# Neurofibromatosis Type 1 Genetic Testing

## Introduction

Neurofibromatosis Type 1 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 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 or Prevalence

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 only 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 patients 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. "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."  

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. Malignant peripheral nerve sheath tumors may develop by malignant transformation of neurofibromas during adolescence or adulthood.

**Diagnosis**

Diagnostic criteria for NF1 were formulated by the National Institute of Health (1988). A full description can be found in the Guidelines and Evidence section.

“Only about half of children with NF1 and no known family history of NF1 meet the NIH criteria for diagnosis by age one year; almost all do by age eight years because many features of NF1 increase in frequency with age. Children who have inherited NF1 from an affected parent can usually be identified within the first year of life because diagnosis requires just one feature in addition to a positive family history. Young children with multiple café au lait spots and no other NF1 features whose parents do not show signs of NF1 on careful physical and ophthalmologic examination should be strongly suspected of having NF1 and followed clinically as though they do.”

NF1 has overlapping clinical features with Legius syndrome, other forms of neurofibromatosis, conditions with café-au-lait and pigmented macules, and overgrowth syndromes.

**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. 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.

NF1 microdeletions are associated with early appearance of numerous cutaneous neurofibromas, severe cognitive abnormalities, somatic overgrowth, large hands and feet, and dysmorphic facial features.
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.\textsuperscript{11}

**Segmental NF**

Segmental 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.\textsuperscript{12} There is an increased risk of malignancies.

**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.\textsuperscript{4}

**Inheritance**

Neurofibromatosis type 1 is inherited in an autosomal dominant fashion. 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.\textsuperscript{13} 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.\textsuperscript{3,7}

**Treatment**

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.\textsuperscript{5} Clinical trials are underway to study new medications for the treatment of tumors common in NF1.

**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.\textsuperscript{3}
Test Information

Introduction

Testing for Neurofibromatosis Type 1 may include NF1 gene sequencing, NF1 deletion/duplication analysis, or known familial mutation analysis.

NF1 sequencing 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 NF1.¹

NF1 deletion/duplication analysis

Large deletions in NF1 are infrequently reported. Deletion/duplication analysis is done as second-tier testing after NF1 sequence analysis.

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.

Segmental NF

Testing of various sample types is available to help identify individuals with segmental 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.”¹⁴

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 (ACMG)

The American College of Medical Genetics and Genomics (ACMG, 2019) stated the following in regard to genetic testing for NF1 in children:⁷

• "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:15

• “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.”

National Institute of Health (NIH)

The diagnostic criteria set forth by the National Institute of Health (NIH Consensus Development Conference, 1988) are met for NF1 in individuals who have at least two or more of following findings:6

• 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)
• A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudoarthrosis
• A first-degree relative with NF1 as defined by the above criteria
**Expert authored review**

"[Genetic] testing is indicated for individuals in whom NF1 is suspected but who do not fulfill the NF1 diagnostic criteria. This is rarely necessary after early childhood. Testing may be useful in a young child with a serious tumor (e.g., optic glioma) in whom establishing a diagnosis on NF1 immediately would affect management. Testing of an adult with NF1 is necessary if prenatal or preimplantation genetic diagnosis in a current or future pregnancy is anticipated. In some families with spinal NF1 or the NF1 c.2970-2972 delAAT pathogenic variant, affected individuals may not meet the NIH diagnostic criteria, especially in childhood. In such families, molecular testing is indicated for diagnosis of at-risk relatives." \(^3\)

"Young children who present with six or more café au lait macules and freckling in axillary or inguinal regions and who have no known family history of NF1 will meet the diagnostic criteria for NF1, but diagnoses of Legius syndrome or constitutional mismatch repair syndrome are also possible and need to be considered especially if no additional findings of NF1 develop with increasing age." \(^3\)

"A multistep pathogenic variant detection protocol that combines analysis of genomic DNA and cDNA (mRNA) and testing for whole-gene or exon copy number changes is recommended if molecular genetic testing is indicated. This approach identifies more than 95% of NF1 pathogenic variants in individuals fulfilling the NIH diagnostic criteria. Because of the variety and rarity of individual pathogenic variants found in people with NF1 and the frequency of pathogenic variants that affect splicing (22%-30%, more than 1/3 of which are not detected by gDNA sequencing), methods that include cDNA sequencing have higher detection rates than methods based solely on analysis of gDNA." \(^3\)

**Criteria**

**Introduction**

Requests for NF1 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 could detect the familial mutation, AND
- NF1 mutation identified in 1st degree biological relative, OR

Prenatal Testing for At-Risk Pregnancies:
• NF1 mutation identified in a previous child or either parent

**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
  - A distinctive osseous lesion (e.g., sphenoid dysplasia or tibial 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, 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.

**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

**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.

**References**

**Introduction**

This guideline cites the following references.


14. UAB School of Medicine Department of Genetics. RNA-based NF1/SPRED1 Testing on Cultured from Affected Tissues. Available at: [https://uab.edu/medicine/genetics/medical-genomics-laboratory/testing-services/nf1-legius-syndrome-and-rasopathies/nf1-spred1-on-affected-tissues](https://uab.edu/medicine/genetics/medical-genomics-laboratory/testing-services/nf1-legius-syndrome-and-rasopathies/nf1-spred1-on-affected-tissues)