Neurofibromatosis Type 1 Genetic Testing

MOL.TS.301.A
v1.0.2025

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:

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- 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]

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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."

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- "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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