Genetic Testing for Limb-Girdle Muscular Dystrophy

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Introduction

Limb-girdle muscular dystrophy (LGMD) 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.

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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 two groups, based on inheritance pattern: LGMD1 (autosomal dominant) and LGMD2 (autosomal recessive).

Incidence or Prevalence

Autosomal recessive LGMD is more common, with an overall prevalence of about 1/15,000.\(^2\) Dominant forms are comparatively rare, representing 10% of LGMD cases.\(^2\) The prevalence of specific LGMD subtypes may differ in certain populations:\(^1\)

- LGMD2C is more common in Roma and Tunisian populations,
- LGMD2A is more common in Southern European, Eastern European, and British populations, and
- LGMD2I is more common in Northern European populations.
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.²

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.² The genes identified so far encode muscle proteins within the sarcomere- sarcolemma- sarcoplasm-extracellular-matrix network.³

Inheritance

LGMD inheritance is typically autosomal with LGMD subtype nomenclature reflecting autosomal dominant inheritance (LGMD1 with subtypes designated by letter), and autosomal recessive inheritance (LGMD2 with subtypes designated by letter). This autosomal inheritance pattern helps distinguish LGMD from the more common X-linked dystrophies (Duchenne, Becker and Emery-Dreifuss).⁴

Diagnosis

Diagnosis of muscular dystrophies is typically based on clinical phenotype and inheritance pattern.³ 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)²
- Onset is not congenital
- Course is progressive

- Biochemical/histological investigation should suggest muscle damage (although findings can be non-specific)³
  - 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.

**Treatment**

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.²

**Test information**

**Introduction**

Testing for LGMD disease may include targeted mutation analysis, gene by gene sequence analysis, or panel testing. Known familial mutation analysis is also available.
Sequence analysis

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.

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. NGS may not perform as well as Sanger sequencing in some applications.

NGS tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

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.

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.

Results may be obtained that cannot be adequately interpreted based on the current knowledge base. 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.

Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion exists for a particular syndrome testing for that syndrome should be performed instead of a broad multi-gene panel.

Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used and in which labs they were performed.

Additionally, 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 clinical significance
LGMD sequence analysis

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.

Deletion/duplication analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

LGMD deletion/duplication analysis

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.

Known familial mutation analysis

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

Guidelines and evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to Limb Girdle Muscular Dystrophy testing.

American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine


• 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)."

**Literature Review**

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%).

Sequencing of 18 LGMD related genes in 35 patients 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. Pathogenic variants confirmed a LGMD-related molecular etiology in 20 patients (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.

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. One study analyzed 50 families with an LGMD type distribution of muscle weakness. 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. 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. A large gene panel may also increase the risk of incidental findings or variant of uncertain clinical significance.

A US study of 4656 patients with clinically suspected LGMD (no prior molecular testing) underwent genetic testing via a 35-gene NGS panel (included LGMD or LGMD-like genes). A molecular diagnosis was established in 27% (N=1259). There was a high prevalence of patients 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%. All patients had normal dystrohin 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 patients with features of LGMD.

A US study of 55 families affected by LGMD demonstrated pathogenic variants in 22 families using exome sequencing. 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 LGMD (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.

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.

Criteria

Introduction

Requests for LGMD testing are reviewed using the following clinical 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 of requested LGMD gene, 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:
  o Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

• Previous Genetic Testing:
  o No redundant previous LGMD related gene sequencing, and
  o No known LGMD related gene mutation in family, AND

• Diagnostic Testing for Symptomatic Individuals:
  o 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
  o 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 patient, AND

• Rendering laboratory is a qualified provider of services per the Health Plan policy.

**LGMD Multi-Gene Diagnostic Panels**

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

• Previous Genetic Testing:
  o No known molecular cause of LGMD (single disease causing mutation in dominant forms or biallelic disease-causing mutations in recessive forms) in family, and
  o 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:
  o Muscle weakness and atrophy not secondary to a neurogenic cause in a limb-girdle distribution, and
  o Member does not have a congenital myopathy, and
  o EMG does not show evidence of a nerve etiology as the primary cause, and
  o 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 patient, AND
  • Rendering laboratory is a qualified provider of services per the Health Plan policy

Billing and Reimbursement Considerations:

For a panel 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, panels 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 genes associated with findings will be reimbursable.

When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, the laboratory will be redirected to the appropriate panel code(s).

References

Introduction

This guideline cites the following references.


