years.[2] If no duodenal polyps are detected, then repeat endoscopy occurs every 3 to 5 years. If duodenal polyps are detected, repeat endoscopy is dependent on the quantity and size of the polyps.[2]

"Chemoprevention may be considered in select patients, but options have not been studied specifically in MAP. Consider referral to a center with expertise for discussion of chemoprevention and surgical options, particularly for patients with a high polyp burden in the remaining rectum after colectomy."[2]

For individuals with a single MUTYH mutation, the recommended surveillance is dependent on the family history of colon cancer.[2]

- Individuals without a history of colorectal cancer and with a first-degree relative with colorectal cancer: colonoscopy screening every 5 years beginning at 40 years or 10 years prior to the age of the first-degree relative's diagnosis, whichever comes first. Colonoscopy may be repeated at more frequent intervals if indicated based on colonoscopy findings.

- Individuals without a history of colorectal cancer and with a second-degree relative with colorectal cancer or if there is no family history of colorectal cancer: there are no specific screening recommendations.

Test information

Introduction

Testing for MAP may include known familial mutation analysis, targeted mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to MAP testing.

American College of Gastroenterology

Evidence-based guidelines from the American College of Gastroenterology (ACG, 2015) stated:[10]

- "Individuals who have a personal history of >10 cumulative colorectal adenomas, a family history of one of the adenomatous polyposis syndromes, or a history of adenomas and FAP-type extracolonic manifestations (duodenal/ampullary adenomas, desmoid tumors (abdominal>peripheral), papillary thyroid cancer, congenital hypertrophy of the retinal pigment epithelium (CHRPE), epidermal cysts, osteomas) should undergo assessment for the adenomatous polyposis syndromes. . . Genetic testing of patients with suspected adenomatous polyposis syndromes should include APC and MUTYH gene mutation analysis."

American Society of Clinical Oncology and European Society for Medical Oncology

The American Society of Clinical Oncology (ASCO, 2015) endorsed the European Society for Medical Oncology (ESMO, 2013) clinical practice guideline on hereditary colorectal cancer syndromes. This guideline stated:[11]

- "Patients with multiple colorectal adenomas (>10), should be considered for germline testing of APC and/or MUTYH."
- "Germline testing of MUTYH can be initiated by screening for the most common mutations (G396D, Y179C) in the white population followed by analysis of the entire gene in heterozygotes. Founder mutations among ethnic groups should be taken into account. For nonwhite individuals, full sequencing of MUTYH should be considered."

American Society of Gastrointestinal Endoscopy

Consensus guidelines from the American Society of Gastrointestinal Endoscopy (ASGE, 2020) recommended:[12]

- ..."genetic counseling and testing in patients with clinical polyposis defined as 10 or more adenomas found on single endoscopy and 20 or more adenomas during their lifetime." [low quality]
- "...genetic counseling and testing in all first-degree relatives of confirmed polyposis syndrome patients. ...suspected AFAP and MAP should be tested at age 18-20 years" [low quality]

National Comprehensive Cancer Network

Guidelines from the National Comprehensive Cancer Network (NCCN, 2023) stated:[2]

- MUTYH testing criteria:
  - At least 10 adenomas
  - Meets criteria for SPS [Serrated Polyposis Syndrome] and some adenomas. (see below)
  - Known deleterious MUTYH mutation(s) in the family
- SPS clinical diagnostic criteria:
  - "≥5 serrated lesions/polyps proximal to the rectum, all being ≥5 mm in size, with ≥2 being ≥10 mm in size."
  - “>20 serrated lesions/polyps of any size distributed throughout the large bowel, with ≥5 being proximal to the rectum.”
  - Note: "any histological subtype of serrated lesion/polyp (hyperplastic polyp, sessile serrated lesion without or with dysplasia, traditional serrated adenoma, and unclassified serrated adenoma) is included in the final polyp count. The polyp count is cumulative over multiple colonoscopies."
- "Siblings of a patient with MAP are recommended to have site-specific testing for the familial pathogenic variants. Full sequencing of MUTYH may be considered in an unaffected parent when the other parent has MAP. If the unaffected parent is found to not have a MUTYH pathogenic variant, genetic testing in the children is not necessary to determine MAP status. If the unaffected parent is not tested, comprehensive testing of MUTYH should be considered in the adult children. If the unaffected parent is found to have one MUTYH pathogenic variant, testing the adult children for the familial MUTYH pathogenic variants is indicated."
- "When colonic polyposis is present only in the proband and/or in siblings, consider recessive inheritance or de novo APC gene mutations … Overall, the decision to order APC, MUTYH, or germline multi-gene testing including these genes should be at the discretion of the clinician."
- All recommendations are category 2A.

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Neurofibromatosis Type 1 Genetic Testing

MOL.TS.301.A
v2.0.2024

Introduction

Neurofibromatosis Type 1 (NF1) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
NF1 Deletion/Duplication Analysis	81479
NF1 Known Familial Mutation Analysis	81403
NF1 Sequencing	81408

Criteria

Introduction

Requests for neurofibromatosis type 1 (NF1) genetic testing are reviewed using the following clinical criteria.

NF1 Known Familial Mutation Analysis

Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Previous Genetic Testing:

- No previous genetic testing of NF1 that would detect the familial mutation, AND
- NF1 mutation identified in 1st degree biological relative, AND

Rendering laboratory is a qualified provider of service per the Health Plan policy.

NF1 Sequencing

Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Previous Genetic Testing:

- No previous genetic testing of NF1, and
- No known pathogenic NF1 mutation in biological relatives, AND

Diagnostic Testing for Symptomatic Individuals:

- The member is suspected to have neurofibromatosis type 1 but the diagnosis is in question because member meets only one of the following:
    - Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals, or
    - Six or more café-au-lait macules over 15 mm in greatest diameter in postpubertal individuals, or
    - Freckling in the axillary or inguinal regions, or
    - Two or more neurofibromas of any type or one plexiform neurofibroma, or
    - Optic glioma, or
    - Two or more Lisch nodules (iris hamartomas) or two or more choroidal abnormalities, or
    - A distinctive osseous lesion (e.g., sphenoid dysplasia or long bone pseudoarthrosis), or
    - The member displays at least two of the following findings:
        - Less than 6 café-au-lait macules of any size
        - One neurofibroma
        - One Lisch nodule or choroidal abnormality, AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the individual, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

NF1 Deletion/Duplication Analysis

- Criteria for NF1 Sequencing are met, AND
- No previous deletion/duplication analysis of NF1, AND
- No mutation detected in full sequencing of NF1, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

NF1 Testing on Tissue Samples

Requests for NF1 testing on café au lait macules or neurofibromas after negative NF1 testing on a blood sample in individuals with a clinical suspicion of segmental NF will be reviewed on a case by case basis.

What is Neurofibromatosis Type 1?

Definition

Neurofibromatosis Type 1 (NF1) is a neurocutaneous condition characterized by the growth of tumors along nerves in the skin, brain, eyes, and other parts of the body and changes in skin pigmentation (café-au-lait macules and freckling).[1]

Incidence

NF1 is one of the most common dominantly inherited genetic disorders. This condition has an incidence at birth of approximately 1 in 2500 to 1 in 3000 individuals.[2]

Symptoms

The signs and symptoms of NF1 develop gradually over time. Initial clinical features of NF1 are café-au-lait macules. These macules increase in size and number with age. Freckling in the axilla and inguinal area (groin) develop later in childhood. Lisch nodules are present in fewer than 50% of affected children under the age of 5 years. However, these benign iris tumors (hamartomas) are present in almost all affected adults.[3]

The spectrum and severity of symptoms vary greatly between individuals with NF1, even in the same family.[4] Skin findings and Lisch nodules may be the only clinical features in some individuals with NF1. Multi-systemic manifestations of NF1 include short stature, macrocephaly, scoliosis, distinctive osseous lesions, learning differences, seizures, and attention deficit hyperactivity disorder (ADHD). Cardiovascular complications include high blood pressure, cerebral and peripheral arterial stenosis, and stroke.[3,5] “Juvenile xanthogranuloma and nevus anemicus are more common than expected in people with NF1 and may be useful in supporting the diagnosis in young children who do not meet the standard diagnostic criteria.” [3]

NF1 is associated with an increased risk of benign tumors, including cutaneous and plexiform neurofibromas, optic glioma, and pheochromocytoma. There is also an increased risk of certain cancers, including malignant peripheral nerve sheath tumors, brain tumors, leukemia, and breast cancer.[6] Malignant peripheral nerve sheath tumors may develop by malignant transformation of neurofibromas during adolescence or adulthood.

Diagnosis

Revised diagnostic criteria for NF1 were formulated by the International Consensus Group on Neurofibromatosis Diagnostic Criteria (2021).[7] A full description can be found in the Guidelines and Evidence section.

"Negative NF1 molecular testing does not rule out a diagnosis of NF1. Some individuals diagnosed with NF1 based on clinical criteria do not have a pathogenic variant detectable by current technology. Many clinical features of NF1 increase in frequency with age, and some individuals who have unequivocal NF1 as adults cannot be diagnosed in early childhood, before these features become apparent."[3]

NF1 has overlapping clinical features with Legius syndrome, other forms of neurofibromatosis, conditions with café-au-lait and pigmented macules, and overgrowth syndromes.[2,3,8]

Genotype-Phenotype Correlations

Only a few clear correlations between specific NF1 mutations and distinct clinical phenotypes have been described.

Individuals with a single amino acid deletion p.Met922del in the NF1 gene have a very mild phenotype with typical pigmentary features of NF1 without cutaneous neurofibromas or other tumors.[9,10] Missense mutations affecting p.Arg1809 are associated with a distinct presentation including pulmonic stenosis, learning disabilities, short stature, and Noonan-like features, in addition to mild NF1 phenotype.[11]

NF1 microdeletions are associated with early appearance of numerous cutaneous neurofibromas, severe cognitive abnormalities, somatic overgrowth, large hands and feet, and dysmorphic facial features.[12]

Individuals with missense mutations in codons 844-848 have a high risk of plexiform and spinal neurofibromas, optic gliomas, skeletal abnormalities, and other malignant tumors.[13]

Segmental NF

Segmental NF1 (also called mosaic NF1) is a rare subtype that results from a post-zygotic mutation in the NF1 gene leading to somatic mosaicism. Neurofibromas, café-au-lait macules, and axillary freckling are typically unilateral and localized to one area of the body, usually following the lines of Blashko.[14] There is an increased risk of malignancies.[13,14]

Cause

Neurofibromatosis Type 1 is caused by mutations in the NF1 gene which produces the protein product, neurofibromin. Neurofibromin functions as a tumor suppressor. NF1 gene mutations lead to defective or missing neurofibromin resulting in uncontrolled cell proliferation and growth of tumors common in NF1.[4]

Inheritance

Neurofibromatosis type 1 is inherited in an autosomal dominant fashion.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Almost half of all NF1 cases are the result of a new or de novo gene mutation. The mutation rate for NF1 is among the highest known for any gene in humans.[15] The remainder of NF1 cases are inherited from an affected parent. Individuals with NF1 have a 50% chance of passing the mutation to their children. Additionally, parents and siblings of known affected individuals have a 50% chance of having the same mutation. Penetrance is virtually complete after childhood; however, there is significant clinical variability.[3,8]

Management

There is no cure for Neurofibromatosis type 1. Long-term management includes multi-system surveillance for potential complications, treatment of bulky tumors and cancers, and therapies and medications for other systemic manifestations.[5] Clinical trials are underway to study new medications for the treatment of tumors common in NF1.

Selumetinib (Koselugo) is an FDA-approved treatment for children 2 years of age and older with neurofibromatosis type 1 and symptomatic, inoperable plexiform neurofibromas.[16]

Survival

The lifespan of individuals with Neurofibromatosis Type 1 is reported to be approximately 8 years less than the general population. The most important causes of early death are malignancy, especially malignant peripheral nerve sheath tumors, and vasculopathy.[3]

Test Information

Introduction

Testing for Neurofibromatosis Type 1 may include known familial mutation analysis, NF1 gene sequencing, or NF1 deletion/duplication analysis.

Sequence Analysis

NF1 sequence analysis may involve a multistep protocol to increase the detection of splicing mutations. This protocol combines sequence analysis in genomic DNA and

cDNA (mRNA). NF1 sequencing variants, such as missense, nonsense, and splice site variants, account for up to 95% of mutations seen in NF1.[3]

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Segmental NF

Testing of various sample types is available to help identify individuals with segmental or mosaic NF1. “RNA-based NF1/SPRED1 testing on cultured cells from affected tissues is offered starting from biopsies of café-au-lait macules (CALM) and/or neurofibromas.”[17]

“Detection of the causal NF1 PVs [pathogenic variants] in individuals with a mosaic/segmental phenotype requires special attention to (1) the sensitivity of the technology used to detect variants, as well as (2) the type of cells to be analyzed in affected tissue if the variant is not detectable in blood, i.e., melanocytes (but not keratinocytes or fibroblasts) from CALMs or Schwann cells from the cutaneous or plexiform neurofibromas.” [7]

Guidelines and Evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to Neurofibromatosis type 1 testing.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2019) stated the following in regard to genetic testing for NF1 in children:[8]

- "The following can be summarized about genetic testing:
  - can confirm a suspected diagnosis before a clinical diagnosis is possible
  - can differentiate NF1 from Legius syndrome
  - may be helpful in children who present with atypical features
  - usually does not predict future complications; and
  - may not detect all cases of NF1; a negative genetic test rules out a diagnosis of NF1 with 95% (but not 100%) sensitivity."

- "There are also other, less common, conditions associated with CALMs [café-au-lait macules]. The condition that could appear most similar to NF1 is Legius syndrome, which is caused by pathogenic variants in SPRED1, which encodes a protein that also functions within the Ras signaling pathway. People with Legius syndrome have multiple CALMs, intertriginous freckling, learning disabilities, and relative macrocephaly that is indistinguishable from findings in mild cases of NF1. Other manifestations of NF1, such as neurofibromas or other tumors, ophthalmologic findings, and skeletal manifestations, are not present in families with Legius syndrome. The absence of neurofibromas in adults with multiple CALMs in an extended pedigree is helpful to establish a diagnosis of Legius syndrome versus NF1, and molecular testing for SPRED1 versus NF1 should be considered in these cases."

The American College of Medical Genetics and Genomics (ACMG, 2018) stated the following in regard to genetic testing for NF1 in adults:[18]

- "In childhood, NF1 genetic testing can quickly establish a diagnosis and relieve anxiety, but that is less likely an issue for adults."
- "Most adults with NF1 are clinically diagnosed in childhood, according to NIH consensus criteria. The criteria are both highly specific and sensitive in adults with NF1."

International Consensus Panel

An international consensus panel (2021) updated the diagnostic criteria set forth by the National Institute of Health in 1988. The panel stated:[7]

In an individual who does not have a parent with NF, two or more of the following must be present:

- Six or more café-au-lait macules >5 mm in greatest diameter in prepubertal individuals and >15 mm in greatest diameter in postpubertal individuals
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axillary and/or inguinal (groin) regions
- Optic glioma
- Two or more Lisch nodules (iris hamartomas) or two or more choroidal abnormalities
- A distinctive osseous lesion such as sphenoid dysplasia, tibial anterolateral bowing, or long bone pseudoarthrosis
- Heterozygous pathogenic NF1 variant present in 50% of apparently normal tissue (e.g: white blood cells)

If an individual has a parent diagnosed with NF based on the criteria above, at least one of the criteria above must be present to merit a diagnosis of NF1.

“As panel testing testing by next-generation sequencing and exome/genome sequencing analysis is ordered with increasing frequency in individuals with a variable set of clinical features, some individuals have been found to carry an NF1 variant (P, LP, VUS) in unaffected tissue such as blood, although NF1 was not clinically suspected. NF1 experts agreed that identification of an NF1 variant alone does not suffice to make a diagnosis of NF1 but does require further clinical and genetic evaluation...”

National Society of Genetic Counselors

The National Society of Genetic Counselors (NSGC, 2020) stated the following regarding genetic testing for NF1:[19]

- “The two primary reasons for targeted genetic testing for NF1, NF2, or SWN are to confirm a diagnosis for management purposes, and to provide information for reproductive decision-making. In familial cases with a known pathogenic variant it is appropriate to offer testing to children as all of these conditions may present in childhood.”

Selected Relevant Publications

An expert authored review (2022) stated:[3]

- "If the phenotypic findings suggest the diagnosis of NF1, single-gene testing may be considered. Sequence analysis of NF1 genomic DNA (gDNA) and/or cDNA (complementary DNA, copied from mRNA) is performed in association with gene-targeted deletion analysis. Because of the frequency of pathogenic variants that affect splicing (22%-30%, more than 1/3 of which are not detected by gDNA sequencing of protein-coding regions), methods that include cDNA sequencing have higher detection rates than methods based solely on analysis of gDNA."
    - "If an NF1 variant is not detected, sequence analysis and deletion/duplication analysis of SPRED1 may be considered in individuals with only pigmentary features of NF1...Clinically distinguishing Legius syndrome from NF1 may be impossible in a young child because neurofibromas and Lisch nodules do not usually arise until later in childhood or adolescence in those with NF1. Examination of the parents for signs of Legius syndrome or NF1 may distinguish the two conditions, but in simplex cases, reevaluation of the individual after adolescence or molecular testing may be necessary to establish the diagnosis." For information on SPRED1 genetic testing, please refer to the guideline *Legius Syndrome Genetic Testing*, as this testing is not addressed here.
    - "Chromosomal microarray analysis (CMA) may be performed instead of sequence analysis to detect NF1 whole-gene deletions if the NF1 microdeletion phenotype is suspected clinically." For information on CMA testing, please refer to the guideline *Chromosomal Microarray Testing For Developmental Disorders (Prenatal and Postnatal)*, as this testing is not addressed here.

- "A karyotype [chromosome analysis] may be considered to look for a translocation or complex cytogenetic abnormality if a clinical diagnosis of NF1 is certain, but no pathogenic variant is found on sequence analysis of NF1 gDNA or cDNA and gene-targeted deletion analysis." For information on chromosome analysis, please refer to the guideline *Chromosome Analysis for Reproductive Disorders, Prenatal Testing, and Developmental Disorders*, as this testing is not addressed here

- "If neither parent of an individual with NF1 has features that meet the clinical diagnostic criteria for NF1 after detailed medical history, physical examination, and ophthalmologic examination, the proband most likely has NF1 as the result of a de novo pathogenic variant. Alternatively, the proband may have NF1 as the result of a disease-causing variant inherited from a parent who is mosaic or, rarely, from a heterozygous parent with incomplete penetrance. If the disease-causing variant has been identified in a child with NF1, targeted molecular testing of the parents can be performed to look for mosaicism and determine if a parent is heterozygous (but apparently unaffected due to incomplete penetrance)."
- "An individual in whom NF1 appears to have arisen as the result of [a] de novo mutation may have somatic mosaicism associated with segmental or unusually mild manifestations of NF1. The risk of a parent with mosaicism for an NF1 pathogenic variant transmitting the disorder to his or her child is less than 50%, but if the pathogenic variant is transmitted, it will be present in every cell in the child's body and the child may be much more severely affected...If neither parent of an individual with NF1 meets the clinical diagnostic criteria for NF1... the risk to the sibs of the affected individual of having NF1 is low but greater than that of the general population because of the possibility of parental germline mosaicism."

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Niemann-Pick Disease Types A and B Genetic Testing

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Introduction

Niemann-Pick disease types A and B genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Acid Sphingomyelinase Enzyme Activity	82657
Genetic Testing for Niemann-Pick Diseases	S3849
SMPD1 Deletion/Duplication Analysis	81479
SMPD1 Known Familial Mutation	81403
SMPD1 Sequencing	81479
SMPD1 Targeted Mutation Analysis	81330

Criteria

Introduction

Requests for Niemann-Pick disease types A and B (NPD-A/NPD-B) genetic testing are reviewed using these criteria.

Niemann Pick Type A or B Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic and Predisposition Testing:

- Niemann Pick A or B family mutation identified in biologic relative(s), OR

- Prenatal Testing:
  - Niemann Pick A or B mutation identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Niemann Pick A or B Targeted Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for Niemann Pick A or B, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Measurement of acid sphingomyelinase (ASM) enzyme activity in peripheral blood lymphocytes or cultured skin fibroblasts (in symptomatic individuals) with negative or equivocal result where suspicion of clinical diagnosis remains high, and
  - Hepatosplenomegaly, and/or
  - Evidence of interstitial lung disease on chest radiograph, and/or
  - Developmental Delay, and/or
  - Cherry Red Maculae, and/or
  - Hyperlipidemia, and/or
  - Thrombocytopenia, OR
- Predisposition/Carrier Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1st degree) diagnosed with Niemann Pick A or B clinically, and no family mutation identified, or
  - Ashkenazi Jewish ancestry and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Niemann Pick A or B Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:

  - If Ashkenazi Jewish, common mutations have been tested and resulted negative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Measurement of acid sphingomyelinase (ASM) enzyme activity in peripheral blood lymphocytes or cultured skin fibroblasts (in symptomatic individuals) with negative or equivocal result where suspicion of clinical diagnosis remains high, and
  - Hepatosplenomegaly, and/or
  - Evidence of interstitial lung disease on chest radiograph, and/or
  - Developmental Delay, and/or
  - Cherry Red Maculae, and/or
  - Hyperlipidemia, and/or
  - Thrombocytopenia, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1st degree) diagnosed with Niemann Pick A or B clinically, and no family mutation identified, and
  - If Ashkenazi Jewish, common mutations have been tested and resulted negative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Niemann Pick A or B Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous large rearrangement testing, and
  - Previous SMPD1 sequencing performed and no mutations found, and
  - No known familial mutation, and
  - Meets criteria for SMPD1 sequencing, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Niemann-Pick disease types A and B?

Definition

Niemann-Pick disease (NPD), also known as acid sphingomyelinase deficiency (ASMD), is a genetic disorder caused by an inability to process lipids (fats), which results in a toxic buildup of lipids in some organs.[1-4] Two types of NPD are caused by a deficiency of the acid sphingomyelinase enzyme: Niemann-Pick Type A (NPD-A) and Niemann-Pick Type B (NPD-B), collectively referred to as NPD-A/NPD-B in this guideline.[1-4]

Incidence

About 1 in 250,000 people have NPD.[1,3] NPD-A is more common in persons of Ashkenazi Jewish descent than in the general population. In the Ashkenazi Jewish population, the frequency of NPD is 1 in 40,000.[1,5]

Symptoms

NPD-A, also called the “neurological” or "neuronopathic" type or infantile neurovisceral ASMD, causes symptoms beginning in infancy. These include an enlarged liver and spleen (hepatosplenomegaly), psychomotor impairment with neurologic deterioration, interstitial lung disease, and eventually a classic cherry-red spot of the retina.[1-4]

NPD-B, also called the “non-neurological” or “non-neuronopathic” type or chronic visceral ASMD, causes some symptoms similar to NPD-A, but symptoms are usually milder and begin later. Additional symptoms include hyperlipidemia (high fat levels in blood) and thrombocytopenia (low platelets).[1,3,4]

A phenotype with intermediate severity is also known as chronic neurovisceral ASMD (intermediate form, NPD-A/B).[3,4] Clinical features in individuals with intermediate form NPD-A/B vary greatly, although all are characterized by the presence of some CNS manifestations

Cause

The SMPD1 gene encodes the acid sphingomyelinase (ASM) enzyme. Gene mutations in the SMPD1 gene lead to reduced or absent sphingomyelinase enzyme activity, causing the symptoms of NPD-A/NPD-B.[1,3,4]

Inheritance

NPD-A/NPD-B is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not

typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Individuals at increased risk to have a child with NPD-A/NPD-B should routinely be offered carrier screening.This includes those with:[5,6]

- Ashkenazi Jewish ancestry (1 in 90 carrier risk)[3,5]
- A family history of NPD-A/NPD-B (regardless of ethnicity)
- A partner who is a known carrier of NPD-A/NPD-B (or affected with the milder type)

Diagnosis

NPD-A/NPD-B is suspected when an individual presents with hepatosplenomegaly, interstitial lung disease, and depending on the subtype, neurological symptoms in infancy or abnormal blood findings.[3,4]

- A diagnosis cannot be made clinically.
- When the diagnosis suspected, acid sphingomyelinase enzyme activity testing should be performed first.[3,4] People with NPD-A or NPD-B usually have less than 10% of normal ASM activity compared to healthy individuals.[3,4]
- SMPD1 targeted mutation analysis tests for four of the most common SMPD1 gene mutations.
  - Three mutations - R496L, L302P, fsP330 - account for 97% of all cases of NPD-A in Ashkenazi Jewish people.[5]
  - The fourth mutation - deltaR608 - is a common cause of NPD-B in people of North African descent.[3]
  - Carrier screening by SMPD1 mutation panel for NPD is widely available as part of an “Ashkenazi Jewish Panel” that includes several other genetic diseases that are more common in this population.
    - For information on Ashkenazi Jewish carrier screening, please refer to the guideline *Ashkenazi Jewish Carrier Screening,* as this testing is not addressed here.
- SMPD1 next generation sequencing (NGS) analyzes the entire coding region of the SMPD1 and is available to detect less common mutations that cannot be detected on a targeted mutation analysis panel. SMPD1 NGS detects more than 95% of all SMPD1 mutations.[3]
- The frequency of deletions/duplications in SMPD1 is unknown.[3]

Management

If possible, individuals should be cared for by a multidisciplinary team with expertise in the condition.[4] The treatment for individuals with NPD-A/NPD-B includes supportive care and therapeutic interventions. These may include:[3,4]

- Nutritional support, which may include a feeding tube.
- Management of disease manifestations such as coagulopathy, liver disease, life-threatening bleeding, pulmonary disease, hyperlipidemia, sleep disturbance and irritability.
- Developmental interventions such as feeding, occupational, and physical therapies.
- Enzyme replacement therapy
    - Enzyme replacement therapy is FDA approved for treatment of non-central nervous system disease manifestations.[3,4] "Olipudase alfa, an enzyme replacement therapy (ERT) using human recombinant acid sphingomyelinase, is indicated as a disease-modifying enzyme replacement therapy for the long-term treatment of noncentral nervous system (CNS) manifestations of ASMD."[4] (Strength of recommendation: 1. Level of evidence: A)
    - "All patients with a confirmed diagnosis of ASMD and significant non-Central Nervous System (CNS) manifestations could be considered for olipudase alfa therapy on an individual basis."[4] (Strength of recommendation: 1. Level of evidence: A)
- Hematopoietic stem cell transplantation
    - Hematopoietic stem cell transplantation has had variable results and has associated morbidity or mortality. It may improve blood counts and hepatosplenomegaly but it not address the neurologic symptoms.[3,4]

Survival

Affected individuals with NPD-A usually do not survive beyond childhood.[1-3]

Affected individuals with NPD-B and intermediate form NPD-A/B can survive to adulthood.[1,3]

Test information

Introduction

Testing for NPD-A/NPD-B may include biochemical studies or genetic testing which may include familial mutation analysis, targeted mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Acid Sphingomyelinase Enzyme Analysis

Measuring ASM enzyme activity in peripheral blood lymphocytes or cultured skin fibroblasts is a reliable way to confirm a suspected case of NPD-A/NPD-B.[3] However, false-negative and inconclusive results are possible.[3] In such cases, genetic testing may be useful to resolve a diagnosis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

SMPD1 known familial mutation testing can be performed for at-risk relatives when the familial mutation is known and is not one of the common mutations.[3]

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to NPD-A/NPD-B genetic testing.[4-6] Professional guidelines generally support NPD-A/NPD-B carrier screening for those at increased risk.[5,6]

American College of Medical Genetics and Genomics

Consensus guidelines from the American College of Medical Genetics and Genomics (ACMG, 2008) recommended routine carrier screening for a group of disorders that includes NPD-A when at least one member of the couple is Ashkenazi Jewish and that couple is pregnant or planning pregnancy.[5]

American College of Obstetricians and Gynecologists

Consensus guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2020) addressed carrier screening and prenatal diagnosis for NPD-A.

- "Individuals with a positive family history of one of these conditions [including NPD-A] should be offered carrier screening for the specific condition and may benefit from genetic counseling." [6]
- Carrier screening for Ashkenazi Jewish people is routinely recommended for some disorders (i.e., Tay-Sachs, Canavan, cystic fibrosis, familial dysautonomia). ACOG states: "Some experts have advocated for a more comprehensive screening panel for those of Ashkenazi descent, including tests for several diseases that are less common (carrier rates 1 in 15 to 1 in 168) [including NPD-A]."[6]
- "If it is determined that this individual [a partner of Ashkenazi Jewish descent] is a carrier, the other partner should be offered screening."[6]
- "If both partners are found to be carriers of a genetic condition, genetic counseling should be offered. Prenatal diagnosis and advanced reproductive technologies to decrease the risk of an affected offspring should be discussed."[6]
- "The prevalence of these disorders [including NPD-A] in non-Jewish populations is unknown, and the sensitivity of these carrier tests in non-Jewish populations has not been established. Because the mutations in other populations may vary, counseling on the residual risks after negative carrier screening can be complicated in non-Jewish individuals. For couples in which one partner is a carrier and the other is of non-Jewish ancestry, genetic counseling may be useful in determining the best approach to risk estimation."[6]

International Niemann-Pick Disease Alliance

The International Niemann-Pick Disease Alliance (INPDA, 2023) published clinical practice guidelines for individuals with NPD-A/NPD-B.[4] They stated the following regarding genetic testing:

- "Genetic testing of SMPD1 gene should be performed to confirm diagnosis in subjects with ASM activity below normal reference intervals and allows genetic counselling. If SMPD1 sequencing is done before assessing ASM activity and the identified variant for 1 or 2 alleles is not known as pathogenic, demonstration of a deficient ASM activity is mandatory to confirm the diagnosis. Measurement of biomarkers can also be helpful." (Strength of recommendation: 1. Level of evidence: B)
- "Once the SMPD1 pathogenic variants have been identified in an affected family member, diagnostic testing of all at-risk family members is warranted to allow for early diagnosis and treatment of ASMD. …" (Strength of recommendation: 1. Level of evidence: A). Genetic testing for the parents of the affected individual is recommended to confirm carrier status and to inform at-risk family members.
- "Prenatal ASMD testing for a pregnancy at increased risk should be offered to all at risk couples, subject to local protocols and laws. Molecular testing for the familial SMPD1 variants using chorionic villus sampling (CVS) or amniotic fluid sampling is the most common means of testing at risk pregnancies. Biochemical prenatal diagnosis by testing of ASM enzyme activity in CVS or cultured amniocytes may also be used for at risk pregnancies." (Strength of recommendation: 2. Level of evidence: B)

Selected Relevant Publications

A 2023 expert-authored review recommended the following testing strategy for diagnosis of an affected person:[3]

- "The diagnosis of ASM deficiency is established by detection of biallelic pathogenic variants in SMPD1 by molecular genetic testing and residual acid sphingomyelinase enzyme activity that is less than 10% of controls (in peripheral blood lymphocytes or cultured skin fibroblasts)."
- Molecular testing approaches include targeted mutation analysis, single-gene testing and multigene panel testing.
- For individuals from populations in which common SMPD1 pathogenic mutations occur (e.g., individuals of Ashkenazi Jewish background with a severe neurodegenerative form of the disease suggestive of NPD-A, individuals of North African descent with NPD-B, or individuals from Chile, Saudi Arabia, and Turkey):
    - Perform targeted analysis for pathogenic mutations.
    - If targeted analysis does not identify both pathogenic mutations in individuals from these populations, sequence analysis of SMPD1 is appropriate.
- For individuals who are not in the populations discussed above:
    - Perform sequence analysis.
    - If no or only one pathogenic mutation is identified, consider gene-targeted deletion/duplication analysis.

References

Introduction

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Niemann-Pick Disease Type C Genetic Testing

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Introduction

Niemann-Pick disease type C genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Genetic Testing for Niemann-Pick Diseases	S3849
NPC1 Deletion/Duplication Analysis	81479
NPC2 Deletion/Duplication Analysis	81479
NPC1 Known Familial Mutation Analysis	81403
NPC2 Known Familial Mutation Analysis	81403
NPC1 Sequencing	81406
NPC2 Sequencing	81404

Criteria

Introduction

Requests for Niemann-Pick disease, type C (NPC) genetic testing are reviewed using these criteria.

Niemann-Pick Disease Type C Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing that would detect the familial mutation, AND

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- Diagnostic and Predisposition Testing:
  - NPC family mutation identified in biologic relative(s), OR
- Carrier Testing:
  - NPC family mutation identified in biologic relative(s), OR
- Prenatal Testing:
  - NPC mutation identified in both biologic parents AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Niemann-Pick C Disease Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for NPC, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Biochemical testing performed showing abnormal biomarkers, and
  - One or more of the following clinical findings:
    - Hepatosplenomegaly and/or liver failure
    - Central hypotonia or low muscle tone characterized by frequent falls and clumsiness
    - Ocular motor abnormalities, especially saccadic eye movements (SEM) and vertical supranuclear gaze palsy
    - Delayed or arrested speech development with or without cognitive impairment
    - Cerebellar
    - Seizures
    - Dystonia
    - Dysphagia, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1st, 2nd, or 3rd degree) diagnosed with NPC clinically, and no family mutation identified, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Niemann-Pick C Disease Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - NPC sequencing performed and no mutations or only one mutation identified, and
  - No previous NPC deletion/duplication analysis, and
  - Meets criteria for NPC sequencing, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

What is Niemann-Pick disease type C?

Definition

Niemann-Pick disease, type C (NPC) is a lipid storage condition that can present at any age, though the classic presentation is in mid-to-late childhood. Symptoms fall into one of three categories: visceral, neurological and psychological.[1]

Prevalence

NPC is pan-ethnic with a prevalence of 1 in 100,000 live births.[1] There are a few populations that have a founder effect, including French Acadians of Nova Scotia, Canada originally from Normandy France;[2] individuals of Hispanic descent in the Upper Rio Grande valley of the United States;[2] and a Bedouin group in Israel.

Symptoms

The presentation of clinical symptoms at each stage is different: [3,4]

- Presentation in the pre/perinatal period varies but most often includes liver disease with prolonged cholestatic jaundice and mild hepatosplenomegaly most frequent. Some cases present with acute liver failure that is independent of pulmonary disease. Fetal ascites/hydrops and thrombocytopenia are also possible.[1]
- Presentation between the ages of 2 months and <2 years most often includes hypotonia and developmental delay, with or without lung and liver disease. Liver disease evidenced by hepatosplenomegaly and/or prolong jaundice is almost always present. Vertical supranuclear gaze palsy (VSGP) may be present but challenging to identify.[1]
- The late infantile form typically presents from age 2 to <6 years with symptoms of clumsiness, ataxic gait and impaired fine motor skills. VSGP is typically present but

may be under recognized. Gelastic cataplexy or sensory deafness may be the first presenting symptoms. A history of neonatal cholestasis and variable visceromegaly and epilepsy may be noted.[1]

- The juvenile form of NPC has onset between 6 and 15 years of age with cognitive impairment, clumsiness, frequent falls, progressive ataxia, and VSGP, seizures, behavioral problems and gelastic cataplexy may be noted.[1]
- Individuals with NPC with onset after 15 years of age usually have an onset of progressive cognitive impairment and higher rates of psychiatric and other neurological symptoms. VSGP is typically present.[1]

Cause

Two genes have been associated with NPC: NPC1 and NPC2.

- The proteins of these genes are thought to work together in the cellular transport of cholesterol and other molecules.
- Most (90-95%) individuals with NPC have at least one identifiable gene mutation in NPC1.[5,6] Only 30 families have been found to have mutations in the NPC2 gene, making mutations in this gene rare (about 4% of NPC cases).[1,2,5]
- There have been over 200 mutations described that cause NPC.[7] Genotype-phenotype correlation is difficult to determine as most individuals are compound heterozygotes; however, there has been observation of some alleles being associated with mild or severe disease.[7-9]
- NPC1 sequence analysis can identify 76% of mutations in the NPC1 gene.[10]
- NPC2 sequence analysis can identify 88% of mutations in the NPC2 gene.[10]
- NPC1 and NPC2 deletion/duplication analysis is available clinically for individuals who test negative on sequence analysis.

Inheritance

NPC is inherited in an autosomal recessive inheritance pattern.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

Once a diagnosis of NPC is suspected clinically, the diagnosis can be confirmed through a combination of biochemical and genetic studies.

- The NPC-suspicion index assists in the diagnosis of adult individuals with NPC, with strong indicators including cognitive and psychiatric symptoms, and the combination of neurological with psychiatric signs is highly suggestive of NPC.[1,11]

Treatment

Healthcare management after diagnosis includes treatment for current symptoms.[10]

- This generally includes medications to prevent the onset of seizures, although treatment of liver disease, sleeping dysfunction or other symptoms should be considered as well.
- There is no definitive therapy available for NPC.
- Bone marrow transplantation (BMT), liver transplantation or the use of cholesterol lowering drugs did not prevent the progression of neurological disease.

Survival

There is wide variability with disease progression and survival rate, which can range from just a few days to, in rare circumstances, 60 years. Most individuals survive between 10-25 years.[12]

Test information

Introduction

Testing for NPC may include biochemical studies or genetic testing which would include known familial mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Biochemical Analysis

The following biochemical studies may be performed for NPC.

Oxysterols (cholesterol oxidation products)

- This testing includes measurement of the oxysterols cholestane-3β, 5α, 6β-triol (C-triol) and 7-ketocholesterol (7-KC) in blood. Both are sensitive markers for NPC. [1,13,14]
- When this testing indicates an individual is affected, the diagnosis must be confirmed by sequence/mutation analysis and if necessary, filipin test.
- Carrier testing is not reliable through biochemical testing.

Filipin biochemical testing

- This testing involves the demonstration of abnormal intracellular cholesterol homeostasis in cultured fibroblasts.[2,15]
- Fibroblasts are cultured in an LDL-enriched medium, and then fixed and stained with a compound called “filipin”. To perform biochemical testing, filipin interacts with unesterified cholesterol to make specific cholesterol-filled complexes in ~80-85% of cases.
- The filipin test is no longer considered a first line test for the diagnosis of NPC. It is still an extremely useful test for cases in which molecular or biochemical results are not conclusive.[1]
- Carrier testing is not available through biochemical testing, as there is overlap of enzyme activity between carriers and non-carriers.
- The biochemical assay can be used for prenatal diagnosis if both mutations are not known.[2]

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to NPC genetic testing.

International Niemann-Pick Disease Registry

Consensus-based diagnostic recommendations available from the International Niemann-Pick Disease Registry (INPDR, 2018), an international, collaborative group of disease experts, stated:[1]

- “Once NPC is suspected clinically, diagnosis can be confirmed by the combination of biochemical and molecular genetic studies.[16] In recent years, several plasma metabolites (cholestane-3β, 5α, 6β-triol, lyso-sphingomyelin isoforms and bile acid metabolites) have emerged as sensitive and specific diagnostic biomarkers for NPC and their study, completed by genetic analyses, should now be considered as the first line laboratory testing.[16,17] The filipin test, although still very useful, is no longer considered as the primary tool."
- “Assessment of biomarkers should be considered as a first-line test to screen for NPC. Three classes of biochemical markers are either currently in use (oxysterols; lyso-SM-509 and lyso-sphingomyelin) or are in development (bile acid derivatives).They can be used alone or in combination to enhance sensitivity and specificity. The diagnosis, however, must in all cases be confirmed by mutation analysis and if necessary, filipin test."
- “Any individual in whom the diagnosis of NPC is considered based on their clinical manifestation and/or abnormal biomarker profile should undergo genetic testing for NPC genes to confirm the diagnosis. Referral to a clinical geneticist or genetic counsellor should be considered upon the diagnosis of NPC."
- “Filipin test is no longer considered a first line test for the diagnosis of NPC. It still remains an extremely useful diagnostic tool in uncertain cases in which biomarkers and/or molecular analysis present inconclusive results and to assess the pathogenicity of novel genetic variants."
- Regarding genetic testing:
    - “Mutation analysis of NPC1 and NPC2 genes is mandatory to confirm the diagnosis of NPC. In addition, it is the only reliable method to diagnose NPC carriers within the family and the highly preferred strategy for prenatal diagnosis." This testing will also expedite identification of potentially pre-symptomatic affected siblings.
    - “Although genotype/phenotype correlations are difficult to establish, some conclusions can be drawn from current evidence."

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Noonan Spectrum Disorder Genetic Testing

MOL.TS.371.A
v2.0.2024

Introduction

Noonan spectrum disorder genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Known Familial Mutation Analysis	81403
Noonan Spectrum Disorder Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Noonan Spectrum Disorders (eg, Noonan syndrome, cardio-facio-cutaneous syndrome, Costello syndrome, LEOPARD syndrome, Noonan-like syndrome), genomic sequence analysis panel, must include sequencing of at least 12 genes, including BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, and SOS1	81442

400 Buckwalter Place Boulevard, Bluffton, SC 29910 (800) 918-8924

Criteria

Introduction

Requests for Noonan spectrum disorder (NSD) genetic testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Known familial mutation in a causative gene in a 1st-degree biologic relative, OR
- Prenatal Testing for At-Risk Pregnancies:
  - Known familial disease-causing mutation identified in a biologic parent or affected sibling of the pregnancy, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Single Gene Sequence Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous testing of the requested gene, and
  - No known NSD mutation in a biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Two or more of the following major features:
    - Hypertrophic cardiomyopathy
    - Congenital pulmonary valve stenosis
    - Electrocardiogram characteristic of NSD associated with the requested gene
    - Facial dysmorphism suggestive of NSD associated with the requested gene
    - Stature less than 3rd percentile for age and gender
    - Pectus carinatum and/or excavatum

        - First-degree relative with known or suspected NSD associated with the requested gene, or
    - One major feature as listed above, in combination with one or more of the following:
        - Other cardiac abnormality suggestive of the Noonan Spectrum disorder associated with the requested gene (e.g., atrial septal defect, ventricular septal defect, branch pulmonary artery stenosis, tetralogy of Fallot, etc.)
        - Stature 3rd to 10th percentile for age and gender
        - Broad thorax/widely-spaced nipples
        - Developmental delay, intellectual disability, or diagnosed learning disability
        - Cryptorchidism
        - Broad or webbed neck
        - Lymphatic dysplasia
        - Coagulopathy confirmed with hematologic studies
        - Skin abnormality characteristic of the NSD associated with the requested gene (e.g. multiple lentigines, follicular keratosis, etc.)
        - Pubertal delay and/or infertility, OR
- Prenatal Testing:
    - Prenatal chromosome study is not diagnostic, and
    - Fetal ultrasound exhibits features of the NSD associated with the requested gene based on the presence of one or more of the following:
        - Nuchal edema (e.g., increased nuchal translucency, increased nuchal fold, or cystic hygroma) and/or hydrops fetalis
        - Pulmonary valve stenosis
        - Hypertrophic cardiomyopathy
        - A combination of TWO or more of the following: Polyhydramnios, distended jugular lymphatic sacs (JLS), pleural effusion, cardiac anomaly, renal anomaly, ascites, facial abnormalities suggestive of a NSD and/or first-degree relative known or suspected to have the associated NSD, and
    - No known cause for the above features (e.g., known genetic disorder, etc), and
    - The requested single gene sequencing test is appropriate due to one or more of following:
        - The requested gene is the only gene known to be associated with the suspected type of NSD (e.g., HRAS for Costello syndrome, etc.)

    - Mutations in the requested gene are the most common cause of the suspected type of NSD (e.g., PTPN11 for classic NS or NSML, etc.)
    - Sequencing of genes more frequently associated with the suspected Noonan Spectrum Disorder have been completed and was not diagnostic, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Multigene Panel Testing

When a multi-gene panel is requested and billed with the appropriate CPT panel code, 81442, the panel will be considered medically necessary when the following criteria are met:

- Genetic Counseling:
    - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
    - No previous NSD panel testing, and
    - No known NSD mutation in a biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
    - Two or more of the following major features:
        - Hypertrophic cardiomyopathy
        - Congenital pulmonary valve stenosis
        - Electrocardiogram characteristic of an NSD
        - Facial dysmorphism suggestive of an NSD
        - Stature less than 3rd percentile for age and gender
        - Pectus carinatum and/or excavatum
        - First-degree relative with known or suspected NSD, or
    - One major feature as listed above, in combination with one or more of the following:
        - Other cardiac abnormality suggestive of the NSD (e.g., atrial septal defect, ventricular septal defect, branch pulmonary artery stenosis, tetralogy of Fallot, etc.)
        - Stature 3rd to 10th percentile for age and gender
        - Broad thorax/widely-spaced nipples
        - Developmental delay, intellectual disability, or diagnosed learning disability
        - Cryptorchidism

        - Broad or webbed neck
        - Lymphatic dysplasia
        - Coagulopathy confirmed with hematologic studies
        - Skin abnormality characteristic of the NSD (e.g., multiple lentigines, follicular keratosis, etc.)
        - Pubertal delay and/or infertility, OR
- Prenatal Testing:
    - Prenatal chromosome study is not diagnostic, and
    - Fetal imaging exhibits features of an NSD based on the presence of one or more of the following:
        - Nuchal edema (e.g. increased nuchal translucency, increased nuchal fold, or cystic hygroma) and/or hydrops fetalis
        - Pulmonary valve stenosis
        - Hypertrophic cardiomyopathy
        - A combination of TWO or more of the following: polyhydramnios, distended jugular lymphatic sacs (JLS), pleural effusion, cardiac anomaly, renal anomaly, ascites, facial abnormalities suggestive of an NSD and/or first-degree relative known or suspected to have the associated NSD, and
    - No known cause for the above features (e.g., known genetic disorder, etc), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Deletion/Duplication Analysis

Deletion/duplication analysis for NSD is not medically necessary due to the extremely low diagnostic yield.

Other Considerations

Broad NSD panels may not be medically necessary when a more targeted test is available and more appropriate based on clinical findings.

The criteria stated in this section applies only to germline diagnostic testing for NSDs. For information on somatic (tumor marker) testing, please refer to the appropriate test-specific guideline or to the guideline *Somatic Mutation Testing - Solid Tumors*, as this testing is not addressed here. For information on non-invasive screening, please refer to the guideline *Non-Invasive Prenatal Screening*, as this testing is not addressed here.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Deletion/Duplication analysis for NSD is not reimbursable.
- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81442*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.
    - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
    - When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement, based on which NSD is most likely:
        - Classic NS: PTPN11, followed by SOS1, RAF1, RIT1 and KRAS if PTPN11 sequencing is negative.
        - CFC syndrome: BRAF, followed by MAP2K1, MAP2K2, and KRAS if BRAF sequencing is negative.
        - NSML/LEOPARD syndrome: PTPN11, followed by RAF1, BRAF, and MAP2K1 if PTPN11 sequencing is negative.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is Noonan spectrum disorder?

Definition

Noonan spectrum disorders (NSDs) are a group of disorders that includes Noonan syndrome (NS), Cardiofaciocutaneous (CFC) syndrome, Noonan syndrome with multiple lentigines (NSML or LEOPARD syndrome), Costello syndrome, Noonan syndrome-like disorder with loose anagen hair, and Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia (JMML). These disorders are often referred to as “RASopathies” due the associated gene products being involved in the Ras/MAPK-pathway.[1-4]

Prevalence

The prevalence of NS is between 1:1,000 and 1:2,500 individuals. Though mild expression of the condition is likely to be overlooked. Other NSDs are relatively rare.[1-4]

Symptoms

NSDs are multisystem disorders characterized by facial features, short stature, cardiovascular abnormalities (particularly pulmonary valve stenosis and hypertrophic cardiomyopathy), and developmental delay of variable degree.[1-4]

Cause

NSDs are associated with mutations in a number of genes involved in the Ras/MAPK-pathway, with genetic overlap between many of the NSD types:[1-4]

- NS: Causative mutations are found in PTPN11 (50%), SOS1 (~10-13%), LZTR1 (~8%), RAF1 (5%), RIT1 (5%), and KRAS (<5%). BRAF, MAP2K1, MRAS, NRAS, RASA2, RRAS2, and SOS2 mutations each account for 4% or fewer cases.
- CFC: Caused by mutations in BRAF (~75%), MAP2K2/MEK2 (~25%), KRAS (<2%), and MAP2K1.
- NMSL or LEOPARD syndrome: Caused by mutations in PTPN11 (90%), RAF1 (<5%), BRAF, and MAP2K1.
- Costello syndrome: Caused by mutations in HRAS (80-90%). This is the only causative gene reported to date.
- Noonan syndrome-like disorder with loose anagen hair: Caused by mutations in SHOC2, particularly a recurrent 4A>G pathogenic variant. Sequencing of SHOC2 will detect a pathogenic variant in ~5% of individuals with NS, most of which have the classic loose anagen hair. This is also caused by mutation in PPP1CB.
- JMML: Caused by mutations in the CBL gene.

Inheritance

Inheritance is autosomal dominant, with the exception of mutations in LZTR1, which can be inherited in either an autosomal dominant or autosomal recessive manner.[1-4]

Individuals with NS and NSML may have an affected parent. In contrast, CFC and Costello syndrome are almost always the result of a de novo mutation.[1-4]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

The diagnosis of an NSD is established with molecular testing, which can be accomplished with the use of a multigene panel or serial single-gene testing. Once the causative mutation in the family has been identified, prenatal diagnosis is possible via CVS or amniocentesis.

Additionally, NSDs are usually diagnosed on clinical grounds based on the presence of key features. Clinical diagnostic criteria are available for NSML. No formal diagnostic criteria exist for NS, CFC or Costello syndrome. The diagnosis should be suspected in individuals with the following:[1-4]

- **NS:**
  - Characteristic facies: "low-set, posteriorly rotated ears with fleshy helices; vivid blue or blue-green irises; and eyes that are often wide-spaced, downslanted, and with epicanthal folds and fullness or droopiness of the upper eyelids (ptosis).
  - Short stature for sex and family background
  - Congenital heart defects, most commonly pulmonary valve stenosis, atrial septal defect, and/or hypertrophic cardiomyopathy
  - Developmental delay of variable degree
  - Broad or webbed neck

- Unusual chest shape with superior pectus carinatum, inferior pectus excavatum
- Widely set nipples
- Cryptorchidism in males
- Lymphatic dysplasias of the lungs, intestines, and/or lower extremities
- Coagulation defects"[1]
- Per a recent expert summary, "No consensus clinical diagnostic criteria for Noonan syndrome have been published."[1] However, diagnostic scoring systems for NS were developed by van der Burgt and published in 2007.[5] These are also embedded in the Dyscerene 2010 guidelines for NS, and similar recommendations were provided by Romano et al 2010 and Roberts et al 2013.[6-8] Each feature has a major finding and minor finding as indicated below. Per the scoring systems, a clinical diagnosis of NS is definitive when an individual has: two major signs OR one major sign plus two minor signs OR three minor signs.

  - Facial
    - Major: typical face dysmorphology
    - Minor: suggestive face dysmorphology
  - Cardiac
    - Major: pulmonary valve stenosis, HOCM [hypertrophic obstructive cardiomyopathy] and/or ECG typical of NS
    - Minor: other defect
  - Height
    - Major: height less than third percentile for age
    - Minor: height less than tenth percentile for age
  - Chest wall
    - Major: pectus carinatum/excavatum
    - Minor: broad thorax
  - Family history
    - Major: first degree relative with definite NS
    - Minor: first degree relative with suggestive NS
  - Other
    - Major: intellectual disability, cryptorchidism, and lymphatic dysplasia
    - Minor: intellectual disability, cryptorchidism, and/or lymphatic dysplasia

- **CFC:**
  - Cardiac features: pulmonic stenosis, atrial septal defects, ventricular septal defects, hypertrophic cardiomyopathy, heart valve anomalies, and rhythm disturbances.
  - Craniofacial features: "high forehead, relative macrocephaly, bitemporal narrowing, hypoplasia of the supraorbital ridges, ocular hypertelorism, telecanthus, downslanting palpebral fissures, epicanthal folds, ptosis, short nose with depressed bridge and anteverted nares, ear lobe creases, low-set ears that may be posteriorly rotated, deep philtrum, cupid's bow configuration of the upper lip, high-arched palate, relative micrognathia."
  - Ectodermal features: characteristic skin, hair, and nail abnormalities.[4]
- **NSML (previously LEOPARD) syndrome:**
  - "Lentigines
  - Cardiac abnormalities, particularly hypertrophic cardiomyopathy
  - Poor linear growth/short stature
  - Pectus deformity"
  - Craniofacial features including widely spaced eyes and ptosis
  - Clinical diagnostic criteria are:
    - "Multiple lentigines plus two of the cardinal features listed above, OR
    - In the absence of lentigines, three of the other cardinal features plus a first-degree relative with NSML"[3]
- **Costello syndrome:**
  - "Prenatal findings: increased nuchal thickness, polyhydramnios (>90%), characteristic ulnar deviation of the wrists, short humeri and femurs, fetal tachycardia (various forms of atrial tachycardia), preterm delivery
  - Postnatal findings: severe postnatal feeding difficulties extending throughout early childhood, failure to thrive, short stature, macrocephaly (relative), coarse facial features, curly or sparse, fine hair
  - Skin: loose and soft skin, increased pigmentation, deep palmar and plantar creases, papillomata of face and perianal region (typically absent in infancy but may appear in childhood), hyperkeratosis and calluses, premature aging, hair loss
  - Musculoskeletal system: diffuse hypotonia, joint laxity, low muscle mass, ulnar deviation of wrists and fingers, splayed fingers resulting in characteristic hand posture, spatulate finger pads, abnormal fingernails, tight Achilles tendons (often developing throughout childhood), positional foot deformity, vertical talus,

kyphoscoliosis, pectus carinatum, pectus excavatum, asymmetric rib cage, developmental hip dysplasia

- Cardiovascular system: cardiac hypertrophy, usually typical hypertrophic cardiomyopathy (i.e., idiopathic subaortic stenosis, asymmetric septal hypertrophy), although other forms (i.e., biventricular) have been reported; congenital heart defect, usually valvar pulmonic stenosis; arrhythmia, usually supraventricular tachycardia"; aortic dilation, mild; hypertension
- "Neurologic: Chiari I malformation which may develop over time, hydrocephalus, syringomyelia, seizures, tethered cord
- Tumors: increased occurrence of malignant solid tumors
- Psychomotor development: developmental delay or intellectual disability, sociable, outgoing personality, findings suggestive of autism spectrum disorder in early infancy that improve by age four years."[2]

Management

Surveillance is indicated for anomalies in any organ system, particularly the cardiovascular system. Heart defects are usually treated the same as in the general population. Developmental delay is addressed by early intervention programs and individualized education strategies. Growth hormone (GH) treatment may be used to increase growth velocity. Coagulation screening, including CBC with differential and PT/PTT, and treatment of serious bleeding problems as needed.[1-4,6,8] Some genotype-phenotype correlations are present, which may help to guide medical management.[9]

Survival

An individual with an NSD can have a normal lifespan. However, lifespan can vary depending on the medical complications, such as cardiovascular defects, present in the affected individual.[1-4]

Test information

Introduction

Testing for NSDs may include known familial mutation analysis, next generation sequencing, or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

- Research has demonstrated that postnatal NGS panel testing in symptomatic individuals has a diagnostic yield of 19-47%.[10-12]
- One study of multigene NSD panel testing in individuals with apparently isolated cardiomyopathy (per clinical information obtained from test requisition forms) demonstrated a detection rate of 0.6%.[13] NSDs are estimated to account for ~6% of pulmonary valve stenosis.[14]
- Approximately 3-15% of fetuses with normal chromosomes and increased nuchal translucency are estimated to have NS.[1]
- Nearly all pathogenic mutations associated with an NSD are detected with sequence analysis. Very rare cases of duplication and/or deletion have been reported in some genes; the yield of such testing is expected to be extremely low.[1-4] There is also some question as to whether these case reports with copy number variation did indeed have a clinical diagnosis of an NSD.[15]
- Roughly 10% of individuals who fit the clinical diagnosis of an NSD do not have an identifiable pathogenic mutation in any of the known genes, suggesting that additional genes are involved.[16]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to NSD genetic testing.

Selected Relevant Publications

A 2023 expert-authored review on CFC stated:[4]

- "Consensus guidelines have been developed for genetic testing strategy for CFC syndrome.
- Based on current published information, sequencing can be approached stepwise.
- A multigene panel for RASopathies/Noonan spectrum disorders that includes BRAF, MAP2K1, MAP2K2, and KRAS and other genes of interest… is usually the preferred initial test.
- If multigene panel testing is not available, serial single-gene testing is recommended, beginning with BRAF, MAP2K1, and MAP2K2, and KRAS; if no pathogenic variants are found follow with sequencing of HRAS (all exons) even though the patient appears to have a clinical diagnosis of CFC syndrome. Individuals who have an HRAS pathogenic variant by definition have Costello syndrome.
- If no pathogenic variant is identified in these genes using sequencing analysis, gene-targeted deletion/duplication analysis or array CGH can be considered. Rare deletions in MEK genes (i.e., MAP2K1 and MAP2K2) may cause phenotypic features that are reminiscent of CFC syndrome."
- More comprehensive genomic testing (when available) including exome sequencing or genome sequencing may be considered if serial single-gene testing (and/or use of a multigene panel that includes BRAF, MAP2K1, MAP2K2, and KRAS) fails to confirm a diagnosis in an individual with features of CFC syndrome."

A 2022 expert-authored review on NS stated:[1]

- “When the phenotypic findings suggest the diagnosis of Noonan syndrome, molecular genetic testing approaches usually include the use of a multigene panel.”
- “Serial single-gene testing can be considered if panel testing is not feasible. Approximately 50% of individuals with NS have a pathogenic missense variant in PTPN11; therefore, single-gene testing starting with PTPN11 would be the next best first test. Appropriate serial single-gene testing if PTPN11 testing is not diagnostic can be determined by the individual's phenotype (e.g., RIT1 if there is hypertrophic cardiomyopathy, LZTR1 if autosomal recessive inheritance is suspected); however, continued sequential single-gene testing is not recommended as it is less efficient and more costly than panel testing.”
- “Since Noonan syndrome occurs through a gain-of-function mechanism and large intragenic deletions or duplications have not been reported, testing for intragenic deletions or duplications is unlikely to result in a diagnosis; however, rare cases have been reported for some genes.”
- “Molecular genetic testing approaches can include a combination of gene-targeted testing (multigene panel) and comprehensive genomic testing (exome sequencing or genome sequencing) depending on the phenotype.”

- "When the diagnosis of Noonan syndrome has not been considered because an individual has atypical phenotypic features or if some but not all characteristic phenotypic features are present (e.g., a "Noonan-like" phenotype), comprehensive genomic testing, which does not require the clinical to determine which gene is likely involved, may be used. Exome sequencing is most commonly used; genome sequencing is also possible."

A 2022 expert-authored review on NSML stated:[3]

- "Molecular genetic testing approaches can include single-gene testing or use of a multigene panel." Single-gene testing should be "based on the order in which a pathogenic variant is most likely to be identified."
- "Although gene-targeted deletion/duplication analysis could be considered, the variant detection frequency is unknown and expected to be extremely low."

A 2019 expert-authored review on Costello syndrome stated:[2]

- “When the clinical findings suggest the diagnosis of Costello syndrome, molecular genetic testing approaches can include single-gene testing or use of a multigene panel.”
- “When the diagnosis of Costello syndrome is not considered because an individual has atypical phenotypic features, comprehensive genomic testing (which does not require the clinician to determine which gene[s] are likely involved) is the best option. Exome sequencing is the most commonly used genomic testing method; genome sequencing is also possible.”

A 2014 expert-authored review on NS made the following recommendations:[17]

- Noonan syndrome should be considered in anyone with two or more of the following:
  - "Characteristic facial features
  - Developmental delay and/or learning disability
  - Heart defect
  - Pubertal delay and/or infertility
  - Short stature
  - Typical chest deformity
  - Undescended testes
  - First-degree relative who has Noonan syndrome or any of the above features"
- "The diagnosis of Noonan syndrome should be considered in all fetuses with a normal karyotype and increased nuchal translucency, especially when cardiac anomaly, polyhydramnios, and/or multiple effusions are observed [Evidence rating: C]."

- "Management of patients with Noonan syndrome is optimized by adherence to age-specific guidelines that emphasize screening and testing for common health issues [Evidence rating: C]. U.S. and United Kingdom age-specific guidelines are available."
- "Referral to a clinical geneticist for assistance in diagnosis and management of Noonan syndrome is helpful [Evidence rating: C]."
- "The appropriateness and sequence of genetic testing should be determined by a clinical geneticist [Evidence rating: C]. Mutation testing will prove a diagnosis in approximately 70% of cases. Mutation testing may benefit a family if reproductive decisions depend on this information."

Gripp KW, et al (2019) stated the following regarding Costello syndrome:[18]

- "Genetic testing coordinated by a genetics professional is important to confirm the diagnosis.
    - HRAS sequencing, or common mutation panel followed by full analysis if common panel is negative.
    - Multi-gene RASopathies panel if diagnosis is unclear or negative HRAS testing.
    - Additional testing may be considered by medical genetics professionals including chromosome microarray and exome testing."

Tafazoli A, et al (2017) stated:[19]

- “All cases should be confirmed by molecular testing for appropriate specific treatments and follow-up procedures in addition to making correct genotype-phenotype correlations…Karyotype and copy number analysis are suggested only in cases with intense neurocognitive involvement and are not performed routinely for patients with typical phenotypes of NS.”

Roberts AE, et al (2013) stated:[7]

- “Genetic testing can be useful in several scenarios. Because the presentation of cardiofaciocutaneous and Costello syndromes overlaps substantially in the first year of life, genotyping can aid diagnosis. If a patient has a mild or atypical presentation, genotyping could establish the diagnosis. For an adult with suspected Noonan syndrome, establishing the molecular genetic cause will enable preimplantation, prenatal, or postnatal testing if desired. The specific genotype of a child with Noonan syndrome is useful to know in order to provide specific guidance—for example, to address the increased prevalence of hypertrophic cardiomyopathy in RAF1-associated Noonan syndrome or short stature and growth hormone abnormalities in PTPN11-associated Noonan syndrome.”

Romano, AA et al (2010) stated:[8]

- "If sequential molecular testing is determined to be indicated (rather than simultaneous chip based analysis):

- PTPN11 sequencing should be performed first, because this gene explains the highest number of cases
- If normal, phenotype should be used to guide the choice of the next gene to sequence
- If developmental delays are absent or mild, CFC syndrome–like skin and hair findings are present, and/or patient is of normal stature, consider SOS1 sequencing
- If HCM is present, consider RAF1 sequencing
- For significant developmental delays or cognitive issues, consider KRAS sequencing
- For sparse, thin, slow-growing hair, consider SHOC2 sequencing
- If a variant is found, consider testing the parents to provide accurate recurrence risks."

- "...routine karyotyping or copy-number analysis is not recommended at this time for typical NS cases. It may be considered for atypical cases or when there is particularly severe neurocognitive involvement."

Special Considerations

There is considerable debate about when genetic testing for an NSD should be pursued in a pregnancy with abnormal ultrasound findings and absence of a known family history. Some authors recommend that testing for NS be undertaken for any pregnancy with an increased nuchal translucency and normal chromosome studies, even if there are no additional associated abnormalities, while others recommend that testing only be performed if there is at least one additional ultrasound finding, such as polyhydramnios, hydrops fetalis, renal anomalies, distended JLS, hydrothorax, cardiac anomalies or ascites.[17,20-28]

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PALB2 Genetic Testing for Breast Cancer Risk

MOL.TS.251.A
v2.0.2024

Introduction

PALB2 genetic testing is addressed by this guideline.

Procedure addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure(s) addressed by this guideline	Procedure code(s)
PALB2 Deletion/Duplication Analysis	81479
PALB2 Known Familial Mutation Analysis	81308
PALB2 Sequencing	81307

Criteria

Introduction

Requests for PALB2 testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous testing that would detect the familial mutation, and
  - Known family mutation in PALB2 identified in 1st, 2nd, or 3rd degree relative(s), AND
- Age 18 years or older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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Full Sequence Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member has had BRCA1/2 analysis and no mutations were found, and
  - Member has not had previous PALB2 sequencing, AND
- Diagnostic Testing in Symptomatic Individuals and Presymptomatic Testing in Asymptomatic individuals:
  - Member has met criteria for BRCA1/2 analysis,** AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**For information on BRCA1/2 testing, please refer to the guideline *BRCA Analysis,* as this testing is not addressed here.

Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member meets above criteria for PALB2 full sequence analysis, and
  - Member has had PALB2 full sequence analysis and no mutations were found, and
  - Member had not had previous PALB2 deletion/duplication analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other Considerations

PALB2 testing may be performed as part of a multigene, multisyndrome panel. For information on multigene, multisyndrome panel testing, please refer to the guideline *Hereditary Cancer Syndrome Multigene Panels*, as this testing is not addressed here.

What is PALB2 genetic testing?

Definition

Breast cancer is the most frequently diagnosed malignancy and one of the leading causes of cancer mortality in women around the world. Hereditary breast cancer accounts for 5% to 10% of all breast cancer cases.[1] Two cancer susceptibility genes, BRCA1 and BRCA2, are implicated in 40-45% of all hereditary breast cancer cases.[1] Other genes have also been identified in the literature as being associated with inherited breast cancer risk, including ATM, BARD1, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11, and TP53.[1-4] PALB2 is a gene that encodes a protein that may be involved in tumor suppression, and is considered a partner and localizer of BRCA2.[2] Mutations in PALB2 increase the chance a person will develop certain cancers and, in particular, female breast cancer.[1-5]

Prevalence

In one study, pathogenic mutations in 12 genes associated with hereditary breast cancer were found in 5.06% of 32,347 women with breast cancer. Of those with a mutation, 0.46% had a mutation in PALB2.[1] Approximately 50 truncating mutations in PALB2 have been detected among families with breast cancer worldwide.[4]

Symptoms

One study analyzing the risk of breast cancer, estimated a relative risk (RR) of 2.3 (95% CI, 1.4 to 3.9) conferred by PALB2 mutations, indicating an approximate two-fold increased risk of developing hereditary breast cancer.[4] A meta-analysis of three studies estimated a relative risk of 5.3 (90% CI, 3.0-9.4).[6] Another study documented a lifetime risk of breast cancer of 32% in women with a PALB2 mutation.[1] Per the National Comprehensive Cancer Network, the absolute risk for breast, ovarian, and pancreatic cancer are quoted as 41-60%, 3-5% and 5-10%, respectively.[7]

Cause

Pathogenic mutations in the PALB2 gene cause the aforementioned associated cancer risks.[1-7]

Diagnosis

The diagnosis is established with identification of a pathogenic mutation in the PALB2 gene.

Inheritance

PALB2 mutations are inherited in an autosomal dominant manner.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

PALB2 mutations inherited in an autosomal recessive manner cause Fanconi Anemia.[7] Testing for Fanconi Anemia is not addressed in this guideline.

Management

Screening and prevention options are available to specifically address the increased risk of cancer in an individual with a PALB2 pathogenic mutation.[7]

Test information

Introduction

PALB2 testing may include known familial mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence
Introduction

This section includes relevant guidelines and evidence pertaining to PALB2 testing.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2021) published a clinical practice resource for management of individuals with PALB2 pathogenic mutations. They stated the following:[8]

- "ACMG recommends:
  - the use of personalized risk estimates (e.g., CanRisk) in guiding clinical management.
  - that PALB2 should be included in breast, ovarian, and pancreas germline cancer gene panels.
  - that PALB2 VUS [variants of uncertain significance] are not used to guide clinical management.
  - prospective collection of clinical data from PALB2 heterozygotes to establish clear metrics on treatment outcome and survival.
  - surveillance for breast cancer should be equivalent to that for BRCA1/2 heterozygotes.
  - risk-reducing mastectomy can be considered as an option. The decision should be guided by personalized risk assessment.
  - ovarian cancer surveillance should not be offered, and risk-reducing salpingo-oophorectomy should include shared decision making and should rarely be considered before the age of 50.
  - pancreatic cancer surveillance should be considered, but ideally as part of a clinical trial.
  - PALB2 heterozygotes should be considered for the same therapeutic regimens and trials as those for BRCA1/2."
- "ACMG does not recommend testing partners of PALB2 heterozygotes in the reproductive setting, unless they are from a country with founder variants or it can be justified by the partner's family history of cancer."

American Society of Breast Surgeons

The American Society of Breast Surgeons (ASBrS, 2019) published a consensus guideline on genetic testing for hereditary breast cancer. They stated the following:[9]

- "Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling and

make recommendations to their patients regarding genetic testing and arrange testing. When the patient's history and/or test results are complex, referral to a certified genetic counselor or genetics professional may be useful. Genetic testing is increasingly provided through multi-gene panels. There are a wide variety of panels available, with different genes on different panels. There is a lack of consensus among experts regarding which genes should be tested in different clinical scenarios. There is also variation in the degree of consensus regarding the understanding of risk and appropriate clinical management of mutations in some genes."

- "Genetic testing should be made available to all patients with a personal history of breast cancer. Recent data support that genetic testing should be offered to each patient with breast cancer (newly diagnosed or with a personal history). If genetic testing is performed, such testing should include BRCA1/BRCA2 and PALB2, with other genes as appropriate for the clinical scenario and family history. For patients with newly diagnosed breast cancer, identification of a mutation may impact local treatment recommendations (surgery and potentially radiation) and systemic therapy. Additionally, family members may subsequently be offered testing and tailored risk reduction strategies."
- "Patients who had genetic testing previously may benefit from updated testing. Every patient being seen by a breast surgeon, who had genetic testing in the past and no pathogenic variant was identified, should be re-evaluated and updated testing considered. In particular, a patient who had negative germline BRCA1 and 2 testing, who is from a family with no pathogenic variants, should be considered for additional testing. Genetic testing performed prior to 2014 most likely would not have had PALB2 or other potentially relevant genes included and may not have included testing for large genomic rearrangements in BRCA1 or BRCA2."
- "Genetic testing should be made available to patients without a history of breast cancer who meet NCCN guidelines. Unaffected patients should be informed that testing an affected relative first, whenever possible, is more informative than undergoing testing themselves. When it is not feasible to test the affected relative first, then the unaffected family member should be considered for testing if they are interested, with careful pre-test counseling to explain the limited value of "uninformative negative" results. It is also reasonable to order a multi-gene panel if the family history is incomplete (i.e., a case of adoption, patient is uncertain of exact type of cancer affecting family members, among others) or other cancers are found in the family history, as described above."

European School of Oncology and European Society of Medical Oncology

The European School of Oncology (ESO, 2022) and the European Society of Medical Oncology (ESMO, 2022) held the fifth International Consensus Conference for Breast Cancer in Young Women leading to the publication of consensus recommendations. The following was stated regarding PALB2 genetic testing:[10]

- "Although BRCA1/2 are the most frequently mutated genes, other additional moderate- to high-penetrance genes may be considered, if deemed appropriate by the geneticist/genetic counselor or if they will impact therapeutic interventions."
- "When a hereditary cancer syndrome is suspected and a mutation in BRCA1/2 has not been identified, multi-gene panel testing may be considered. Practice should be guided by high quality national/international guidelines."
- "As commercially available multi-gene panels include different panels of genes, the choice of the specific panel and quality-controlled laboratory is crucial."
- "For BRCA1/2 mutation carriers and others at high risk based on family history or predisposing mutations in other genes (e.g. p53, PALB2, CHEK2, ATM) and for those at increased risk because of a personal history of therapeutic radiation, annual surveillance with MRI and mammography with or without ultrasound is recommended."

European Society of Medical Oncology

The European Society for Medical Oncology (ESMO, 2022) stated the following regarding risk reduction and screening strategies for individuals with a PALB2 mutation:[11]

- "BRRM [bilateral risk-reducing mastectomy] is the most effective method for reducing breast cancer risk among BRCA1/2 PV [pathogenic variant] carriers. High-risk carriers who may wish to consider RRM are those with PVs in other high-risk genes: TP53, PTEN, STK11, CDH1 and PALB2. For these rare and less known PVs, RRM should be discussed after careful consideration of individualised risk assessment"
- "Women with HBOC [hereditary breast and ovarian cancer] should be offered intensified screening if they do not opt for RRM."
- "In the presence of a BRCA1, BRCA2, or PALB2 PVs, intensified screening should start at age 30, or 5 years younger than the youngest family member with breast cancer."
- "Annual screening intervals are recommended, except for BRCA1 where 6-monthly screening should be considered."
- "RRBSO [risk-reducing bilateral salpingo-oophorectomy] may be considered for postmenopausal women with a PALB2 PV."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) included breast, ovarian, and pancreatic cancer risk and management recommendations for individuals with a pathogenic/likely pathogenic germline PALB2 mutation in a table located in their Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic guideline. However, it is noted that, “The inclusion of a gene in this table below does not imply

endorsement either for or against multi-gene testing for moderate-penetrance genes." Recommendations included:[7]

- Breast cancer:
  - "Screening: Annual mammogram and breast MRI with contrast at 30 y."
  - "Risk reduction: Discuss option of RRM [risk-reducing mastectomy]."
- Ovarian cancer:
  - "Risk reduction: Consider RRSO [risk-reducing salpingo oophorectomy] at age >45 y"
- Pancreatic cancer:
  - "Emerging data have examined the efficacy of pancreatic cancer screening in select individuals at increased risk for exocrine pancreatic cancer."
  - "These studies have typically started screening with contrast-enhanced MRI/magnetic resonance cholangiopancreatography (MRCP) and/or endoscopic ultrasound (EUS) in such high-risk individuals."
  - For individuals with a pathogenic/likely pathogenic germline variant in a pancreatic cancer susceptibility gene, such as PALB2, NCCN recommended the following:
    - "Consider pancreatic cancer screening beginning at age 50 years (or 10 years younger than the earliest exocrine pancreatic cancer diagnosis in the family, whichever is earlier) for individuals with exocrine pancreatic cancer in ≥1 first- or second-degree relatives from the same side of (or presumed to be from the same side of) the family as the identified P/LP [pathogenic/likely pathogenic] germline variant.

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PancraGEN

MOL.TS.271.A
v2.0.2024

Introduction

PancraGEN testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
PancraGEN	81479

Criteria

Introduction

Requests for PancraGEN testing are reviewed using the following criteria

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What are pancreatic cysts?

Definition

Pancreatic cysts are reported as incidental findings in 3 to 13% of individuals undergoing abdominal imaging procedures. Four of the most common types of pancreatic cysts are serous cystadenomas (SCA), solid-pseudopapillary neoplasms (SPN), mucinous cystic neoplasms (MCN), and intraductal papillary mucinous

neoplasms (IPMN).[1]

- Overall, considering all types of pancreatic cysts, the risk of cancer is very low (<1% per year), but with different risks based on the histologic type of cyst and its clinical characteristics. Given that most cysts do not progress to cancer, and that pancreatic surgery has a high rate of morbidity and mortality, conservative management is recommended for the vast majority of patients.[1,2]
- Clinicians typically rely on imaging, cytology, and fluid chemistry to assess the malignancy risk of pancreatic cysts.
- In cases where an individual's diagnosis based on conventional pathologic and imaging approaches is inconclusive, PancraGEN has been proposed as an adjunctive risk stratification tool to provide additional clarifying information to inconclusive results of standard diagnostic tools, including imaging, carcinoembryonic antigen (CEA), cytology, and clinical risk factors.[3-5]

Test information

Introduction

According to the test manufacturer, PancraGEN provides molecular results for DNA quantity and quality, specific oncogene point mutations (in codons 12 and 13 of KRAS and codon 201 of GNAS), and information on loss of heterozygosity at 10 loci [3p (VHL, OGG1), 10q (PTEN, MXI1), 17p (TP53), 18q (SMAD4, DCC), 9p (CDN2A/B), 17q (RNF43, NME1), 21q (PSEN2, TFF1), 1p (RUNX3, CMM1, L-MYC), 5q (MCC, APC), and 22q (NF2)] in order to stratify patients according to their risk for progression to malignancy.[6-10]

- The test requires specimens of pancreatobiliary fluid, pancreatic masses, or pancreatic tissue usually obtained by endoscopic ultrasound (EUS) guided fine needle aspiration (FNA).[6,11]
- The PancraGEN report categorizes patients into one of four groups: low risk category that supports surveillance (a. benign; b. statistically indolent) or high risk category that supports treatment intervention decisions (c. statistically higher risk; d. aggressive).[6-10]
- This test is intended to determine a patient's risk of cancer progression and assess the best course of treatment. Based on test results, low-risk patients with benign cysts may benefit from early disease surveillance and avoidance of invasive surgical resection, while higher risk patients with aggressive cysts can receive proper surgical treatment for malignant lesions.[6-10]

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to PancraGEN testing. Of note, the current National Comprehensive Cancer Network Guidelines for Pancreatic Adenocarcinoma (NCCN, 2023) did not make any recommendations regarding risk stratification via molecular profiling of pancreatic cysts.[12]

American College of Gastroenterology

The American College of Gastroenterology (ACG, 2018) published comprehensive guidelines for the diagnosis and management of pancreatic cysts. Although these guidelines did not include molecular analysis as part of the routine analysis of all pancreatic cysts, the authors stated: “A number of DNA, RNA, protein, and metabolomic markers have been evaluated in cyst fluid. The majority of these are still early in development and not yet ready for translation into clinical practice. However, analysis of DNA mutations in cyst fluid has shown promise in identifying IPMNs and MCNs.”[2]

National Institute of Health and Clinical Excellence

The National Institute for Health and Clinical Excellence (NICE, 2018) stated the following regarding evaluation of pancreatic cysts:[13]

- "Offer a pancreatic protocol CT scan or magnetic resonance cholangiopancreatography (MRI/MRCP) to people with pancreatic cysts. If more information is needed after one of these tests, offer the other one.
- Refer people with any of these high-risk features for resection:
    - obstructive jaundice with cystic lesions in the head of the pancreas
    - enhancing solid component in the cyst
    - a main pancreatic duct that is 10 mm diameter or larger
- Offer EUS after CT and MRI/MRCP if more information on the likelihood of malignancy is needed, or if it is not clear whether surgery is needed.
- Consider fine-needle aspiration during EUS if more information on the likelihood of malignancy is needed.
- When using fine-needle aspiration, perform carcinoembryonic antigen (CEA) assay in addition to cytology if there is sufficient sample.
- For people with cysts that are thought to be malignant, follow the recommendations on staging."

Selected Relevant Publications

A small base of evidence comprised of a few clinical studies evaluated the correlation between genetic testing using the PancraGen test and histologic evaluation of pancreatic tissue samples (including cytology specimens).[14-26]

Overall, the quality of the evidence base is low, consisting primarily of retrospective studies comparing the diagnostic performance of PancraGen with conventional testing methods. It is not clear if PancraGEN would perform well in a broad, general population of patients with pancreatic cysts. Small sample sizes may lead to imprecise estimates of test accuracy. The reported diagnostic performance values vary widely and were often not accompanied by confidence intervals. Included confidence intervals were wide, suggesting a lack of precision.

Additional well-designed clinical studies are needed to assess the clinical utility of PancraGEN testing in patients with pancreatic cysts.

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PCA3 Testing for Prostate Cancer

MOL.TS.215.A
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Introduction

PCA3 testing for prostate cancer is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
PCA3 Score	81313

Criteria

Introduction

Requests for PCA3 testing are reviewed using these criteria.

Prostate cancer antigen testing (PCA3) is medically necessary in males with ALL of the following:

- Age >50 years, and
- One or more previous negative prostate biopsies, and
- Continued clinical suspicion of prostate cancer based on digital rectal exam (DRE) or elevation of prostate specific antigen (PSA) of >3 ng/mL, and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, and
- Atypical small acinar proliferation (ASAP) was NOT identified on the most recent biopsy.

What is prostate cancer antigen 3 (PCA3)?

Definition

Prostate cancer antigen 3 (PCA3) is a non-protein-coding messenger RNA (mRNA) that is highly overexpressed in >95% prostate cancer tissue compared with normal prostate tissue or benign prostatic hyperplasia.[1]

Test information

Introduction

The strong association between PCA3 mRNA levels and prostate cancer led to the development of a urinary assay to measure this analyte to aid in cancer detection.[1]

PCA3 Testing for Prostate Cancer Detection

- Following a digital rectal examination, first-void urine is collected, rapidly processed, and the mRNAs for the PCA3 gene and the PSA gene are quantified. A PCA3 score is calculated from the ratio of PCA3 RNA to PSA RNA.
- A high (>25) PCA3 Score indicates an increased likelihood of a positive biopsy. A low (<25) PCA3 Score is associated with a decreased likelihood of a positive biopsy.[2]
- A multi-center study which included a total of 466 men found that at a score cutoff of 25 for men with at least one previous negative biopsy, PCA3 demonstrated 77.5% sensitivity, 57.1% specificity, and negative and positive predictive values of 90% and 33.6%, respectively. Men with a PCA3 score of <25 were 4.56 times more likely to have a negative repeat biopsy than men with a score of >25.[3]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to PCA3 testing.

American Urological Association

The American Urological Association (AUA, 2023) guideline on the early detection of prostate cancer stated:[4]

- "When screening for prostate cancer, clinicians should use PSA as the first screening test. (Strong Recommendation; Evidence Level: Grade A)"
- "For people with a newly elevated PSA, clinicians should repeat the PSA prior to a secondary biomarker, imaging, or biopsy. (Expert Opinion)"
- "Clinicians may use adjunctive urine or serum markers when further risk stratification would influence the decision regarding whether to proceed with biopsy. (Conditional Recommendation; Evidence Level: Grade C)"
- "After a negative negative biopsy, clinicians may use blood, urine, or tissue-based biomarkers selectively for further risk stratification if results are likely to influence the decision regarding repeat biopsy or otherwise substantively change the patient's management. (Conditional Recommendation; Evidence Level: Grade C) Blood, urine, or tissue-based biomarkers may provide additional information for risk

stratification in patients with a prior negative biopsy and with ongoing suspicion for GG2+ prostate cancer."

- "While there are a plethora of serum, urine, tissue, and imaging biomarkers to assess the likelihood of high-grade prostate cancer, there is little knowledge on comparative effectiveness, how they may complement or supplement each other, and how various stepwise algorithms perform."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2023) guidelines for prostate cancer early detection recognized the FDA-approved use of PCA3 testing and stated:[5]

- "Results were reported from an NCI Early Detection Research Network (EDRN) validation study of the PCA3 urinary assay in 859 individuals scheduled for a diagnostic prostate biopsy in 11 centers. The primary outcomes were reported at a PPV of 80% (95% CI, 72%–86%) in the initial biopsy setting and an NPV of 88% (95% CI, 81%–93%) in the repeat biopsy setting. Based on the data, use of PCA3 in the repeat biopsy setting would reduce the number of biopsies by almost half, and 3% of men with a low PCA3 score would have high-grade prostate cancer that would be missed. In contrast, the risk of high-grade disease in men without prior biopsy with a low PCA3 is 13%. Thus, the panel believes that this test is not appropriate to use in the initial biopsy setting."
- "The FDA has approved the PCA3 assay to help decide, along with other factors, whether a repeat biopsy in men aged 50 years or older with one or more previous negative prostate biopsies is necessary. This assay is recommended for men with previous negative biopsy in order to avoid repeat biopsy by the Molecular Diagnostic Services Program (MolDX) and is therefore covered by CMS (Centers for Medicare & Medicaid Services) in this setting. The panel also includes PCA3 as an option in the post-biopsy setting."

U.S. Food and Drug Administration

The U.S Food and Drug Administration (FDA, 2012) approved the Progensa PCA3 assay with the following intended use:[6]

- “The PROGENSA PCA3 Assay is indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 Assay results.”
- "The Clinical Study only included men who were recommended by urologists for repeat biopsy. Therefore, the performance of the PROGENSA PCA3 Assay has not been established in men for whom a repeat biopsy was not already recommended."
- "Black Box Warning: The PROGENSA PCA3 Assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with

ASAP on their most recent biopsy should be treated in accordance with current medical guidelines."

Selected Relevant Publications

Data from many peer-reviewed publications suggest that PCA3 gene testing, when used with other patient information, may help address some of the well-known challenges urologists face, such as identifying prostate cancers while reducing unnecessary repeat biopsies.[7-9]

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Polymerase Gamma (POLG) Related Disorders Genetic Testing

MOL.TS.276.A
v2.0.2024

Introduction

Polymerase gamma (POLG) related disorders genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
POLG Deletion/Duplication Analysis	81479
POLG Full Gene Sequencing	81406
POLG Known Familial Mutation Analysis	81403

Criteria

Introduction

Requests for genetic testing for polymerase gamma (POLG)-related disorders, including Alpers-Huttenlocher syndrome (AHS), childhood myocerebrohepatopathy spectrum (MCHS), myoclonic epilepsy myopathy sensory ataxia (MEMSA), ataxia neuropathy spectrum (ANS), autosomal dominant progressive external ophthalmoplegia (adPEO), or autosomal recessive progressive external ophthalmoplegia (arPEO), are reviewed using these criteria.

Known POLG Family Mutation Testing

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Diagnostic Testing for Symptomatic Individuals
  - No previous genetic testing of POLG that would detect the familial mutation, and
  - If adPEO is suspected:

- Clinical examination is consistent with a diagnosis of adPEO, and
- POLG mutation identified in 1st degree biological relative, OR

- If AHS, MCHS, MEMSA, ANS, or arPEO is suspected:
  - Clinical examination is consistent with a diagnosis of AHS, MCHS, MEMSA, ANS, or arPEO, and
  - Two POLG mutations identified in a sibling, or
  - One POLG mutation identified in both parents

POLG Full Gene Sequencing

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous POLG sequencing, and
  - No known POLG mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - If adPEO is suspected:
    - Clinical examination is consistent with a diagnosis of adPEO, and
    - Genetic testing is needed to confirm the diagnosis, OR
  - If AHS, MCHS, MEMSA, ANS, or arPEO is suspected:
    - Clinical examination is consistent with a diagnosis of AHS, MCHS, MEMSA, ANS, or arPEO, and
    - Genetic testing is needed to confirm the diagnosis, OR
  - If evaluating the risk for valproate-induced hepatic toxicity:
    - The member has epilepsy, and
    - There is suspicion for a POLG-related disorder based on the presence of at least one of the following:
      - unexplained encephalopathy, or

- refractory epilepsy, or
- status epilepticus at presentation, or
- developmental delays, or
- psychomotor regression, or
- axonal sensorimotor neuropathy, or
- myopathy and/or hypotonia, or
- progressive spastic paraparesis, or
- renal tubular acidosis, or
- sensorineural hearing loss, or
- cyclic vomiting, or
- pancreatitis, or
- cerebellar ataxia, or
- ophthalmoplegia and/or ptosis, or
- complicated migraine with occipital aura, and

▪ The member is currently on Depakene (valproate) or Depakote ER (divalproex sodium) therapy, or the use of one of these medications is being proposed.

POLG Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for POLG Full Gene Sequencing is met, AND
- If adPEO is suspected:
  - No mutations found on POLG Full Gene Sequencing, OR
- If AHS, MCHS, MEMSA, ANS, or arPEO is suspected:
  - No mutations or only one mutation found on POLG Full Gene Sequencing, OR
- If evaluating the risk for valproate-induced hepatic toxicity:
  - No mutations or only one mutation found on POLG Full Gene Sequencing

What are POLG-related disorders?

Definition

"POLG-related disorders" is a term used to describe medical conditions caused by mutations in the POLG gene. This is a wide spectrum of conditions that may involve multiple organ systems and have variable severity and age at onset.[1,2]

Prevalence

Although Alpers-Huttenlocher syndrome (AHS) is clinically reported to occur in 1/51,000 individuals, disease frequency calculated based on prevalence of the most common POLG mutations may be as high as 1/10,000.[1]

Symptoms

There are 6 main phenotypes attributed to POLG mutations. Most affected individuals have some features ascribed to each phenotype, but rarely have all.

- Alpers- Huttenlocher syndrome (AHS):[3,4]
  - Most common symptoms
    - refractory seizures
    - psychomotor regression
    - liver disease
  - Other possible symptoms
    - migraine with visual auras
    - cortical blindness
    - hypotonia
    - ataxia
    - extrapyramidal movements
    - peripheral neuropathy
    - progressive spastic paraparesis
    - renal tubular acidosis
    - hearing loss
    - cyclic vomiting
    - pancreatitis
  - Development is often normal until disease onset, which is typically before 4 years of age. However, congenital static encephalopathy and juvenile-onset have also been described.[2] When seizure etiology is unknown, valproic acid

must be used with extreme caution, as it can precipitate liver dysfunction and/or failure in AHS.[5,6]

- Childhood myocerebrohepatopathy spectrum (MCHS):[7]
  - Most common / presenting symptoms
    - failure to thrive
    - lactic acidosis
    - developmental delay
    - encephalopathy
    - dementia
    - myopathy
    - hypotonia
  - Other possible symptoms
    - liver failure
    - renal tubular acidosis
    - pancreatitis
    - cyclic vomiting
    - hearing loss
  - MCHS is a rapidly progressive disease with a fatal outcome that usually presents between the first few months of life and 3 years. MCHS has a similar presentation to AHS, however severe myopathy, specific liver pathology, and nonspecific brain MRI brain findings (diffuse atrophy) help differentiate MCHS from AHS. In addition, seizures are less prominent and more easily controlled in MCHS compared to AHS.
- Myoclonic epilepsy myopathy sensory ataxia (MEMSA):[8]
  - Common symptoms
    - epilepsy
    - myopathy
    - ataxia without ophthalmoplegia
  - MEMSA has also been known as spinocerebellar ataxia with epilepsy (SCAE). Disease onset typically occurs in adolescence and presents with cerebellar and sensory ataxia. Epilepsy usually follows, with refractory seizures leading to a progressive encephalopathy.
- Ataxia neuropathy spectrum (ANS):[9]

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- Common symptoms
  - migraine headaches
  - ataxia
  - neuropathy (sensory, motor, or mixed)
  - encephalopathy with seizures
  - psychiatric disturbance
- Other possible symptoms
  - myoclonus
  - blindness
  - hearing loss
  - liver failure (varying severity)
- Disease onset ranges between adolescence and adulthood. Migraine headaches may be the first presenting symptom and precede the other symptoms by many years. Clinical myopathy is very rare. The encephalopathy is often milder than AHS and more slowly progressive. ANS was previously referred to as mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO).

• Autosomal recessive progressive external ophthalmoplegia (arPEO):[10]
- Common symptoms
  - Progressive weakness of the extraocular eye muscles resulting in ptosis and ophthalmoparesis without associated systemic involvement.
  - Apparently isolated PEO can present with additional symptoms later in life.
- Onset is typically in adulthood.

• Autosomal dominant progressive external ophthalmoplegia (adPEO):[1,9]
- Common symptoms
  - progressive weakness of the extraocular eye muscles resulting in ptosis and ophthalmoparesis
  - generalized myopathy
  - sensorineural hearing loss
  - axonal neuropathy
  - ataxia
  - depression
  - Parkinsonism

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    - hypogonadism
    - cataracts
  - Previously, adPEO was called Chronic Progressive External Ophthalmoplegia plus (CPEO+).
- Onset of the POLG-related disorders can range from infancy to late adulthood. Younger individuals typically present with seizures and lactic acidosis.[11] Later in life, the most common presenting symptoms are myopathy, chronic progressive external ophthalmoplegia (CPEO), and sensory ataxia.[11] Liver failure may also occur, particularly with exposure to the antiepileptic drug, valproic acid.[1,5,6]

Cause

POLG-related disorders are caused by mutations in the POLG gene. POLG codes for a subunit of DNA polymerase protein that replicates and repairs mitochondrial DNA (mtDNA). Disease-causing mutations can affect polymerase activity, processing, DNA binding, or subunit association.[1]

Inheritance

POLG-related disorders can be inherited in an autosomal recessive or autosomal dominant pattern.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

AHS, MCHS, MEMSA, ANS, and arPEO are inherited in an autosomal recessive inheritance pattern, while adPEO is inherited in an autosomal dominant pattern. A case of arPEO caused by digenic inheritance of POLG and TWNK mutations has been reported.[1]

Diagnosis

As no clinical diagnostic criteria exist, genetic testing of POLG is required to confirm clinical suspicion of a disorder in this spectrum.

Management

Management is supportive and based on presenting symptoms and typically involves referral for speech therapy, physical therapy, and occupational therapy. Respiratory and nutritional support are provided as needed.

Any medications metabolized by hepatic enzymes should be carefully dosed to avoid liver toxicity. Certain antiepileptic drugs should be avoided due to the risk for precipitating or accelerating liver disease.[1]

Occurrence of dehydration, fever, anorexia and infection can create physical stress and hasten medical deterioration. These events should be avoided as much as possible.

Survival

The range of survival is broad and is largely dependent on the presenting phenotype, age at onset, and the occurrence of secondary complications.

Test information

Introduction

Testing for POLG-related disorders may include known familial mutation analysis, next generation sequencing, or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Sequence analysis for this group of disorders is typically limited to full sequencing of the POLG gene only, although POLG may appear on multigene panels for mitochondrial-related disorders.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Given that clinical diagnostic criteria do not exist, genetic testing of POLG is required in order to confirm the diagnosis of a POLG-related disorder.[1]

- For individuals with suspected adPEO, identification of one POLG mutation is required to confirm the diagnosis.
- For individuals presenting with clinical features consistent with one of the five other phenotypes, identification of two (biallelic) mutations is required to confirm the diagnosis.

While biochemical analyses of an affected tissue may be informative, they are not sensitive or specific enough to definitively diagnose a POLG-related disorder. Muscle biopsy can be completely normal in children and adults with a POLG-related disorder and in clinically unaffected tissue.[12]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to POLG-related disorders genetic testing.

European Federation of Neurological Sciences/European Neurological Society

The European Federation of Neurological Sciences/European Neurological Society (EFNS/ENS, 2014) consensus guidelines on the diagnosis and management of chronic ataxias in adulthood recommended POLG testing in the following evaluation of individuals with autosomal recessive cerebellar ataxia:[13]

- “Step 1: mutation analysis of the FRDA gene for Friedreich’s ataxia (although one can refrain from this in the case of severe cerebellar atrophy), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, creatine kinase (CK) and a-fetoprotein. Also consider doing nerve conduction studies/EMG (presence versus absence of peripheral neuropathy, axonal versus demyelinating) and referral to an ophthalmologist (retinitis pigmentosa, cataract, cherry red spot etc.) (Table S2) (good practice point).”

- “Step 2: mutation analysis of the SACS, POLG, Aprataxin (APTX) and SPG7 genes (taking into account specific phenotypes, as given in Table S2), and biochemical testing for white cell enzymes, phytanic acid and long chain fatty acids (good practice point).”
- “Step 3: referral to a specialized centre, e.g. for skin or muscle biopsy targeted at diagnoses such as Niemann - Pick type C, recessive ataxia with coenzyme Q deficiency [aarF domain containing kinase 3 (ADCK3)/autosomal recessive spinocerebellar ataxia 9 (SCAR9)] and mitochondrial disorders, or for extended genetic screening using gene panel diagnostics (good practice point).”

Mitochondrial Medicine Society

Although not specific to genetic testing for POLG, the Mitochondrial Medicine Society (MMS, 2015)[14] developed consensus recommendations for the diagnosis and management of mitochondrial disease. Testing strategies, including strategies for genetic testing, were discussed. Recommendations for testing included:

- “When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole-exome sequencing should be considered.”

US Food and Drug Administration

The Food and Drug Administration (FDA) stated that Depakene (valproic acid) capsules and oral solution (2020), Depakote ER (divalproex sodium) extended-release tablets (2023), Depakote (divalproex sodium) delayed-release tablets (2020), and Depakote Sprinkles Capsules (2023) are contraindicated for individuals known to have mitochondrial disorders caused by POLG mutations and children under two years of age who are clinically suspected of having a mitochondrial disorder:[15-18]

- "Valproate-induced acute liver failure and liver-related deaths have been reported in patients with hereditary neurometabolic syndromes caused by mutations in the gene for mitochondrial DNA polymerase γ (POLG) (e.g., Alpers-Huttenlocher Syndrome) at a higher rate than those without these syndromes. Most of the reported cases of liver failure in patients with these syndromes have been identified in children and adolescents."[15]
- "POLG-related disorders should be suspected in patients with a family history or suggestive symptoms of a POLG-related disorder, including but not limited to unexplained encephalopathy, refractory epilepsy (focal, myoclonic), status epilepticus at presentation, developmental delays, psychomotor regression, axonal sensorimotor neuropathy, myopathy, cerebellar ataxia, ophthalmoplegia, or complicated migraine with occipital aura. POLG mutation testing should be performed in accordance with current clinical practice for the diagnostic evaluation

of such disorders. The A467T and W748S mutations are present in approximately 2/3 of patients with autosomal recessive POLG-related disorders."[16]

- "There is an increased risk of valproate-induced acute liver failure and resultant deaths in patients with hereditary neurometabolic syndromes caused by DNA mutations of the mitochondrial DNA Polymerase γ (POLG) gene (e.g. Alpers Huttenlocher Syndrome). Depakote Sprinkle Capsules is contraindicated in patients known to have mitochondrial disorders caused by POLG mutations and children under two years of age who are clinically suspected of having a mitochondrial disorder. … In patients over two years of age who are clinically suspected of having a hereditary mitochondrial disease, Depakote Sprinkle Capsules should only be used after other anticonvulsants have failed. This older group of patients should be closely monitored during treatment with Depakote Sprinkle Capsules for the development of acute liver injury with regular clinical assessments and serum liver testing. POLG mutation screening should be performed in accordance with current clinical practice."[17]

Selected Relevant Publications

An expert-authored review (updated 2018) suggested the following testing strategy for those with a known or suspected diagnosis of a POLG related disorder:[1]

- “POLG-related disorders comprise a continuum of broad and overlapping phenotypes that can be distinct clinical entities or consist of a spectrum of overlapping phenotypes.”
- “Clinical diagnostic criteria do not exist. The diagnosis of most POLG-related disorders is established in a proband by identification of biallelic pathogenic variants in POLG by molecular genetic testing. The diagnosis of adPEO is established in a proband by identification of a heterozygous pathogenic variant in POLG by molecular genetic testing.”
- “Sequence analysis of POLG is performed first and followed by gene-targeted deletion/duplication analysis if no pathogenic variant is found.”
- “Sequence analysis of TWNK (formerly C10orf2 or PEO1) may be considered in persons with a suspected autosomal recessive POLG-related disorder but in whom only one POLG pathogenic variant was identified by single-gene testing, to investigate the possibility of digenic inheritance.”
- “A multigene panel that includes POLG, TWNK (formerly C10orf2 or PEO1), and other genes of interest may be considered.”

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Introduction

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POLG

Prader-Willi Syndrome Genetic Testing

MOL.TS.217.A
v2.0.2024

Introduction

Prader-Willi syndrome genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Chromosomal Microarray [BAC], Constitutional	81228
Chromosomal Microarray [CGH], Constitutional	S3870
Chromosomal Microarray [SNP], Constitutional	81229
Chromosome 15 Uniparental Disomy	81402
Cytogenomic (Genome-wide) Analysis for Constitutional Chromosomal Abnormalities; Interrogation of Genomic Regions for Copy Number and Loss-of-heterozygosity Variants, Low-pass Sequencing Analysis	81349
FISH Probe for 15q11-q13 Deletion	88271
Imprinting Center Defect Analysis	81479
Imprinting Center Known Familial Mutation Analysis	81403
SNRPN/UBE3A Methylation Analysis	81331

Criteria

Introduction

Requests for Prader-Willi syndrome genetic testing are reviewed using these criteria.

Imprinting Center (IC) Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous IC defect analysis testing that would detect the familial mutation, AND
- Family History:
  - Familial IC defect mutation known in blood relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

SNRPN Methylation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous SNRPN methylation analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Neonatal hypotonia and feeding problems (i.e., poor suck), OR
  - Developmental delay/intellectual disability, with some combination of the following:
    - Neonatal hypotonia, or
    - Feeding problems (i.e., poor suck) or poor growth in infancy, or
    - Obesity and/or food-related behavior problems (i.e., hyperphagia; obsession with food), or
    - Characteristic facial features, or
    - Hypogonadism AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Deletion Analysis (FISH Analysis for 15q11-q13 Deletion or Chromosomal Microarray)

- Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Testing:
  - No previous 15q11-q13 deletion analysis, and
  - No previous chromosomal microarray, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Neonatal hypotonia and feeding problems (i.e., poor suck), OR
  - Developmental delay/intellectual disability, with some combination of the following:
    - Neonatal hypotonia, or
    - Feeding problems (i.e., poor suck) or poor growth in infancy, or
    - Obesity and/or food-related behavior problems (i.e., hyperphagia; obsession with food) or
    - Characteristic facial features, or
    - Hypogonadism, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Chromosome 15 Uniparental Disomy (UPD)

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - No previous chromosome 15 UPD studies, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Meets clinical criteria for SNRPN methylation analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Imprinting Center (IC) Defect Analysis

- Genetic Counseling:

  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - Previous chromosome 15 UPD studies negative, and
  - No previous IC analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Meets clinical criteria for SNRPN methylation analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

What is Prader-Willi syndrome?

Definition

Prader-Willi syndrome (PWS) is a multi-system genetic disorder that is due to a loss of specific genes on chromosome 15. Infants present with low muscle tone (hypotonia) and feeding difficulties which can result in failure to thrive. In the childhood years, children with Prader-Willi syndrome develop an increased appetite with decreased satiety which, without proper management, results in obesity and an increased risk of type 2 diabetes. Cognitive impairment and behavioral problems are usually present in addition to an increased risk for specific medical diagnoses.[1]

Prevalence

The prevalence is estimated to be 1/10,000 to 1/30,000.[1]

Symptoms

Prader-Willi syndrome is characterized by:[1,2]

- Decreased muscle tone (hypotonia) and feeding difficulties in early infancy
- Strabismus
- Insatiable appetite in childhood that often results in obesity
- Developmental delay
- Short stature
- Behavior problems
- Small hands and feet
- Underdeveloped genitalia and infertility

Cause

The features of Prader-Willi syndrome are caused when the Prader-Willi critical region (PWCR) on chromosome 15 is only inherited from the mother and there is no copy from the father.

Prader-Willi syndrome can be caused by a chromosome deletion, uniparental disomy (two copies of the maternal chromosome), or imprinting center (IC) defect. There are several genetic tests available that can help diagnose Prader-Willi syndrome.[1-4]

Diagnosis

If an individual has all of the clinical findings denoted below at the indicated age, diagnostic testing is recommended.[1,5] Prader-Willi syndrome is established in individuals who have abnormal DNA methylation analysis consistent with absence of the paternal contribution of the PWCR.[1]

Neonatal period

- Hypotonia with poor suck

One month to two years

- Hypotonia with poor appetite and poor suck
- Developmental delay

Two to six years

- Hypotonia with history of poor suck
- Developmental delay

Six year to 12 years

- History of hypotonia with poor suck
- Developmental delay
- Excessive eating and, if uncontrolled, central obesity

13 years to adulthood

- Cognitive impairment which is most often mild intellectual disability
- Excessive eating and, if uncontrolled, central obesity
- Hypothalamic hypogonadism and/or typical behavior problems

Determination of recurrence risk following a diagnosis of PWS may require additional genetic testing of the individual and testing of one or both parents depending on the identified molecular cause.[4]

Management

Individuals with Prader-Willi syndrome have age-specific medical needs. Some of the more common treatments and management include: [1]

Infancy

- Ensuring adequate nutrition through feeding support
- Physical therapy for improved muscle strength
- Screening for strabismus
- Managing cryptorchidism through hormonal and surgical treatments
- Growth hormone treatment may be initiated in infancy

Childhood through adulthood

- Monitoring of daily food intake
- Determining if calcium and vitamin D supplementation is indicated
- Encouraging physical activity
- Growth hormone replacement therapy
- Evaluating for sleep disturbance
- Educational planning
- Addressing behavioral concerns with applied behavioral analysis therapy, behavior management strategies, and/or medication
- Assessing for hypothyroidism
- Assessing for scoliosis

Teenage years

- Sex hormone replacement at puberty as indicated

Adulthood

- Housing in a group home familiar with the needs of individuals with PWS to regulate behavior and weight management
- Growth hormone may help with maintaining muscle bulk
- Evaluate for possible osteoporosis every two years

Survival

Obesity and the associated complications contribute to the higher mortality rate in individuals with Prader-Willi syndrome. The current death rate is 1.25% per year and is lower than previous reports. The decrease is attributed to improved management. [1]

Test information

Introduction

Testing for Prader-Willi syndrome may include known familial mutation analysis, SNRPN methylation analysis, chromosomal microarray, FISH analysis for 15q11-q13 deletion, chromosome 15 uniparental disomy (UPD), or imprinting center defect analysis.

Known Familial Mutation Analysis: Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing. Analysis for known familial mutations typically includes only the specific mutation identified in the family, but if available, a targeted mutation panel that includes the familial mutation(s) may be performed.

SNRPN/UBE3A Methylation Analysis: This test is typically the first test in the evaluation of both Angelman syndrome (AS) and Prader-Willi syndrome (PWS). It will detect about 80% of individuals with AS and greater than 99% of individuals with PWS. However, DNA methylation analysis does not identify the underlying cause, which is important for determining the risk to future siblings. This risk ranges from less than 1% to up to 50%, depending on the genetic mechanism. Follow-up testing for these causes may be appropriate.

Chromosomal Microarray or FISH Analysis for 15q11-q13 Deletion: If DNA methylation analysis for AS or PWS is abnormal, deletion analysis is typically the next step. Approximately 70% of cases of both AS and PWS have a deletion in one copy of chromosome 15 involving the 15q11.2-q13 region. FISH (fluorescence in situ hybridization) analysis and chromosomal microarray (CMA, array CGH) can detect such deletions. If CMA has already been done, FISH is not likely to be necessary.

Chromosome 15 Uniparental Disomy (UPD): If DNA methylation analysis is abnormal but deletion analysis is normal, UPD analysis may be an appropriate next step for evaluation of both AS and PWS. About 28% of PWS cases are due to maternal UPD (both chromosome 15s are inherited from the mother). About 7% of cases of AS are due to paternal UPD (both chromosome 15s are inherited from the father). Both parents must be tested to diagnose UPD.

Imprinting Center Defect Analysis: This test may be considered in the evaluation of AS and PWS when methylation is abnormal, but FISH (or array CGH) and UPD studies are normal. Individuals with such results are presumed to have an imprinting defect. An abnormality in the imprinting process has been described in a minority of cases. However, imprinting center deletions may be familial, and if familial, the recurrence risk can be up to 50%.

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Prader-Willi syndrome testing.

Prader-Willi Syndrome Association

The Prader-Willi Syndrome Association (PWSA, 2023) stated the following in regards to PWS genetic testing and diagnosis.[3]

- "The physical examination and history are very important parts of making the diagnosis and should be done before genetic testing. All hypotonic children in the Neonatal Intensive Care Unit (NICU) who do not have a diagnosis should be tested for PWS."
- "All persons suspected of having PWS should be tested with a DNA methylation analysis. This test detects nearly all (>99%) cases of PWS."

Selected Relevant Publications

An expert-authored review (2023) stated the following regarding testing for Prader-Willi syndrome:[1]

- Methylation-specific analysis … can establish the diagnosis of PWS by identification of maternal-only imprinting at 15q11.2-q13 but cannot identify the cause of the abnormal DNA methylation."
- Additional testing is necessary to establish the mechanism of disease and recurrence risk.
- This review recommended the following test strategy:
    - Methylation analysis and deletion analysis (Oligo-SNP Array) as first-tier testing.
        - If methylation is normal and deletion analysis is abnormal but does not include the SNORD116 gene cluster, workup for a chromosomal abnormality may be considered.
    - Absence of heterozygosity (AOH) analysis of chromosome 15: If only the maternal methylated imprint is present but deletion testing is normal, AOH analysis is recommended.
    - DNA polymorphism analysis: If only the maternal methylated imprint is present but deletion and AOH analysis are normal, DNA polymorphism analysis is recommended.

References

Introduction

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PTEN Hamartoma Tumor Syndromes Genetic Testing

MOL.TS.223.A
v2.0.2024

Introduction

PTEN hamartoma tumor syndromes (PHTS) genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Genomic Unity PTEN Analysis	0235U
PTEN Deletion/Duplication Analysis	81323
PTEN Known Familial Mutation Analysis	81322
PTEN Sequencing	81321

Criteria

Introduction

Requests for PTEN hamartoma tumor syndromes (PHTS) testing are reviewed using the following criteria.

PTEN Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic and Predisposition Testing:
  - Known deleterious family mutation in PTEN identified in 1st, 2nd, or 3rd degree biologic relative(s), AND

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- Rendering laboratory is a qualified provider of service per the Health Plan policy.

PTEN Sequencing with Promoter Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous sequencing of PTEN, AND
- Diagnostic Testing for Symptomatic Individuals
  - Personal history of ANY of the following:
    - Bannayan Riley-Ruvalcaba (BRR) syndrome; or
    - Adult Lhermitte-Duclos disease (LDD); or
    - Autism spectrum disorder and macrocephaly; or
    - At least two biopsy-proven trichilemmomas; or
    - At least two major criteria** (one must be macrocephaly); or
    - Three major criteria** without macrocephaly; or
    - One major** and at least three minor criteria***; or
    - Four or more minor criteria***, OR
- Predisposition testing for Presymptomatic/Asymptomatic Individuals:
  - At-risk person with a family history of:
    - A relative (includes first-degree relative or more distant relatives if the first-degree relative is unavailable or unwilling to be tested) with a clinical diagnosis of Cowden syndrome or BRR (no previous genetic testing); and
    - One major** OR two minor criteria*** in the at-risk person, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

PTEN Deletion/Duplication Analysis:

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Sequence analysis of PTEN has been performed and resulted negative, and

- No previous deletion/duplication testing, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Criteria for testing purposes are:

**Major:	***Minor:
• Breast cancer • Endometrial cancer • Follicular thyroid cancer • Multiple GI hamartomas or ganglioneuromas • Macrocephaly (at least 97th percentile: 58cm in adult women and 60cm in adult men) • Macular pigmentation of glans penis • Mucocutaneous lesions: one biopsy-proven trichilemmoma, multiple palmoplantar keratoses, multifocal or extensive oral mucosal papillomatosis, multiple cutaneous facial papules (often verrucous)	• Autism spectrum disorder • Colon cancer • ≥ 3 esophageal glycogenic acanthoses • Lipomas • Intellectual disability (IQ≤75) • Papillary or follicular variant of papillary thyroid cancer • Thyroid structural lesions (e.g., adenoma, nodule(s), goiter) • Renal cell carcinoma • Single GI hamartoma or ganglioneuroma • Testicular lipomatosis • Vascular anomalies (including multiple intracranial developmental venous anomalies)

Other Considerations

PHTS testing may be performed as part of a multigene, multisyndrome panel. For information on multigene, multisyndrome panel testing, please refer to the guideline *Hereditary Cancer Syndrome Multigene Panels*, as this testing is not addressed here.

What is PTEN hamartoma tumor syndrome?

Definition

PTEN hamartoma tumor syndrome (PHTS) is used to describe the group of conditions caused by PTEN mutations that include hamartomatous growths: Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome and Proteus-like syndrome, and autism spectrum disorder with macrocephaly.[1]

Prevalence

The prevalence is unknown. The prevalence of CS was previously estimated to be 1 in 200,000 individuals, although this is likely low due to underdiagnosis.[1]

Symptoms

Historically, these conditions have been considered clinically distinct but share an underlying genetic etiology, and show some overlap in families.[1]

- **Cowden syndrome** (CS) is characterized by an increased risk for benign and malignant tumors of the breast, endometrium, and thyroid (non-medullary).[1,2]
    - Other common features include macrocephaly and growths on the skin or mucous membranes (mucocutaneous lesions). The lifetime risk for breast cancer is 25-50% with an average age at diagnosis of 38-46 years.[1] However, a 2012 publication by Tan et al. reports that this lifetime risk may be as high as 85%, particularly in individuals with PTEN promoter mutations.[3]
    - The lifetime risk for thyroid cancer can range from 10% to as high as 35%.[1,3] If it occurs, thyroid cancer is usually follicular. It is rarely papillary and is never medullary. Benign thyroid growths are also found in up to 75% of individuals with CS.[1] "However, the high frequency of thyroid disease in the general population means that when taken on their own, thyroid neoplasms have a low predictive value for identifying mutations carriers."[4]
    - Endometrial cancer has an estimated 5-10% lifetime risk, although this is not well-defined.[1] Tan et al. reports a lifetime risk of up to 28%.[3]
    - The gastrointestinal polyp risk (often colonic) in patients with CS may be 80% or higher and the lifetime risk for colorectal cancer is estimated to be 9%.[3]
    - Early onset colorectal cancer has been reported in 13% of patients with PTEN associated CS indicating earlier and more frequent colonoscopy is warranted in this population.[3,5,6]
    - Additionally, an increased lifetime risk for kidney cancer (approximately 34%) and melanoma (about 5-6%) has been reported.[1-3]
- **Lhermitte-Duclos disease** (LDD) is a rare, benign tumor of the cerebellum called dysplastic gangliocytoma that may present in childhood or adulthood.[1,2] Most adult-onset LDD is caused by a PTEN mutation even when no other signs of CS are present.[1]
- **Bannayan-Riley-Ruvalcaba syndrome** (BRRS) is a genetic disorder characterized by macrocephaly, multiple benign intestinal polyps (hamartomatous type), lipomas, colored spots on the tip of the penis (pigmented macules of the glans penis), and hemangiomas. Some people with BRRS have intellectual disability and/or birth defects. There may be an increased risk for several types of cancer, including breast, thyroid and endometrial.[2]

- **Proteus and Proteus-like syndromes** are highly variable conditions characterized by overgrowth of several different tissues usually in a patchy asymmetric pattern (mosaic) that is often present from birth but gets worse over time.[1] Clinical signs and symptoms include connective tissue and epidermal nevi (hamartomatous growths), ovarian cystadenomas, parotid monomorphic adenomas, lipomas, capillary/venous/lymphatic malformations, and a characteristic facial dysmorphology.
- **Autism spectrum disorder with macrocephaly** (defined as >2.5 SDs above the age mean or ≥97th percentile) may be caused by a mutation in the PTEN gene.[1]
- **Juvenile polyposis of infancy** may be caused by mutations in PTEN. In this condition, juvenile polyposis is diagnosed before six years of age and the phenotype may be similar to BRRS. “GI manifestations (bleeding, diarrhea, & protein-losing enteropathy) are often severe."[1]

Cause

Pathogenic mutations in the PTEN gene cause PHTS.

- Up to 80% of people with a clinical diagnosis of CS have a PTEN mutation in the coding region.[1] Ten percent of individuals with CS have a PTEN mutation in the promotor region.[1]
- The majority of CS cases are simplex. Approximately 10-50% of individuals with CS have an affected parent.[1] De novo PTEN pathogenic variants occur in 10-44% of individuals with PHTS.
- Nearly all individuals with a PTEN mutation will develop symptoms (complete penetrance).[1,2]
- Up to 71% of individuals with a clinical diagnosis of BRRS have a PTEN mutation.[1] Up to 50% of individuals with Proteus-like syndrome and 20% of individuals with Proteus syndrome have a PTEN mutation.[1] An estimated 10-20% of all individuals with ASD/macrocephaly have a PTEN mutation.[1,7] The likelihood may be greater if other family members have signs and symptoms in the PHTS spectrum.

Inheritance

PHTS are autosomal dominant disorders.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Diagnosis

The diagnosis of PHTS can be established with the identification of a pathogenic mutation in the PTEN gene.

- Sequence analysis of the PTEN gene will detect a mutation in about 80% of people with a clinical diagnosis of CS and 60% of people with a clinical diagnosis of BRRS.[1]
    - **Sequencing of the promoter region** will detect an additional 10% of PTEN mutations that cause CS.[1] As such, it is important to determine whether or not the selected laboratory includes PTEN promoter analysis in their testing.
- The likelihood of identifying a deletion or duplication in people with clinically diagnosed CS is unknown but expected to be relatively low.[1] About 11% of people with BRRS have large PTEN gene deletions.[1]

Clinical diagnostic criteria have been developed. A clinical diagnosis of PHTS is based on the major and minor criteria in the table below.[2]

An operational diagnosis of CS is established if an individual meets any of the following criteria:

- Three or more major criteria* (one must include macrocephaly, Lhermitte-Duclos disease, or GI hamartomas); or
- Two major* and three minor** criteria

If an individual meets the clinical criteria noted above or has a PTEN pathogenic mutation, the family members would meet criteria for an operational diagnosis of CS if they meet one of the following criteria:

- Two major criteria* with or without minor criteria; or
- One major* and two minor criteria**; or
- Three minor** criteria

The major and minor criteria for a clinical diagnosis of PHTS are:[2]

Major:*	Minor:**

• Breast cancer
• Endometrial cancer
• Follicular thyroid cancer
• Three or more GI hamartomas (including ganglioneuromas but excluding hyperplastic polyps)
• Adult Lhermitte-Duclos disease
• Macrocephaly (at least 97th percentile: 58cm in adult women and 60cm in adult men)
• Macular pigmentation of glans penis
• Mucocutaneous lesions:
  • At least three trichilemmomas (at least one biopsy proved)
  • At least three acral keratoses
  • At least three mucocutaneous neuromas
  • At least three oral papillomas that are biopsy proven or diagnosed by a dermatologist
	• Autism spectrum disorder
	• Colon cancer
	• At least three esophageal glycogenic acanthoses
	• At least three lipomas
	• Intellectual disability (IQ of 75 or less)
	• Renal cell carcinoma
	• Testicular lipomatosis
	• Papillary or follicular variant of papillary thyroid cancer
	• Thyroid structural lesions (e.g., adenoma, nodule(s), goiter)
	• Vascular anomalies (including multiple intracranial developmental venous anomalies)

Management

People with CS need heightened cancer surveillance starting at age 18 years. This may begin earlier if warranted: “For individuals with a family history of a particular cancer type at an early age, screening should be considered five to ten years prior to the youngest diagnosis in the family”.[1] The exception is children should have a yearly thyroid ultrasound starting at age 7 years and skin check with physical examination.[1] Because of the overlap in clinical phenotypes, people with other PTEN-related conditions are advised to follow the same heightened cancer surveillance guidelines as for CS.[8,9]

Survival

Given the phenotypic spectrum of PHTS and underdiagnosis, especially of individuals with non-classic phenotypes, the prognosis for individuals with PHTS is unknown. The increased risk for malignant tumors is the largest factor impacting survival.

Test information

Introduction

Testing for PHTS may include known familial mutation analysis, next generation sequencing, and/or deletion/duplication analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to PHTS testing.

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2013) issued consensus practice guidelines on the genetics evaluation of autism. They proposed an evaluation scheme with three tiers. The first tier included routine studies such as chromosome analysis and fragile X genetic testing. PTEN gene testing is

recommended as a second tier test when the head circumference is greater than 2.5 SDs above the mean (if no diagnosis is made via first tier testing).[10]

National Comprehensive Cancer Network

Evidence-based guidelines (Category 2A) from the National Comprehensive Cancer Network (NCCN, 2023) support the use of PTEN genetic testing in those with clinical features or a family history. They recommended PTEN genetic testing in any of the following situations:[2]

- Family history of a known PTEN mutation [PTEN known familial mutation testing is appropriate]
- Individual with a personal history of Bannayan-Riley-Ruvalcaba syndrome (BRRS)
- Individual meeting clinical diagnostic criteria for CS/PHTS
- Individual not meeting clinical diagnostic criteria for CS/PHTS with a personal history of any of the following:
    - Adult-onset Lhermitte Duclos disease (cerebellar dysplastic gangliocytoma)
    - Autism spectrum disorder and macrocephaly (greater than or equal to 97th percentile)
    - Two or more biopsy proven trichilemmomas
    - Macrocephaly and at least one other major*** criteria
    - Three major*** criteria without macrocephaly
    - One major*** and three or more minor**** criteria
    - Four or more minor**** criteria
- At-risk relative of someone clinically diagnosed with Cowden syndrome or BRRS (who has not had genetic testing), when the at-risk relative has at least one major*** or two minor**** criteria. Ideally, the at-risk person is a first-degree relative (parent, sibling, child) of someone clinically diagnosed, but testing more distant relatives is acceptable if closer relatives are not available or willing to have testing.
- Affected individuals with pathogenic/likely pathogenic variant identified on tumor genomic testing that may have implications if also identified on germline testing. "This should prompt a careful evaluation of personal and family history of the individual to determine the yield of germline sequencing. Somatic PTEN P/LP [pathogenic/likely pathogenic] variants are common in many tumor types in absence of a germline P/LP variant." For information on germline testing after somatic testing, please refer to the guideline *Hereditary (Germline) Testing After Tumor (Somatic) Testing*, as this testing is not addressed here.

The major and minor criteria to determine appropriateness of genetic testing are:

***Major:	****Minor:
• Breast cancer • Endometrial cancer • Follicular thyroid cancer • Multiple GI hamartomas or ganglioneuromas • Macrocephaly (at least 97th percentile: 58 cm in adult women and 60 cm in adult men) • Macular pigmentation of glans penis • Mucocutaneous lesions: one biopsy-proven trichilemmoma, multiple palmoplantar keratoses, multifocal or extensive oral mucosal papillomatosis, multiple cutaneous facial papules (often verrucous)	• Autism spectrum disorder • Colon cancer • 3 or more esophageal glycogenic acanthoses • Lipomas • Intellectual disability (IQ less than or equal to 75) • Papillary or follicular variant of papillary thyroid cancer • Thyroid structural lesions (e.g., adenoma, nodule(s), goiter) • Renal cell carcinoma • Single GI hamartoma or ganglioneuroma • Testicular lipomatosis • Vascular anomalies (including multiple intracranial developmental venous anomalies)

Note These NCCN defined major and minor criteria for genetic testing do not fully align with the major and minor criteria required for a clinical diagnosis.

US Multi-Society Task Force on Colorectal Cancer

The US Multi-Society Task Force on Colorectal Cancer issued a consensus statement on the diagnosis and management of hamartomatous polyposis syndromes that stated:[11]

- “We recommend patients with any of the following undergo a genetic evaluation: 2 or more lifetime hamartomatous polyps, a family history of hamartomatous polyps, or a cancer associated with a hamartomatous polyposis syndrome in first or second-degree relatives. Genetic testing (if indicated) should be performed using a multigene panel test. (Strong recommendation, low quality of evidence)”

Selected Relevant Publication

An expert-authored review of the PTEN hamartoma syndromes stated:[1]

- "Sequence analysis of PTEN is performed first and followed by gene-targeted deletion/duplication analysis if no pathogenic variant is found. If a pathogenic

variant is not identified with deletion/duplication analysis, perform sequence analysis of the PTEN promoter region for variants that decrease PTEN gene expression."

- “The most serious consequences of PHTS relate to the increased risk of cancers including breast, thyroid, endometrial, renal, and to a lesser extent, colon. In this regard, the most important aspect of management of any individual with a PTEN pathogenic variant is increased cancer surveillance to detect any tumors at the earliest, most treatable stages.”

References

Introduction

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Somatic Mutation Testing

MOL.TS.230.C
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Introduction

Somatic mutation testing in solid tumors and hematological malignancies is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
ABL1 Mutation Analysis	81170
ABL1 Targeted Mutation Analysis	81401
APC Sequencing	81201
ASXL1 Full Gene Sequencing	81175
ASXL1 Mutation Analysis	81176
Aventa FusionPlus	0444U
BCR-ABL1 detection, major breakpoint	81206
BCR-ABL1 detection, minor breakpoint	81207
BCR-ABL1 detection, other breakpoint	81208
BCR-ABL1 major and minor breakpoint fusion transcripts	0016U
BRAF V600 Targeted Mutation Analysis	81210
BRCA1/2 Sequencing	81163
BRCA1 Sequencing	81165
BRCA2 Sequencing	81216
BTK gene analysis	81233
CALR Exon 9 Mutation Analysis	81219
CCND1/IGH (t(11;14)) Translocation Analysis, Major Breakpoint	81168
CEBPA Full Gene Sequencing	81218
clonoSeq	0364U

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Procedures addressed by this guideline	Procedure codes
CRCdx RAS Mutation Detection Kit	0471U
EGFR Targeted Mutation Analysis	81235
EZH2 Common Variant(s) (e.g. codon 646)	81237
EZH2 Full Gene Sequencing	81236
FISH Analysis for t(9;22) BCR-ABL1	88271
FLT3 Internal Tandem Duplication MRD-Invivoscribe	0046U
FLT3 Mutation Analysis (internal tandem duplication variants)	81245
FLT3 Mutation Analysis (tyrosine kinase domain variants)	81246
FoundationOne CDx	0037U
Guardant360 TissueNext	0334U
Hematolymphoid Neoplasm Molecular Profiling	81450
IDH1 Mutation Analysis	81120
IDH2 Mutation Analysis	81121
IGH@/BCL2 (t(14;18)) Translocation Analysis, Major Breakpoint Region (MBR) and Minor Cluster Region (mcr) Breakpoints	81278
JAK2 Exons 12 to 15 Sequencing	0027U
JAK2 Mutation	0017U
JAK2 Targeted Mutation Analysis (e.g exons 12 and 13)	81279
JAK2 V617F Mutation Analysis	81270
KIT D816 Targeted Mutation Analysis	81273
KIT Targeted Sequence Analysis	81272
KRAS Exon 2 Targeted Mutation Analysis	81275
KRAS Targeted Mutation Analysis, Additional Variants	81276
LeukoStrat CDx FLT Mutation Assay	0023U
MGMT Promoter Methylation Analysis	81287

Procedures addressed by this guideline	Procedure codes
MI Cancer Seek - NGS Analysis	0211U
MLH1 Sequencing	81292
Molecular Tumor Marker Test	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
Molecular Tumor Marker Test	88271
MPL Common Variants (e.g. W515A, W515K, W515L, W515R)	81338
MPL Mutation Analysis, Exon 10	81339
MRDx® BCR-ABL Test	0040U
MSH2 Sequencing	81295
MSH6 Sequencing	81298
MSK-IMPACT	0048U
MyAML NGS- Invivoscribe	0050U
myChoice CDx	0172U
MYD88 Mutation Analysis	81305
MyMRD NGS Panel	0171U
NPM1 MRD- Invivoscribe	0049U
NPM1 Mutation Analysis	81310
NRAS Exon 2 and Exon 3 Analysis	81311
NTRK1 Translocation Analysis	81191
NTRK2 Translocation Analysis	81192
NTRK3 Translocation Analysis	81193
NTRK Translocation Analysis	81194

Procedures addressed by this guideline	Procedure codes
Oncomine Dx Target Test (NSCLC)	0022U
oncoRevealTM DX Lung and Colon Cancer Assay	0448U
Oncotype MAP PanCancer Tissue Test	0244U
PALB2 Sequencing	81307
PDGFRA Targeted Sequence Analysis	81314
PGDx Elio Tissue Complete	0250U
PIK3CA Targeted Sequence Analysis	81309
PLCG2 Common Variants (e.g. R665W, S707F, L845F)	81320
PMS2 Sequencing	81317
Praxis Extended RAS Panel	0111U
PTEN Sequencing	81321
RUNX1 Mutation Analysis	81334
SF3B1 Common Variants (e.g. A672T, E622D, L833F, R625C, R625L)	81347
Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis	81445
Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, microsatellite instability	81457
Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, copy number variants and microsatellite instability	81458
Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements	81459

Procedures addressed by this guideline	Procedure codes
Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis	81455
Solid Tumor Expanded Panel	0379U
SRSF2 Common Variants (e.g. P95H, P95L)	81348
TERT Targeted Sequence Analysis	81345
therascreen FGFR RGQ RT-PCR Kit	0154U
therascreen PIK3CA RGQ PCR Kit	0155U
TP53 Sequencing	81351
TP53 Targeted Sequence Analysis	81352
U2AF1 Common Variants (e.g. S34F, S34Y, Q157R, Q157P)	81357
xT CDx (Tempus)	0473U
ZRSR2 Common Variants (e.g. E65fs, E122fs, R448fs)	81360

Criteria

Introduction

Requests for molecular somatic mutation testing in solid tumors and hematological malignancies are reviewed using these criteria.

Medical necessity criteria differ based on the type of testing being performed (i.e., tests for individual genes separately chosen based on the cancer type, versus pre-defined panels of genes) and how that testing will be billed (one or more individual gene-specific procedure codes, specific panel procedure codes, or unlisted procedure codes).

Note This guideline addresses molecular markers only. It is intended to address DNA and RNA markers that are present on tumor marker panels, including microsatellite instability (MSI) when requested as part of panel. It does not address

immunohistochemistry (IHC) or other markers that may be detected through other methods such as FISH, chromosomal microarray, routine chromosome analysis, etc.

Individual Tumor Markers

When separate procedure codes will be billed for individual tumor markers (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), each individually billed tumor marker test will be evaluated separately for medical necessity. The following criteria will be applied:

- The member has a tumor type that will benefit from information provided by the requested tumor marker test based on at least one of the following:
  - All criteria are met from an eviCore test-specific guideline, if one is available, or
  - An oncology therapy FDA label requires results from the tumor marker test to effectively or safely use the therapy for the member's cancer type, or
  - NCCN guidelines include the tumor marker test in the management algorithm for that particular cancer type and all other requirements are met (specific pathology findings, staging, etc.); however, the tumor marker must be explicitly included in the guidelines and not simply included in a footnote as an intervention that may be considered

Note If five or more individually billed tumor marker tests are under review together (a "panel") and the member meets criteria for 5 or more individual tumor markers on an NGS panel, the panel will be approved. However, the laboratory will be redirected to use a panel CPT code for billing purposes (e.g. 81445, 81450, or 81455).

Companion Diagnostic (CDx) Tumor Marker Panels

Somatic mutation companion diagnostic assay panels are considered medically necessary when the member meets ALL of the following criteria:

- Member has a diagnosis of cancer, AND
- Treatment with a medication for which there is an FDA-approved companion diagnostic assay is being considered, AND
- FDA approval for the CDx being requested must include the member's specific cancer type as an approved indication, AND
- FDA label for the drug and indication being considered states companion diagnostic testing is necessary for patient selection, AND
- Member has not had previous somatic and/or germline testing that would have identified the genetic change required to prescribe medication under consideration, AND
- Family History:

  - Member does not have a close (1st or 2nd degree) biological relative with a known germline mutation in a gene that is a target of the requested companion diagnostic test (e.g. known familial mutation in BRCA1/2 and requested test is myChoice CDx), or
  - Member has a close (1st or 2nd degree) biological relative with a known germline mutation in a gene that is a target of the requested companion diagnostic test (e.g. known familial mutation in BRCA1/2 and requested test is myChoice CDx), and the member's germline test was negative, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Multigene Tumor Marker Panel Testing

When a multigene panel is being requested and will be billed with a single panel CPT code (e.g. 81445, 81450, or 81455), the panel will be considered medically necessary when the following criteria are met:

- The member is a candidate for a targeted therapy associated with a specific tumor biomarker(s) or disease site and has a diagnosis of one of the following cancers:
  - Advanced, metastatic solid tumor
  - Recurrent cutaneous melanoma
  - Non-small cell lung cancer
  - Recurrent pancreatic cancer
  - Epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer
  - Recurrent or unresectable salivary gland tumors
  - Myeloproliferative disease
  - Multiple myeloma
  - Systemic mastocytosis, OR
- The member has a confirmed or suspected diagnosis of acute myeloid leukemia (AML), OR
- The member has a confirmed or suspected diagnosis of myelodysplastic syndrome (MDS), OR
- The member has a diagnosis of cancer with at least 5 tumor markers included in the panel that individually meet criteria for the member's tumor type based on the medical necessity criteria for individual tumor markers listed above, OR
- Tumor mutational burden (TMB) testing is recommended in the NCCN management algorithm for the member's particular cancer type and all other requirements are met (specific pathology findings, staging, etc.); however, TMB testing must be explicitly included in the guidelines and not simply included in a footnote as an intervention that may be considered

Note If the member meets criteria for less than 5 of the individual tumor markers in the panel, the panel will not be reimbursed. The laboratory will be redirected to billing for individual tests for which the member meets criteria.

clonoSEQ

clonoSEQ testing is medically necessary for initial assessment of dominant clonal sequences and once for response assessment after primary treatment for members diagnosed with one of the following:

- Acute lymphoblastic leukemia (ALL), or
- Chronic lymphocytic leukemia (CLL), or
- Multiple myeloma (MM), AND

Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other Considerations

- For hematological malignancies, panels over 50 genes are considered not medically necessary as they are excessive.
- For information on tumor markers assayed by liquid biopsy, please refer to the guideline *Liquid Biopsy Testing*, as this testing is not addressed here.
- For information on MSI performed outside of a somatic mutation panel, please refer to the guideline *Microsatellite Instability and Immunohistochemistry Testing in Cancer*, as this testing is not addressed here.
- For information on testing for germline (inherited) mutations in genes related to hereditary cancer syndromes (e.g. Hereditary Breast and Ovarian Cancer, Lynch syndrome, etc), please refer to the appropriate eviCore test-specific guideline, as this testing is not addressed here. Although some of the same genes may be tested for inherited or acquired (somatic) mutations, this guideline addresses only testing for acquired mutations.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

When otherwise reimbursable, the following limitations will apply:

- For hematological malignancies, panels over 50 genes, typically billed with CPT code 81455, are not reimbursable.
    - If the laboratory's testing platform consists of more than 50 genes, yet a panel of 5 to 50 genes is considered medically necessary based on the above criteria, the laboratory can choose to bill using a panel procedure code (e.g., 81450) that represents a smaller number of genes on the panel.
- TMB testing may be considered an eligible tumor marker only when testing is performed by NGS on a solid tumor with a panel size of >667 Kb (typically more than 50 genes and billed with 81455, or an applicable code).
- Multigene panels will only be considered for reimbursement when billed with an appropriate panel CPT code (e.g. 81445, 81450, 81455, a PLA code, etc.)*.
- Only one somatic mutation biomarker panel will be considered for reimbursement per occurrence of cancer.
    - If multiple CDx biomarker panels are ordered simultaneously based on FDA label requirements, only one panel will be considered for reimbursement. Additional unique biomarkers from the second panel may be considered for reimbursement if appropriate single marker or single gene procedure codes are billed.
    - If a biomarker panel was previously performed and an additional panel is being requested, only testing for the medically necessary, previously untested biomarkers will be reimbursable. Therefore, only the most appropriate procedure codes will be considered for reimbursement.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are somatic mutation tests?

Definition

Somatic mutation tests are broadly defined here as any test that measures changes in DNA, RNA, or chromosomes found in malignant tissue that is used to make cancer management decisions.

- Somatic mutation tests are increasingly useful for therapy selection. Many cancer therapies are targeted at particular gene functions (therapeutic targets) and some require information about tumor genetics to use the therapies effectively (companion diagnostics). In these cases, NCCN as well as the FDA have outlined somatic testing that is recommended for specific cancers and the associated treatment implications.[1-5]

Test information

Somatic Mutation Testing

The specific methodology used to identify somatic mutations is dependent upon the type of mutation being investigated.

- DNA mutations are generally detected through direct analysis of individual mutations, portions of a gene, a whole gene, panels of genes, or the entire exome.
- Chromosome abnormalities, such as translocations or deletions, may be detected through direct visualization of the chromosomes (karyotyping), in situ hybridization of probes (e.g., FISH) to detect deletions or duplications that are too small to see directly, or by DNA-based methods (hybridization arrays or sequencing) that identify deletions or translocation breakpoints.
- Gene expression profiling simultaneously measures the amount of RNA being made by many genes. Expression patterns may be used to predict the type of cancer present, the aggressiveness of the malignancy, and therapies that are likely to be effective.

The efficiency of next generation sequencing (NGS) has led to an increasing number of large, multi-gene somatic mutation panels. Given that malignancies can have multiple and unexpected genetic changes, these panels may provide physicians with information about therapeutic targets that would not otherwise be considered.

Tumor Mutation Burden (TMB) Testing

Tumor mutational burden (TMB) is a quantitative measure of the number of mutations in the genome of a solid tumor "sometimes defined as the total number of non-synonymous point mutations per coding area of a tumor genome".[5] High TMB, typically defined as ≥10 mut/Mb for formalin-fixed paraffin-embedded (FFPE) tumor tissue, is thought to be a useful marker in predicting tumor response to immune checkpoint inhibitor therapies and is often used as a type of biomarker.[5-8] "Panel sizes >667 Kb are necessary to maintain adequate PPA [positive percent agreement] and NPA [negative percent agreement] for calling TMB high versus TMB low across the range of cut-offs used in practice."[8]

While TMB testing can be completed by whole exome sequencing (WES), this method tends to be high cost and requires an extensive analysis and data management.[6] As a result, NGS testing through targeted panels has become a preferred method for

measuring TMB; however, this allows for variation among panels, including "sample input, tumor content, panel size, gene content, quality control (QC), NGS platform, and bioinformatics pipeline, which may influence TMB estimates and lead to inconsistent TMB calculation and reporting."[8] This has resulted in the need to align variability between TMB assays, which led to the formation of the Friends of Cancer Research (Friends) TMB Harmonization Consortium, which is made up of "diagnostic manufacturers, academics, pharmaceutical companies, the National Cancer Institute (NCI), Frederick National Laboratory for Cancer Research, and the FDA.[8]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to somatic mutation testing.

College of American Pathologists

The College of American Pathologists, in collaboration with the Association for Molecular Pathology, American Society of Clinical Oncology, and patient advocacy group Fight Colorectal Cancer (CAP/AMP/ASCO/FCC, 2022) examined the best methodology for testing for MSI and published the following recommendations:[9]

- "For patients with CRC being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR for the detection of DNA mismatch repair defects. Although MMR-IHC or MSI by PCR are preferred, pathologists may use a validated MSI by NGS assay for the detection of DNA mismatch repair defects. Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency." (Strong Recommendation)
- "For patients with gastroesophageal and small bowel cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR over MSI by NGS for the detection of DNA mismatch repair defects. Note: This recommendation does not include esophageal squamous cell carcinoma." (Strong Recommendation)
- "For patients with endometrial cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA mismatch repair defects." (Strong Recommendation)
- "For patients with cancer types other than CRC, GEA [gastroesophageal adenocarcinoma], small bowel, and endometrial being considered for immune checkpoint inhibitor therapy, pathologists should test for DNA mismatch repair, although the optimal approach for the detection of mismatch repair defects has not been established. Note: Assays must be adequately validated for the specific cancer type being tested with careful consideration of performance characteristics of MMR-IHC and MSI by NGS or PCR for the detection of DNA mismatch repair defects." (Conditional Recommendation)

- "For all cancer patients being considered for immune checkpoint inhibitor therapy based on defective mismatch repair, pathologists should not use TMB as a surrogate for the detection of DNA mismatch repair defects. If a tumor is identified as TMB-High, pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to mismatch repair deficiency." (Strong Recommendation)

European Society of Medical Oncology

The European Hematology Association and European Society for Medical Oncology (EHA/ESMO, 2021) clinical guidelines for multiple myeloma (MM) stated:[10]

- The detection of clonal plasma cells is obligatory at diagnosis.
- The confirmation of minimal residual disease (MRD) negativity is obligatory at response.
- "The use of MRD to drive treatment decisions is under investigation, e.g. whether maintenance/continuous therapy in MRD-negative patients can be stopped or whether treatment needs to be changed in MRD-positive patients, especially in high-risk MM. The results of several phase III trials in the field will clarify the role of MRD in making decisions about therapy in MM."

The Friends TMB Harmonization Consortium

The Friends TMB Harmonization Consortium reported the following:[8]

- "At a TMB cutoff of 10[mut/Mb], the panels assayed have a theoretical NPA [negative percent agreement] of at least 95%, with a theoretical NPA falling <95% for panel sizes under 667 Kb," supporting the claim that "a sufficiently sized panel is required to maintain reasonable PPA [positive percent agreement] of panel TMB measurements"
- An observed "substantial acceleration of the decrease in PPA of panels at critical intersections of small panel sizes and low TMB cut-offs", supporting the claim that "small panels are insufficient to maintain adequate PPA and NPA for calling TMB high versus TMB low across the range of cut-offs for positivity likely to be used in practice"

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN) provided the following guidance on somatic mutation testing.

Solid Tumors:

- NCCN Guidelines for Treatment of Cancer by Site provided detailed guidelines on the use of individual tumor markers for each cancer type addressed.[2,4,11-16]
- NCCN made the following recommendations specifically for using multi-gene panels in the evaluation of non-small cell lung cancer (NSCLC): “The NCCN

NSCLC Guidelines Panel strongly advises broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is defined as molecular testing that identifies all biomarkers identified in NSCL-19 [gene rearrangements in ALK, NTRK1/2/3, RET, and ROS1, BRAF V600E mutation, certain EGFR mutations, KRAS G12C mutation, and MET exon 14 skipping mutation], in either a single assay or a combination of a limited number of assays and optimally identifies emerging biomarkers. Tiered approaches based on low prevalence of co-occurring biomarkers are acceptable. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC." [2]

- NCCN made the following recommendations specifically for using multi-gene panels in the evaluation of metastatic colorectal cancer: "All patients with metastatic colorectal cancer should have tumor tissue genotyped for RAS (KRAS and NRAS) and BRAF mutations individually or as part of an NGS panel."[11]
- NCCN made the following recommendation for cutaneous melanoma: "For initial presentation of stage IV disease or clinical recurrence, obtain tissue to ascertain alterations in BRAF, and in the appropriate clinical setting, KIT from either biopsy of the metastasis (preferred) or archival material if the patient is being considered for targeted therapy. Broader genomic profiling (eg, larger NGS panels, BRAF non-V600 mutations) is recommended if feasible, especially if the test results might guide future treatment decisions of eligibility for participation in a clinical trial. If BRAF single-gene testing was the initial test performed, and is negative, clinicians should strongly consider larger NGS panels to identify other potential genetic targets (eg, KIT, BRAF non-V600)."[12]
- NCCN made the following recommendation for epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer, prior to selection of systemic therapy for refractory or recurrent disease "Validated molecular testing should be performed in a CLIA-approved facility using the most recent available tumor tissue. Tumor molecular analysis is recommended to include, at a minimum, tests to identify potential benefit from targeted therapeutics that have tumor-specific or tumor-agnostic benefit including, but not limited to, BRCA1/2, HR status, MSI, TMB, NTRK if prior testing did not include these markers."[14]
- NCCN made the following recommendation for ampullary adenocarcinoma: "Tumor/somatic molecular profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for potentially actionable somatic findings including, but not limited to: fusions (ALK, NRG1, NTRK, ROS1, FGFR2, and RET), mutations (BRAF, BRCA1/2, KRAS, and PALB2), amplifications (HER2), microsatellite instability (MSI) mismatch repair deficiency deficiency (dMMR), or tumor mutational burden (TMB) via an FDA-approved and/or validated next-generation sequencing (NGS)-based assay."[15]
- NCCN made the following recommendation for distantly metastatic salivary gland tumors: "Targeted systemic therapy is increasingly becoming an option for patients

with distantly metastatic salivary gland tumors. NGS and other biomarker tests should be used to evaluate AR, NTRK, HRAS, PIK3CA, TMB, and HER2 status."[16]

- NCCN made the following recommendation for locally advanced/metastatic pancreatic adenocarcinoma: "Tumor/somatic molecular profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for potentially actionable somatic findings including, but not limited to: fusions (ALK, NRG1, NTRK, ROS1, FGFR2, and RET), mutations (BRAF, BRCA1/2, KRAS, and PALB2), amplifications (HER2), microsatellite instability (MSI), mismatch repair deficiency (dMMR), or tumor mutational burden (TMB) via an FDA-approved and/or validated next-generation sequencing (NGS)-based assay."[13]

Hematological Malignancies:

- NCCN Guidelines for Treatment of Cancer by Site provided detailed guidelines on the use of individual markers for each cancer type addressed.[4]
- NCCN stated that for individuals with acute lymphoblastic leukemia (ALL), molecular characterization by "comprehensive testing by next-generation sequencing (NGS) for gene fusions and pathogenic mutations is recommended" for determining risk and planning treatment.[17]
- NCCN stated that for individuals with cytopenia when myelodysplasia is suspected, "genetic testing for somatic mutations (i.e., acquired mutations) in genes associated with myelodysplastic syndromes (MDS) is highly recommended." [18]
- NCCN stated that for individuals being evaluated for and with acute myeloid leukemia (AML): "The field of genomics in myeloid malignancies and related implication in AML are evolving rapidly. Mutations should be tested in all patients. Multiplex gene panels and targeted next-generation sequencing (NGS) analysis are recommended for the ongoing management of AML and various phases of treatment."[19]
- NCCN stated that for individuals with chronic lymphocytic leukemia (CLL): "Evidence from clinical trials suggests that undetectable MRD in the peripheral blood after the end of treatment is an important predictor of treatment efficacy. … MRD evaluation should be performed using an assay with a sensitivity of 10-4 according to the standardized ERIC method or standardized NGS method."[20]

U.S. Food and Drug Administration

Some FDA labels require results from biomarker tests to effectively or safely use the therapy for a specific cancer type.[3] A list of all Pharmacogenomic Biomarkers included in FDA labeling and associated implications can be found here. While these tumor marker tests generally consist of a single biomarker, some larger panels of biomarkers are also included in the FDA labeling.

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Spinal Muscular Atrophy Genetic Testing

MOL.TS.225.A
v2.0.2024

Introduction

Genetic testing for spinal muscular atrophy is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Genomic Unity SMN1/2 Analysis	0236U
SMN1 Gene Analysis; Dosage/Deletion Analysis (e.g., carrier testing), includes SMN2 Analysis, if performed	81329
SMN1 Full Gene Sequencing	81336
SMN1 Known Familial Mutation Analysis	81337
SMN2 Dosage/Deletion Analysis	81479
SMN2 Targeted Mutation Analysis (c.859G>C)	81479

Criteria

Introduction

Requests for genetic testing for spinal muscular atrophy (SMA) are reviewed using these criteria.

SMN1 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy) , AND
- Previous Genetic Testing:

  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Known familial mutation(s) in biological relative, OR
- Carrier Screening
  - Known familial mutation(s) in biological relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

SMN1 Exon 7 Deletion

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of the SMN1 gene, AND
- Diagnostic Testing:
  - Child with hypotonia and weakness (generally symmetrical, proximal more than distal), or
  - Young adult (through twenties) onset of weakness more severely affecting the legs than arms (may be associated with frequent falls, difficulty with stairs), and
  - No obvious signs of a different neurological disorder, OR
- Prenatal Testing:
  - Both parents are carriers of an SMA mutation (at least one of which is an exon 7 deletion mutation), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Note Carrier Screening: SMN1 exon 7 deletion testing is not suitable for carrier screening. SMN1/SMN2 dosage analysis is the required test. Please see that section for required medical necessity criteria.

SMN1/SMN2 Deletion/Dosage Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:

- No previous genetic testing of the SMN1 gene in the carrier testing setting, AND

- Diagnostic Testing:
  - Infants with an abnormal result on newborn screening and the diagnosis of SMA is still uncertain, or
  - Index of suspicion for SMA remains high based on:
    - Proximal greater than distal weakness, and
    - Normal creatine kinase (CK), OR

- Carrier Screening:
  - Be of reproductive age, and
  - Have potential and intention to reproduce, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Note Prenatal Testing: SMN1/SMN2 Dosage Analysis is not suitable for preimplantation/prenatal diagnosis. Other forms of SMA testing may be indicated based on the mutation status of parents. Please see those sections for guidance.

SMN1 Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Genetic Testing:
  - SMN1 exon 7 deletion testing did not reveal a homozygous SMN1 deletion or SMN1/SMN2 gene dosage analysis identified a single copy of SMN1 exon 7 in the diagnostic setting, or
  - SMN1/SMN2 gene dosage analysis did not confirm carrier status of an exon 7 deletion in the carrier testing setting, AND

- Diagnostic Testing:
  - Individual is suspected to have compound heterozygous SMA based previous test results, and
  - Proximal greater than distal weakness, and
  - Normal creatine kinase (CK), OR

- Carrier Screening:

- Have one of the following increased risk indications with a noninformative SMN1/SMN2 gene dosage analysis result:
  - Have a reproductive partner who is a carrier of SMA, or
  - Have a reproductive partner with SMA, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Note Prenatal Testing: SMN1 full gene sequencing is not generally necessary for preimplantation/prenatal diagnosis as parental mutation status should have already been determined with SMN1 exon 7 deletion testing +/- SMN1 known familial variant analysis.

SMN2 Deletion/Dosage Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member meets the following criteria:
  - Member has a genetically confirmed diagnosis of SMA, and
  - Member has a diagnosis of either SMA Type 1 or SMA Type 2, and
  - Member has not had previous SMN2 copy number analysis performed, and
  - Documentation is provided that SMN2 copy number is needed to obtain insurance approval for medication being considered for treatment, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

SMN2 Targeted Mutation Analysis (c.859G>C)

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member meets the following criteria:
  - Member has a genetically confirmed diagnosis of SMA, and
  - Member has a diagnosis of either SMA Type 1 or SMA Type 2, and
  - Member has not had previous c.859G>C analysis performed, and
  - Documentation is provided that c.859G>C analysis is needed to obtain insurance approval for medication being considered for treatment, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Exclusions

SMN2 gene copy analysis for the purpose of predicting SMA prognosis is not medically necessary.

Targeted analysis of c.859G>C for the purpose of predicting SMA prognosis is not medically necessary.

What is Spinal Muscular Atrophy?

Definition

Spinal muscular atrophy (SMA) is a severe, inherited neuromuscular disease.[1-3] SMA is caused by loss of lower motor neurons (anterior horn cells) in the spinal cord, resulting in progressive symmetrical muscle weakness and atrophy.[1-3]

Incidence

The incidence and carrier frequencies are dependent on ethnicity. SMA affects 1/7,829 to 1/18,808 people.[3] The carrier frequency ranges from 1/45 to 1/100.[3] SMA is present in all ethnic groups.[1-3]

Symptoms

SMA is commonly divided into five clinical subtypes based on age of onset and clinical course. While genetic testing has shown these clinical subtypes are not completely distinct, they are still widely used, and include:[1-3]

- Prenatal onset form (“Type 0” proposed): characterized by polyhydramnios, decreased fetal movements, breech presentation, arthrogryposis multiplex congenita, and respiratory failure at birth.
- Type I (infantile or Werdnig-Hoffmann type): most common form (60-70% of cases). It presents before 6 months of age and the cause of death is often respiratory failure. Affected children have severe, generalized weakness and do not ever sit without support.
- Type II (intermediate type): causes muscle weakness with onset after 6 months, although children often are able to sit alone and can survive through childhood. Intelligence is normal.
- Type III (juvenile, Kugelberg-Welander type): milder. Onset ranges from infancy to youth, but affected people usually walk unassisted albeit with frequent falls or trouble with stairs. Intelligence is normal.
- Type IV (adult type): much later onset with muscle weakness generally presenting at 20-30 years of age. People may or may not become wheelchair dependent and have normal intelligence.

Cause

SMA is caused by mutations in the SMN1 gene.

- Large gene deletions (exon 7 +/- exon 8) cause SMA in the vast majority (95-98%) of affected individuals.[3]
- The remaining 2-5% of individuals with SMA have a deletion in one copy of the SMN1 gene and a different mutation in the other.[3]

The clinical severity of SMA can be influenced by the number of copies a person has of the SMN2 gene.[3] The SMN2 gene is almost identical to SMN1 and is located on the same chromosome. SMN2 gene mutations do not cause SMA. In fact, about 15% of unaffected people have no copies of the SMN2 gene. Individuals may have between 0-5 copies of SMN2 and SMN2 has been shown to modify the disease severity in people with SMA.

Although a higher copy number of SMN2 (usually 3 or more) is generally associated with a milder phenotype, SMA is still a highly variable disease. It is difficult to use SMN2 copy number to reliably predict the clinical manifestations of SMA in an affected person because other modifying factors not yet fully delineated are likely contributing to the variability in clinical presentation.[3] Identifying SMN2 copy number greater than 3 is technically challenging, sometimes inaccurate, and may require repeat testing for confirmation.[4]

Other potential genetic modifiers have been identified; however, the significance of these potential modifiers is yet to be determined.[5]

Inheritance

SMA is an autosomal recessive disorder.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

About 2% of individuals with SMA have a de novo (new) mutation in one of their two SMN1 genes. In this case, only one parent is a carrier of SMA.[3]

About 4% of carriers have two copies of SMN1 on a single chromosome. These individuals with two copies of SMN1 on one chromosome (a [2+0] genotype) are misdiagnosed as non-carriers by the SMN1 dosage test (i.e., a false negative test result).[3]

Diagnosis

The diagnosis of SMA is established in a proband with a history of motor difficulties, evidence of motor unit disease on physical examination, and identification of biallelic pathogenic variants in SMN1 on molecular genetic testing.[3] Most states include SMA testing with newborn screening, which enables earlier diagnosis and treatment for affected individuals.[6]

Carrier screening for SMA is recommended preconceptionally or prenatally.[7] Asymptomatic carriers typically have one intact copy of the SMN1 gene and one SMN1 gene with the common deletion. However, some unaffected carriers have two intact copies of the SMN1 gene. These may be on the same chromosome with no intact SMN1 gene on the other chromosome. Carriers of rare mutations and those carrying two SMN1 genes on the same chromosome will not be detected by gene dosage analysis. Therefore, a negative gene dosage analysis result reduces the carrier risk but cannot completely rule out that a person is an SMA carrier.[3,8] The detection rate of carrier screening varies based on ethnicity, ranging from 71% in African Americans to 95% in Caucasians.[2]

Management

Since 2016, three medications for SMA have met FDA Approval. Spinraza/nusinersen, Zolgensma/onasemnogene abeparvovec-xioi, and Evrysdi/risdiplam are used to treat disease manifestations for specific types of SMA. These treatments have the best efficacy when treatment is started before symptoms appear. Onset of symptoms may be prevented or delayed; however, long-term effects of these treatments are unknown.[3] For symptomatic individuals, treatment and care are best coordinated through a multidisciplinary team. Care may include support for feeding, neuromuscular, pulmonary, gastrointestinal, and skeletal symptoms.[3]

Survival

Treatment with Spinraza/nusinersen, Zolgensma/onasemnogene abeparvovec-xioi, and/or Evrysdi/risdiplam impacts the natural history of SMA with longer survival. Historically, the survival of individuals with SMA with supportive care only has correlated with the subtype:[3]

- Prenatal onset form: survival less than 6 months
- Type I: median survival 8-10 months
- Type II: approximately 70% of affected individuals are alive at 25 years
- Types III and IV: normal life span

Test information

Introduction

Testing for SMA may include known familial mutation analysis, deletion analysis, gene dosage analysis, sequencing, or targeted mutation analysis.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

SMN1 Exon 7 Deletion

Diagnostic testing in an affected individual begins with deletion or copy number analysis, which will identify a deletion of exon 7 in the SMN1 gene. For most affected individuals, both SMN1 genes will be missing exon 7. If one or both SMN1 genes do not have an exon 7 deletion, SMN1 gene sequencing can be considered.

SMN1/SMN2 Deletion/Dosage Analysis

SMN1/SMN2 deletion/dosage analysis is performed by multiplex ligation-dependent probe amplification (MLPA), quantitative polymerase chain reaction (qPCR) or next generation sequencing (NGS) to determine the number of full copies of the SMN1/SMN2 genes. Dosage analysis is commonly performed in the diagnostic testing of affected individuals and in carrier testing.[3,8]

SMN1 Sequencing

SMN1 sequencing is typically performed in reflex, when one or no deletions are identified by deletion/dosage analysis in a symptomatic individual. About 2-5% of affected individuals fall into this group. Sequencing detects the other mutation in virtually all cases.[2,3]

SMN2 Deletion/Dosage Analysis

SMN2 deletion/dosage analysis is performed in individuals following SMN1 genetic testing that established a diagnosis of SMA. SMN2 genetic testing serves to provide a better understanding of the expected severity and to determine eligibility for certain treatments.[3]

SMN2 Targeted Mutation Analysis (c.859G>C)

The c.859G>C mutation in SMN2 is positive modifier variant.[3] This testing may be indicated when treatment is being considered.

Guidelines and evidence

Introduction

The following section includes guidelines and evidence pertaining to SMA testing.

Diagnostic Testing

The following organizations have published guidelines regarding diagnostic testing for SMA.

European Neuromuscular Centre

The 218th European Neuromuscular Centre (ENMC, 2017) workshop revisited the consensus statement that was published in 2007 from the International Standard of Care Committee for Spinal Muscular Atrophy.[8] They stated the following regarding testing for SMA:[9]

- "There was consensus that genetic testing is the first line investigation when this condition is suspected in a typical case and that muscle biopsy or electromyography should not be performed in a typical presentation."[9]
- "There was also consensus that, at variance with previous recommendations, the current gold standard is SMN1 deletion/mutation and SMN copy number testing, with a minimal standard of SMN1 deletion testing. Other areas concerning the value of SMN2 copy number were more controversial and a further Delphi round was planned to complete the task."[9]

International Conference on the Standard of Care for Spinal Muscular Atrophy

An international consensus statement (2018) provided recommendations regarding the diagnosis and management of SMA and stated:[10]

- Clinical suspicion for SMA is often based on hypotonia with progressive symmetric proximal weakness affecting legs more than arms and sparing of facial muscles along with normal creatinine kinase (CK) levels. Electromyography is typically not needed in individuals with SMA type 1 and 2, nor is muscle biopsy.
- Genetic testing is considered a first line investigation when SMA is suspected. Multiplex ligation-dependent probe amplification (MLPA), quantitative polymerase chain reaction (qPCR) or next generation sequencing (NGS) that allow for quantitative analysis of SMN1 and SMN2 is recommended. SMN2 copy number inversely correlates with disease severity and may be required for inclusion in therapy.
- If neither full SMN1 copy is present, a diagnosis of SMA may be made. If the phenotype is suggestive of SMA, but one or both full copies of the SMN1 gene are present, SMN1 gene sequencing is recommended. If SMN1 genetic testing is unable to make a diagnosis, "other motor neuron diseases should be considered."

U.S. Secretary of Health and Human Services (HHS)

The U.S. Secretary of HHS released a national guideline (HHS, 2023) that made recommendations related to which disorders should be included in the state universal newborn screening programs, which includes screening for SMA.[11] The recommended follow-up for an abnormal newborn screening result is SMN1 and SMN2 gene dosage testing.[12]

Carrier Testing

The following organizations have published guidelines regarding carrier testing for SMA.

American College of Medical Genetics and Genomics

The American College of Medical Genetics (ACMG, 2021) released an educational practice resource on carrier screening. This consensus statement asserted that general population carrier screening should be ethnicity and family history agnostic.[13] To accomplish this, screening all individuals in the prenatal/preconception period for autosomal recessive and X-linked conditions with a carrier frequency of >1/200 was suggested, including SMA.

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2017; Reaffirmed 2023) stated the following in regard to carrier testing for SMA in an updated Committee Opinion:[7]

- "Screening for spinal muscular atrophy should be offered to all women who are considering pregnancy or are currently pregnant."

Treatments

The FDA has approved use of Spinraza (nusinersen), Zolgensma (onasemnogene abeparvovec-xioi), and Evrysdi (risdiplam) in individuals with SMA.

Spinraza (nusinersen)

Spinraza (nusinersen) is FDA approved for use in individuals with SMA. While the FDA label does not require SMN2 copy number analysis, the study of 121 affected individuals on which FDA approval was based used the following inclusion criteria:[14]

- 5q SMN1 homozygous gene deletion or mutation or compound heterozygous mutation
- 2 copies of the SMN2 gene (98% of enrolled individuals had 2 copies of SMN2)
- Onset of SMA symptoms at or before 6 months of age
- No hypoxemia at baseline screening at age 7 months or younger

Zolgensma (onasemnogene abeparvovec-xioi)

Zolgensma (onasemnogene abeparvovec-xioi) is FDA approved for use in individuals with SMA who are full-term to 2 years old. While the FDA label does not require SMN2 copy number analysis, the study of the 21 affected individuals on which FDA approval was based used the following inclusion criteria:[15]

- Confirmed bi-allelic SMN1 gene deletions
- 2 copies of the SMN2 gene
- Onset of SMA symptoms before 6 months of age
- Absence of the c.859G>C positive modifier variant in exon 7 of the SMN2 gene

Evrysdi (risdiplam)

Evrysdi/risdiplam is FDA approved for use in individuals with SMA at any age.

- Clinical studies included infantile onset SMA and later onset SMA. The overall findings of these studies support the effectiveness of Evrysdi in SMA patients of any age and appear to support the early initiation of treatment with Evrysdi.[16]
- Infantile onset SMA study enrolled patients with genetic confirmation of homozygous deletion or compound heterozygosity predictive of loss of function of the SMN1 gene, and two SMN2 gene copies.
- Later onset SMA study enrolled 180 non-ambulatory patients with Type 2 (71%) or Type 3 (29%) SMA. The median age of patients at the start of treatment was 9.0 years (range 2 to 25), and the median time between onset of initial SMA symptoms and first treatment was 102.6 months (range 1 to 275).

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Spinocerebellar Ataxia Genetic Testing

MOL.TS.311.A
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Introduction

Spinocerebellar ataxia genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
ATXN1 gene analysis, evaluation to detect abnormal (eg,expanded) allele	81178
ATXN2 gene analysis, evaluation to detect abnormal (eg,expanded) allele	81179
ATXN3 gene analysis, evaluation to detect abnormal (eg,expanded) allele	81180
ATXN7 gene analysis, evaluation to detect abnormal (eg,expanded) allele	81181
ATXN8 gene analysis, evaluation to detect abnormal (eg, expanded) alleles	81182
ATXN10 gene analysis, evaluation to detect abnormal (eg, expanded) alleles	81183
CACNA1A gene analysis; evaluation to detect abnormal (eg, expanded) alleles	81184
CACNA1A gene analysis; full gene sequence	81185
CACNA1A gene analysis; known familial variant	81186
Genomic Unity CACNA1A Analysis	0231U
PPP2R2B gene analysis, evaluation to detect abnormal (eg, expanded) alleles	81343
SCA multigene panel	81479
TBP gene analysis, evaluation to detect abnormal (eg, expanded) alleles	81344

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Criteria

Introduction

Requests for spinocerebellar ataxia (SCA) testing are reviewed using these criteria.

Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Presymptomatic Testing for Asymptomatic Individuals:
  - Member is 18 years of age or older, and
  - Known disease-causing mutation in SCA gene identified in 1st or 2nd degree relative(s), OR
- Diagnostic Testing for Symptomatic Individuals:
  - Known disease-causing mutation in SCA gene identified in 1st or 2nd degree relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Single Gene Testing

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous testing of requested gene(s), and
  - No mutation identified by previous analysis, if performed, and
  - No known familial mutation in a gene known to cause ataxia, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individual has been diagnosed with cerebellar ataxia, and
  - Medical history points to the specific subtype of SCA requested (e.g. age of onset, distinguishing features present, etc), AND
- Documentation from ordering provider indicating how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), AND

- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation, etc), AND
- Family history is consistent with an autosomal dominant inheritance pattern (including simplex cases), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Multigene Panel Testing

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous testing of requested genes, and
  - No mutation identified by previous analysis, if performed, and
  - No known familial mutation in a gene known to cause ataxia, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individual has been diagnosed with cerebellar ataxia, regardless of age of onset, AND
- Documentation from ordering provider indicating how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), AND
- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation, etc), AND
- Family history is consistent with an autosomal dominant inheritance pattern (including simplex cases), AND
- Medical history does not point to a specific genetic diagnosis for which a more focused test or panel would be appropriate, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

Other considerations

- For information on broader hereditary ataxia panel testing, please refer to the guideline *Hereditary Ataxia Multigene Panel Genetic Testing*, as this testing is not addressed here.
- Gene panels that are specific to SCA will be considered for medical necessity according to the criteria outlined in this guideline. Test methodology should be appropriate to the disease-causing mutations that are commonly reported for the

disorder in question (e.g., sequencing-only panels will not detect triplet repeat or large deletion/duplication mutations).

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

Gene panels that are specific to SCA will be eligible for reimbursement according to the criteria outlined in this guideline.

Any individual gene or multi-gene panel is only reimbursable once per lifetime.

When otherwise reimbursable, the following limitations apply:

- When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81479*).
- When use of a panel code is not possible, each billed component procedure will be assessed independently.
- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
- When the test is billed with multiple stacked procedure codes, only the following genes may be considered for reimbursement:
    - ATXN1 (SCA1)
    - ATXN2 (SCA2)
    - ATXN3 (SCA3)
    - CACNA1A (SCA6)
    - ATXN7 (SCA7)
    - TBP (SCA17)

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What is spinocerebellar ataxia?

Definition

Spinocerebellar ataxias (SCA) are a group of autosomal dominant ataxias that have a range of phenotypes. There are various subtypes of SCA, which are denoted by numbers (e.g. SCA1, SCA3, etc.)

Prevalence

The prevalence of autosomal dominant cerebellar ataxias, as a whole, is 1-5:100,000.[1] SCA3 is the most common autosomal dominant form of ataxia. This is followed by SCA1, SCA2, SCA6, and SCA7.[1] The prevalence of specific subtypes of SCA vary by region. SCA3 is most common is Portugal.[1]

Symptoms

Although the specific phenotype of each subtype varies, most individuals with SCA have “progressive adult-onset gait ataxia (often with hand dysmetria) and dysarthria associated with cerebellar atrophy on brain imaging.”[1] The age of onset for the different subtypes also overlaps, which it makes it difficult to distinguish between subtypes based on clinical phenotype only.[1,2] See the table below for the various subtypes of SCA and the associated clinical features.

Cause

SCAs are caused by mutations in one of numerous genes. See the table below for the various subtypes of SCA and the associated genes.

Inheritance

SCAs are autosomal dominant disorders. Anticipation is observed in some of the SCAs. This means that as the disease passes through generations, the severity can increase and the age of onset can decrease.

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Diagnosis

Molecular genetic testing can be used to establish a specific diagnosis, which aids in understanding the prognosis and risk assessment for family members.[1]

Management

Treatment of ataxia is largely supportive, and includes the use of canes and walkers for ambulation, speech therapy, and other assistive devices.[1]

SCA subtype	Gene Associated	Clinical Features
SCA1	ATXN1	Progressive cerebellar ataxia, dysarthria, deterioration of bulbar functions, pyramidal signs, peripheral neuropathy[2,3]
SCA2	ATXN2	Progressive ataxia and dysarthria, nystagmus, slow saccadic eye movements, peripheral neuropathy, decreased DTRs, dementia[2,4]
SCA3	ATXN3	Gait problems, speech difficulties, clumsiness, visual blurring, diplopia, hyperreflexia, progressive ataxia, nystagmus, dysarthria, pyramidal and extrapyramidal signs; lid retraction, nystagmus, decreased saccade velocity; amyotrophy fasciculations, sensory loss[2,5]
SCA4	16q22.1	Sensory axonal neuropathy, deafness; may be allelic with 16q22-linked SCA[2]
SCA5	SPTBN2	Early onset, slow course[2]
SCA6	CACNA1A	Progressive cerebellar ataxia, dysarthria, nystagmus, sometimes episodic ataxia, very slow progression[2,6]

SCA subtype	Gene Associated	Clinical Features
SCA7	ATXN7	Progressive cerebellar ataxia, dysarthria, dysphagia, cone-rod and retinal dystrophy with progressive central visual loss resulting in blindness[2,7]
SCA8	ATXN8	Principally cerebellar ataxia, slowly progressing ataxia, scanning dysarthria, truncal instability, hyperactive tendon reflexes, decreased vibration sense; rarely, cognitive impairment[2,8]
SCA10	ATXN10	Progressive cerebellar ataxia, scanning dysarthria, dysphagia, upper-limb ataxia, generalized motor seizures and/or complex partial seizures, most families are of Native American background[2,9]
SCA11	TTBK2	Progressive cerebellar ataxia, abnormal eye signs (jerky pursuit, horizontal and vertical nystagmus), mild, remain ambulatory[2,10]
SCA12	PPP2R2B	Slowly progressive ataxia; action tremor in the 30s; hyperreflexia; subtle Parkinsonism possible; cognitive/psychiatric disorders including dementia[2]
SCA13	KCNC3	Ranges from progressive childhood-onset cerebellar ataxia, cerebellar dysarthria, occasional seizures to adult-onset progressive ataxia, mild intellectual disability, short stature[2,11]

SCA subtype	Gene Associated	Clinical Features
SCA14	PRKCG	Progressive cerebellar ataxia, dysarthria, nystagmus, axial myoclonus, cognitive impairment, tremor, sensory loss, Parkinsonian features including rigidity and tremor[2,12]
SCA15	ITPR1	Progressive gait and limb ataxia, ataxic dysarthria, titubation, upper limb postural tremor, mild hyperreflexia, gaze-evoked nystagmus, and impaired vestibuloocular reflex gain[2,13]
SCA16	SCA16	Head tremor; reported in one Japanese family[2]
SCA17	TBP	Ataxia, dementia, mental deterioration; occasional chorea, dystonia, myoclonus, epilepsy; Purkinje cell loss, intranuclear inclusions with expanded polyglutamine[2,14]
SCA18	7q22-q32	Ataxia with early sensory/motor neuropathy, nystagmus, dysarthria, decreased tendon reflexes, muscle weakness, atrophy, fasciculations, Babinski responses[2]
SCA19/22	KCND3	Slowly progressive, rare cognitive impairment, myoclonus, hyperreflexia[2]

SCA subtype	Gene Associated	Clinical Features
SCA20	11q12.2-11q12.3	Progressive ataxia, dysarthria, palatal tremor (myoclonus), and/or abnormal phonation clinically resembling spasmodic adductor dysphonia, hyperreflexia, bradykinesia; calcification of the dentate nucleus.[2,15]
SCA21	TMEM240	Mild cognitive impairment[2]
SCA23	PDYN	Dysarthria, abnormal eye movements, reduced vibration and position sense; reported in one Dutch family; neuropathology[2]
SCA25	SCA25	Sensory neuropathy; reported in one French family[2]
SCA26	EEF2	Dysarthria, irregular visual pursuits; reported in one Norwegian-American family; MRI: cerebellar atrophy[2]
SCA27	FGF14	Early-onset tremor; dyskinesia, cognitive deficits; reported in one Dutch family[2]
SCA28	AFG3L2	Young-adult onset, progressive gait and limb ataxia resulting in coordination and balance problems, dysarthria, ptosis, nystagmus, and ophthalmoparesis, increased tendon reflexes; reported in two Italian families[2,16]
SCA29	ITPR1	Learning deficits, infant-onset hypotonia, motor delays[2,17]

SCA subtype	Gene Associated	Clinical Features
SCA30	4q34.3-q35.1	Hyperreflexia[2]
SCA31	BEAN1	Normal sensation[2]
SCA35	TGM6	Hyperreflexia, Babinski responses; spasmodic torticollis[2]
SCA36	NOP56	Late-onset, slowly progressive cerebellar syndrome typically associated with sensorineural hearing loss, muscle atrophy and denervation, especially of the tongue, as well as pyramidal signs, muscle fasciculations, hyperreflexia[2]
SCA37	DAB1	Adult onset, abnormal vertical eye movements, dysarthria, dysmetria, dysphagia[1,18]
SCA38	ELOVL5	Adult onset, axonal neuropathy[1]
SCA40	CCDC88C	Adult onset, brisk reflexes, spasticity[1]
SCA41	TRPC3	Adult onset, uncomplicated ataxia[1]
SCA42	CACNA1G	Mild pyramidal signs, saccadic pursuit[1]

Survival

The SCAs are a group of progressive disorders with a range of phenotypes. Specific symptoms and a genetically determined diagnosis can assist with determining predicted survival and prognosis.

Test Information

Introduction

Testing for SCA may include known familial mutation analysis, repeat expansion

analysis, next generation sequencing, deletion/duplication analysis, and/or multigene panel testing. Test methods vary by gene of interest.

Known Familial Mutation Analysis

Analysis for known familial mutations is typically performed by nucleotide repeat expansion analysis. Some mutations may require Sanger sequencing or deletion/duplication analysis.

Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

Repeat Expansion Analysis

Several of the SCAs are caused by repeat expansions. Testing for these conditions is performed by expansion analysis to identify the number of repeats. Expansion analysis can be performed for diagnostic testing, presymptomatic testing, as well as prenatal testing.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and Evidence

Introduction

This section includes relevant guidelines and evidence pertaining to SCA testing.

European Federation of Neurological Sciences

The European Federation of Neurological Sciences (EFNS, 2014) stated the following with regard to testing for autosomal dominant cerebellar ataxia:[19]

- "In the case of a family history that is compatible with an autosomal dominant cerebellar ataxia, screening for SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 is recommended (Level B). In Asian patients, DRPLA should also be tested for."
- "If mutation analysis is negative, we recommend contact with or referral to a specialized clinic for reviewing the phenotype and further genetic testing (good practice point)"
- "In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA6, and DRPLA (in Asian patients) (level B), the step one panel of the recessive ataxia workup, i.e. mutation analysis of the FRDA gene (level B), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, CK, and alpha-fetoprotein."

Selected Relevant Publications

de Silva R, Greenfield J, Cook A, et al. (2019) stated that as part of the diagnostic evaluation for progressive ataxias, genetic tests should include: [20]

- "Genetic tests for FRDA, SCA 1, 2, 3, 6, 7 (12,17) and FXTAS"

Hadjivassiliou M, Martindale J, Shanmugarajah P, et al (2017) stated the following with regard to testing for hereditary ataxias:[21]

- "We have shown that patients with early onset idiopathic ataxia (irrespective of family history) are much more likely to have a genetic aetiology (81%) than those with late onset idiopathic ataxia (55%). One possible selection criterion for genetic testing is early onset ataxia. Additional selection criteria may include the presence of other clinical features, for example, 91% of patients with histologically suspected/genetically confirmed mitochondrial disease had ataxia with other clinical features (eg, deafness, diabetes, myoclonus, etc) and only 9% pure ataxia."
- "Furthermore, the presence of severe cerebellar atrophy without any clinical correlation and with well-preserved spectroscopy of the cerebellum often suggests that the ataxia is long standing (maybe even early onset) and slowly progressive. Such patients should therefore be offered genetic testing. The pattern of cerebellar involvement on MR spectroscopy may also direct to a particular diagnosis. Most genetic ataxias involve both the hemispheres and the vermis while the majority of immune-mediated acquired ataxias (eg, gluten ataxia, anti-GAD ataxia and primary autoimmune cerebellar ataxia) have a predilection for the vermis."

Jayadev S and Bird T (2013) stated the following:[2]

The "differential diagnosis of hereditary ataxia includes acquired, nongenetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, and paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung, and the idiopathic degenerative disease multiple system atrophy (spinal muscular atrophy). The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available."

- Regarding establishing the diagnosis of hereditary ataxias:
  - "Detection on neurological examination of typical clinical signs including poorly coordinated gait and finger/hand movements, dysarthria (incoordination of speech), and eye movement abnormalities such as nystagmus, abnormal saccade movements, and ophthalmoplegia."
  - "Exclusion of nongenetic causes of ataxia."
  - "Documentation of the hereditary nature of the disease by finding a positive family history of ataxia, identifying an ataxia-causing mutation, or recognizing a clinical phenotype characteristic of a genetic form of ataxia."
- Regarding testing when the family history suggests autosomal dominant inheritance:
  - "An estimated 50–60% of the dominant hereditary ataxias can be identified with highly accurate and specific molecular genetic testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, and DRPLA; all have nucleotide repeat expansions in the pertinent genes."
  - "Because of broad clinical overlap, most laboratories that test for the hereditary ataxias have a battery of tests including testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA14, and SCA17. Many laboratories offer them as two groups in stepwise fashion based on population frequency, testing first for the more common ataxias, SCA1, SCA2, SCA3, SCA6, and SCA7. Although pursuing multiple genes simultaneously may seem less optimal than serial genetic testing, it is important to recognize that the cost of the battery of ataxia tests often is equivalent to that of an MRI. Positive results from the molecular genetic testing are more specific than MRI findings in the hereditary ataxias. Guidelines for genetic testing of hereditary ataxia have been published."
  - "Testing for the less common hereditary ataxias should be individualized and may depend on factors such as ethnic background (SCA3 in the Portuguese, SCA10 in the Native American population with some exceptions [Fujigasaki et al., 2002]); seizures (SCA10); presence of tremor (SCA12, fragile X-associated tremor/ataxia syndrome); presence of psychiatric disease or chorea (SCA17); or uncomplicated ataxia with long duration (SCA6, SCA8, and SCA14). Dysphonia and palatal myoclonus are associated with calcification of the dentate nucleus of cerebellum (SCA20)."

- "If a strong clinical indication of a specific diagnosis exists based on the affected individual's examination (e.g., the presence of retinopathy, which suggests SCA7) or if family history is positive for a known type, testing can be performed for a single disease."

- Regarding testing for a simplex case:
  - "If no acquired cause of the ataxia is identified, the probability is ~13% that the affected individual has SCA1, SCA2, SCA3, SCA6, SCA8, SCA17, or FRDA, and mutations in rare ataxia genes are even less common."
  - "Other possibilities to consider are a de novo mutation in a different autosomal dominant ataxia, decreased penetrance, alternative paternity, or a single occurrence of an autosomal recessive or X-linked disorder in a family such as fragile X-associated tremor/ataxia syndrome."
  - "Although the probability of a positive result from molecular genetic testing is low in an individual with ataxia who has no family history of ataxia, such testing is usually justified to establish a specific diagnosis for the individual's medical evaluation and for genetic counseling."
  - "Always consider a possible nongenetic cause such as multiple system atrophy, cerebellar type in simplex cases."

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SCA

Thoracic Aortic Aneurysms and Dissections (TAAD) Panel Genetic Testing

MOL.TS.227.A
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Introduction

Thoracic aortic aneurysms and dissection (TAAD) panel genetic testing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Aortic Dysfunction or Dilation Duplication/ Deletion Analysis Panel	81411
Aortic Dysfunction or Dilation Genomic Sequence Analysis Panel	81410
TAAD Gene Analysis	81400 81401 81402 81403 81404 81405 81406 81407 81408 81479
TAAD Known Familial Mutation Analysis	81403

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Criteria

Introduction

Requests for thoracic aortic aneurysms and dissection (TAAD) genetic testing are reviewed using these criteria.

Known Familial Mutation Analysis for TAAD

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing that would detect the familial mutation, AND
- Diagnostic or Predisposition Testing for Symptomatic or Presymptomatic Individuals:**
  - TAAD family mutation in 1st degree biological relative, AND
- Rendering laboratory is a qualified provider for service per the Health Plan policy.

****NOTE:** Since symptoms may occur in childhood, testing of children who are at-risk for a pathogenic mutation may be considered.

Sequencing Panel for TAAD

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous panel testing for TAAD, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Cardiology examination consistent with a diagnosis of TAAD, and
  - Clinical features are not sufficiently specific to suggest a single condition, and
  - The results of the test will directly impact the diagnostic and treatment options that are recommended for the member, AND
- Rendering laboratory is a qualified provider for service per Health Plan policy.

Deletion/Duplication Analysis for TAAD

- Criteria for TAAD Genetic Testing Sequencing panel met, AND

- No mutations found in TAAD Sequencing panel, AND
- No previous deletion/duplication analysis for TAAD, AND
- Rendering laboratory is a qualified provider for service per Health Plan policy.

Other Considerations

- This guideline addresses testing specifically for TAAD. For information on additional indications, please refer to the guideline *Hereditary Connective Tissue Disorder Testing*.
- For information on Marfan syndrome testing, please refer to the guideline *Marfan Syndrome Genetic Testing*, as this testing is not addressed here.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Any individual gene or multi-gene panel is only reimbursable once per lifetime.
- When otherwise reimbursable, the following limitations apply:
    - When a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code.
        - Gene panels that are specific to TAAD that include the following genes will be eligible for reimbursement according to the criteria outlined in this guideline: FBN1, TGFBR1, TGFBR2, COL3A1, MYH11, ACTA2, SLC2A10, SMAD3, and MYLK. This sequencing panel will only be considered for reimbursement when billed under an appropriate panel CPT code (e.g., 81410*).
        - Duplication/deletion panels will only be considered for reimbursement when billed under an appropriate panel CPT code (e.g., 81411*).
    - When use of a panel code is not possible, each billed component procedure will be assessed independently.

- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
- When a TAAD multi-gene panel is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
  - TGFBR2
  - TGFBR1
  - ACTA2
  - SMAD3

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

What are thoracic aortic aneurysms and dissections (TAAD)?

Definition

The major cardiac problems seen in individuals with Thoracic Aortic Aneurysms and Dissections (TAAD) are aneurysm of the aorta, typically the aortic root and ascending aorta, and aortic dissections.[1]

Prevalence

Thoracic aortic aneurysm is seen in approximately 1% of the population.[2] In the absence of a known inherited syndrome, 20% of individuals with TAAD will have a positive family history.[1]

Symptoms

Most aneurysms are asymptomatic; however, If undetected and untreated, they can lead to aortic dissection, which is a life-threatening condition.[1,2] The individual's age at the time of aortic dissection and the severity of the disease can vary.[1]

Cause

To date, at least 37 genes have been identified in association with TAAD.[2] Some of these genes are associated with specific genetic conditions that may require additional management or surveillance. Medical management, including timing of surgery, may

differ based on the underlying genetic etiology.[2-4] In many cases, a careful clinical examination by a specialist familiar with clinical features of these conditions can help to point toward one condition. In these cases, testing for gene(s) associated with a single condition would be most appropriate.

TAAD can be a symptom in several genetic syndromes, including:

- **Marfan syndrome (MFS)** – MFS is an autosomal dominant disorder that affects connective tissue in many parts of the body.[5] MFS is caused by mutations in the FBN1 gene. Diagnostic criteria, called the Ghent criteria, exists for Marfan syndrome. Major manifestations of the disease include aortic enlargement and ectopia lentis. Other features include, but are not limited to, bone overgrowth and joint laxity, long arms and legs, scoliosis, sternum deformity (pectus excavatum or carinatum), long thin fingers and toes, dural ectasia (stretching of the dural sac), hernias, stretch marks on the skin, and lung bullae. Symptoms can present in males or females at any age. Symptoms typically worsen over time. Infants who present with symptoms typically have the most severe disease course.[5]
- **Loeys-Dietz syndrome (LDS)** - LDS is an autosomal dominant disorder that affects many parts of the body.[6] LDS is mostly caused by mutations in either the TGFBR1 gene (20-25%) or TGFBR2 gene (55-60%). However, a small percentage of people with LDS may have mutations in SMAD2 (1-5%), SMAD3 (5-10%), TGFB2 (5-10%), or TGFB3 (1-5%). Major manifestations of this condition include "vascular findings (cerebral, thoracic, and abdominal arterial aneurysms and/or dissections), skeletal manifestations (pectus excavatum or pectus carinatum, scoliosis, joint laxity, arachnodactyly, talipes equinovarus, cervical spine malformation and/or instability), craniofacial features (widely spaced eyes, strabismus, bifid uvula/ cleft palate, and craniosynostosis that can involve any sutures), and cutaneous findings (velvety and translucent skin, easy bruising, and dystrophic scars)." [6] Given that there is no clinical diagnostic criteria established for LDS, genetic testing can help with the diagnosis.[6]
- **Vascular Ehlers-Danlos syndrome (vEDS or EDS type IV)** – EDS type IV is an autosomal dominant condition. It is caused by mutations in the COL3A1 gene. Major manifestations of this condition include "arterial, intestinal, and/or uterine fragility; thin, translucent skin; easy bruising; characteristic facial appearance (thin vermilion of the lips, micrognathia, narrow nose, prominent eyes); and an aged appearance to the extremities, particularly the hands." [7] Many adults present with the following symptoms: vascular dissection or rupture, gastrointestinal perforation, or organ rupture. Infants and children may present with congenital dislocation of the hips, clubfoot, pneumothorax, and/or recurrent joint subluxation or dislocation.[7]
- **Heritable Thoracic Aortic Disease (HTAD)** – HTAD describes those with TAAD who have absence of a known syndrome (e.g., Marfan syndrome, vEDS, LDS) and have a positive family history of TAAD.[1] 30% of those with HTAD will have a causative pathogenic variant identified in one of the known HTAD-related genes (including ACTA2, BGN, COL3A1, FBN1, FOXE3, LOX, MAT2A, MFAP5, MYH11, MYLK, PRKG1, TGFB2, TGFB3, TGFBR1, TGFBR2, SMAD3).[1,2]

Inheritance

Inherited forms of TAAD are most commonly autosomal dominant.[1] Not everyone who inherits a pathogenic variant in a gene associated with TAAD will develop an aortic aneurysm or dissection.

Autosomal recessive and X-linked patterns of inheritance have been reported for some associated genes.[2]

Diagnosis

TAAD can be diagnosed by various imaging studies, including echocardiography, computed tomography (CT) and MRI.[1] Genetic testing can be helpful to determine if there is an underlying genetic condition causing the TAAD.

Management

TAAD is managed with medications and regular imaging to assess the extent of aortic dilatation.[3] Surgical repair of the aorta may be necessary in some cases to help prevent aortic dissection.[1]

Survival

Survival depends on the occurrence of aortic dissection and the comorbidities that may be associated with an underlying genetic syndrome.

Test information

Introduction

Testing for TAAD may include known familial mutation analysis, next generation sequencing, deletion/duplication testing, and/or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and

insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

The proportion of pathogenic TAAD mutations that are gene deletions or duplications is not well described.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

Many laboratories offer testing for at least 9 genes that have been associated with TAAD in their panels, including the genes that cause MFS, LDS, EDS type IV and HTAD. Detection rates of expanded panels vary by laboratory and depend on the genes included and the methods used for testing.[1]

Testing multiple genes, without supporting clinical features, has the potential to obtain results which may be hard to interpret. The chance that a variant of uncertain significance will be found increases as more genes are tested. However, given that many of the symptoms of conditions associated with TAAD overlap, if a person presents with overlapping features of more than one condition, a panel approach should be considered.

If features of a specific genetic disorder that is associated with TAAD are present, more targeted testing may be appropriate. For example, if an individual has TAAD and ectopia lentis, focused testing for Marfan syndrome (FBN1 sequencing and deletion/duplication analysis) is most appropriate.[1]

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to TAAD genetic testing.

American Heart Association and American College of Cardiology

The American Heart Association (AHA, 2022) and American College of Cardiology (ACC, 2022) published clinical practice guidelines for the diagnosis and management of aortic disease. They stated the following regarding genetic evaluation and family screening:[8]

- Risk factors for familial thoracic aortic disease (TAD), also known as heritable thoracic aortic disease (HTAD), were outlined as:
  - "TAD and syndromic features of Marfan syndrome, Loeys-Dietz syndrome, or vascular EDS syndrome
  - TAD presenting at <60 years
  - A family history of either TAD or peripheral/intracranial aneurysms in a first- or second-degree relative
  - A history of unexplained sudden death at a relatively young age in a first- or second-degree relative"
- "In patients with aortic root/ascending aortic aneurysms or aortic dissection, obtaining a multigenerational family history of TAD, unexplained sudden deaths, and peripheral and intracranial aneurysms is recommended."
- "In patients with aortic root/ascending aortic aneurysms or aortic dissection and risk factors for HTAD, genetic testing to identify pathogenic/likely pathogenic variants (ie, mutations) is recommended."
- "In patients with an established pathogenic or likely pathogenic variant in a gene predisposing to HTAD, it is recommended that genetic counseling be provided and the patient's clinical management be informed by the specific gene and variant in the gene."
- " In patients with TAD who have a pathogenic/likely pathogenic variant, genetic testing of at-risk biological relatives (ie, cascade testing) is recommended. In family members who are found by genetic screening to have inherited the pathogenic/likely pathogenic variant, aortic imaging with TTE (if aortic root and ascending aorta are adequately visualized, otherwise with CT or MRI) is recommended."
- " In a family with aortic root/ascending aortic aneurysms or aortic dissection, if the disease-causing variant is not identified with genetic testing, screening aortic imaging of at-risk biological relatives (ie, cascade testing) is recommended."
- "In patients with aortic root/ascending aortic aneurysms or aortic dissection, in the absence of either a known family history of TAD or pathogenic/likely pathogenic variant, screening aortic imaging of first-degree relatives is recommended."
- "In patients with acute type A aortic dissection, the diameter of the aortic root and ascending aorta should be recorded in the operative note and medical record to inform the management of affected relatives."

Canadian Cardiovascular Society

The Canadian Cardiovascular Society (2014) stated the following:[9]

- "We recommend screening for TAD-associated genes in non-BAV aortopathy index cases to clarify the origin of disease and improve clinical and genetic counseling (Strong Recommendation, Moderate Quality Evidence)."
- "We recommend complete aortic imaging at initial diagnosis and at 6 months for patients with LDS or a confirmed genetic aortopathy (e.g., TGFBR1/2, TGFB, SMAD3, ACTA2, or MYH11) to establish if enlargement is occurring (Strong Recommendation, Moderate-Quality Evidence)."
- "We recommend that genetic counselling and testing be offered to first-degree relatives of patients in whom a causal mutation of a TAD-associated gene is identified. We recommend that aortic imaging be offered only to mutation carriers (Strong Recommendation, Low-Quality Evidence)."

Cardiac Society of Australia and New Zealand

The Cardiac Society of Australia and New Zealand (CSANZ) Cardiovascular Genetic Disease Council (2017) stated:[10]

- "A definitive molecular genetic diagnosis can clarify an equivocal clinical picture or result in a diagnosis in an apparently phenotypically normal individual. It is unknown at this stage what proportion of patients with these different genetic mutations will develop aortic dilatation or dissection. Identification of a causal mutation allows for the provision of accurate genetic counseling, the screening of at-risk family members and offers the possibility of accurate prenatal or preimplantation genetic diagnosis."
- "Molecular confirmation of a suspected clinical diagnosis is increasingly important for guiding patient management. As an example, an individual who looks marfanoid will have more extensive arterial imaging screening if identified to have a SMAD3 mutation as opposed to an FBN1 mutation."
- "Many clinical laboratories offer a multi-gene MFS/LDS/ familial TAAD panel that includes FBN1 and numerous other genes associated with aortic aneurysm and dissection disorders. This approach may be advantageous, given the known clinical and genetic heterogeneity of these disorders."
- "The clinical picture of non-syndromic aortopathies remains to be fully elucidated, and therefore the optimal extent and frequency of vascular imaging is unclear. We would err on the side of caution and suggest imaging the entire vasculature, at least at baseline, in non-syndromic individuals with a genetic mutation."
- "If there is a clear genetic diagnosis, then first-degree relatives should be offered predictive testing. If the screened relative does not have the familial mutation they can be released from screening. We advocate erring on the side of caution with respect to screening echocardiography of at-risk relatives." Screening is advised in the following relatives:

a) “All family members who share the familial mutation and who therefore should be under clinical care, not screening”

b) “At-risk family members where a clinical genetic diagnosis exists”

c) “At-risk family members where no clinical genetic diagnosis is made but the dissection occurred in a young individual without an apparent risk factor e.g. long standing hypertension”

European Society of Cardiology

The European Society of Cardiology (ESC, 2014) stated the following:[11]

- "Once a familial form of TAAD is highly suspected, it is recommended to refer the patient to a geneticist for family investigation and molecular testing." (Class I, Level C)

National Working Group on Bicuspid Aortic Valve and Thoracic Aortic Aneurysm

An expert consensus recommendation published on behalf of the National Working Group on Bicuspid Aortic Valve (BAV) and Thoracic Aortic Aneurysm (TAA) stated the following regarding cardiogenetic care for individuals with thoracic aortic disease and their first-degree relatives:[12]

- High-risk groups for genetic predisposition are defined as thoracic aneurysm (equal to or greater than 45 mm) or dissection:
  - Age at diagnosis <50 years, or
  - Age at diagnosis 50-60 years, no hypertension, or
  - Positive family history, or
  - Syndromic features
- "If no specific syndrome features are present, next-generation sequencing (NGS) of multiple genes (associated with TAA) is the most efficient and cost-effective method."
- "If a disease-causing mutation has been identified in the proband, the working group recommends offering presymptomatic genetic testing to relatives. This is best undertaken using a stepwise approach called "cascade screening"."
- Screening of first-degree relatives for familial TAA:
  - "Cardiovascular screening of mutation carriers should take place at or in close collaboration with an academic center, according to gene-specific management guidelines."
  - If no disease-causing mutation has been identified in the proband, screening should be offered to all first-degree relatives (parents, siblings, and children) starting at age 25 years or 10 years before the youngest case in the family using

transthoracic echocardiography (TTE), baseline computed tomography (CT), or magnetic resonance imaging (MRI). If normal, repeat every 5 years. Discontinue at age 65 years or if first screening >60 years.

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Human Platelet and Red Blood Cell Antigen Genotyping

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v2.0.2024

Introduction

Molecular testing of red blood cell or human platelet antigens in individuals to determine alloimmunization status or risk is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
BLOODchip® ID CORE XT	0084U
Gene analysis (Human Platelet Antigen 1) for common variant	81105
Gene analysis (Human Platelet Antigen 2) for common variant	81106
Gene analysis (Human Platelet Antigen 3) for common variant	81107
Gene analysis (Human Platelet Antigen 4) for common variant	81108
Gene analysis (Human Platelet Antigen 5) for common variant	81109
Gene analysis (Human Platelet Antigen 6) for common variant	81110
Gene analysis (Human Platelet Antigen 9) for common variant	81111
Gene analysis (Human Platelet Antigen 15) for common variant	81112
Fetal RHD genotyping using maternal plasma (e.g. SensiGene)	81403
Navigator ABO Blood Group NGS	0221U
Navigator ABO Sequencing	0180U
Navigator CO Sequencing	0181U

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Procedure addressed by this guideline	Procedure code
Navigator CROM Sequencing	0182U
Navigator DI Sequencing	0183U
Navigator DO Sequencing	0184U
Navigator FUT1 Sequencing	0185U
Navigator FUT2 Sequencing	0186U
Navigator FY Sequencing	0187U
Navigator GE Sequencing	0188U
Navigator GYPA Sequencing	0189U
Navigator GYPB Sequencing	0190U
Navigator IN Sequencing	0191U
Navigator JK Sequencing	0192U
Navigator JR Sequencing	0193U
Navigator KEL Sequencing	0194U
Navigator KLF Sequencing	0195U
Navigator LU Sequencing	0196U
Navigator LW Sequencing	0197U
Navigator Rh Blood Group NGS	0222U
Navigator RHD/C/E Sequencing	0198U
Navigator SC Sequencing	0199U
Navigator XK Sequencing	0200U
Navigator YT Sequencing	0201U
PreciseType HEA Test	0001U
PrecisionBlood Red Cell Antigen Genotyping	0246U
RBC antigen analysis	81479
RHD Deletion analysis	81403
Versiti Red Cell Genotyping Panel	0282U

Criteria

Introduction

Requests for molecular testing for tissue antigen typing are reviewed using the

following criteria.

Human Platelet Antigen (HPA) Genotyping

Testing for human platelet antigens through molecular genotyping is medically necessary for individuals with clinical indications as outlined here.

- Member has at least one of the following:
  - Post-transfusion purpura 5-10 days after a blood transfusion, or
  - Suspected Neonatal Alloimmune Thrombocytopenia (NAIT)/ Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) based on clinical presentation during pregnancy or neonatal period, or
  - Pregnancy or newborn with suspected or diagnosed NAIT/FNAIT, or
  - Female partner had a previous child with NAIT/FNAIT and is known to be alloimmunized, or
  - Fetus with suspected NAIT/FNAIT based on clinical presentation (ie: intracranial bleeding on ultrasound), and fetal diagnostic testing is medically necessary, or
  - Previous child with NAIT/FNAIT and there is a risk for this disorder in a current pregnancy based on parental HPA genotypes, and prenatal risk assessment is desired, or
  - Platelet refractoriness despite receiving HLA matched platelets, or
  - Platelet refractoriness in the context of being unable to find compatible platelets for transfusion, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other considerations

Targeted HPA genotyping is not medically necessary when assessed as part of a pharmacogenomics or coagulopathy workup.

The procedure codes billed for HPA genotyping (including, but not limited to ITGB3 and ITGA2B) are not medically necessary outside of those indications outlined above, including for use in pharmacogenomics panels or to assess other cardiovascular disease states.

For information on pharmacogenomics panels, please refer to the guideline *Pharmacogenomic Testing for Drug Toxicity and Response*.

Red Blood Cell (RBC) Antigen Genotyping

Testing for red blood cell antigens through molecular genotyping is considered medically necessary when the member has a documented risk for red blood cell alloimmunization as outlined here.

- One of the following criteria must be met:
  - Member has weak D antigen on serology, or
  - Member is pregnant and has erythrocyte antibodies identified, or
  - Member is the parent of a pregnancy or newborn suspected of having or at risk for Hemolytic Disease of the Fetus and Newborn (HDFN), or
  - Pregnancy or newborn is suspected of having or at risk for Hemolytic Disease of the Fetus and Newborn (HDFN), or
  - Member has warm autoantibodies, or
  - Member is receiving certain monoclonal antibody therapies (such as anti CD38 therapy), or
  - Member has a blood disorder requiring frequent transfusions (such as sickle cell disease, some forms of thalassemia, autoimmune hemolytic anemia, or myelodysplasia), or
  - Member has a result from a traditional serology (hemagglutination) assay that is not consistent with the antibody that they are expressing, or
  - Member has evidence of an antigen that cannot be detected, or is not easily detected, by traditional hemagglutination (including the Dombrock antigen, complex Rh phenotypes, Fy silencing mutations, and MNS system mutations), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other considerations

Although most genotyping tests should only be performed once per lifetime, it may be medically necessary to repeat RBC antigen genotyping in some individuals. These requests will be reviewed on a case by case basis.

Fetal RhD Genotyping Using Maternal Plasma

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

Genotyping is reimbursable once per lifetime.

What are tissue antigens?

Definition

An antigen is a substance (protein, sugar, or lipid) that is on the surface of a cell. Red blood cell antigens are on the surface of red blood cells (RBC), while human platelet antigens (HPA) are on the surface of platelets.

Individuals can be exposed to red blood cell or human platelet antigens that they do not have on their cells through blood transfusion or pregnancy. Once exposed, they may become alloimmunized to these antigens and mount an immune response to them if they are presented again (e.g., during future transfusions).[1,2]

If subsequent antigen exposure occurs during pregnancy, the fetus/newborn is at risk for serious disease.

- Red Blood Cell Antigens: Fetuses and newborns of alloimmunized mothers are at risk for developing Hemolytic Disease of the Fetus and Newborn (HDFN). Symptoms include high output cardiac failure and kernicterus.[3,4]
- Human Platelet Antigens: Fetuses and newborns of alloimmunized mothers are at risk for developing Fetal and Neonatal Alloimmune Thrombocytopenia.(FNAIT). Symptoms include thrombocytopenia and intracranial, gastrointestinal, or genitourinary hemorrhage.[5,6] Unlike HDFN, FNAIT can occur in a first pregnancy.[5,6]

Test information

Introduction

Laboratory work-up of alloimmunization may include serology (antibody and/or antigen analysis) and molecular analysis.

Human Platelet Antigen (HPA) Genotyping

Molecular testing for human platelet antigens is typically performed in specialized reference laboratories via laboratory developed tests. Testing typically consists of targeted genotyping for specific, well-described gene variants.

Red Blood Cell (RBC) Antigen Genotyping

Molecular testing for red blood cell antigens is typically performed in specialized reference laboratories via laboratory developed tests, but RBC antigen panels may also be performed on FDA-approved instrument platforms. Testing may consist of targeted genotyping for specific gene variants, gene sequencing, or deletion analysis.

Table: Selected Red Blood Cell Antigens and Corresponding Genes

RBC antigen names, abbreviations, and genes

Red Blood Cell Blood Group Name	Antigen Abbreviation	Gene
RH	RHD/C/E	RHCE / RHD
ABO	ABO	ABO
Colton	CO	AQP1
Cromer	CROM	CD55
Diego	DI	SLC4A1
Dombrock	DO	ART4
H	FUT1	FUT1
Se	FUT2	FUT2
Duffy	FY	ACKR1
Gerbich	GE	GYPC
MN	GYPA	GYPA
Ss	GYPB	GYPB
Indian	I	CD44
Kidd	JK	SLC14A1
Junior	JR	ABCG2
Kell	KEL	KEL
Lutheran inhibitor	KLF	KLF1
Lutheran	LU	BCAM
Landsteiner-Wiener	LW	ICAM4
Scianna	SC	ERMAP

Red Blood Cell Blood Group Name	Antigen Abbreviation	Gene
Kell (X-linked)	XK	XK
YT	YT	ACHE
Knops	KN	CR1
Vel	Vel	SMIM1

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to human platelet and red blood cell antigen genotyping.

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2018) Practice Bulletin 192 Management of Alloimmunization During Pregnancy made the following recommendations after maternal antibodies are identified:[3]

- "The initial management of a pregnancy involving an alloimmunized patient is determination of the paternal erythrocyte antigen status."
- "The fetal genotype should be assessed when the paternal genotype is thought to be heterozygous or is unknown."

ACOG Practice Bulletin 181 Prevention of Rh D Alloimmunization (2017) stated:[4]

- "All pregnant women should be tested at the time of the first prenatal visit for ABO blood group and the Rh D type and screened for the presence of erythrocyte antibodies."
- "If Rh D antibodies are present because of sensitization, anti-D immune globulin is not beneficial, and management should proceed in accordance with protocols for Rh D-alloimmunized pregnancies."
- "If paternity is certain and the father is known to be Rh D negative, antenatal prophylaxis is unnecessary."
- "Despite the improved accuracies noted with noninvasive fetal RHD genotyping, cost comparisons with current routine prophylaxis of anti-D Immunoglobulin at 28 weeks of gestation have not shown a consistent benefit and, thus, this test is not routinely recommended."

Regarding maternal weak D phenotype on serology, ACOG Bulletin 181 (2017) stated:[4]

- "An attractive solution to this problem [maternal weak D phenotype] is to perform molecular genetic RHD typing in weak D phenotype individuals as suggested by the Work Group on RHD Genotyping."
- "Clinicians are advised to administer Rh D immune globulin to patients with weak D blood type in appropriate clinical situations, by the same rationale as that for Rh D typing blood donors, until further scientific and economic studies are available."

American Society of Hematology

The American Society of Hematology (ASH, 2020) stated the following in their guidelines for transfusion support for sickle cell disease:[1]

- "The ASH guideline panel *recommends* prophylactic red cell antigen matching for Rh (C, E or C/c, E/e) and K antigens over only ABO/RhD matching for patients with SCD (all genotypes) receiving transfusions (strong recommendation based on moderate certainty in the evidence about effects)."
- "The ASH guideline panel *suggests* an extended red cell antigen profile by genotype or serology over only ABO/RhD typing for all patients with SCD (all genotypes) at the earliest opportunity (optimally before the first transfusion) (conditional recommendation based on very low certainty in the evidence about effects)."
- "Extended red cell antigen matching (Jk^a, Jk^b, Fy^a, Fy^b, S/s) may provide further protection from alloimmunization."

In a 2014 Mini Review, the ASH stated:[2]

- "One to two percent of all patients who receive transfusions develop antibodies to RBC antigens."
- Between 10 and 30% of patients receiving chronic transfusions are alloimmunized, typically before the 15^{th} transfusion.
- "Once alloimmunization occurs, the likelihood of additional antibody responses is also relatively high. In surgical, pregnant, and non–hematologic malignancy patients, once RBC antibodies have been induced, 20 percent to 25 percent of patients form additional antibodies after subsequent transfusions and thus become multiply alloimmunized."
- In this review, ASH lists the following scenarios in which red blood cell antigen genotyping may be helpful:
    - Hemoglobinopathy patients at baseline,
    - Alloimmunized patients who are expected to need additional transfusions,
    - Alloimmunized patients with a co-existing autoantibody,
    - Patients who have been recently transfused,
    - Prenatal diagnosis in pregnancies at risk for hemolytic disease of the newborn.

Regarding platelet refractoriness, ASH (2020) recommended ordering HLA/HPA antibody screening tests and either platelet crossmatching or HLA/HPA matched platelets in individuals with thrombocytopenia, repeated poor response to platelet transfusion, and HLA/HPA antibodies.[7]

British Committee for Standards in Haematology

In a 2017 guideline on red cell transfusion in sickle cell disease, the British Committee for Standards in Haematology stated:[8]

- "An extended phenotype (or genotype) including C, c, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s should be performed on all patients at baseline. If the patient is S- s-, then U typing should be performed (Milkins et al, 2013). If the patient has not been transfused within 3 months then this can be undertaken serologically, otherwise the genotype needs determination by molecular techniques (Chou & Westhoff, 2011; Milkins et al, 2013) through an appropriate reference laboratory."
- "Select ABO extended Rh and K matched units negative for the relevant antigen(s) to which there are current or historical antibodies."
- "If the identity of the new alloantibody is in doubt despite further specialist testing, consider providing extended antigen matched blood (if serological phenotyping cannot be used because of the presence of transfused donor red blood cells, the sample should be sent to an appropriate reference laboratory for molecular red cell genotyping)."

In a 2017 guideline on the use of platelet transfusions the British Committee for Standards in Haematology stated:[9]

- Post-transfusion purpura (PTP) is "a rare condition associated with severe thrombocytopenia following blood transfusion and caused by antibodies against platelet-specific antigens. Bleeding can be serious and fatal". The condition usually occurs 5-10 days after transfusion.
- "Management is based on individual case reports and case series."
- "Current practice is to transfuse high dose intravenous immunoglobulin without waiting for the results of laboratory investigations, with random donor platelets reserved to control severe bleeding."

In a 2021 guideline on the management of sickle cell disease in pregnancy, the British Committee for Standards in Haematology stated:[10]

- "If transfusion is needed, pregnant women with SCD should be given ABO-compatible, extended Rh- and Kell-matched, CMV-negative units. If there are clinically significant red cell antibodies (current or historical) then the red cells selected should be negative for the corresponding antigens (1C)."

College of American Pathologists and AABB

A College of American Pathologists (CAP) and AABB Work Group on RHD Genotyping (2015) made the following recommendation regarding genotyping individuals with a weak D phenotype on serology:[11]

- "The Work Group recommends that RHD genotyping be performed whenever a discordant RhD typing result and/or a serological weak D phenotype is detected in patients, including pregnant women, newborns and potential transfusion recipients. It is anticipated that the immediate benefit will be fewer unnecessary injections of RhIG and increased availability of RhD-negative RBCs for transfusion."

The AABB reiterated on their website:[12]

- "RHD genotyping is recommended whenever a weak D phenotype is detected by routine Rh blood typing of pregnant women and other females of childbearing potential. The Work Group rates this as strong recommendation, based on high-quality evidence from observational studies (1A)."

International Collaboration for Transfusion Medicine

The International Collaboration for Transfusion Medicine Guidelines (ICTMG, 2019) guideline on fetal and neonatal alloimmune thrombocytopenia stated:[13]

- "If there is clinical suspicion of fetal and neonatal alloimmune thrombocytopenia (FNAIT), manage as FNAIT without waiting for laboratory confirmation (moderate evidence, strong recommendation)."
- "Fetal HPA typing, preferably using non-invasive methods, if adequately quality assured, should be performed during pregnancy when the father is unknown, unavailable for testing or heterozygous for the implicated antigen (moderate evidence, strong recommendation)."
- "Antenatal IVIG administration to the mother, commencing at 12–16 weeks gestation, should be offered to all women in a subsequent pregnancy with maternal fetal incompatibility who have had a previous fetus or neonate with FNAIT-related ICH (very low evidence, strong recommendation)."

Newborn Services Clinical Guideline: Auckland District Health Board

The Auckland, New Zealand District Health Board points to the Starship Child Health (2019) clinical management guideline on neonatal alloimmune thrombocytopenia, which stated the following regarding FNAIT:[5]

- "Neonatal Alloimmune Thrombocytopenia (NAIT) results from maternal human platelet antibodies (HPA) against fetal platelet antigens inherited from the father. In contrast to rhesus haemolytic disease, platelet allo-immunization can occur during the first pregnancy."
- "Definitive diagnosis of NAIT depends on parental testing."

- Maternal and paternal genotyping is recommended in this clinical guideline. If paternity is uncertain or no paternal sample is available, fetal genotyping is recommended.

Royal College of Obstetricians and Gynaecologists

In a 2019 guideline addressing pregnancies at risk for alloimmune thrombocytopenia, the Royal College of Obstetricians and Gynaecologists stated:[14]

- There is no evidence to support routine screening for pregnancies at risk of FNAIT (Fetal and Neonatal Alloimmune Thrombocytopenia).
- "IVIg in pregnancy is safe and likely to be effective. It seems reasonable to start therapy at 16–18 weeks of gestation in an at-risk pregnancy."

Selected Relevant Publications

Multiple review articles have addressed human platelet antigen genotyping, specifically with regard to Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT).

A review by Winklehorst and colleagues (2017) stated:[15]

- "When FNAIT is suspected, or in case of a family member with FNAIT, diagnostic work-up should ideally include HPA genotyping of mother, father, and child to establish possible HPA incompatibilities, as well as serological testing (maternal–paternal serum crossmatch, and a maternal platelet antibody screening)."
- "If, in case of suspicion due to an affected family member, after the HPA-typing, the pregnant woman turns out to be HPA-1a negative, the HPA-1a type of father and, in case of paternal heterozygosity, consequently fetus can be determined."
- "Adequate diagnosis does not only contribute to adequate management in the index cases, but is just as important for taking adequate measures in subsequent pregnancies to prevent bleeding complications."
- "When the father is homozygous, every consecutive pregnancy is incompatible and therefore the fetus is at risk. When the father is heterozygous, fetal genotyping has to be performed."

A review by Mella and colleagues (2015) stated:[16]

- "Approximately 80% of pregnancies affected by NAIT have maternal antibodies that are directed against platelet antigen HPA-1a with the remaining 20% being affected by the other HPA types. Studies have shown that approximately 98% of Caucasian women express HPA-1a (genotype HPA-1a/HPA-1a or HPA1a/HPA1b) and about 2% of Caucasian women are HPA-1a negative (genotype HPA-1b/HPA-1b). The second most common platelet antigen causing NAIT in Caucasians is HPA-5b antigen, followed by HPA-1b and HPA-15."

- "In at-risk pregnancies, mothers are antigen negative (most commonly HPA-1b) and fathers are either antigen-positive homozygous (genotype HPA-1a/1a) or heterozygous (genotype HPA-1a/1b)."
- "If the parental genotypes are different and the mother has specific antibodies to the putative antigen, then the pregnancy is at risk for NAIT and fetal/neonatal antigen typing would then be indicated."

A review by Peterson and colleagues (2013) stated:[17]

- "Some have argued that it may be cost-effective to perform such screening routinely and offer special case management to the 10% of HPA-1a-negative women who produce antibody (Husebekk et al, 2009) but at the present time this is not practiced in the absence of a family history of NAIT, e.g., in a sister."
- "A first affected neonate with NAIT in a family is normally identified when clinical signs of bleeding are evident at or shortly after birth and a platelet count confirms isolated thrombocytopenia."

Platelet Refractoriness

A review by Stanworth et al. (2015) stated the following regarding platelet refractoriness:[18]

- "If there are poor responses to HLA-selected platelet transfusions, the reasons should be sought including poor HLA compatibility of the selected product, non-immune platelet consumption and HPA and ABO incompatibility."
- "Depending on the results of these investigations, the appropriate management could be the use of ABO-identical or HPA-selected platelet concentrates if the specificity of the HPA anti-bodies can be identified."

Fetal RhD Genotyping Using Maternal Plasma

The overall certainty of the body of evidence regarding the diagnostic accuracy of fetal RhD genotyping in maternal plasma samples of RhD- women is low.[19,20] The body of evidence primarily evaluates nonalloimmunized women and women with singleton pregnancies. There is a high risk of bias among the studies due to concerns about the index test, standards, flow and timing, as well as eligibility criteria and selection used. There are also limited studies on the impact of fetal RhD genotyping using maternal plasma on patient health outcomes or clinical decision making in both nonalloimmunized and alloimmunized women.

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Tissue of Origin Testing for Cancer of Unknown Primary

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v2.0.2024

Introduction

Tissue of origin testing for cancer of unknown primary is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Oncology (Tissue of Origin), Microarray Gene Expression Profiling of Greater than 2000 genes (e.g. Tissue of Origin Testing)	81504
Oncology (Tumor of Unknown Origin), mRNA, Gene Expression Profiling of Real-time RT-PCR of 92 Genes to Classify Tumor into Main Cancer Type and Subtype (e.g. CancerTYPE ID)	81540
Unlisted Molecular Testing for Tumor of Unknown Origin	81479

Criteria

Introduction

Requests for tissue of origin testing for cancer of unknown primary are reviewed using the following criteria.

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.

- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What is cancer of unknown primary testing?

Definition

In order to determine the most effective treatment regimen for an individual with cancer it is important to identify the cancer cell type.[1]

- When a cancer is found in one or more metastatic sites but the primary site is not known, it is called a cancer of unknown primary (CUP) or an occult primary cancer.[2] This happens in a small portion of cancers.
- The most commonly used techniques to identify tissue of origin (TOO) for CUP include light microscopy, immunohistochemistry (IHC) staining and computed tomography (CT) or positron emission tomography (PET) imaging. [1,3] However, conventional methods have had poor success.[4,5]
- With advances in technology, some laboratory tests utilize gene expression profiling or other molecular techniques in cancer cells. Ramaswamy et al. found that a cancer-intrinsic gene expression pattern distinguished primary from metastatic adenocarcinomas.[6] By comparing the pattern of gene expression in the CUP sample to the patterns seen with other known types of cancer, a CUP may be identified as belonging to a particular cancer type. Survival, quality of life (QOL), and/or disease symptoms may improve in some cases if the site and type of primary origin can be accurately detected and appropriate therapy administered early in the disease course.[7,8]

Test information

Introduction

A number of different companies and approaches are being utilized to diagnose metastatic neoplasms for individuals with CUP, typically using gene expression analysis.

A representative example of a tissue-of-origin test, CancerTYPE ID (Biotheranostics, Inc), is a gene expression test designed to identify the most likely tissue of origin from 50 tumor types in individuals with cancer of unknown primary.[9] "CancerTYPE ID uses real-time RT-PCR to measure the expression of 92-genes in the patient's tumor and classifies the tumor by matching the gene expression pattern to a database of over 2,000 known tumor types and subtypes...The test reports a molecular diagnosis of the cancer type with the highest probability match, as well as a list of tumor types that may be ruled out with 95% confidence."[9]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to tissue of origin testing.

European Society for Medical Oncology

The European Society for Medical Oncology (ESMO, 2023) Clinical Practice Guideline for the diagnosis, treatment and follow-up of cancer of unknown primary stated the following:[10]

- "Despite a promising pilot study, two randomised trials failed to demonstrate superiority of gene expression profiling-based 'site-specific' therapy over standard empiric ChT with either carboplatine–paclitaxel or cisplatine–gemcitabine, respectively. Consequentially, no recommendation for the use of gene expression profiling-based 'site-directed' therapy can currently be provided."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network Guidelines in Oncology: Occult Primary (NCCN, 2024) stated the following regarding tissue of origin testing:[11]

- "Gene sequencing to predict tissue of origin is not recommended."
- "...the clinical benefit of using molecular profiling to guide treatment decisions in CUP remains to be determined."
- "Currently there is no evidence of improved outcomes with the use of site-specific therapy guided by molecular testing in patients with CUP."
- "While there may be a diagnostic benefit to GEP [gene expression profiling], a clinical benefit has not been demonstrated. Consequently, the panel does not currently recommend use of gene sequencing to predict tissue of origin. Until more robust outcomes and comparative effectiveness data are available, pathologists and oncologists must collaborate on the judicious use of IHC and GEP on a case-by-case basis, with the best possible individualized patient outcome in mind."

National Institute for Health and Care Excellence

The National Institute for Health and Care Excellence (NICE, 2023) clinical guideline for metastatic malignant disease of unknown primary origin stated that further research is required to determine whether gene-expression-based profiling "could be beneficial addition to standard management in CUP."[12]

Select Relevant Publications

In systematic reviews of cancer of unknown primary site, gene-profiling diagnosis was noted to have high sensitivity, but additional prospective studies were deemed

necessary to establish whether outcomes for individual's with cancer are improved by its clinical use.[1,13-22]

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VeriStrat Testing for NSCLC TKI Response

MOL.TS.232.A
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Introduction

VeriStrat testing for NSCLC TKI response is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedure addressed by this guideline	Procedure code
VeriStrat	81538

Criteria

VeriStrat Testing is not currently supported in clinical practice guidelines for the treatment of advanced NSCLC and the published evidence does not independently meet the criteria for coverage for this indication.

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

What is VeriStrat testing for non-small cell lung cancer?

Definition

The aim of the VeriStrat® test is to assess overall prognosis in advanced NSCLC and to predict treatment response to TKIs, single agent chemotherapy, and/or PDL1 inhibitors.[1,2]

- NSCLC is any type of cancer of the lung epithelial cells that is not classified as small-cell lung cancer.[3]
- Although associated with cigarette use and smoke exposure, NSCLC can be diagnosed in individuals who have never smoked.[3]
- Treatment selection in NSCLC may be guided by molecular genetic testing:
    - Approximately 15-25% of individuals with NSCLC have activating mutations in the EGFR gene. These individuals display improved progression-free survival following treatment with EGFR TKI therapy, such as erlotinib, afatinib, or osimertinib.[4-6]
    - Another 5-9% of individuals with NSCLC have ALK or ROS-1 rearrangements and are treated with inhibitors, including crizotinib (Xalkori).[6,7]
    - An additional 10% of individuals with NSCLC harbor alterations that are also amenable to FDA approved inhibitors including: activating BRAF or ERBB2 (HER2) mutations, MET amplification or exon 14 skipping mutations, or fusions involving RET, NTRK1, NTRK2, or NTRK3.[6]
- For the remaining approximately 50% of individuals who are negative for these targetable alterations, other therapies are used as first-line treatment (including chemotherapy and/or PDL1 inhibitors).[2,6] However, for individuals who fail front-line therapy, EGFR inhibitors can be considered as a potential option.[8,9] This applies in particular to individuals whose tumors express an increased number of copies of EGFR (even without EGFR mutations).[9,10]

Test information

Introduction

VeriStrat is a proprietary, serum-based proteomic test designed to be an adjunct to a conventional clinical workup and combined with the individual's clinical history, other diagnostic tests, and clinicopathologic factors.[1]

- The test has been developed to measure an individual's immune response to NSCLC and help determine if an individual may have a more aggressive cancer. VeriStrat is currently marketed as part of the IQLung treatment guidance.[1]
- The VeriStrat test result is reported as good, poor, or indeterminate.[1] The results are also intended to set individual expectations, facilitate a discussion about prognosis, improve knowledge to potentially reduce anxiety, and improve quality of life.[1]
    - **VSGood results:** A good result indicates that an individual is more likely to benefit from standard of care (SOC) treatment and have better overall survival (OS).[1]

- **VSPoor results:** A poor result indicates that an individual will likely have decreased OS and may benefit from alternative treatment strategies such as novel combination of therapies, NGS testing for rare mutations, non-platinum based regimens, and/or palliative care.[1]
- **Indeterminate results:** In rare instances (< 2%), a test result of indeterminate is reported, indicating that a VSGood or VSPoor classification could not be confirmed.

- VeriStrat is not a replacement for assays designed to detect targetable oncogenic drivers (including EGFR, BRAF, ALK, ROS, MET, RET, or NTRK1/2/3).

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to VeriStrat testing for NSCLC TKI response.

National Comprehensive Cancer Network

Previous National Comprehensive Cancer Network (NCCN) guidelines for the treatment of NSCLC supported the use of proteomic tests to evaluate potential therapies in advanced NSCLC. However, likely due to technical advances, availability of next generation sequencing testing for solid tumors, and treatment options, available current NCCN (2023) guidelines no longer incorporate these proteomic tests into their NSCLC evaluation algorithms.[11]

- Previous eviCore criteria (VeriStrat Testing for NSCLC TKI Response) were largely based on the 2015 NCCN Guidelines. These recommended proteomic testing for individuals with advanced NSCLC who were either EGFR wild type or had an unknown mutation status. For these individuals, the NCCN stated that those with a “Poor” result should not be offered second-line erlotinib therapy.
- In contrast, current NCCN guidelines for NSCLC no longer include specific recommendations for proteomic testing; there is no mention of proteomic testing or the use of VeriStrat for NSCLC.

Selected Relevant Publications

The available peer-reviewed clinical validity studies assessed the predictive performance of VeriStrat-directed erlotinib therapy compared with chemotherapy in individuals who were either EGFR wild type or had an unknown EGFR mutation status and had progressed after first-line treatment. These studies do not align with the NCCN treatment pathway for individuals with EGFR wild-type or unknown EGFR status with NSCLC and progression after first-line treatment. The NCCN treatment pathways do not include erlotinib as a recommended agent in either case. For lung cancers with unknown mutational status, NCCN stated that these should be treated as though they

do not harbor driver oncogenes.[11] Therefore, to definitively establish clinical validity and predictive power, studies are needed that evaluate VeriStrat in the context of randomized controlled trials evaluating guideline-recommended therapies for NSCLC.

The overall evidence base for predictive use is also characterized by several study design limitations.[12-33] For example, VeriStrat was not used to determine treatment in the available studies and the majority of the study authors reported that treatment selection was based on standard of care. In addition, a “VSGood” result claims to identify individuals with NSCLC who are EGFR wild-type but still likely to benefit from EGFR-TKI therapy. Yet the clinical validity studies did not consistently test for EGFR variants and, consequently, the true relationship between VeriStrat results, EGFR status, and survival cannot be definitively understood.

Similar flaws to those observed in the publications assessing response to EGFR inhibitors were also observed in publications addressing more recently approved targeted therapies, including PDL1 inhibitors.

For VeriStrat to demonstrate clinical validity in individuals with NSCLC in light of the NCCN guidelines and some of the original design limitations, additional studies supporting its performance are required.

Direct clinical utility studies were not identified in the scientific literature. Examples of these would include prospective studies comparing survival outcomes in individuals who had targeted treatment selected either by VeriStrat classification or through other standard variant analysis methods (such as next-generation sequencing).

Regarding the prognostic ability of VeriStrat, the majority of the available evidence predicting disease outcomes included retrospective clinical validity studies which evaluated the test in individuals with advanced NSCLC who were treatment-naïve or had either failed first-line treatment or had a recurrence. To infer how well VeriStrat performed as a prognostic test, these studies examined the degree of association between VSGood or VSPoor scores and survival outcomes. Overall, this evidence base demonstrating the performance of VeriStrat as a prognostic test is of low quality.

A number of individual study limitations were observed that weakened the strength of the evidence base. This includes the VeriStrat score not being used to determine treatment and the variability in testing for activating variants. Also, the adjustments for variant status in survival analyses were inconsistently reported and the relationship between VeriStrat scores and overall survival (OS) as well as progression-free survival (PFS) in study populations with unknown mutational status was not clear.

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VeriStrat

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Exome Sequencing

MOL.TS.235.C
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Introduction

Exome sequencing is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Exome Sequencing (e.g., unexplained constitutional or heritable disorder or syndrome)	81415
Exome Sequencing, Comparator (e.g., parent(s), sibling(s))	81416
Exome Sequencing Re-evaluation (e.g., updated knowledge or unrelated condition/syndrome)	81417
Genomic Unity Exome Plus Analysis - Comparator	0215U
Genomic Unity Exome Plus Analysis - Proband	0214U

Criteria

Introduction

Requests for exome sequencing are reviewed using these criteria.

Exome Sequencing

- Exome sequencing (ES) is considered medically necessary when ALL of the following criteria are met:
  - The member has not had previous exome sequencing performed, AND
  - The member has not had previous genome sequencing performed, AND

- The patient and family history have been evaluated by a Board-Certified or Board-Eligible Medical Geneticist, AND
  - A clinical letter detailing the evaluation by a Geneticist is provided which includes ALL of the following information:
    - Differential diagnoses, and
    - Testing algorithm, and
    - Previous tests performed and results, and
    - A genetic etiology is the most likely explanation, and
    - Recommendation that exome sequencing is the most appropriate test, and
    - Predicted impact on member's plan of care, AND

- Patient is <21 years of age, AND
- A genetic etiology is considered the most likely explanation for the phenotype, based on ONE of the following:
  - Unexplained epileptic encephalopathy (onset before three years of age) and no prior epilepsy multigene panel testing performed, OR
  - Global developmental delay (significant delay in younger children, under age 5 years, in at least two of the major developmental domains: gross or fine motor; speech and language; cognition; social and personal development; and activities of daily living) following formal assessment by a developmental pediatrician or neurologist, OR
  - Moderate/severe/profound intellectual disability (defined by Diagnostic and Statistical Manual of Mental Disorders [DSM-5] criteria, diagnosed by 18 years of age) following formal assessment by a developmental pediatrician or neurologist, OR
  - Multiple congenital abnormalities defined by ONE of the following:
    - Two or more major anomalies affecting different organ systems*, or
    - One major and two or more minor anomalies affecting different organ systems*, OR
  - TWO of the following criteria are met:
    - major abnormality affecting at minimum a single organ system*, and/or
    - formal diagnosis of autism, and/or

- symptoms of a complex neurodevelopmental disorder (e.g., self-injurious behavior, reverse sleep-wake cycles, dystonia, ataxia, alternating hemiplegia, neuromuscular disorder), and/or
- severe neuropsychiatric condition (e.g., schizophrenia, bipolar disorder, Tourette syndrome), and/or
- period of unexplained developmental regression, and/or
- laboratory findings suggestive of an inborn error of metabolism, AND

- Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
- Clinical presentation does not fit a well-described syndrome for which first tier testing (e.g., single gene testing, comparative genomic hybridization [CGH]/chromosomal microarray analysis [CMA]) is available, AND
- Multiple targeted panels are appropriate based on the member's clinical presentation, AND
- There is a predicted impact on health outcomes including:
  - Application of specific treatments, or
  - Withholding of contraindicated treatments, or
  - Surveillance for later-onset comorbidities, or
  - Initiation of palliative care, or
  - Withdrawal of care, AND
- A diagnosis cannot be made by standard clinical work-up, excluding invasive procedures such as muscle biopsy, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

* Major structural abnormalities are generally serious enough as to require medical treatment on their own (such as surgery) and are not minor developmental variations that may or may not suggest an underlying disorder.

Prenatal Exome

Testing of a fetus using exome sequencing is considered medically necessary when ALL of the following criteria are met:

- Standard diagnostic genetic testing (CMA and/or karyotype) of the fetus has been performed and is uninformative
- Testing is performed on direct amniotic fluid/chorionic villi, cultured cells from amniotic fluid/chorionic villi or DNA extracted from fetal blood or tissue
- At least one of the following is present:

- Multiple fetal structural anomalies affecting unrelated organ systems
- Fetal hydrops of unknown etiology
- A fetal structural anomaly affecting a single organ system and family history strongly suggests a genetic etiology

Genomic Unity Exome Plus Analysis (CPT: 0214U and 0215U)

The member meets the above criteria for exome sequencing, AND

The member meets criteria for whole mtDNA sequencing based on current eviCore guideline *Mitochondrial Disorders Genetic Testing* AND

The member has not had previous whole mtDNA sequencing analysis performed

Other considerations

- Exome deletion/duplication analysis (typically billed with 81228 or 81229) is considered experimental, investigational, or unproven (E/I/U).
- ES is considered E/I/U for screening for genetic disorders in asymptomatic or pre-symptomatic individuals.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- ES is not reimbursable for screening for genetic disorders in asymptomatic or pre-symptomatic individuals.
- Exome deletion/duplication analysis (typically billed with 81228 or 81229) is not reimbursable.
- ES will be considered for reimbursement only when billed with an appropriate CPT code:
  - 81415 should be billed for the proband. 81415 should only be billed when analyzing the entire exome sequence, rather than a targeted set of genes.
  - 81416 should be billed when a comparator exome is performed. A trio of the proband and both parents is generally preferred, although other family members

may be more informative based on the clinical presentation. A maximum of two units of 81416 will be considered for reimbursement.

- Re-evaluation of a previously obtained exome due to updated clinical information or expanded scientific knowledge or for the purpose of evaluating a patient for an unrelated condition/syndrome on a different date of service will be considered for reimbursement only when billed using 81417. 81417 is not reimbursable for reflex from targeted to full exome.

- 81415 is not reimbursable for a targeted exome analysis (e.g. XomeDxSlice custom gene panel completed on a single exome platform). The appropriate GSP panel code, unlisted code (e.g. 81479), or Tier 1 or Tier 2 code(s) must be billed.
- 81415 will be reimbursable once per lifetime.

What is exome sequencing?

Definition

Exome sequencing (ES/WES) utilizes DNA-enrichment methods and massively parallel nucleotide sequencing to identify disease-associated variants throughout the human genome.

- ES has been proposed for diagnostic use in individuals who present with complex genetic phenotypes suspected of having a rare genetic condition, who cannot be diagnosed by standard clinical workup, or when features suggest a broad differential diagnosis that would require evaluation by multiple genetic tests.
- The standard approach to the diagnostic evaluation of an individual suspected of having a rare genetic condition may include combinations of radiographic, biochemical, electrophysiological, and targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel.[1]
- ES may be appropriate if initial testing is unrevealing, there is no single-gene or panel test available for the particular condition, or a rapid diagnosis for a critically-ill child is indicated.[2-5]
- Identifying a molecularly confirmed diagnosis in a timely manner for an individual with a rare genetic condition can have a variety of health outcomes,[2-12] including:

  - guiding prognosis and improving clinical decision-making, which can improve clinical outcome by
    - application of specific treatments as well as withholding of contraindicated treatments for certain rare genetic conditions
    - surveillance for later-onset comorbidities
    - initiation of palliative care
    - withdrawal of care

- reducing the financial & psychological impact of diagnostic uncertainty and the diagnostic odyssey (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved)
- informing genetic counseling related to recurrence risk and prenatal or preconception (utilizing in-vitro fertilization with preimplantation genetic diagnosis) diagnosis options
- allowing for more rapid molecular diagnosis than a sequential genetic testing approach

Test information

Introduction

Exome sequencing is limited to the DNA sequence of coding regions (exons) and flanking intronic regions of the genome, which is estimated to contain 85% of heritable disease-causing variants. Results of testing with ES include known pathogenic variants definitely associated with disease or a variant of uncertain significance (VUS).[13,14]

- Pathogenic variants that can be identified by ES include missense, nonsense, splice-site, and small deletions or insertions.
- At the present time, ES typically fails to detect certain classes of disease-causing variants, such as structural variants (e.g., translocations, inversions), abnormal chromosome imprinting or methylation, some mid-size insertions and deletions (ca. 10-500 bp), trinucleotide repeat expansion mutations, deeper intronic mutations, and low-level mosaicism. The current evidence base evaluating ES to specifically identify deletions/duplications for any disease or condition is very limited, consisting mostly of small case reports [15-19] and case reviews or small uncontrolled studies.[20,21]
- ES has the advantage of decreased turnaround time and increased efficiency relative to Sanger sequencing of multiple genes.
- ES is associated with technical and analytical variability, including uneven sequencing coverage, gaps in exon capture before sequencing, as well as variability in variant classification based on proprietary filtering algorithms and potential lack of critical clinical history or family samples.[22]

Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to exome sequencing.

American College of Medical Genetics and Genomics

The American College of Medical Genetics (ACMG, 2012) stated the following regarding the clinical application of exome and genome testing:[23]

- "WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:"
  - “The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.”
  - “A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.”
  - “A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.”
  - “A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.”
  - “Prenatal diagnosis by genomic (i.e., next-generation whole-exome or whole-genome) sequencing has significant limitations. The current technology does not support short turnaround times, which are often expected in the prenatal setting. There are high rates of false positives, false negatives, and variants of unknown clinical significance. These can be expected to be significantly higher than seen when array CGH is used in prenatal diagnosis.”
- The following are recommended pretest considerations:
  - “Pretest counseling should be done by a medical geneticist or an affiliated genetic counselor and should include a formal consent process.”
  - “Before initiating WGS/WES, participants should be counseled regarding the expected outcomes of testing, the likelihood and type of incidental results that could be generated, and what results will or will not be disclosed.”
  - “As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing. In many cases, findings will include variants of unknown significance that might be the subject for research; in such instances a protocol approved by an institutional review board must be in place and appropriate prior informed consent obtained from the participant.”

The American College of Medical Genetics (ACMG, 2013) stated the following regarding informed consent for exome and genome testing:[24]

- “Before initiating GS/ES, counseling should be performed by a medical geneticist or an affiliated genetic counselor and should include written documentation of consent from the patient.”

- “Incidental/secondary findings revealed in either children or adults may have high clinical significance for which interventions exist to prevent or ameliorate disease severity. Patients should be informed of this possibility as a part of the informed consent process.”
- “Pretest counseling should include a discussion of the expected outcomes of testing, the likelihood and type of incidental results that may be generated, and the types of results that will or will not be returned. Patients should know if and what type of incidental findings may be returned to their referring physician by the laboratory performing the test.”
- “Patients should be counseled regarding the potential benefits and risks of GS/ES, the limitations of such testing, potential implications for family members, and alternatives to such testing.”
- "GS/ES is not recommended before the legal age of majority except for
    - Phenotype-driven clinical diagnostic uses;
    - Circumstances in which early monitoring or interventions are available and effective; or
    - Institutional review board-approved research."
- “As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing.”
- “Patients should be informed as to whether individually identifiable results may be provided to databases, and they should be permitted to opt out of such disclosure.”
- “Patients should be informed of policies regarding re-contact of referring physicians as new knowledge is gained about the significance of particular results.”

The American College of Medical Genetics (ACMG, 2021) published an updated guideline for the reporting of secondary findings (SF) in clinical exome and genome sequencing. They stated:[25]

- “ The overall goal of the SFWG [Secondary Findings Working Group] is to recommend a minimum list of genes that places limited excess burden on patients and clinical laboratories while maximizing the potential to reduce morbidity and mortality when ES/GS is being performed.”
- “Variants of uncertain significance should not be reported in any gene.”
- “It is important to reiterate here that use of the SF results should not be a replacement for indication-based diagnostic clinical genetic testing.”
- A table of “ACMG SF v3.0 gene and associated phenotypes recommended for return as secondary findings from clinical exome and genome sequencing” was provided
- “Given the increase in uptake of clinical ES/GS, the ACMG SFWG and BOD [Board of Directors] have agreed the list of recommended genes should now be updated annually.”

The American College of Medical Genetics and Genomics (ACMG, 2020) issued an educational Points to Consider Statement addressing good process, benefits, and limitations of using exome sequencing in the prenatal setting.[26]

Evidence for the clinical utility of ES in individuals with multiple congenital anomalies and/or a neurodevelopmental phenotype includes numerous large case series. Relevant outcomes include improved clinical decision making (e.g., application of specific treatments, withholding of contraindicated treatments, changes to surveillance), changes in reproductive decision making, and resource utilization. ES serves as a powerful diagnostic tool for individuals with rare genetic conditions in which the specific genetic etiology is unclear or unidentified by standard clinical workup.[10,27-32]

- The average diagnostic yield of ES is 20-40% depending on the individual's age, phenotype, previous workup, and number of comparator samples analyzed.[8,11,27,33] Among individuals with a pathogenic or likely pathogenic findings by ES, 5-7% received a dual molecular diagnosis (i.e., two significant findings associated with non-overlapping clinical presentations).[27,33]
- The use of family trio ES reduces the rate of uncertain findings, adds to the clinical sensitivity with regard to the interpretation of clinically novel genes, and increases the diagnostic utility of ES. For example, in three publications the positive rate ranges from 31-37% in patients undergoing trio analysis compared to 20-23% positive rate among proband-only ES.[5,27,34,35]
- Re-evaluation of previously obtained exome sequence has the potential for additional diagnostic yield because of constant expansions of existing variant databases, as well as periodic novel gene discovery.[36-38]
- A 2020 systematic evidence-based review by the ACMG on "outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability" stated:[39]
    - "There is evidence that ES/GS for patients with CA/ DD/ID informs clinical and reproductive decision-making, which could lead to improved outcomes for patients and their family members. Further research is needed to generate evidence regarding health outcomes to inform robust guidelines regarding ES/GS in the care of patients with CA/DD/ID."[39]

ACMG (2021) published a clinical guideline on the use of exome and genome sequencing in the pediatric population that stated:[40]

- "We strongly recommend ES [exome sequencing] and GS [genome sequencing] as a first-tier or second-tier test (guided by clinical judgment and often clinician–patient/family shared decision making after CMA or focused testing) for patients with one or more CAs prior to one year of age or for patients with DD/ID with onset prior to 18 years of age."
- "Consistent with existing guidelines/recommendations/position statements, patients with clinical presentations highly suggestive of a specific genetic diagnosis should undergo targeted testing first."

- "Isolated autism without ID or congenital malformation is formally out of scope for this recommendation but evaluation of exome/genome studies is ongoing."
- Diagnostic yield of genome-wide sequencing was determined to be outside the scope of the systematic evidence review.

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2018; Reaffirmed 2023) stated the following in a technology assessment for modern genetics in obstetrics and gynecology:[41]

- "The American College of Medical Genetics and Genomics recommends considering whole-exome sequencing when specific genetic tests available for a phenotype, including targeted sequencing tests, have failed to arrive at a diagnosis in a fetus with multiple congenital anomalies suggestive of a genetic disorder."

The 2020 guidelines for management of stillbirth stated:[42]

- "Microarray is the preferred method of evaluation for these reasons but, due to cost and logistic concerns, karyotype may be the only method readily available for some patients. In the future, whole exome sequencing or whole genome sequencing may be part of the stillbirth workup, but it is not currently part of the standard evaluation."

American College Obstetricians and Gynecologists and Society for Maternal Fetal Medicine

A joint statement, the American College of Obstetricians and Gynecologists (ACOG, 2016) and the Society for Maternal Fetal Medicine (SMFM, 2016) stated the following regarding prenatal ES.[43]

- "The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published."

International Society for Prenatal Diagnosis

The International Society for Prenatal Diagnosis (2022) updated position statement on the use of prenatal genome-wide sequencing stated:[44]

- "Although wider integration of genome-wide sequencing into prenatal care is now considered appropriate for specific indications, it remains a complex test, particularly when used clinically for prenatal diagnosis of fetuses with suspected genetic disorders."
- "There is still limited genotype-phenotype correlation for the genetic disorders identified in the fetal period. Since ultrasound and/or MRI imaging is frequently limited, the fetal phenotypes of many conditions have not been well described and new fetal phenotypes for conditions recognized postnatally are now being identified."

- "There is no universal consensus on the management of IF [incidental findings] and SF [secondary findings] and each center should convey their policy detailing whether they are or are not reported, and if reported what is included for parents and fetus."
- Data support benefit of prenatal genomic analysis for clinical indications such as multiple congenital anomalies with a negative microarray and previous undiagnosed fetus with major or multiple anomalies. Routine prenatal genomic testing (by parental request) is not supported by the current body of evidence.

International Society for Prenatal Diagnosis, Society for Maternal Fetal Medicine, and Perinatal Quality Foundation

A joint statement from the International Society for Prenatal Diagnosis (ISPD, 2018), the Society for Maternal Fetal Medicine (SMFM, 2018), and the Perinatal Quality Foundation (PQF, 2018) on prenatal ES stated:[45]

- "The routine use of prenatal [genome wide] sequencing as a diagnostic test cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls. Prospective studies with adequate population numbers for validation are needed…. Currently, it is ideally done in the setting of a research protocol. Alternatively, sequencing may be performed outside a research setting on a case-by-case basis when a genetic disorder is suspected for which a confirmatory genetic diagnosis can be obtained more quickly and accurately by sequencing. Such cases should be managed after consultation with and under the expert guidance of genetic professionals working in multidisciplinary teams with expertise in the clinical diagnostic application of sequencing, including interpretation of genomic sequencing results and how they translate to the prenatal setting, as well as expertise in prenatal imaging and counseling."
- "There is currently limited genotype-phenotype correlation for the genetic disorders identified in the fetal period because ultrasound imaging is frequently limited, and the fetal phenotypes of many conditions have not been well described."

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Genome Sequencing

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Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis	81425
Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)	81426
Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)	81427
Genomic Unity Whole Genome Analysis - Comparator	0213U
Genomic Unity Whole Genome Analysis - Proband	0212U
Praxis Combined Whole Genome Sequencing and Optical Genome Mapping	0267U
Praxis Whole Genome	0265U
RCIGM Rapid Whole Genome Sequencing	0094U
RCIGM Rapid Whole Genome Sequencing, Comparator Genome	0425U
RCIGM Ultra-Rapid Whole Genome Sequencing	0426U

Criteria

Introduction

Requests for genome sequencing (GS) are reviewed using the following criteria.

Genome Sequencing

Genome sequencing (GS) is considered medically necessary when ALL of the following criteria are met:

- The member has not had previous exome sequencing performed, AND
- The member has not had previous genome sequencing performed, AND
- The patient and family history have been evaluated by a Board-Certified or Board-Eligible Medical Geneticist, AND
- A clinical letter detailing the evaluation by a Geneticist is provided which includes ALL of the following information:
    - Differential diagnoses, and
    - Testing algorithm, and
    - Previous tests performed and results, and
    - A genetic etiology is the most likely explanation, and
    - Recommendation that genome sequencing is the most appropriate test, and
    - Predicted impact on member's plan of care, AND
- Patient is <21 years of age, AND
- A genetic etiology is considered the most likely explanation for the phenotype, based on ONE of the following:
    - Unexplained epileptic encephalopathy (onset before three years of age) and no prior epilepsy multigene panel testing performed, OR
    - Global developmental delay (significant delay in younger children, under age 5 years, in at least two of the major developmental domains: gross or fine motor; speech and language; cognition; social and personal development; and activities of daily living) following formal assessment by a developmental pediatrician or neurologist, OR
    - Moderate/severe/profound intellectual disability (defined by Diagnostic and Statistical Manual of Mental Disorders [DSM-5] criteria, diagnosed by 18 years of age) following formal assessment by a developmental pediatrician or neurologist, OR
    - Multiple congenital abnormalities defined by ONE of the following:
        - Two or more major anomalies affecting different organ systems*, or

        - One major and two or more minor anomalies affecting different organ systems*, OR
    - TWO of the following criteria are met:
        - major abnormality affecting at minimum a single organ system*, and/or
        - formal diagnosis of autism, and/or
        - symptoms of a complex neurodevelopmental disorder (e.g., self-injurious behavior, reverse sleep-wake cycles, dystonia, ataxia, alternating hemiplegia, neuromuscular disorder), and/or
        - severe neuropsychiatric condition (e.g., schizophrenia, bipolar disorder, Tourette syndrome), and/or
        - period of unexplained developmental regression, and/or
        - laboratory findings suggestive of an inborn error of metabolism, AND
- Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
- Clinical presentation does not fit a well-described syndrome for which first tier testing (e.g., single gene testing, comparative genomic hybridization [CGH]/chromosomal microarray analysis [CMA]) is available, AND
- Multiple targeted panels are appropriate based on the member's clinical presentation, AND
- There is a predicted impact on health outcomes including:
    - Application of specific treatments, or
    - Withholding of contraindicated treatments, or
    - Surveillance for later-onset comorbidities, or
    - Initiation of palliative care, or
    - Withdrawal of care, AND
- A diagnosis cannot be made by standard clinical work-up, excluding invasive procedures such as muscle biopsy, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

*Major structural abnormalities are generally serious enough as to require medical treatment on their own (such as surgery) and are not minor developmental variations that may or may not suggest an underlying disorder.

CPT: 0212U, 0213U, 0265U

- The member meets the above criteria for genome sequencing, AND

- The member meets criteria for whole mtDNA sequencing based on current eviCore guideline, *Mitochondrial Disorders Genetic Testing,* AND
- The member has not had previous whole mtDNA sequencing analysis performed, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Rapid Whole Genome Sequencing (rWGS)

The following criteria apply for individuals who are **inpatient** at the time of testing.

rWGS is considered medically necessary for the evaluation of acutely-ill infants 12 months of age or younger when ALL of the following criteria are met:

- The member has not had previous exome sequencing performed, AND
- The member has not had previous genome sequencing performed, AND
- The patient and patient's family history have been evaluated by a Board Certified or Board-Eligible Medical Geneticist, AND
- The etiology of the infant's features is not known and a genetic etiology is considered a likely explanation for the phenotype, based on EITHER of the following:
    - Multiple congenital abnormalities affecting unrelated organ systems, or
    - TWO of the following criteria are met:
        - abnormality affecting at minimum a single organ system
        - encephalopathy
        - symptoms of a complex neurodevelopmental disorder (e.g., dystonia, hemiplegia, spasticity, epilepsy, hypotonia)
        - family history strongly suggestive of a genetic etiology, including consanguinity
        - laboratory findings suggestive of an inborn error of metabolism
        - abnormal response to therapy, AND
- Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection, isolated prematurity), AND
- Clinical presentation does not fit a well-described syndrome for which rapid single-gene or targeted panel testing is available, AND
- A diagnosis cannot be made in a timely manner by standard clinical evaluation or laboratory testing, excluding invasive procedures such as muscle biopsy, AND
- Predicted impact on health outcomes, including immediate impact on medical management based on the molecular results, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Exclusions and Other Considerations:

- Trio samples are preferred.
- rWGS is considered not medically necessary in individuals with the following diagnoses:
  - Isolated transient neonatal tachypnea
  - Isolated unconjugated hyperbilirubinemia
  - Isolated hypoxic ischemic encephalopathy with clear precipitating event
  - Isolated meconium aspiration
  - Isolated prematurity
  - Infection/sepsis with normal response to therapy
- GS or rWGS used for prenatal diagnosis is considered not medically necessary.
- GS and rWGS are considered E/I/U for screening for genetic disorders in asymptomatic or pre-symptomatic individuals.
- GS combined with Optical Genome Mapping (e.g. 0267U) is considered experimental, investigational, or unproven (E/I/U).

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Prenatal diagnosis by genome sequencing (GS) is not reimbursable.
- GS is not reimbursable for screening for genetic disorders in asymptomatic or pre-symptomatic individuals.
- Genome deletion/duplication analysis (typically billed with 81228 or 81229) is not separately reimbursable.
- GS will be considered for reimbursement only when billed with an appropriate CPT code:
  - 81425 should be billed for the proband. 81425 should only be billed when analyzing the entire genome sequence, rather than a targeted set of genes.

  - 81426 should be billed when a comparator genome is performed. A trio of the proband and both parents is generally preferred, although other family members may be more informative based on the clinical presentation. A maximum of two units of 81426 will be considered for reimbursement.
  - 81427 should be billed for re-evaluation of a previously obtained genome due to updated clinical information or expanded scientific knowledge or for the purpose of evaluating a patient for an unrelated condition/syndrome on a different date of service. 81427 is not reimbursable for reflex from targeted to full genome.

- 81425 is not reimbursable for a targeted genome analysis. If targeted analysis is performed, the appropriate GSP panel code, unlisted code (e.g. 81479), or Tier 1 or Tier 2 code(s) must be billed instead.
- 81425 will be reimbursable once per lifetime.

What is genome sequencing?

Definition

Genome sequencing (WGS or GS) utilizes DNA-enrichment methods and massively parallel nucleotide sequencing to identify disease-associated variants throughout the human genome.

- GS has been proposed for diagnostic use in individuals who present with complex genetic phenotypes suspected of having a rare genetic condition, who cannot be diagnosed by standard clinical workup, or when features suggest a broad differential diagnosis that would require evaluation by multiple genetic tests.
- The standard approach to the diagnostic evaluation of an individual suspected of having a rare genetic condition may include combinations of radiographic, biochemical, electrophysiologic, and targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel.[1]
- Broad genomic testing is typically not an appropriate first-tier test, but can be appropriate if initial testing is unrevealing, or if there is no single-gene or panel test available for the particular condition.[2]
- Identifying a molecularly confirmed diagnosis in a timely manner for an individual with a rare genetic condition can have a variety of health outcomes,[2-9] including:
  - guiding prognosis and improving clinical decision-making, which can improve clinical outcome by
    - application of specific treatments as well as withholding of contraindicated treatments for certain rare genetic conditions
    - surveillance for later-onset comorbidities
    - initiation of palliative care

    - withdrawal of care
  - reducing the financial and psychological impact of diagnostic uncertainty and the diagnostic odyssey (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved)
  - informing genetic counseling related to recurrence risk and prenatal or preconceptional (utilizing in-vitro fertilization with preimplantation genetic diagnosis) diagnosis options
  - allowing for more rapid molecular diagnosis than a sequential genetic testing approach

Test information

- Both coding (exons) and noncoding (introns) regions are analyzed by GS.[10] Often, coding regions are first analyzed by GS. If no pathogenic mutations are found, the noncoding regions are then analyzed.[10]
- Pathogenic variants that can be identified by GS include missense, nonsense, splice-site, and small deletions or insertions. “Data can also be examined for copy-number variants (CNVs) or structural variants that may either be outside of the coding regions or more easily detected using GS due to increased quantitative accuracy.”[10]
- GS currently is “the most costly technology with the least average depth of coverage, although these limitations are likely to diminish in the future.”[10]

Guidelines and evidence

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2021) published a guideline on the use of exome and genome sequencing in the pediatric population that stated:[11]

- “We strongly recommend ES [exome sequencing] and GS [genome sequencing] as a first-tier or second-tier test (guided by clinical judgment and often clinician–patient/family shared decision making after CMA or focused testing) for patients with one or more CAs prior to one year of age or for patients with DD/ID with onset prior to 18 years of age.”
- “Consistent with existing guidelines/recommendations/position statements, patients with clinical presentations highly suggestive of a specific genetic diagnosis should undergo targeted testing first.”
- “Isolated autism without ID or congenital malformation is formally out of scope for this recommendation but evaluation of exome/genome studies is ongoing.”

- Diagnostic yield of genome-wide sequencing was determined to be outside the scope of the systematic evidence review.

ACMG (2012) stated the following regarding informed consent for exome and genome testing:[12]

- “Before initiating GS/ES, counseling should be performed by a medical geneticist or an affiliated genetic counselor and should include written documentation of consent from the patient.”
- “Incidental/secondary findings revealed in either children or adults may have high clinical significance for which interventions exist to prevent or ameliorate disease severity. Patients should be informed of this possibility as a part of the informed consent process.”
- “Pretest counseling should include a discussion of the expected outcomes of testing, the likelihood and type of incidental results that may be generated, and the types of results that will or will not be returned. Patients should know if and what type of incidental findings may be returned to their referring physician by the laboratory performing the test.”
- "GS/ES is not recommended before the legal age of majority except for:
    - Phenotype-driven clinical diagnostic uses
    - Circumstances in which early monitoring or interventions are available and effective; or
    - Institutional review board–approved research."
- “As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing.”
- “Patients should be as to whether individually identifiable results may be provided to databases, and they should be permitted to opt out of such disclosure.”
- “Patients should be informed of policies regarding re-contact of referring physicians as new knowledge is gained about the significance of particular results.”

ACMG (2021) published guidelines for the reporting of incidental findings in clinical exome and genome sequencing that stated:[13,14]

- “Variants classified as likely pathogenic and pathogenic variants should be reported. Variants of uncertain significance, likely benign, and benign variants should not be reported as a secondary finding.”
- This guideline includes a table of “ACMG SF v3.0 genes and associated phenotypes recommended for return from clinical exome and genome sequencing”.

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2018; Reaffirmed 2023) stated the following in a technology assessment for modern genetics in obstetrics and gynecology:[15]

- "The American College of Medical Genetics and Genomics recommends considering whole-exome sequencing when specific genetic tests available for a phenotype, including targeted sequencing tests, have failed to arrive at a diagnosis in a fetus with multiple congenital anomalies suggestive of a genetic disorder."

The 2020 guidelines for management of stillbirth stated:[16]

- "Microarray is the preferred method of evaluation for these reasons but, due to cost and logistic concerns, karyotype may be the only method readily available for some patients. In the future, whole exome sequencing or whole genome sequencing may be part of the stillbirth workup, but it is not currently part of the standard evaluation."

American College Obstetricians and Gynecologists and Society for Maternal Fetal Medicine

A joint statement, the American College of Obstetricians and Gynecologists (ACOG, 2016) and the Society for Maternal Fetal Medicine (SMFM, 2016) stated the following regarding prenatal ES.[17]

- "The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published."

International Society for Prenatal Diagnosis

The International Society for Prenatal Diagnosis (2022) updated position statement on the use of prenatal genome-wide sequencing stated:[18]

- “Although wider integration of genome-wide sequencing into prenatal care is now considered appropriate for specific indications, it remains a complex test, particularly when used clinically for prenatal diagnosis of fetuses with suspected genetic disorders.”
- “There is still limited genotype-phenotype correlation for the genetic disorders identified in the fetal period. Since ultrasound and/or MRI imaging is frequently limited, the fetal phenotypes of many conditions have not been well described and new fetal phenotypes for conditions recognized postnatally are now being identified.”
- “There is no universal consensus on the management of IF [incidental findings] and SF [secondary findings] and each center should convey their policy detailing whether they are or are not reported, and if reported what is included for parents and fetus.”

- Data support benefit of prenatal genomic analysis for clinical indications such as multiple congenital anomalies with a negative microarray and previous undiagnosed fetus with major or multiple anomalies. Routine prenatal genomic testing (by parental request) is not supported by the current body of evidence.

International Society for Prenatal Diagnosis, Society for Maternal Fetal Medicine, and Perinatal Quality Foundation

A joint statement from the International Society for Prenatal Diagnosis (ISPD, 2018), the Society for Maternal Fetal Medicine (SMFM, 2018), and the Perinatal Quality Foundation (PQF, 2018) on prenatal ES stated:[19]

- "The routine use of prenatal [genome wide] sequencing as a diagnostic test cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls. Prospective studies with adequate population numbers for validation are needed…. Currently, it is ideally done in the setting of a research protocol. Alternatively, sequencing may be performed outside a research setting on a case-by-case basis when a genetic disorder is suspected for which a confirmatory genetic diagnosis can be obtained more quickly and accurately by sequencing. Such cases should be managed after consultation with and under the expert guidance of genetic professionals working in multidisciplinary teams with expertise in the clinical diagnostic application of sequencing, including interpretation of genomic sequencing results and how they translate to the prenatal setting, as well as expertise in prenatal imaging and counseling."
- "There is currently limited genotype-phenotype correlation for the genetic disorders identified in the fetal period because ultrasound imaging is frequently limited, and the fetal phenotypes of many conditions have not been well described."

Selected Relevant Publications

The clinical utility of prenatal genomic sequencing is currently lacking. Multiple recent reviews cite a need for further research to better understand the impact of testing in the clinical setting.[20-23] Future studies are needed to "better understand which pregnancies benefit most and how to prioritise cases in a way that maximizes benefit" and to "determine the extent to which prenatal genomic sequencing results actually alter perinatal care."[23,24]

Prenatal genomic sequencing aims to improve reproductive decision-making, allow for more informed pregnancy and perinatal management options, and reduce morbidity and mortality.[22,23] However, there is a paucity of well-designed studies examining the use of prenatal WGS, and routine usage cannot be recommended due to insufficient data and a need to address several complex clinical scenarios that may arise during clinical use.[21,23,24]

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Administrative Guidelines

Medical Necessity Review Information Requirements

MOL.AD.304.A
v2.0.2024

Introduction

This guideline addresses the minimum information needed to perform a medical necessity review of laboratory testing.

Description

In order to accurately and effectively conduct medical necessity reviews, certain information is necessary when the case is submitted. This guideline outlines the information that is required to conduct a medical necessity review.

This information must be provided before applicable medical necessity criteria can be applied. If the below information is not received, the testing will be denied, as medical necessity cannot be determined.

Criteria

The following information must be submitted to perform a medical necessity review for any test:

- Details about the test being performed (test name, description, and/or unique identifier), and
- Laboratory that will be performing the test, and
- All CPT codes and units that will be billed related to the entire test, and
- Clinical information, which may include:
    - All information required by applicable policy, or
    - Test indication, including any applicable signs and symptoms or other reasons for testing, and
    - Any applicable test results (laboratory, imaging, pathology, etc.), and
    - Any applicable family history, and
    - How test results will impact patient care

When procedure codes are not provided with the request, code(s) will be assigned by the eviCore Laboratory Management Program based on one of the following methods:

- Any documentation provided with the request and/or publicly available on the laboratory's website will be used to assign the code(s), or

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- If documentation is neither provided with the request, nor readily available on the laboratory's website, the most appropriate code(s) will be assigned according to relevant clinical guidelines or the guideline *Laboratory Procedure Code Requirements*, or
- If appropriate code(s) are unable to be identified with the above methods, an unlisted molecular pathology code (81479) will be assigned as a placeholder

Information Requirements

Date of Service and Authorization Period Effective Date

MOL.AD.314.A
v2.0.2024

Introduction

This guideline addresses the date of service (DOS) and effective date of the authorization period for laboratory testing.

Description

The DOS for a laboratory test or service is generally deemed to be either the date of specimen collection or the date of retrieval for archived specimens. This guideline outlines the rules for establishing the laboratory test DOS and the resultant effective date of the authorization for testing.

Criteria

The following rules and definitions outline a laboratory test or service billing DOS:

- Date of Service (DOS)
  - The DOS for clinical diagnostic laboratory tests or services is generally the date the specimen is collected (collection date).[1]
  - An archived specimen is defined as a previously collected specimen that has been stored for more than 30 calendar days prior to testing (e.g. a tumor sample obtained from previous biopsy, isolated DNA that has been in frozen storage, etc.). The DOS for archived specimens is the date the specimen was removed from storage (retrieval date).[2]
  - Specimens stored for 30 days or less are required to use the date the specimen was collected (collection date) for the DOS.
- Authorization Effective Date
  - The effective date of the authorization for testing is established by the DOS, as determined by the collection or retrieval date (see above criteria).
  - Tests or services submitted for medical necessity determination prior to the DOS will use the case determination date as the authorization effective date.
    - Case determination date is defined as the decision date of the medical necessity determination.
- Authorization Time Period

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  - The time period of the authorization (i.e., the number of days between the authorization effective date and the expiration date) is established per health plan policy or regulatory authority.
- Medical Necessity Determinations
  - Medical necessity determinations are conducted using coverage criteria for tests or services outlined within the appropriate clinical guideline.
  - The DOS of the requested tests or services determine whether eviCore's clinical guidelines will be used (DOS on or after the health plan's effective date for utilization management services by eviCore) or the health plan's policies will be used (DOS prior to the health plan's effective date for utilization management services by eviCore).
  - The DOS will also be used to establish which version of a guideline is used for the medical necessity determination, based upon the specific guideline version's effective date.
- Billable Event
  - Standard laboratory billing practices define the billable event at the time when valid test results are generated AND the test report is provided to the ordering physician.
  - Depending on the test, the assay may take multiple days for results to generate.
  - Consequently, pre-service requests for medical necessity determination are permitted at any time prior to claim submission to the health plan (the billable event).

References

Introduction

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DOS and Effective Date

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Laboratory Procedure Code Requirements

MOL.AD.391.A
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Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Molecular Pathology	81105 - 81479
Molecular Multianalyte Assays with Algorithmic Analyses (MAAA)	81490 - 81599; Molecular* administrative MAAA codes (ending in M)
Molecular Proprietary Laboratory Analyses (PLA)	Molecular* PLA codes (ending in U)
Molecular Infectious Testing	Molecular tests* within range 87149 – 87912 0500T G0476
Molecular HCPCS Codes	S3800 - S3870 G0452, G0327, G9143 U0001-U0004
Molecular Cytopathology Procedures (Flow Cytometry, In Situ Hybridization)	88120 - 88121 88182 - 88199
Cytogenetics	88230 - 88299
Molecular Surgical Pathology Procedures (Immunohistochemistry, In Situ Hybridization)	88341 - 88344 88360 - 88361 88364 –88377 88380 - 88388
Other Molecular Codes	86152 86153

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Note *Generally defined as codes that include “DNA”, “RNA”, “nucleic acid”, “genotype”, “phenotype” or related language in the code description.

Description

The administrative handling of procedure coding by the eviCore Laboratory Management Program is addressed by this guideline. It is intended to augment other clinical and administrative guidelines and does not represent all possible procedure code requirements. The assessment of medical necessity of tests requested with these codes is addressed separately.

What Are Laboratory Procedure Codes

Introduction

Common Procedural Terminology (CPT) codes are five-digit codes developed by the American Medical Association (AMA), and intended to report a wide range of tests and procedures.

The AMA issues guidance regarding the appropriate use of CPT codes in the AMA CPT Professional manual. CPT codes that represent lab testing are generally published in the Pathology and Laboratory section and/or Appendix O of the CPT manual.[1]

The Healthcare Common Procedure Coding System (HCPCS) is the system used by the Centers for Medicare & Medicaid Services (CMS) to ensure consistent coding of claims for Medicare and other health insurance programs. Level I of the HCPCS simply utilizes the CPT coding system. In contrast, level II of the HCPCS includes alpha-numeric codes that describe drugs, supplies, and services that are not addressed by the CPT codes. These level II codes are developed and maintained by CMS. Various HCPCS codes beginning with "G", "P", "Q", "S", and "U" may apply to laboratory procedures. These codes are allowed to be used by non-Medicare insurers.[2]

Guidelines and Evidence

Introduction

This section includes relevant billing and coding guidance for laboratory testing.

The National Correct Coding Initiative (NCCI)

CMS provides Pathology/Laboratory Services coding guidance in chapter 10 of the NCCI Policy Manual, which is often broadly adopted by other non-CMS payers.[3]

The NCCI Policy Manual's general guidance for laboratory procedure codes includes, but is not limited to, the following:

- "Providers/suppliers shall report the HCPCS/CPT code that describes the procedure performed to the greatest specificity possible. A Healthcare Common Procedure Coding System/Current Procedural Terminology (HCPCS/CPT) code shall be reported only if all services described by the code are performed. A provider/supplier shall not report multiple HCPCS/CPT codes if a single HCPCS/CPT code exists that describes the services. This type of unbundling is incorrect coding."[3]
- "HCPCS/CPT codes include all services usually performed as part of the procedure as a standard of medical/surgical practice. A provider/supplier shall not separately report these services simply because HCPCS/CPT codes exist for them." [3]
- "The "CPT Manual" also includes coding instructions which may be found in the "Introduction", individual chapters, and appendices. In individual chapters, the instructions may appear at the beginning of a chapter, at the beginning of a subsection of the chapter, or after specific CPT codes. Providers/suppliers should follow "CPT Manual" instructions unless the CMS has provided different coding or reporting instructions."[3]
- "Medicare does not pay for duplicate testing. Multiple tests to identify the same analyte, marker, or infectious agent shall not be reported separately. For example, it would not be appropriate to report both direct probe and amplified probe technique tests for the same infectious agent." [3]
- "If a laboratory procedure produces multiple reportable test results, only a single HCPCS/CPT code shall be reported for the procedure. If there is no HCPCS/CPT code that describes the procedure, the laboratory shall report a miscellaneous or unlisted procedure code with a single unit of service." [3]

Procedure Coding Guidelines by Category

The AMA organizes their laboratory CPT codes into the categories listed below.[1]

- CPT Codes 80047-80081: Organ or Disease Oriented Panels
- CPT Codes 80143-80299: Drug Assay
- CPT Codes 80305-83992: Therapeutic Drug Assays
- CPT Codes 80400-80439: Evocative/Suppression Testing Procedures
- CPT Codes 80503-80506: Pathology Clinical Consultations
- CPT Codes 81000-81099: Urinalysis Procedures
- CPT Codes 81105-81408; 81479: Molecular Pathology
- CPT Codes 81410-81471: Genomic Sequencing Procedures (GSP) and Other Molecular Multianalyte Assays
- CPT Codes 0002M-81599: Multianalyte Assays with Algorithmic Analyses (MAAA)
- CPT Codes 82009-84999: Chemistry Procedures
- CPT Codes 85002-85999: Hematology and Coagulation Procedures

- CPT Codes 86000-86849: Immunology Procedures
- CPT Codes 86850-86999: Transfusion Medicine Procedures
- CPT Codes 87003-87999: Microbiology Procedures
- CPT Codes 88000-88099: Anatomic Pathology Procedures
- CPT Codes 88104-88199: Cytopathology Procedures
- CPT Codes 88230-88299: Cytogenetic Studies
- CPT Codes 88300-88399: Surgical Pathology Procedures
- CPT Codes 88720-88749: In Vivo [eg, Transcutaneous] Laboratory Procedures
- CPT Codes 89049-89240: Other Pathology and Laboratory Procedures
- CPT Codes 89250-89398: Reproductive Medicine Procedures
- CPT Codes ending in "U": Proprietary Laboratory Analyses (PLA) Codes

A comprehensive review of all laboratory and pathology procedure coding guidelines is beyond the scope of this guideline. A discussion of coding guidelines that have particular relevance for eviCore's management of these procedure code categories can be found below.

Molecular Pathology Procedures (81105-81408; 81479)

Tier 1 Molecular Pathology Codes (81105-81383) represent gene-specific and genomic procedures.[1] Only one specific molecular pathology procedure is associated with each code.

Tier 2 Molecular Pathology CPT Codes (81400-81408) are a set of CPT codes designed to represent the level of technical and interpretive effort required for a large number of molecular and genomic tests that have not been assigned a unique CPT code (i.e., are not addressed by Tier 1, GSP, MAAA, or PLA codes). Specific tests, or analytes, are assigned to these Tier 2 codes by the AMA and cannot be self-assigned by the laboratory.

The AMA codebook states that 81403 may be used to represent known familial mutation analysis when a Tier 1 code is not available; see the guideline *Genetic Testing for Known Familial Mutations* for more details on this type of test.

Unlisted Molecular Pathology Code 81479 is assigned to molecular and genomic test procedures that are not addressed by a Tier 1, Tier 2, or Genomic Sequencing Procedure (GSP) code.

The AMA codebook states the following regarding the use of these codes:

- "The molecular pathology codes include all analytical services performed in the test (eg, cell lysis, nucleic acid stabilization, extraction, digestion, amplification, and detection). Any procedures required prior to cell lysis (eg, microdissection, codes 88380 and 88381) should be reported separately."[1]

- For Tier 2 codes in particular: "Use the appropriate molecular pathology procedure level code that includes the specific analyte listed after the code descriptor. If the analyte tested is not listed under one of the Tier 2 codes or is not represented by a Tier 1 code, use the unlisted molecular pathology procedure code, 81479."[1]

The NCCI Manual states the following regarding the use of these codes:

- "Molecular pathology procedures (e.g., CPT codes 81161-81408) include all aspects of sample preparation, cell lysis, internal measures to assure adequate quantity of DNA or RNA, and performance of the assay. These procedures include DNA analysis and/or RNA analysis."[3]
- "A Tier 1 or Tier 2 molecular pathology procedure CPT code should not, in general, be reported with a genomic sequencing procedure, molecular multianalyte assay, multianalyte assay with algorithmic analysis, or proprietary laboratory analysis CPT code where the CPT code descriptor includes testing for the analyte described by the Tier 1 or Tier 2 molecular pathology code."[3]

When multiple Tier 1, Tier 2, and/or unlisted codes are billed together, this is considered a panel. The *Genetic Testing by Multigene Panels* guideline contains additional coding guidance for this type of procedure.

Genomic Sequencing Procedures (GSP) (81410-81471)

GSP codes represent DNA or RNA sequence analysis methods that simultaneously assay multiple genes or genetic regions relevant to a specific clinical situation (e.g., multi-gene panels), typically via next generation sequencing (NGS) or massively parallel sequencing (MPS).[1]

The AMA codebook states the following regarding the use of GSP codes:

- "The analyses listed below represent groups of genes that are often performed by GSPs; however, the analyses may also be performed by other molecular techniques (polymerase chain reaction [PCR] methods and microarrays). These codes should be used when the components of the descriptor(s) are fulfilled regardless of the technique used to provide the analysis, unless specifically noted in the code descriptor."[1]
- "When all of the components of the descriptor are not performed, use individual Tier 1 codes, Tier 2 codes, or 81479 (Unlisted molecular pathology procedure)."[1]

See guideline *Genetic Testing by Multigene Panels* for additional panel guidance.

Multianalyte Assays with Algorithmic Analyses (MAAA) (0002M-81599)

MAAA codes represent procedures that incorporate different types of assays, in combination with an algorithmic analysis and other patient information (if applicable), to generate a numeric score or probability. These tests are typically unique to a single laboratory.[1]

The AMA codebook states the following regarding the use of MAAA codes:

- "The results of individual component procedure(s) that are inputs to the MAAAs may be provided on the associated laboratory report, however these assays are not reported separately using additional codes."[1]
- "All MAAA codes are listed in Appendix O along with the procedure's proprietary name. In order to report a MAAA code, the analysis performed must fulfill the code descriptor and, if proprietary, must be the test represented by the proprietary name listed in Appendix O. When a specific MAAA procedure is not listed below or in Appendix O, the procedure must be reported using the Category | MAAA unlisted code (81599)."[1]
- "These codes encompass all analytical services required (eg, cell lysis, nucleic acid stabilization, extraction, digestion, amplification, hybridization and detection) in addition to the algorithmic analysis itself. Procedures that are required prior to cell lysis (eg, microdissection, codes 88380 and 88381) should be reported separately."[1]

Microbiology Procedures (87003-87999)

These codes are used to identify microorganisms such as viruses, bacteria, and other infectious agents. Some of these procedures involve the use of molecular diagnostic testing with nucleic acid probes.[1]

The NCCI manual states the following regarding the use of these codes:

- "With one exception, CMS policy prohibits separate payment for testing for a single microorganism from an anatomic site by more than one methodology. For example, if a physician performs tests for cytomegalovirus antigen at an anatomic site by immunoassay (CPT code 87332) and by nucleic acid direct probe (CPT code 87495), only one of these codes may be reported for the testing. If a culture independent diagnostic testing method is positive for a microorganism, it may be medically reasonable and necessary to additionally culture the microorganism for drug sensitivity testing or (rarely) for community surveillance identification.[3]

Proprietary Laboratory Analyses (PLA) (CPT Codes ending in "U")

PLA codes are used to describe proprietary clinical laboratory analyses that can only be provided by a single laboratory or set of providing laboratories.[1]

The AMA CPT Professional codebook states the following regarding PLA codes:

- "When a PLA code is available to report a given proprietary laboratory service, that PLA code takes precedence. The service should not be reported with any other CPT code(s) and other CPT code(s) should not be used to report services that may be reported with that specific PLA code." [1]
- "These codes encompass all analytical services required for the analysis (eg, cell lysis, nucleic acid stabilization, extraction, digestion, amplification, hybridization and detection). For molecular analyses, additional procedures that are required prior to

cell lysis (eg, microdissection [codes 88380 and 88381]) may be reported separately."[1]

Criteria: Procedure Code Review

Introduction

All procedure codes included in the Laboratory Management Program may be subject to specific coding requirements. The following define many, but not all, of the most commonly applied coding requirements under this program. Any procedure codes that do not meet these criteria will not be reimbursable, even if medical necessity criteria for the associated test(s) are met. Exceptions to these requirements will be handled on a case-by-case basis.

Correct Coding Requirements

Any procedure codes managed by the program will be subject to the requirements outlined below.

- Any test-specific coding requirements, which are generally addressed in the Billing and Reimbursement Considerations section of applicable clinical guidelines, must be met.
- NCCI coding guidance is generally adopted by eviCore in the absence of coding requirements addressed in test-specific guidelines.
- All procedure code(s) must be an accurate representation of the associated test(s). This includes:
    - The procedure code(s) must be in effect on the date of service associated with the case review or claim (please refer to the *Date of Service and Authorization Period Effective Date* guideline for details about how this date is determined for prior authorizations).
    - The test must fulfill all of the minimum requirements of the AMA/CMS code description. This includes, but is not limited to, specimen type, test content, and test methodology (e.g. sequencing, deletion/duplication analysis, targeted mutation analysis, etc.).
    - Proprietary codes (e.g., PLA codes and proprietary MAAA codes) will only be accepted when used by the single laboratory or set of providing laboratories to which the AMA has assigned the code. The code will not be accepted for use by a different laboratory, even if their test is similar in nature.
    - Tier 2 Codes (81400-81408) will only be accepted when the AMA has specifically assigned the test to the Tier 2 code. Laboratories may not self-assign tests to Tier 2 codes that are not specifically listed as analytes by the AMA.

  - GSP codes (81410-81471) will only be accepted for panels that include the minimum gene content required per the AMA descriptor. When this gene list is directly preceded by the word "including", all of the specified genes must be included on the associated panel. When the gene list is directly preceded by the abbreviation "e.g.", these genes are considered examples, and do not need to be included on the panel.
  - When a specific code that accurately describes the test is not available, the appropriate unlisted/miscellaneous procedure code should be used (e.g., 81479, 81599, etc.).

- Add-on codes (e.g. 81266, 88185, etc.) will be addressed as follows:
  - The add-on code(s) will only be reimbursed when billed with the appropriate primary code(s) on the same date of service by the same provider.
  - If none of the primary code(s) meet medical necessity criteria, any accompanying add-on code(s) will not be separately reimbursable.
- When multiple codes are submitted that address the same test content for the same date of service, only the most appropriate code(s) will be eligible for reimbursement, and the redundant/overlapping code(s) will not be reimbursable. Codes meeting medical necessity requirements will be prioritized for approval in the following manner:
  - Any guidance provided in the applicable clinical guideline will be followed, when available.
  - A proprietary code (e.g. PLA code or proprietary MAAA code) will be prioritized over non-proprietary codes when available for the providing laboratory.
  - An appropriate test-specific code will be prioritized over a non-specific code for a single gene/analyte test (e.g., a tier 1 code is prioritized over an unlisted code).
  - For procedures with multiple components, a single code will be prioritized over a combination of codes. See also the *Genetic Testing by Multi-Gene Panels* guideline for acceptable panel codes.
- When a prior authorization request is submitted for a group of procedure codes and at least one procedure code requires prior authorization, all submitted procedure codes that are under management by the Program (in any form) will be reviewed regardless of the authorization requirements for each code.

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Special Circumstances Influencing Coverage Determinations

MOL.AD.364.A
v2.0.2024

Introduction

eviCore healthcare performs independent Healthcare Technology Assessments (HTA) to assess analytical validity, clinical validity, and clinical utility of laboratory testing. These HTAs are used as the foundation for eviCore's coverage determinations and medical necessity criteria. However, there may be special circumstances, including state and federal legislation, which may override or supplement eviCore criteria. This guideline outlines special circumstances that may impact coverage determinations for certain laboratory testing.

Special Circumstances

Federal Legislation

Preventive Services Addressed by the Affordable Care Act

eviCore's position is that the Affordable Care Act does not preclude eviCore's laboratory management program from determining the medical necessity of preventive services.

While private health plans must provide coverage for such preventive services without cost sharing, these tests may be subject to medical necessity requirements. A list of preventive services covered under the regulation can be found at https://www.healthcare.gov/preventive-care-benefits.

Section 2713 of the Public Health Service Act (PHS Act), added by the Patient Protection and Affordable Care Act, as amended, states that:[1]

- "Section 2713 of the PHS Act requires coverage without cost sharing of certain preventive health services by non-grandfathered group health plans and health insurance coverage."
- "[T]o the extent not specified in a recommendation or guideline, a plan or issuer may rely on the relevant evidence base and established reasonable medical management techniques to determine the frequency, method, treatment, or setting for the provision of a recommended preventive service."

Therefore, eviCore's managed procedure codes for a Health Plan are subject to medical necessity requirements, even if the requested test is considered a preventive service.

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State Mandates

Autism Screening

eviCore's position is that because autism is a diagnosis that is made clinically based on an individual's symptoms, genetic testing is not required by state mandate unless explicitly stated.

According to the National Conference of State Legislatures (NCSL), “[m]ost states require insurers to provide coverage for the treatment of autism.” [2]

Such state mandates typically apply to the diagnosis, screening, and/or treatment of autism, for which genetic testing is not relevant, as autism is diagnosed through evaluation of an individual’s development and behaviors by an appropriate specialist (such as neurodevelopmental pediatrician or developmental-behavioral pediatrician).[3] While genetic testing may identify an underlying genetic cause for the individual’s autism, it does not diagnose autism. In addition, there is not a specific genetic test to diagnose autism, as there are numerous genetic syndromes which may include autism as a component of the condition. For example, a child who has a clinical diagnosis of autism may have genetic testing to determine if there is an underlying genetic condition, such as Fragile X Syndrome, that may explain the child’s autism. However, the genetic test is not required to make a diagnosis of autism or to treat the child’s autism. For information on the medical necessity criteria that must be met for coverage of genetic testing for autism, please refer to the guideline *Autism, Intellectual Disability, and Developmental Delay Genetic Testing*.

Delaware State Mandate: House Bill 319

House Substitute No. 1 for HB 319 "An Act to Amend Title 18 of the Delaware Code Relating to Experimental Treatment Health Insurance Coverage" states that no individual or group policy or health insurance contract:[4]

- "...shall deny coverage, payment, or reimbursement for a National Coverage Determination Service on the basis that the treatment is experimental or investigational. ... "National Coverage Determination Service" as used in this section shall mean a service, item, or test which receives reimbursement from the Centers for Medicare and Medicaid Services pursuant to the Social Security Act 1869 (f)."

A synopsis of the Delaware General Assembly House Bill 319 (HB319) states:[4]

- "This legislation creates a benchmark for determining when a treatment or service is no longer experimental or investigational. Essentially, when Medicare determines that a treatment is safe for its population, commercial insurers in Delaware may no longer deny coverage on that basis. This will remove inconsistencies for properly-evidenced treatments between payers."

Therefore the state of Delaware prohibits denial of a service as investigational/experimental if the service is coverable under CMS National Policy

outlined in an National Coverage Determination (NCD) or National Coverage Analysis (NCA). If eviCore has an experimental, investigational, or unproven (E/I/U) coverage determination for the billed procedure code(s) and CMS has a current NCD or NCA with specific coverage of that procedure code, eviCore will apply the CMS national coverage policy for non-Medicare members identified as Delaware residents. This policy application may occur during pre-service review, through automated claim edits (such as enforcing ICD requirements), or through post-service medical necessity review.

Applicable Laws

States are increasingly addressing the coverage and management of certain laboratory tests. eviCore monitors evolving legislation to ensure compliance.

These bills can generally be grouped into 3 categories:

- Broad Biomarker Bills: these bills generally govern biomarker testing across medical diagnoses
- Cancer-Specific Biomarker Bills: these bills govern biomarker testing for individuals with a medical diagnosis of cancer
- Other State Bills: these bills are specific to a type of clinical test (e.g. BRCA testing), or address the requirement for prior authorization in specific circumstances

The eviCore process for assessing these types of bills differs, and is detailed below.

Broad Biomarker Bills

The following is a list of broad biomarker bills that are applicable to laboratory testing at the time of this guideline. These bills generally address tests performed specifically for diagnosis, treatment, management, or monitoring of a disease or condition. Note that this excludes screening tests or any test that is not focused on a specific diagnosis, management, or treatment decision.

State	Bill	Effective Date	Line(s) of Business
Arizona	HB 2144[5]	January 1, 2023	Commercial
California	SB 496[6]	July 1, 2024	Commercial; Medicaid
Georgia	HB 85[7]	July 1, 2023	Commercial
Illinois	HB 1779[8]	January 1, 2022	Commercial
Kentucky	HB 180[9]	January 1, 2024	Commercial; Medicaid
Louisiana	SB 104[10]	January 1, 2024	Commercial
Maryland	HB 1217[11]	January 1, 2024	Commercial

State	Bill	Effective Date	Line(s) of Business
New Mexico	HB 73[12]	January 1, 2024	Commercial
New York	S01196A[13]	April 1, 2024	Commercial; Medicaid
Oklahoma	SB 513[14]	January 1, 2024	Commercial
Rhode Island	HB 7587[15]	January 1, 2024	Commercial
Texas	SB 989[16]	January 1, 2024	Commercial; Medicaid

Decision hierarchy criteria

When making medical necessity determinations subject to broad biomarker bill requirements, eviCore will employ the following strategy for both utilization management case reviews and automated claim edit application:

- Apply eviCore criteria and approve if possible.
- If not approvable under eviCore criteria, approve if consistent with coverable indications based on Medicare National Coverage Determinations (NCDs), Medicare Local Coverage Determinations (LCDs), U.S. Food and Drug Administration (FDA) approved tests, FDA cleared tests, indicated tests for a drug that is approved by the FDA, nationally recognized clinical practice guidelines, and/or consensus statements as defined by applicable legislation.

Cancer-Specific Biomarker Bills

The following is a list of biomarker bills specific to cancer tests that are applicable to laboratory testing at the time of this guideline. Note that this excludes screening tests or any test that is not focused on a specific diagnosis, management, or treatment decision.

State	Bill	Effective Date	Line(s) of Business
Arkansas	HB 1121[17]	July 31, 2023	Commercial
Louisiana	SB 84[18]	January 1, 2022	Commercial
Nevada	AB 155[19]	October 1, 2023	Commercial

Decision hierarchy criteria

When making medical necessity determinations subject to cancer specific biomarker bill requirements, eviCore will employ the following strategy for both utilization management case reviews and automated claim edit application:

- Purpose of the requested test is for one or more of the following:
  - early detection of cancer
  - diagnosis of cancer
  - treatment of cancer
  - appropriate management of cancer
  - ongoing monitoring of cancer
- Apply eviCore criteria and approve if possible.
- If not approvable under eviCore criteria, approve if consistent with coverable indications based on Medicare NCDs, Medicare LCDs, FDA approved tests, FDA cleared tests, indicated tests for a drug that is approved by the FDA, national guidelines, and/or consensus statements

Other Applicable Bills

The following is a list of bills applicable to laboratory testing at the time of this guideline.

- Arkansas (AR)
  - AR HB 1042 requires that health insurance issued in AR and health insurance covering AR residents on or after January 1, 2023 provide prostate cancer screening coverage for "at least one (1) screening per year for any man forty (40) years of age or older according to the National Comprehensive Cancer Network guidelines."[20]
    - In order to comply with this legislation, eviCore will use the screening requirements published in HB 1042 when evaluating the medical necessity of prostate cancer screening tests for members who live in AR or have health insurance under AR jurisdiction.
- California (CA)
  - CA SB 535 prohibits insurers that contract in CA on or after July 1, 2022 from requiring prior authorization for biomarker testing for enrollees with advanced or metastatic stage 3 or 4 cancer. Biomarker testing is defined as "a diagnostic test, such as single or multigene, of the cancer patient's biospecimen, such as tissue, blood, or other bodily fluids, for DNA or RNA alterations, including phenotypic characteristics of a malignancy, to identify an individual with a subtype of cancer, in order to guide patient treatment." The prior authorization exemption only applies to biomarker testing necessary for an FDA-approved therapy.[21]
    - In order to comply with this legislation, a minimum amount of information must be registered with eviCore to document that the member is exempt from the prior authorization process.

- Connecticut (CT)
  - CT SB 358 requires that health insurance issued in CT on or after January 1, 2023 provide coverage for "Genetic testing of the breast cancer gene one, breast cancer gene two, any other gene variant that materially increases the insured's risk for breast and ovarian cancer or any other gynecological cancer to detect an increased risk for breast and ovarian cancer when recommended by a health care provider in accordance with the United States Preventive Services Task Force recommendations for testing." USPSTF recommendations state "[t]esting for BRCA1/2 mutations should be done when an individual has personal or family history that suggests an inherited cancer susceptibility, when an individual is willing to see a health professional who is suitably trained to provide genetic counseling and interpret test results, and when test results will aid in decision -making."[22,23]
    - eviCore's guidelines are consistent with the USPSTF requirements and can be used as published to determine coverage.
- Illinois (IL)
  - IL HB 2109 requires that health insurance issued in IL on or after January 1, 2022 provide "coverage for medically necessary comprehensive cancer testing and testing of blood or constitutional tissue for cancer predisposition testing." According to the bill, "Comprehensive cancer testing" includes, but is not limited to, the following forms of testing: (1) Targeted cancer gene panels. (2) Whole-exome genome testing. (3) Whole-genome sequencing. (4) RNA sequencing. (5) Tumor mutation burden." Also, "Testing of blood or constitutional tissue for cancer predisposition testing includes, but is not limited to, the following forms of testing: (1) Targeted cancer gene panels. (2) Whole-exome genome testing. (3) Whole-genome sequencing."[24]
    - As eviCore's guidelines are evidence-based, they are consistent with these requirements, and can be used as published to determine coverage.
  - IL HB 5334 requires that health insurance issued in IL on or after January 1, 2024 provide coverage for the cost of BRCA1 and BRCA2 genetic testing when recommended by a health care provider in accordance with the United States Preventive Services Task Force's (USPSTF) recommendations for testing. USPSTF recommendations state "[t]esting for BRCA1/2 mutations should be done when an individual has personal or family history that suggests an inherited cancer susceptibility, when an individual is willing to see a health professional who is suitably trained to provide genetic counseling and interpret test results, and when test results will aid in decision -making."[23,25]
    - eviCore's guidelines are consistent with the USPSTF requirements and can be used as published to determine coverage.
  - IL HB 3817 requires that beginning January 1, 2024, the State Employee Group Insurance Program "shall provide coverage for diagnosis and treatment of infertility, including, but not limited to, in vitro fertilization, uterine embryo lavage,

embryo transfer, artificial insemination, gamete intrafallopian tube transfer, zygote intrafallopian tube transfer, and low tubal ovum transfer. The coverage required shall include procedures necessary to screen or diagnose a fertilized egg before implantation, including, but not limited to, preimplantation genetic diagnosis, preimplantation genetic screening, and prenatal genetic diagnosis."[26]

- eviCore will determine the medical necessity of laboratory infertility services for this membership group in the following manner:
  - Preimplantation Genetic Diagnosis: The eviCore Preimplantation Genetic Screening and Diagnosis guideline addresses the medically necessary indications for preimplantation genetic diagnosis. It is compliant with the legislation and will used to determine coverage for these services.
  - Preimplantation Genetic Screening: Coverable for those seeking diagnosis and treatment of infertility as defined in the legislation.
  - Prenatal Diagnosis: Coverable when related to preimplantation genetic screening or diagnosis (i.e., to confirm such results) for those seeking diagnosis and treatment of infertility as defined in the legislation.

- Louisiana (LA)
  - LA SB 154 requires that health insurance issued in LA on or after January 1, 2023 provide coverage for "traditional whole genome sequencing, rapid whole genome sequencing, and other genetic and genomic screening that includes individual sequencing, trio sequencing for a parent or parents of the infant, and ultra-rapid sequencing for an infant who is one year of age or younger, is receiving inpatient hospital services in an intensive care unit or in a pediatric care unit, and has a complex illness of unknown etiology."[27]
    - In order to comply with this legislation, eviCore will use the medical necessity criteria published in SB 154 when making medical necessity determinations for critically ill infants (as defined in the regulations) who have health insurance under LA jurisdiction.
- Pennsylvania (PA)
  - PA SB 8 requires that health insurance issued in PA on or after January 1, 2024 provide "coverage for BRCA-related genetic counseling and genetic testing… The minimum coverage required shall include all costs associated with…a genetic laboratory test of the BRCA1 and BRCA2 genes for individuals assessed to be at an increased risk, based on a clinical risk assessment tool, of potentially harmful mutations in the BRCA1 or BRCA2 genes due to a personal or family history of breast or ovarian cancer."[28]
    - In order to comply with this legislation, when reviewing cases related to BRCA1/2 testing for members with PA jurisdiction, eviCore will consider the results of clinical risk assessment tools and approve if these results indicate a lifetime risk of breast cancer greater than 20%.

- Washington (WA)
    - WSR 21-16-076, effective July 1, 2022, applies to health insurers that contract in WA as well as residents living in the state of Washington. "The purpose of WSR 21-16-076 "is to update the state board of health's (board) existing rules outlining prenatal screenings and diagnostic tests required to be covered by certain payers to align with current clinical standards and best practices."[29]
        - In order to comply with this regulation, eviCore will use the medical necessity criteria published in WSR 21-16-076 when making medical necessity determinations for the specific prenatal screening and diagnostic testing described within the regulation.
    - WA HB 1689 prohibits insurers that contract in WA on or after January 1, 2023 from requiring prior authorization for biomarker testing for enrollees with stage 3 or 4 cancer; or recurrent, relapsed, refractory, or metastatic cancer.163 Biomarker testing must "be recommended in the latest version of nationally recognized guidelines or biomarker compendia." The biomarker test must also be a covered service and prescribed by an in-network provider.[30]
        - In order to comply with this legislation, a minimum amount of information must be registered with eviCore to document that the member is exempt from the prior authorization process.

Health Plan Exclusions

Benefit Exclusions

eviCore performs medical necessity determination for any laboratory test that is within the delegated scope of management for the Health Plan (see each Plan's managed procedure code list). However, health plans set varying limitations and exclusions; eviCore's medical necessity review does not take such member-specific benefits into account. Therefore, a medical necessity approval is not a guarantee of payment. Please see the Certificate of Coverage for detail regarding benefit limitations or exclusions (e.g. screening, fertility benefits).

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Unique Test Identifiers for Non-Specific Procedure Codes

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Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
MOPATH PROCEDURE LEVEL 1	81400
MOPATH PROCEDURE LEVEL 2	81401
MOPATH PROCEDURE LEVEL 3	81402
MOPATH PROCEDURE LEVEL 4	81403
MOPATH PROCEDURE LEVEL 5	81404
MOPATH PROCEDURE LEVEL 6	81405
MOPATH PROCEDURE LEVEL 7	81406
MOPATH PROCEDURE LEVEL 8	81407
MOPATH PROCEDURE LEVEL 9	81408
UNLISTED MOLECULAR PATHOLOGY	81479
UNLISTED MAAA	81599

Description

This policy provides instruction on how to submit a unique test identifier when a procedure code is billed that does not adequately describe the performed molecular or genomic test referred to here as “non-specific procedure codes.”

Given the large and rapidly increasing number of molecular and genomic tests, many tests do not have unique procedure codes and are instead billed with non-specific procedure codes. These non-specific procedure codes generally fall into one of the following categories.

Tier 2 codes

Tier 2 Molecular Pathology codes (81400-81408) are a set of CPT codes designed to represent the level of technical and interpretive effort required for a large number of molecular and genomic tests that have not been assigned a unique CPT code

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(i.e., are not addressed by Tier 1, GSP, MAAA, PLA, etc. codes). Specific tests, or analytes, are assigned to these Tier 2 codes by the AMA a few times yearly and cannot be self-assigned by the laboratory.

The AMA publishes a set of gene abbreviations or analyte identifiers, called claim designation codes, for each test assigned to a Tier 2 code. These codes are intended to provide billing transparency such that the combination of a Tier 2 code and the applicable claim designation code on a claim form are reasonably specific to the test performed. Where the test is specific to a gene, the claim designation code is generally the standard gene name. The claim designation codes are published in the annual AMA CPT Professional codebook.[1]

Unlisted codes

If a molecular or genomic test has not been assigned to any test-specific or Tier 2 CPT code, those tests are generally billed under one of the following unlisted codes:

- 81479: Unlisted molecular pathology procedure
- 81599: Unlisted multianalyte assay with algorithmic analysis

The proper unlisted code depends on the nature of the test, but most molecular tests are best described by 81479 or 81599.

There is no publicly-available, widely-adopted source of unique codes for tests billed under unlisted codes.

The Palmetto MolDX program requires that most molecular tests be registered with the program and obtain a unique identifier (McKesson Z-Code or Palmetto Test Indicator) for the purposes of claim processing.[2] However, this identifier is both lab and test-specific and is currently primarily utilized by only certain Medicare jurisdictions.

Criteria

Unique test identifier assignment

Tier 2 AMA claim designation codes

For tests billed under a Tier 2 CPT code, the unique test identifier is the same as the original claim designation code published by the AMA when available, provided the claim designation code described only a single test assigned to that Tier 2 code. In the event that the same claim designation code described more than one test assigned to the same Tier 2 code, eviCore assigned a unique code (not the original AMA claim designation code) to at least one of these tests. When the AMA has not published a claim designation code, a unique code is developed by eviCore. No separate registration or notification process is required on the part of the laboratory.

Tier 2 special cases

Tier 2 code 81403 allows for known familial variant testing to be billed without specific gene assignment. The unique test identifier for known familial variants not otherwise specified is generally either: "KFMNOS" or the AMA assigned claim designation code for the gene if one exists with the addition of “KFM” (e.g., ATM and ATMKFM).

Unlisted codes

For tests billed under unlisted procedure codes, a unique code will be developed by eviCore. No separate registration or notification process is required on the part of the laboratory.

Obtaining a unique test identifier

When a medical necessity review is performed for a test that will be billed under a non-specific procedure code, billing instructions will include the appropriate unique test identifier if required in the determination communication.

If a medical necessity review is not performed for a test that will be billed under a non-specific procedure code, a unique test identifier can be obtained by contacting eviCore through the phone number provided by the health plan. However, most non-specific procedure codes require medical necessity determination. If pre-service medical necessity determination is required and not obtained, that requirement will take precedence over any other billing requirements.

Billing tests using non-specific procedure codes

When a unique test identifier is provided in the medical necessity determination communication, it must be included on the claim regardless of medical necessity review requirements or determination outcome. Enter the unique test identifier in one of the following narrative fields based on the type of claim being submitted:

Claim type	Electronic claim	Paper claim
Professional	837P: Enter in the 2400 SV101-7 field (Line Item Description) associated with the non-specific CPT code. Each non-specific CPT code should have a unique identifier in the associated field.	CMS-1500: Enter in box 24 in the shaded line above the service line that contains the non-specific CPT code. Each non-specific CPT code should have a unique identifier entered above it. Each test identifier should have the qualifier “ZZ” appended at the beginning (e.g., ZZBRAF) to assist in recognition of the code.

Claim type	Electronic claim	Paper claim
Institutional	837I: Enter in the 2400 SV202-7 field (Line Item Description) associated with the non-specific CPT code. Each non-specific CPT code should have a unique identifier in the associated field.	UB-04: Enter in box 80 (Remarks). Only a single non-specific CPT code should be billed per claim form due to the limitations of a single descriptive field. The test identifier should have the qualifier “ZZ” appended at the beginning (e.g., ZZBRAF) to assist in recognition of the code.

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Glossary

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Term	Definition
adenoma	An ordinarily benign neoplasm of epithelial tissue. If an adenoma becomes cancerous, it is known as an adenocarcinoma.
adenomatous polyposis coli	Adenomatous polyposis coli (APC) is a gene located on chromosome 5q. Inherited APC gene mutations are associated with Familial Adenomatous Polyposis (FAP) and Attenuated FAP. Most colorectal cancer polyps have mutations in both copies of the APC gene, even in people that don't have FAP.
adjuvant therapy	When discussing cancer treatment, adjuvant therapy is given after a primary treatment (like surgery) to increase the chances of a cure. Adjuvant therapy may include chemotherapy, radiation therapy, hormone therapy, or biological therapy.
adverse drug reaction	A harmful or unpleasant reaction to a drug that generally means the drug should be prescribed differently or avoided.
aerobic exercise	Any physical activity that causes the heart to pump faster and harder and breathing to quicken. Strengthens the heart muscle and may also help lower high blood pressure and increase good cholesterol.
AFAP	Attenuated FAP (AFAP) is a form of FAP characterized by a less dramatic proliferation of polyps (between 20-99 cumulative polyps) and age of onset for colorectal cancer of approximately 50 years. Polyps generally localize to the proximal (right-sided) colon. The American Gastroenterological Association (AGA) recommends genetic testing once a person has developed 20 or more cumulative polyps.
AFP	Short for “alpha-fetoprotein”, a substance found in pregnant women's blood. High levels of AFP are associated with risk for spina bifida and abdominal wall defects.
amniotic fluid	The protective fluid that surrounds the developing baby. This fluid fills the amniotic sac, or “bag of water” inside the mother's uterus.
ancestry	Can be represented by a family tree showing how biological family members are related to each other. It is sometimes used interchangeably with “lineage.”

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Term	Definition
anemia	A condition caused by too little oxygen in the blood, usually caused by too little hemoglobin or too few red blood cells
angina	Pain, pressure, or a feeling of indigestion in the chest caused by too little oxygen-rich blood reaching the heart. Usually caused by coronary artery disease.
anticipation	A way certain genetic diseases are inherited that causes them to get worse over the generations.
anticoagulant	Medications that prevent the blood from clotting -- often call “blood thinners.”
anticonvulsant drug	Medications used to prevent or treat seizures. Common anticonvulsant drugs include Dilantin, Zarontin, Klonopin, Valium, Tegretol, Depakote and others.
antidepressant	A medication used to prevent or treat depression. Current antidepressants categories include SSRIs, MAOIs, tricyclics, tetracyclics, and others.
antipsychotic	Medications used to treat schizophrenia, schizoaffective disorder, bipolar disorder and other conditions that distort a person's grasp of reality
antiretroviral	A medication used to treat a retrovirus infection, such as HIV
APOB	A gene for the protein that normally helps deliver LDL cholesterol to the liver to be broken down. An APOB gene mutation causes a person not to clear LDL from the body as well as usual and it builds up. APOB mutations are one cause of familial hypercholesterolemia, although LDLR mutations are the most common.
Apolipoprotein B100	ApoB100 is short for apolipoprotein B100. It is a normal protein that is a major part of “bad” cholesterol. High ApoB100 is a strong risk factor for heart disease.
aromatase inhibitor	A class of drugs used to treat postmenopausal women who have hormone-dependent breast cancer. AIs work by blocking the enzyme aromatase responsible for converting androgen to estrogen. This limits the amount of estrogen available to promote breast cancer growth.
arrhythmia	Any variation from the normal heart rate or rhythm. The heart might beat faster than usual (tachycardia), slower than usual (bradycardia), or with an unusual pattern.
artery	Blood vessels that carry oxygen-rich blood throughout the body. The coronary arteries carry blood to the heart muscle.

Term	Definition
Ashkenazi Jewish	Jewish people whose ancestors are from Eastern Europe -- mostly Germany, Poland, Russia, and some parts of France. Whereas Sephardic Jewish people have ancestry from Spain, Portugal, parts of France, Italy, North Africa, and the Middle East. Most American Jews are Ashkenazi.
atherosclerosis	A disease caused by plaque buildup inside the arteries that limits blood flow. Also called hardening of the arteries.
autosomal dominant	A pattern of inheritance where only one gene from a pair isn't working properly and causes the condition. Anyone with an autosomal dominant condition has a 50% chance of passing on the nonworking gene -- and, therefore, the condition -- to each child.
autosomal recessive	Describes a pattern of inheritance where both genes from a pair must be working abnormally to cause the condition. People with one abnormal and one normally working gene don't have the condition and are called carriers. When both parents are unaffected carriers of a condition, there is a 25% chance to have an affected child with each pregnancy.
average woman	The “average woman” is someone picked at random from the general public.
Beta-thalassemia	An inherited blood disorder that causes anemia, which is a shortage of red blood cells. This disorder causes lower than usual amounts of oxygen in the blood.
b-hCG	Short for “beta-human chorionic gonadotropin”, this substance is known as the pregnancy hormone. It is produced by the placenta.
biopsy	The process of removing tissue from living patients for diagnostic evaluation.
black box warning	A warning required by the U.S. Food and Drug Administration (FDA) on the package inserts of some prescription drugs. These are the strongest warnings from the FDA about a significant risk for serious or life-threatening complications of a drug. Black box refers to the heavy black line surrounding the warning.
blood clot	Proteins change liquid blood into a solid blood clot usually in response to an injury to prevent further blood loss. Imbalance in the clotting proteins can lead to too little or too much clotting (thrombosis). When an abnormal clot forms, it can block blood flow and cause tissue damage or death.

Term	Definition
blood clotting factor	Proteins and enzymes in the blood that control changing liquid blood into a solid blood clot. Imbalance of these factors can cause too little or too much clotting.
blood transfusion	Transferring blood or components of blood, such as blood plasma, into a patient.
blood vessel	The channels that carry blood throughout the body: arteries, veins and capillaries
bone marrow transplant	A procedure that replaces diseased or damaged bone marrow with healthy bone marrow. The damaged bone marrow may be destroyed by chemotherapy or radiation. The healthy bone marrow can come from the patient or a donor.
bowel preparation	Purging and cleansing of the bowel of fecal and other matter to assure clear evaluation of the bowel.
BRCA1	A gene located on chromosome 17 that normally produces a protein to help restrain cell growth. A harmful change in BRCA1 may predispose a person toward developing breast and/or ovarian cancer.
BRCA2	A gene located on chromosome 13 that normally produces a protein to help to restrain cell growth. A harmful change in BRCA2 may predispose a person toward developing breast and/or ovarian cancer.
breast MRI	MRI uses powerful magnets and radio waves to create detailed pictures of the breast and surrounding tissues. It provides clear pictures of parts of the breast that are difficult to see clearly on ultrasound or mammogram, but it's not a replacement for mammography.
cancer	A disease where abnormal cells grow and divide without control. Cancer cells can invade nearby tissues and spread through the bloodstream and lymphatic system to other parts of the body (called metastasis).
carbohydrate	Carbohydrates are the most abundant nutrients we eat and are broken down by the liver into glucose (sugar) to provide energy.
carcinoma	A cancer that begins in the skin or tissues that line or cover internal organs.
cardiomyopathy	A heart muscle disease that usually leads to a weakened heart muscle and a reduced ability to pump blood effectively. Any damage to the heart muscle can cause cardiomyopathy. Recognized causes include genetic factors, heart attack, alcoholism, and certain viral infections.

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Term	Definition
carrier	A person who has one copy of a changed gene and one normal copy of that gene.
CBC	An abbreviation for “complete blood count”. A standard test that provides information including the white blood cell count, red blood cell count, amount of hemoglobin, platelet count and more.
CCR5-tropic	A form of HIV virus that uses a protein on the outside of a cell, called the CCR5 receptor, to enter and infect the cell.
CD4 cells	A kind of white blood cell, also called “helper T cells”, which help protect the body against infection. These are the cells that the HIV virus infects.
cell	The basic building block of the tissues and organs in the body. Most cells have a complete copy of our genetic code and all cells are made by copying existing cells.
chelation therapy	Treatment to remove iron from the body using a chemical that attaches to heavy metals inside the body to remove them.
chemoprevention	The administration of any chemical or drug to treat a disease or condition and limit its further progress, or to prevent the condition from ever occurring.
cholesterol	A waxy, fat-like substance used by the body to make hormones, vitamin D, and other important substances. Eating too much cholesterol increases the risk of heart disease.
chromosome	A threadlike strand of DNA that carries genes and transmits hereditary information. Each chromosome can contain hundreds or thousands of individual genes. The number of chromosomes in the normal human cell is 46 (23 pairs).
chromosome translocation	A genetic condition where material from one chromosome breaks off and sticks to another chromosome, or switches places with a part of another chromosome. There are different types of translocations, and they can have different effects on health and development.
CHRPE	Congenital Hypertrophy of Retinal Pigmented Epithelium - a benign eye abnormality common in those with FAP.
close relative	A close relative is defined as a mother, father, sister, brother or child.
colectomy	The surgical removal of the colon. A total colectomy is the surgical removal of the colon and rectum. A subtotal colectomy is the surgical removal of the colon or portions of the colon only (not rectum).

Term	Definition
colon	Another name for the large intestine; the section of the large intestine extending from the cecum to the rectum. An adult colon is approximately five to six feet in length and is responsible for absorbing water and forming, storing, and expelling waste.
colonoscopy	A procedure that examines the entire rectum and colon. A colonoscope is a long, flexible, lighted tube with a tiny lens on the end used to directly examine the whole colon and look for the presence of growths. Colonoscopy is the most effective way to evaluate the inside of your entire colon for the presence of colorectal cancer or polyps. This procedure is considered “invasive,” because it requires sedation and the insertion of the colonoscope through the rectum.
colorectal cancer	Cancer that occurs in the rectum or the colon.
Comprehensive Analysis	Comprehensive Analysis is the most complete BRCA test. It looks at all the coding DNA of the BRCA1 and BRCA2 genes, to see if there are any changes or mutations. It can find: changes that are known to cause cancer, changes that are harmless, and changes whose link to cancer is unknown.
congenital heart defect	A problem with the structure of the heart, or the vessels connected to it, which is present from birth. Many types of heart defects exist. They can affect how the blood flows through the heart, or its rhythm.
corneal arcus	Also called “arcus cornealis”. An accumulation of cholesterol around the cornea (the clear front surface of the eye) that causes a grey ring around the colored part of the eye. May be a normal feature of aging, but may also be a sign of unusually high cholesterol levels.
CXCR4-tropic	A form of HIV virus that uses a protein on the outside of a cell, called the CXCR4 receptor, to enter and infect the cell.
CYP1A2	An enzyme involved in the metabolism of many drugs, including caffeine. Some people have a form of CYP1A2 that is particularly susceptible to tobacco smoke and may have adverse reactions when taking drugs metabolized by CYP1A2 while smoking.
CYP2C19	An enzyme involved in the metabolism of many drugs, including several ulcer and reflux drugs. Variants in the gene can cause adverse reactions to drugs metabolized by CYP2C19.

Term	Definition
CYP2C9	An enzyme involved in the metabolism of many drugs, including warfarin and celecoxib. and several anti-inflammatories. Variants in the gene can cause adverse reactions to drugs metabolized by CYP2C9.
CYP2D6	An enzyme involved in the metabolism of many drugs, including codeine, tamoxifen, and several antidepressants. Variants in the gene can cause adverse reactions to drugs metabolized by CYP2D6.
cytochrome P450	Cytochrome P450, abbreviated CYP450, is a large family of drug metabolizing enzymes, including CYP1A2, CYP2C9, CYP2C19, and CYP2D6.
de novo mutation	A mutation that is not running in the family yet, but occurs when a gene is damaged at conception. A de novo mutation can also then be passed on to one's children.
Desmoid tumor	Fibrous growth identified generally in the abdominal area associated with FAP and AFAP.
detection rate	Also called “sensitivity”. Refers to the likelihood that a test will actually find the condition that it is looking for. If a test has a 90% detection rate, it will find 90% (9 out of 10) of people with the condition. Most tests don't have a 100% detection rate, so you should pay attention to detection rates to understand the limitations of any test you consider.
diabetes	A disease that causes you to have too much glucose (sugar) in your blood because of a problem with the hormone insulin. People with diabetes either can't make insulin (type I) or they can't use it well enough (type II).
DNA	Stands for “deoxyribonucleic acid”. The chemical inside the nucleus of the cell that encodes the genetic instructions passed from generation to generation. Genes are made of DNA.
DNA replication	The duplication process of genetic material.
drug interaction	When a drug reacts with another drug (prescribed, over-the-counter, herbs, supplements, etc.), food, or other environmental exposure to cause an altered response. The effect may be an increased or decreased response or an adverse drug reaction.
environment	When talking about what causes disease, environment refers to basically everything that isn't controlled by genetics. Environment can include what we eat, physical activity, medications we take, chemicals we are exposed to, our physical surroundings, and countless other factors.

Term	Definition
enzyme	A protein made by the body that encourages a biochemical reaction. Humans make hundreds of different enzymes from the instructions in our genes. If any one enzyme isn't working normally, it can cause a disease.
epithelium	Membranous tissue constructed of one or more layers of cells that cover the internal and external surfaces of the body and its organs.
ethnic background	The geographical and racial identity of a person's ancestors
ethnic group	A group of people whose ancestors lived in the same region of the world, and thus, who share a common genetic background
ethnicity	A group of people who frequently share some common ancestry and are, therefore, more likely to share certain genetic traits or mutations. May be based on descending from the same geographical location, a shared religion, a tribal connection, or other cultural practices. People often belong to more than one ethnic group.
extensive metabolizer	Extensive metabolizers have two “normal” drug metabolism genes. They make the average amount of enzyme and usually have normal drug response. Most people are extensive metabolizers. People have many drug metabolism genes and can be different kinds of metabolizers for each.
false negative	A test result that is read as negative when the disease is present.
false positive	A test result that is read as positive when the disease is not present.
familial adenomatous polyposis	Familial Adenomatous Polyposis (FAP) is an inherited condition that causes the formation of hundreds to thousands of precancerous polyps within the colon, often before age 20. FAP is usually caused by an inherited mutation in one copy of the APC gene.
familial hypercholesterolemia	An inherited condition that causes people to have very high levels of LDL, or “bad”, cholesterol and a high risk for heart disease if not aggressively treated with cholesterol-lowering drugs.
family history	Family history may refer to whether or not you have any biological relative with a specific condition. It may also refer to the collective medical histories of all of your biological relatives. An accurate family history is one of the most important tools available to predict and prevent conditions that you may be at risk for.

Term	Definition
FDA	U.S. Food and Drug Administration, a department of the federal government, that regulates drugs, foods, some tests, medical devices, and other things that may impact public health and safety.
fecal immunochemical test	Fecal immunochemical test (FIT) is a test, similar to FOBT, to check for hidden blood in the stool. Blood may signal cancer or one of many non-cancer related causes of bleeding.
fecal occult blood test	Fecal occult blood test (FOBT) is a test to check for hidden blood in the stool. The presence of blood in stool may be a sign of cancer or one of the many non-cancer related causes of bleeding (e.g. hemorrhoids).
fibrate	A group of drugs that work to lower your “bad” (LDL) cholesterol by reducing your triglycerides (another type of fat) and raising your “good” (HDL) cholesterol. Commonly prescribed fibrates include fenofibrate (brand name examples include: Antara, Fenoglide, Lipofen, Lofibra, TriCor, Triglide, and Lipidil) and gemfibrozil (brand name: Lopid).
flexible sigmoidoscopy	Procedure used to examine the rectum and lower third of the colon. A sigmoidoscope is a long, flexible, slender tube with a lens on the end used to visualize a portion of the colon to look for the presence of growths.
functional	Functional refers to genes or proteins that are not affected by genetic changes that disrupt their normal structure or behavior.
gastrointestinal tract	The digestive system, consisting of the esophagus, stomach, small intestine and large intestine.
gene	A piece of DNA that acts as an instruction to the body for how to make a specific protein (enzyme, hormone, etc.). Genes are inherited, passed from parent to child.
gene sequencing	A genetic test that is considered the gold standard for finding genetic changes known as mutations.
genetic	Refers to any trait that is inherited, or passed from generation to generation through genes. These traits may range from having specific diseases to our response to certain drugs to simply our physical characteristics, like eye and hair color.
genetic condition	A genetic condition is any disease, disorder, syndrome, or trait that is caused, at least in part, from alterations in genes or chromosomes.

Term	Definition
genetic counseling	Genetic counseling is a process to help people learn about, cope with, and manage their risk of genetic disorders. This risk may be uncovered because the person is diagnosed with a condition, has a family history, has an affected child, and/or has an abnormal genetic test result.
genetic counselor	A healthcare professional with specialized training in how the science of genetics relates to medical care. A genetic counselor can evaluate your personal and family history, identify any risk factors for birth defects or genetic conditions, and help you understand and make decisions about testing or other options you may have.
genetic discrimination	Treatment or consideration based on genetic status or category rather than individual merit or actual conditions.
genetic modifier	A gene that changes how another gene is expressed.
genetic predisposition	Any condition in which genetic make-up leaves the individual more susceptible to disease.
genetic test	A specific type of laboratory test that is designed to find out if a person has a genetic disorder, is a carrier of a genetic disease, or has a predisposition to develop a genetic problem. Genetic testing can look at chromosomes, genes, or proteins -- depending on the specific condition being tested.
genomics	The study of the genome and its significance to pathology and disease.
genotype	The version of genes a person, organism, or cancer has.
genotyping	Tests that look specifically at the genetic information of a person, organism, or cancer. These tests may predict a certain characteristic (“phenotype”) but don't actually test for that characteristic.
glucose	A form of sugar made from carbohydrates we eat that the body uses for energy. Too much glucose in their blood may be a sign of diabetes.
HBB	A gene involved in making a piece of a protein called hemoglobin. Genetic changes, or mutations, in the HBB gene can cause sickle cell disease and beta-thalassemia.
HDL	High density lipoprotein cholesterol. Also called the “good” cholesterol. High HDL lowers the risk for heart disease.
HDL2	A subtype of HDL (the “good” cholesterol). HDL2 is the “best” cholesterol because high levels give you the most protection against heart disease -- even more than just high total HDL.

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Term	Definition
HDL3	A subtype of HDL (the “good” cholesterol). HDL3 is not as good for you as other types of HDL. Some studies show that high levels of HDL3 may actually increase your risk for heart disease.
heart	A muscular organ whose primary job is to pump blood to all parts of the body.
heart attack	When the blood supply to part of the heart muscle is suddenly blocked. The heart muscle may be damaged or start to die if blood doesn't return quickly.
heart disease	A general term for any condition that threatens the heart's ability to function normally. Because coronary artery disease (plaque buildup that may cause a heart attack) is by far the most common type, it is often just called heart disease.
hemochromatosis	A condition in which too much iron builds up in the body, which can lead to organ damage.
hemoglobin	A protein found in red blood cells that carries oxygen throughout the body
hemoglobin analysis	A test that measures the different types of hemoglobin in the blood. It is used to diagnose diseases caused by abnormal hemoglobin, such as sickle cell anemia.
hereditary	Genetically transmitted -- or capable of being transmitted -- from parent to child.
hereditary nonpolyposis colorectal cancer	Hereditary non-polyposis colorectal cancer (HNPCC) is an inherited disorder in which there is a tendency to develop colorectal cancer without a significant number of polyp precursors. HNPCC is specifically associated with inherited mutations in five mismatch repair genes.
HFE gene	The HFE gene makes a protein that regulates how much iron your body absorbs from your diet.
high performance liquid chromatography	A laboratory procedure that can separate a liquid mixture into its individual compounds. As an example, this procedure is used is to separate different kinds of hemoglobins in a person's blood.
HNPCC-related cancer	Other primary cancers included in an inherited cancer syndrome because of the increased prevalence in syndrome carriers. In addition to colon cancer, HNPCC-related cancers include cancer of the endometrium, ovary, stomach, kidney/urinary tract, brain, biliary tract, central nervous system and small bowel.

Term	Definition
hormone	Chemical messengers made mostly in our glands that influence our growth and development, sexual function, reproduction, mood, and metabolism. Hormone medications include oral contraceptive pills, patches or rings; hormonal treatments for infertility; hormone replacement therapy; or serum estrogen modifiers (sometimes taken to treat or prevent certain forms of cancer).
human immunodeficiency virus	A retrovirus that attacks the human immune system, thus affecting the body's ability to fight off the organisms that cause disease. HIV is the cause of acquired immune deficiency syndrome or AIDS.
hypertension	Blood pressure that stays at 140/90 mmHg or higher over a period of time. Average blood pressure is about 120/80 mmHg.
IDL	Intermediate density lipoprotein -- a type of “bad” cholesterol. High IDL increases the risk for heart disease even more than just high total LDL levels. IDL is under strong genetic control so close relatives of someone with high IDL should also consider testing.
in vitro fertilization	A laboratory procedure in which sperm fertilize eggs outside the body in a laboratory setting to facilitate pregnancy. The fertilized egg is then placed in the woman's uterus for implantation.
inherited	Any trait that is passed from generation to generation through our genes. These traits may range from having a specific disease to how we respond to certain drugs to simply our physical characteristics, like eye and hair color.
inhibin A	A substance made by the placenta during pregnancy and found in the mother's blood. Also abbreviated “DIA.”
insulin	A hormone that helps glucose, the sugar used by the body for energy, get into the cells that need it. When you don't make enough insulin or you can't use insulin effectively, you are likely to develop diabetes.
intermediate metabolizer	Intermediate metabolizers have a drug metabolism gene that doesn't work properly. They make less of the enzyme coded for by those genes, but usually make enough to process most drugs. People have many drug metabolism genes and can have be different kinds of metabolizers for each.

Term	Definition
iron overload	A condition in which higher-than-usual amounts of iron collect in the tissues of the body. Over time, iron overload can damage organs like the liver and cause problems like diabetes.
K-RAS	A gene that when mutated contributes to converting a normal cell into a cancerous cell.
LDL	Low-density lipoprotein cholesterol. Also called the “bad” cholesterol. High LDL increases the risk of heart disease.
LDLR	Stands for low density lipoprotein receptor. The LDLR gene normally makes a protein that helps to remove LDL ((bad≈ cholesterol) from the blood. An LDLR gene mutation causes a person not to get rid of LDL as quickly and it builds up. LDLR mutations are the most common cause of familial hypercholesterolemia.
leukemia	A cancer that starts in blood-forming tissue, such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the bloodstream.
lifestyle	In talking about health conditions, lifestyle generally refers to factors within your control like diet, physical activity, smoking, alcohol use, and use of other preventive health measures.
lipid	A fat that acts as a source of energy and helps the body use certain vitamins. Cholesterol and triglycerides are examples of lipids. High lipid levels increase the risk for heart disease and diabetes and may be caused by eating too much fat, alcohol use, inactivity, inherited conditions, and certain medications and disease.
lipoprotein a	Lp(a) stands for lipoprotein a -- a type of “bad” cholesterol. High Lp(a) increases the risk of heart disease 2 to 10 times more than just high total LDL levels and may cause heart disease earlier than usual. Drug therapy is usually needed. Lp(a) is under strong genetic control so close relatives of someone with high Lp(a) should also consider testing.
liver	An organ involved in a wide range of functions, including helping with digestion and the detoxification of chemicals.
liver biopsy	A surgical procedure that removes a small piece of liver so it can be examined in a lab.
lymphoma	Cancer that begins in the cells of the immune system.
maintenance dose	The amount of drug that is needed over the long-term to reach a stable, therapeutic response.

Term	Definition
malignant	Cancerous. Malignant tumors, or cancer, have the ability to invade adjacent tissues and spread throughout the body. Thus, malignant tumors can become life threatening.
mammogram	An X-ray picture of the breast. The x-ray images make it possible to detect tumors that cannot be felt. They can also find microcalcifications that may signal the presence of cancer.
maraviroc	The generic name of Selzentry, a drug used to treat HIV infection that only works in people whose HIV uses a specific receptor (CCR5) to infect the cell.
maternal serum screening test	A blood test that looks at the levels of certain substances in a pregnant woman's blood. These tests are used to find the risk for having certain birth defects. They can't tell for sure whether a pregnancy has a birth defect.
MCH	An abbreviation for “mean corpuscular hemoglobin”. The average amount of hemoglobin in the average red blood cell. The normal range for the MCH is 27 - 32 picograms. MCH is a standard part of a CBC (complete blood count) test.
MCV	An abbreviation for “mean corpuscular volume”. The average size of a red blood cell. The normal range for the MVC is 80 - 100 femtoliters. MVC is a standard part of the CBC (complete blood count) test.
Mediterranean	Someone whose ancestors come from one of the countries bordering the Mediterranean Sea. These countries include but are not limited to: Spain, southern France, Italy, and Greece.
metabolic syndrome	Also called “insulin resistance”. A combination of factors (like abnormal cholesterol, abdominal obesity, high blood sugar, and high blood pressure) that increases the risk of getting both heart disease and diabetes.
metabolism or metabolize	The way drugs and other substances are broken down for use in the body and elimination.
metastasis	The spread of cancer from one part of the body to another.
methylation	A process by which a methyl group is added to the DNA base cytosine. This process often decreases the amount of gene product that is made. For example, tumor suppressor genes are often methylated which decrease their function and lead to cancer.
mlh1	A mismatch repair (MMR) gene located on chromosome 3. Mutations in MLH1 are associated with Lynch syndrome (also called HNPCC) and greatly increase the chance of cancer -- especially colon.

Term	Definition
MMR gene	Mismatch repair gene, a gene that functions as a part of the “spell check” system of a cell. Mutations in MMR genes are involved in causing some hereditary cancer syndromes.
morbidity	A diseased state.
MSH2	A mismatch repair (MMR) gene located on chromosome 2. Mutations in MLH1 are associated with Lynch syndrome (also called HNPCC) and greatly increase the chance of cancer -- especially colon.
multifactorial inheritance	Conditions that are caused by an interaction between more than one gene and environmental (non-genetic) factors. Most common human diseases seem to be multifactorial, including diabetes, heart disease, mental illness, and most birth defects. A family history of a multifactorial condition usually increases the risk for other relatives.
multiple myeloma	Cancer that begins in the cells of the immune system.
multisite	Multisite Testing looks for the three BRCA gene mutations that cause 80% to 90% of all hereditary breast and ovarian cancers in Ashkenazi Jewish people. This test gives you a clear result: either you have one of these three mutations, or you don't. If you don't, it is possible to have a different BRCA mutation that was not tested for.
mutation	A change in the DNA code that may cause a gene not to function in the normal way.
newborn screening	Testing that is done routinely after birth, to look for serious developmental, genetic and metabolic disorders. This testing is done so that important medical treatments or other actions can start before symptoms develop.
niacin	Also called “nicotinic acid”. Part of vitamin B3 found in foods like meat, fish, milk, eggs, green vegetables, and grains. Niacin supplements increase HDL, lower Lp(a), and to a lesser degree, lower LDL cholesterol. Common brand names include: Niacor, Niaspan, Nicolar, Nicotinex Elixir, and Slo-Niacin.
non-invasive procedure	Procedures that do not require insertion of an instrument or device through the skin or a bodily orifice for diagnosis or treatment.
Noonan syndrome	A genetic disorder that causes abnormal development of many parts of the body. It can be caused by a defect in one of four different genes (KRAS, PTPN11, RAF1, SOS1). Noonan syndrome may be inherited from a parent who has the condition, or may happen by chance in a pregnancy.

Term	Definition
obesity	Having a high amount of body fat. Usually defined by a body mass index (BMI) of 30 or higher.
omega 3-fatty acid	Also called “fish oil”. Omega-3 fatty acids from eating oily fish or taking fish oil supplements may lower triglycerides, slow the buildup of plaque in the arteries, and raise HDL (“good”) cholesterol. Too much omega-3 fatty acid is dangerous, so you should always talk to your doctor before starting supplements.
organs	A grouping of tissue that works together to perform a common function. Examples of organs include: stomach, lungs, and liver.
osteoma	Benign, bony tumors often on the skull or mandible (sometimes a clinical finding with FAP patients).
over-the-counter	OTC or over-the-counter drugs can be bought without a prescription. OTC drugs still carry certain risks and may interact with other drugs.
P-53	A gene which normally regulates the cell cycle and protects the cell from damage to its genome. Mutations in this gene cause cells to develop cancer.
PAPP-A	Short for “pregnancy-associated plasma protein A”, a substance found in pregnant women's blood. Low levels of PAPP-A at 8-14 weeks of pregnancy have been associated with risk for Down syndrome and pregnancy complications.
pedigree	A diagram of biological relationships that usually includes information on each relative's medical history.
premenopausal	The time when a women is entering menopause until it is complete -- often defined as from the time periods become irregular until 12 months after the last period.
phenotype	Characteristics that can be seen or measured and are often the result of genes and environment working together. Examples include things like eye color, weight, IQ, cholesterol levels, or drug response.
phenotyping	Tests that measure specific traits or characteristics that can be caused by genes and/or environmental factors. This is in contrast to genotype testing that only looks at genetic information.
placebo	A phony treatment or “sugar pill”. Researchers often compare people taking a drug with those taking a placebo to better measure the real effects of the drug.

Term	Definition
placenta	Also called the afterbirth, the placenta is the tissue that connects the developing baby to the mother's uterus. It develops as part of the pregnancy and has the same DNA as the developing baby. The placenta allows for the exchange of nutrients, waste and gases between the developing baby and the mother.
plaque	Related to heart disease, plaque is the buildup of cholesterol, calcium, and other substances on the inside walls of the arteries causing the arteries to be more narrow and less flexible.
plasma	The liquid part of the blood that carries blood cells and other components
polymorphism	Natural differences in a DNA sequence that are usually common and do not cause disease
polyp	A usually non-cancerous growth or tumor protruding from the lining of an organ, such as the colon. Left untreated, polyps have an increased risk of becoming cancerous.
poor metabolizer	Produce inactive drug metabolism enzyme or no enzyme at all. Poor metabolizers may have a reduced response or no response and may have increased side effects
poor metabolizer	Poor metabolizers have a pair of drug metabolism genes that don't work properly. They make very little or none of the enzyme coded for by that pair of genes. This causes slower metabolism or the inability to process certain drugs. People have many drug metabolism genes and can be different kinds of metabolizers for each.
postmenopausal	The time in a woman's life after menopause is complete -- often defined as starting 12 months after the last period.
pre-cancerous	Condition of the tissue, such as a polyp, that can turn into a cancer if not treated or removed.
preconception	Generally considered the period of time when a person is planning pregnancy but has not yet conceived (become pregnant).
pre-diabetes	Diagnosed when glucose (sugar) levels are higher than normal, but not high enough to make the diagnosis of diabetes -- usually a fasting glucose of 100 to 125 mg/dL or a glucose of 140 to 199 mg/dL after glucose tolerance test.
predisposition	Any condition, genetic or other, that renders an individual more susceptible to disease.

Term	Definition
preimplantation genetic diagnosis	A technique used with in vitro fertilization to test early-stage embryos for disease-causing genes, so that embryos without the disease-causing genes can be implanted in the mother's uterus.
prenatal diagnosis	Testing for diseases in the fetus or embryo before it is born.
presymptomatic	The stage prior to an individual presenting with symptoms that are clinically relevant to the disease in question.
prophylactic bilateral mastectomy	A risk-reducing treatment where both breasts, as well as some of the surrounding tissue, are surgically removed in order to keep cancerous cells from forming.
prophylactic bilateral oophorectomy	A risk-reducing treatment where ovaries are surgically removed in order to keep cancerous cells from forming; recommended after childbearing is complete.
protein	Large, complex molecules made of amino acids that form body structures, enzymes, hormones, and antibodies. Proteins are all made based on the instructions in our genes. The amino acids we need to make new proteins are consumed in the protein we eat or made by the body.
protein(s)	The molecules that form the body, allow it to grow, and regulate how it works. Our bodies make the proteins we need using the instructions from our genes.
receptor	A protein on the surface of a cell that only binds with certain other molecules. When this happens, a cellular process can occur.
rectum	The last portion of the digestive tract, at the end of the colon.
red blood cells	A cell in the blood that carries oxygen to all parts of the body. Also called an erythrocyte.
risk factor	Anything that increases the chance of developing a certain disease or having a child with a specific condition. Risk factors might include your family history, lifestyle, other health conditions, blood test results, age, gender, and countless other factors.
sarcoma	A cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissues.
screening	In medicine, screening generally refers to a test or exam that is reasonably simple, inexpensive, and harmless that can be given to a large group of people in order to find a smaller group with a higher-than-average chance for a certain condition. These people will sometimes have more specific testing or be treated early before symptoms appear.

Term	Definition
selective estrogen receptor modulator	Selective Estrogen Receptor Modulator (SERM) is a hormone-like drug that affects multiple tissues by interacting with receptors for the hormone estrogen. A particular SERM may have estrogen-like effects in some tissues and anti-estrogen effects in others.
Selzentry	The brand name of maraviroc, a drug used to treat HIV infection that only works in people whose HIV uses a specific receptor (CCR5) to enter the cell.
sequencing	A lab method that looks at each DNA nucleotide (A,T,G, and C) in a piece of DNA for differences (mutations) from the usual DNA sequence. A more labor intensive and expensive test that is often used when the specific mutations that cause a disease aren't known.
serum CA-125	A blood test used in an effort to detect ovarian cancer.
serum ferritin	A protein your body makes when it stores iron.
siblings	Brothers and/or sisters.
sickle cell disease	An inherited disorder in which the red blood cells have an abnormal crescent shape that affects blood flow. This disorder causes anemia because the abnormal blood cells don't survive long.
sickle/beta-thalassemia	A disease that occurs when someone inherits a sickle-cell anemia gene mutation from one parent and a beta-thalassemia gene mutation from the other parent. Symptoms are usually very similar to sickle cell disease.
side effect	An unintended and usually undesired reaction to a drug or treatment.
Single Site	Single Site Testing looks for just one BRCA mutation. This test can only be done for people who know the DNA sequence of a BRCA mutation that is running in their family. This test gives you a clear result: Either you have the mutation that was tested for or you don't.
southeast Asian	Someone whose ancestors come from one of the countries south of China and east of India. These countries include but are not limited to: Vietnam, Cambodia, Laos, Burma, or Indonesia.
spleen	An organ in the abdomen that supports the immune system, destroys and filters out old blood cells, and holds a reserve of blood cells. People can live without a spleen.
sporadic	In reference to cancer, this means a cancer not caused by hereditary genetic mutations. Most cancers are sporadic.

Term	Definition
statin	A group of drugs that lower the amount of cholesterol made naturally by the liver. When diet and exercise changes aren't enough, statins are often the first choice for drug therapy. Commonly prescribed statins include: Lovastatin (Mevacor, Altoprev), Pravastatin (Pravachol), Simvastatin (Zocor), Fluvastatin (Lescol), Atorvastatin (Lipitor), and Rosuvastatin (Crestor).
Stevens-Johnson syndrome	An allergic reaction to a drug or infection that causes flu-like symptoms, skin wounds, and may affect other organs like the eyes and mouth.
stroke	Caused by a sudden lack of blood supply and oxygen to the brain. Usually happens because either a blood clot blocks a blood vessel in the brain (ischemic stroke) or a blood vessel breaks and bleeds into the brain (hemorrhagic stroke).
symptom	Any sign that a person has a condition or disease. Symptoms, like headache, fever, fatigue, nausea, vomiting, and pain, may not be specific but together point to an underlying cause.
symptoms	Changes or signs that are caused by or accompany a disease or condition. Symptoms are the evidence of that underlying disease or condition. Symptoms can be used to help diagnose a problem.
tamoxifen	A drug commonly used to treat patients with breast cancer, certain other cancers, and those at high risk for breast cancer. It works by interfering with the activity of the hormone estrogen, which feeds the growth of many, but not all breast cancers.
toxic epidermal necrolysis	A life-threatening allergic reaction started by certain drugs, infections, illnesses, and unknown factors. TEN can cause large areas of the skin to peel away, flu-like symptoms, and other complications. The condition gets worse quickly and usually requires hospitalization.
transferrin saturation	The percentage of transferrin (a protein that carries iron in the blood) that is currently carrying iron.
translocation	A genetic condition where material from one chromosome breaks off and sticks to another chromosome, or switches places with a part of another chromosome. There are different types of translocations, and they can have different effects on health and development.
transvaginal ultrasound	A type of ultrasound done by inserting an ultrasound probe into the vagina. This allows a view of a woman's reproductive organs, including the uterus, ovaries, cervix, and vagina.

Term	Definition
triglycerides	A type of energy-rich fat. High triglycerides (over 200mg/dL) increase the risk for heart disease and stroke.
tropism	The specific cell types that a virus can recognize and infect.
tumor	An abnormal mass of tissue that results from excessive cell division. Tumors may be benign (not cancerous) or malignant (cancerous).
Turner syndrome	A genetic condition in which a girl or woman does not have the usual pair of two X chromosomes. Instead, some or all of her cells are missing an X chromosome, or part of an X chromosome. Symptoms are variable but usually include short stature and infertility.
ultra metabolizer	Have more than two functional copies of a drug metabolism gene, and produce a larger-than-normal amount of enzyme. Ultra metabolizers may have a reduced or no response and may have increased side effects
ultrarapid metabolizer	Ultrarapid metabolizers have extra copies of a gene involved in drug metabolism, so they make more enzyme than the average person. This results in faster metabolism of drugs processed by that enzyme.
umbilical cord	The cord that connects the developing baby to the placenta, which is attached to the mother's uterus. The umbilical cord carries oxygen- and nutrient-rich blood to the developing baby.
unconjugated estriol	One of the three main estrogens produced by the body. Low levels of this substance are associated with risk for certain birth defects, including Down syndrome and trisomy 18. Also abbreviated “uE3.”
variant	Gene variations contribute to diversity and make people unique. When a certain form of a gene is seen in at least 1% of people, but not most people, it is called a variant. Variants may also increase or decrease a person's risk for certain genetic diseases but usually don't cause the disease themselves.
vein	Blood vessels that carry blood low in oxygen back to the heart.
virtual colonoscopy	A method of examining the colon by taking a series of X-rays (called a CT scan) and using a high-powered computer to reconstruct 2-D and 3-D pictures of the interior surfaces of the colon from these X-rays.

Term	Definition
VKORC1	A gene that tells the body how to make vitamin K epoxide reductase (VKOR), an enzyme important in forming blood-clotting factors. A common VKORC1 gene variant (-1639G>A) puts people at increased risk for complications when taking warfarin at standard doses.
VLDL	Very low density lipoprotein -- a type of “bad” cholesterol. High VLDL increases the risk for plaque buildup in the arteries and heart disease.
VLDL3	A subtype of VLDL (a “bad” cholesterol). High VLDL3 increases heart disease risk the most and is a risk factor even when total cholesterol levels are normal. Diet and exercise changes are very effective for lowering VLDL3.
warfarin	The most commonly prescribed drug for preventing harmful blood clots from forming or from growing larger. Belongs to a class of drugs called anticoagulants or “blood thinners.”
white blood cells	A cell found in the blood whose primary job is to defend the body against infection.
xanthoma	Fat buildup that looks like a yellow lump under the skin, most commonly on the heels, hands, elbows, other joints, feet, and buttocks. Especially common in people with inherited high cholesterol like familial hypercholesterolemia.'

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