Genetic Testing for Neurofibromatosis Type 1 or 2

Effective: December 1, 2024

Next Review: September 2025
Last Review: October 2024

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Neurofibromatoses (NF) are autosomal dominant genetic disorders associated with tumors of the peripheral and central nervous systems. The potential benefit of genetic testing for NF is to confirm the diagnosis in an individual with suspected NF who does not fulfill clinical diagnostic criteria or to determine future risk of NF in asymptomatic at-risk relatives.

MEDICAL POLICY CRITERIA

I. *NF1, NF2, and SPRED1* genetic testing for neurofibromatosis may be considered **medically necessary** when any of the following criteria are met:
   A. The diagnosis is clinically suspected due to signs and symptoms of the disease, but a clinical diagnosis has not been made; or
   B. In at-risk relatives with no signs of disease, when a first-, second-, or third-degree relative has been diagnosed with neurofibromatosis.

II. Genetic testing for neurofibromatosis type 1 or 2 is considered **not medically necessary** if a clinical diagnosis of the disorder has already been made.

III. Genetic testing for neurofibromatosis type 1 or 2 for all other indications is considered **investigational**.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

REQUIRED DOCUMENTATION:

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
6. Medical records related to this genetic test
   - o History and physical exam
   - o Conventional testing and outcomes
   - o Conservative treatment provided, if any

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

BACKGROUND

NEUROFIBROMATOSIS TYPE 1

Neurofibromatosis Type 1 (NF1) is one of the most common dominantly inherited genetic disorders, with an incidence at birth of 1 in 3,000 individuals.

Clinical Characteristics

The clinical manifestations of NF1 show extreme variability, between unrelated individuals, among affected individuals within a single family, and within a single person at different times in life.

NF1 is characterized by multiple café-au-lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, and iris Lisch nodules. Segmental NF1 is limited to one area of the body. Many individuals with NF1 only develop cutaneous manifestations of the disease and Lisch nodules.

Cutaneous Manifestations

Café-au-lait macules occur in nearly all affected individuals, and intertriginous freckling occurs in almost 90%. Café-au-lait macules are common in the general population, but when more than six are present, NF1 should be suspected. Café-au-lait spots are often present at birth and increase in number during the first few years of life.

Neurofibromas

Neurofibromas are benign tumors of Schwann cells that affect virtually any nerve in the body and develop in most people with NF1. They are divided into cutaneous and plexiform types. Cutaneous neurofibromas, which develop in almost all people with NF1, are discrete, soft, sessile, or pedunculated tumors. Discrete cutaneous and subcutaneous neurofibromas are rare before late childhood. They may vary from a few to hundreds or thousands, and the rate of development may vary greatly from year to year. Cutaneous neurofibromas do not carry a risk of malignant transformation but may be a major cosmetic problem in adults.

Plexiform neurofibromas, which occur in about half of individuals with NF1, are more diffuse growths that may be locally invasive. They can be superficial or deep and, therefore, the extent cannot be determined by clinical examination alone; magnetic resonance imaging (MRI) is the method of choice for imaging plexiform neurofibromas.[1] Plexiform neurofibromas represent a major cause of morbidity and disfigurement in individuals with NF1. They tend to develop and grow in childhood and adolescence and stabilize throughout adulthood. Plexiform neurofibromas can compress the spinal cord or airway and can transform into malignant peripheral nerve sheath tumors. Malignant peripheral nerve sheath tumors occur in approximately 10% of affected individuals.[1]

Central Nervous System Tumors

Optic gliomas, which can lead to blindness, develop in the first six years of life. Symptomatic optic gliomas usually present before six years of age with loss of visual acuity or proptosis, but they may not become symptomatic until later in childhood or adulthood.

While optic pathway gliomas are particularly associated with NF1, other central nervous system tumors occur at higher frequency in NF1, including astrocytomas and brainstem gliomas.

Other Findings

Other findings in NF1 include:

- Intellectual disability occurs at a frequency about twice that in the general population, and features of autism spectrum disorder occur in up to 30% of children with NF1.
- Musculoskeletal features include dysplasia of the long bones, most often the tibia and fibula, which is almost always unilateral. Generalized osteopenia is more common in people with NF1 and osteoporosis is more common and occurs at a younger age than in the general population.[1]
- Cardiovascular involvement includes the common occurrence of hypertension. Vasculopathies may involve major arteries or arteries of the heart or brain and can have serious or fatal consequences. Cardiac issues include valvar pulmonic stenosis, and congenital heart defects and hypertrophic cardiomyopathy may be especially frequent in individuals with *NF1* whole gene deletions.[1] Adults may develop pulmonary hypertension, often in association with parenchymal lung disease.
- Lisch nodules are innocuous hamartomas of the iris.

Diagnosis

Although the clinical manifestations of NF1 are extremely variable and some are age-dependent, the diagnosis can usually be made on clinical findings, and genetic testing is rarely needed.[1]

Clinical diagnostic criteria were developed by the National Institutes of Health (NIH) but were revised in 2021 by an international consensus guideline committee to account for phenotypic and genotypic features of NF1 and mosaic NF1.[2]

The diagnostic criteria for NF1 are met when an individual who does not have a parent diagnosed with NF1 and has two or more of the following features:[2]

- Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
- Freckling in the axillary or inguinal regions
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Optic pathway glioma
- Two or more iris Lisch nodules identified by slit lamp examination or two or more choroidal abnormalities (defined as bright, patchy nodules imaged by optical coherence tomography/near-infrared reflectance imaging.
- A distinctive osseous lesion such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of a long bone

A heterozygous pathogenic *NF1* variant with a variant allele fraction of 50% in apparently normal tissue such as white blood cellsThe diagnostic criteria for NF1 are also met if the individual is a child of a parent who meets the diagnostic criteria specified in above merits a diagnosis of NF1 if one or more of the criteria above are present.

The diagnostic criteria for mosaic NF1 are met when an individual has any of the following features present:

- A pathogenic heterozygous NF1 variant with a variant allele fraction of significantly less than 50% in apparently normal tissue such as white blood cells AND one other NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for NF1)
- An identical pathogenic heterozygous NF1 variant in two anatomically independent affected tissues (in the absence of a pathogenic NF1 variant in unaffected tissue)
- A clearly segmental distribution of café-au-lait macules or cutaneous neurofibromas AND
    - Another NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for NF1) OR
    - Child fulfilling diagnostic criteria for NF1
- Only one NF1 diagnostic criterion from the following list
    - Freckling in the axillary and inguinal region
    - Optic pathway glioma
    - Two or more Lisch nodules or two or more choroidal abnormalities
    - Distinctive osseous lesion typical for NF1
    - Two or more neurofibromas or more plexiform neurofibroma AND a child fulfilling the criteria for NF1

Approximately half of the children with NF1 and no known family history of NF1 met previous diagnostic criteria for the clinical diagnosis by age one year. Almost all do by eight years of age because many features of NF1 increase in frequency with age. Children who have inherited NF1 from an affected parent can usually be diagnosed within the first year of life because the diagnosis requires one diagnostic clinical feature in addition to a family history of the disease.

This feature is usually multiple café-au-lait spots, present in infancy in more than 95% of individuals with NF1.[1]

Young children with multiple café-au-lait spots and no other features of NF1 who do not have a parent with signs of NF1 should be suspected of having NF1 and should be followed clinically as if they do.[3] A definitive diagnosis of NF1 can be made in most children by four years of age using the diagnostic criteria.[1]

Genetics

NF1 is caused by dominant loss-of-function variants in the *NF1* gene, which is a tumor suppressor gene located at chromosome 17q11.2 that encodes neurofibromin, a negative regulator of RAS activity. About half of affected individuals have it as a result of a de novo NF1 variant. Penetrance is virtually complete after childhood, however expressivity is highly variable.

The variants responsible for NF1 are very heterogeneous and include nonsense and missense single nucleotide changes, single base insertions or deletions, splicing variants (≈30% of cases), whole gene deletions (≈5% of cases), intragenic copy number variants, and other structural rearrangements. Several thousand pathogenic *NF1* variants have been identified; however, none is frequent.[1]

Management

Patient management guidelines for NF1 have been developed by the American Academy of Pediatrics, the National Society of Genetic Counselors, and other expert groups.[1, 4]

After an initial diagnosis of NF1, the extent of the disease should be established, with personal medical history and physical examination and particular attention to features of NF1, ophthalmologic evaluation including slit lamp examination of the irides, developmental assessment in children, and other studies as indicated on the basis of clinically apparent signs or symptoms.[1]

Surveillance recommendations for an individual with NF1 focus on regular annual visits for skin examination for new peripheral neurofibromas, signs of plexiform neurofibroma or progression of existing lesions, checks for hypertension, other studies (e.g., MRI) as indicated based on clinically apparent signs or symptoms, and monitoring of abnormalities of the central nervous system, skeletal system, or cardiovascular system by an appropriate specialist. In children, recommendations include annual ophthalmologic examination in early childhood (less frequently in older children and adults) and regular developmental assessment.

Long-term care goals for individuals with NF1 are early detection and treatment of symptomatic complications.

It is recommended that radiotherapy is avoided because radiotherapy in individuals with NF1 may be associated with a high risk of developing a malignant peripheral nerve sheath tumor within the field of treatment.

LEGIUS SYNDROME

Clinical Characteristics

A few clinical syndromes may overlap clinically with NF1. In most cases, including Proteus syndrome, Noonan syndrome, McCune-Albright syndrome, and LEOPARD syndrome, patients will be missing key features or will have features of the other disorder. However, the Legius syndrome is a rare autosomal-dominant disorder characterized by multiple café-au-lait macules, intertriginous freckling, macrocephaly, lipomas, and potential attention-deficit/hyperactivity disorder. Misdiagnosis of Legius syndrome as NF1 might result in overtreatment and psychological burden on families about potential serious NF-related complications.

Diagnosis

The diagnostic criteria for Legius syndrome are met when an individual does not have a parent diagnosed with Legius syndrome if the following criteria are present:[2]

- 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

The diagnostic criteria for Legius syndrome are also met when the individual is a child of a parent who meets the diagnostic criteria specified above merits a diagnosis of Legius syndrome if one or more of the criteria above are present

The diagnostic criteria for mosaic Legius syndrome are met when an individual has any of the following features present:

- A heterozygous pathogenic *SPRED1* variant with a variant allele fraction of significantly less than 50% in apparently normal tissue such as white blood cells AND six or more café-au-lait macules
- An identical pathogenic heterozygous *SPRED1* variant in two independent affected tissues (in the absence of pathogenic *SPRED1* variant in unaffected tissue)
- A clearly segmental distribution of café-au-lait macules AND a child fulfilling the criteria for Legius syndrome

Genetics

Legius syndrome is associated with pathogenic loss-of-function variants in the *SPRED1* gene on chromosome 15, which is the only known gene associated with Legius syndrome.

Management

Legius syndrome typically follows a benign course and management generally focuses on treatment of manifestations and prevention of secondary complications.[5] Treatment of manifestations includes behavioral modification and/or pharmacologic therapy for those with attention-deficit/hyperactivity disorder; physical, speech, and occupational therapy for those with identified developmental delays; and individualized education plans for those with learning disorders.

NEUROFIBROMATOSIS TYPE 2

NF2 is also known as neurofibromatosis type 2 or NF2-related schwannomatosis, bilateral acoustic neurofibromatosis or central neurofibromatosis [6]. It is estimated that NF2occurs in 1 in 33,000 individuals.

Clinical Characteristics

NF2- is characterized by development of multiple benign nerve sheath tumors called schwannomas, particularly affecting the vestibular nerve [7]. Individuals with NF2 typically present with bilateral vestibular schwannomas and associated symptoms include tinnitus, hearing loss, and balance dysfunction.[8] The average age of onset is 18 to 24 years, and almost all affected individuals develop bilateral vestibular schwannomas by age 30 years. Affected individuals may also develop schwannomas of other cranial and peripheral nerves, ependymomas, meningiomas, and, rarely, astrocytomas. The most common ocular finding, which may be the first sign of NF2, is posterior subcapsular lens opacities; they rarely progress to visually significant cataracts.

Most patients with NF2 present with hearing loss, which is usually unilateral at onset. Hearing loss may be accompanied or preceded by tinnitus. Occasionally, features such as dizziness or imbalance are the first symptom.[9] A significant proportion of cases (20% to 30%) present with an intracranial meningioma, spinal, or cutaneous tumor. The presentation in pediatric populations may differ from adult populations, in that, in children, vestibular schwannomas may account for only 15% to 30% of initial symptoms.[9]

Diagnosis

The diagnostic criteria for NF2 were recently updated by an International Expert Consensus Panel[6]. This update incorporates advances in understanding genotypic and phenotypic features of NF2-related schwannomatosis, as well as other ways to differentiate between NF2 and schwannomatosis. NF2 does not require genetic testing if clinical criteria are met.

The diagnosis of NF2is usually based on clinical findings, with diagnosis depending on presence of one of the following diagnostic criteria:

- Bilateral vestibular schwannomas
- An identical NF2 pathogenic variant in at least 2 anatomically distinct NF2 related tumors including schwannoma, meningioma, and/or ependymoma. (Note: If the variant allele fraction in unaffected tissues is clearly <50%, the diagnosis would be mosaic NF2-related schwannomatosis.
- Either 2 Major Criteria below OR 1 Major Criteria AND 2 minor criteria
    - Major Criteria:
        - Unilateral vestibular schwannoma
        - First-degree non-sibling relative with NF2-related schwannomatosis
        - Two or more meningiomas
        - Germline NF2 pathogenic variant (Note: If the variant allele fraction is clearly <50, the diagnosis would be mosaic NF2-related schwannomatosis.
    - Minor Criteria:
        - Single meningioma
        - >1 type of tumor ependymoma, meningioma or schwannoma (each distinct tumor counts as one minor criterion)
        - Juvenile subcapsular or cortical cataract, retinal hamartoma, epiretinal membrane in a person <40 years

Genetics

NF2 is inherited in an autosomal-dominant manner; approximately 50% of individuals have an affected parent, and the other 50% have NF2 as a result of a de novo variant.[8]

Between 25% and 33% of individuals with NF2 caused by a de novo variant have somatic mosaicism. Variant detection rates are lower in simplex cases and in an individual in the first generation of a family to have NF2 because they are more likely to have somatic mosaicism. Somatic mosaicism can make clinical recognition of NF2 difficult and results in lower variant detection rates. Clinical recognition of NF2 in these patients may be more difficult because these individuals may not have bilateral vestibular schwannomas. Variant detection rates may also be lower because molecular genetic test results may be normal in unaffected tissue (e.g., lymphocytes), and molecular testing of tumor tissue may be necessary to establish the presence of somatic mosaicism.[1]

Evaluation of At-Risk Relatives

Early identification of relatives who have inherited the family-specific *NF2* variant allows for appropriate screening using MRI for neuroimaging and audiologic evaluation, which result in earlier detection and improved outcomes.[8] Identification of at-risk relatives who do not have the family-specific *NF2* variant eliminates the need for surveillance.

SCHWANNOMATOSIS

Schwannomatosis (also referred to as gene-schwannomatosis)[6] is a rare condition defined as multiple schwannomas without vestibular schwannomas that are diagnostic of NF2.[8] Broadly, schwannomatosis encompasses four subcategories including *SMARCB1*-related schwannomatosis, *LZTR1*-related schwannomatosis, 22q-related schwannomatosis, and schwannomatosis-NOS (not otherwise specified)[6]. Individuals with schwannomatosis may develop intracranial, spinal nerve root, or peripheral nerve tumors. Familial cases are inherited in an autosomal-dominant manner, with highly variable expressivity and incomplete penetrance. Clinically, schwannomatosis is distinct from NF1 and NF2, although some individuals eventually fulfill diagnostic criteria for NF2. *SMARCB1* or *LZTR1* variants account for approximately 70-80% of familial schwannomatosis but only approximately 30% of sporadic cases[10].

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Lab tests for NF are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of these tests.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[11] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used

terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

The evaluation of a genetic test focuses on three main principles:

1. Analytic validity (technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent);
2. Clinical validity (diagnostic performance of the test [sensitivity, specificity, positive and negative predictive values] in detecting clinical disease); and
3. Clinical utility (how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes).

This evidence review focuses on the clinical validity and utility of genetic testing for neurofibromatosis.

CLINICAL VALIDITY

Neurofibromatosis Type 1

Detecting variants in the *NF1* gene is challenging because of the gene’s large size, the lack of variant hotspots, and the wide variety of possible lesions.

A multistep variant detection protocol has identified more than 95% of *NF1* pathogenic variants in individuals who fulfill NIH diagnostic criteria.[1] The protocol involves sequencing of both messenger RNA (complementary DNA [cDNA]) and genomic DNA, and testing for whole *NF1* deletions (e.g., by multiplex ligation-dependent probe amplification [MLPA]) because whole gene deletions cannot be detected by sequencing. Due to the wide variety and rarity of individual pathogenic variants in NF1, sequencing of cDNA increases the detection rate of variants from approximately 61% with genomic DNA sequence analysis alone[12] to greater than 95% with sequencing for both cDNA and genomic DNA and testing for whole gene deletions.

Table 1 summarizes several studies conducted on various populations, using various testing techniques to detect NF1 and SPRED variants. Below is a detailed description of two of the studies with high variant detection rates.

Sabbagh (2013) reported on a comprehensive analysis of constitutional *NF1* variants in unrelated, well-phenotyped index cases with typical clinical features of NF1 who enrolled in a French clinical research program.[13] The 565 families in this study (n=1,697 individuals) were enrolled between 2002 and 2005; 1,083 fulfilled NIH diagnostic criteria for NF1. A comprehensive *NF1* variant screening (sequencing of both cDNA and genomic DNA, as well as large deletion testing by MLPA) was performed in 565 individuals, one from each family, who had a sporadic variant or who represented the familial index case. A *NF1* variant was identified in 546, for a variant detection rate of 97%. A total of 507 alterations were identified at the cDNA and genomic DNA levels. Among these 507 alterations, 487 were identified using only the genomic DNA sequencing approach, and 505 were identified using the single cDNA sequencing approach. MLPA detected 12 deletions or duplications that would not have been detected by sequencing. No variant was detected in 19 (3.4%) patients, two of whom had a

SPRED1 variant, which is frequently confused with NF; the remainder might have been due to an unknown variant of the *NF1* locus.

Valero (2011) developed a method for detecting *NF1* variants by combining an RNA-based cDNA-polymerase chain reaction variant detection method and denaturing high-performance liquid chromatography with MLPA.[14] Their protocol was validated in a cohort of 56 patients with NF1 (46 sporadic cases, 10 familial cases) who fulfilled NIH diagnostic criteria. A variant was identified in 53 cases (95% sensitivity), involving 47 different variants, of which 23 were novel. After validation, the authors implemented the protocol as a routine test and subsequently reported the spectrum of *NF1* variants identified in 93 patients from a cohort of 105. The spectrum included a wide variety of variants (nonsense, small deletions or insertions and duplications, splice defects, complete gene deletions, missense, single exon deletions and duplications, and a multi-exon deletion), confirming the heterogeneity of the *NF1* gene variants that can cause NF1.

Table 1. Diagnostic Performance of Genetic Testing for Suspected NF1

Study	N	Population	Test Description	Detection Results
Spurlock (2009)[15]	85	Patients with NF1-like phenotypes (mild), with negative *NF1* testing	PCR sequencing of *SPRED1*	6 *SPRED* variants
Valero (2011)[14]	56	46 sporadic cases, 10 familial cases fulfilling NIH diagnostic criteria	Method combining RNA-based cDNA-PCR variant detection and DHPLC with MLPA	95% (53/56) patients had *NF1* variant
Sabbagh (2013)[13]	565	Unrelated, well-phenotyped index cases with typical clinical features of NF1	*NF1* variant screening (sequencing of both cDNA and genomic DNA, as well as large deletion testing by MLPA)	97% (546/565) patients had *NF1* variant
Zhu (2016)[16]	32	NF1 patients (plus 120 population match controls)	PCR sequencing of *NF1* gene, followed by MLPA	93.8% (30/32) patients had *NF1* variant
Zhang (2015)[17]	109	Patients with NF1-like phenotypes	Sanger sequencing, MLPA, and cDNA of *NF1,* in sequence; followed by Sanger sequencing and MLPA of *SPRED1* if all others negative (n=14)	*NF1* variant in: • 89% (89/100) of NF1 probands 93% (70/75) of patients met NIH criteria for NF1
Bianchessi (2015)[18]	293	Patients meeting NIH NF1 criteria	MLPA, aCGH, DHPLC, and Sanger sequencing, in sequence, of *NF1*	70% had *NF1* variant
	150	Patients with NF1-like symptoms without meeting NIH criteria	MLPA, aCGH, DHPLC, and Sanger sequencing, in sequence, of *NF1*	22% had *NF1* variant
	61	Patients meeting NIH criteria	MLPA followed by RNA sequencing of *NF1*	87% had *NF1* variant
	9	Patients with NF1-like symptoms without meeting NIH criteria	MLPA followed by RNA sequencing of *NF1*	33.3% had *NF1* variant

Study	N	Population	Test Description	Detection Results
Cali (2017)[19]	79	Patients in Italy with suspected or clinically diagnosed NF1	NGS using Ion Torrent PGM Platform followed by MLPA and calculation of mosaicism percentage using Sanger sequencing	73 variants in 79 NF1 patients
Giugliano (2019)[20]	281	Child patients referred and evaluated using NIH criteria	*NF1* and *SPRED1* analyzed at cDNA level, MLPA, PCR sequencing, validated by Sanger sequencing	85.1% (239/281) causative variant: 73.3% *NF1*, 2.8% *SPRED1*, 8.9% different gene
Angelova-Toshkina (2022)[21]	75	Children with suspected or clinically diagnosed NF1	Retrospective chart review comparing 1988 NIH diagnostic criteria and revised 2021 diagnostic criteria. Genetic testing methods were not described.	59% met 1988 NIH criteria and 75% met revised 2021 criteria. Additional patients met revised criteria due to a pathogenic NF1 variant being found.

aCGH: array comparative genomic hybridization; cDNA: complementary DNA; DHPLC: denaturing high-pressure liquid chromatography; MLPA: multiplex ligation-dependent probe amplification; NF1: neurofibromatosis type 1; NGS: next-generation sequencing; NIH: National Institutes of Health; PCR: polymerase chain reaction.

Genotype-Phenotype Correlations

NF1 is characterized by extreme clinical variability between unrelated individuals, among affected individuals within a single family, and even within a single person with NF1 at different times in life. Two clear correlations have been observed between certain NF1 alleles and consistent clinical phenotypes[1]:

1. A deletion of the entire *NF1* gene is associated with large numbers and early appearance of cutaneous neurofibromas, more frequent and severe cognitive abnormalities, somatic overgrowth, large hands and feet, and dysmorphic facial features.[1, 22, 23]
2. A three-base pair in-frame deletion of exon 17 is associated with typical pigmentary features of NF1, but no cutaneous or surface plexiform neurofibromas.[24]

Also, missense variants of *NF1* p.Arg1809 have been associated with typical NF1 findings of multiple café-au-lait macules and axillary freckling but the reduced frequency of NF1-associated benign or malignant tumors.[25, 26] In a cohort of 136 patients, 26.2% of patients had features of Noonan syndrome (i.e., short stature, pulmonic stenosis) present in excess.

In the Sabbagh (2013) study described above, authors evaluated genotype-phenotype correlations for a subset of patients.[13] This subset, which included 439 patients harboring a truncating (n=368), in-frame splicing (n=36), or missense (n=35) *NF1* variant, was evaluated to assess the contribution of intragenic NF1 variants (vs large gene deletions) to the variable expressivity of NF1. Their findings suggested a tendency for truncating variants to be associated with a greater incidence of Lisch nodules and a larger number of café-au-lait spots compared with missense variants.

However, other studies reported no associations between variant type and phenotype.[16, 27, 28]

Legius Syndrome

Pasmant (2009) described a cohort of 61 index cases meeting the NIH clinical diagnosis of NF1 but without a *NF1* variant detectable who were screened for germline loss-of-function variants in the *SPRED1* gene, located on 15q13.2.[29] *SPRED1* variants were detected in 5% of patients with NF1 features, which were characterized by café-au-lait macules and axillary and groin freckling but not neurofibromas and Lisch nodules. The authors characterized a new syndrome (Legius syndrome) based on the presence of a heterozygous *SPRED1* variant.

Messiaen (2009) described a separate cohort of 22 NF1 variant-negative probands who met NIH clinical criteria for NF1 with a *SPRED1* loss-of-function variant and participated in genotype-phenotype testing with their families.[30] Forty patients were found to be *SPRED1* variant-positive, 20 (50%, 95% confidence interval [CI] 34% to 66%) met NIH clinical criteria for NF1, although none had cutaneous or plexiform neurofibromas, typical NF osseous lesions, or symptomatic optic pathway gliomas. The authors also reported on an anonymous cohort of 1,318 samples received at a university genomics laboratory for NF1 genetic testing from 2003 to 2007 with a phenotypic checklist of NF-related symptoms filled out by the referring physician. In the anonymous cohort, 26 pathogenic *SPRED1* variants in 33 probands were identified. Of 1,086 patients fulfilling NIH criteria for a clinical diagnosis of NF1, a *SPRED1* variant was identified in 21 (1.9%, 95% CI 1.2% to 2.9%).

Neurofibromatosis Type 2

At least 200 different *NF2* variants have been described, most of which are point mutations. Large deletions of *NF2* represent 10% to 15% of *NF2* variants. When variant scanning is combined with deletion and duplication analysis of single exons, the variant detection rate approaches 72% in simplex cases and exceeds 92% for familial cases.[8] Wallace et al (2004) conducted *NF2* variant scanning in 271 patient samples (245 lymphocyte DNA, 26 schwannoma DNA).[31] The overall *NF2* variant detection rate was 88% among familial cases and 59% among sporadic cases. Evans et al (2007) analyzed a database of 460 families with *NF2* and 704 affected individuals for mosaicism and transmission risks to offspring.[32] The authors identified a variant in 84 (91%) of 92 second-generation families, with a sensitivity of greater than 90%. Other studies have reported lower variant detection rates, which likely reflects the inclusion of more mildly affected individuals with somatic mosaicism.[8]

Genotype-Phenotype Correlations

Intrafamilial variability is much lower than interfamilial variability, and the phenotypic expression and natural history of the disease are similar within families with multiple members with NF2.[33]

Frameshift or nonsense variants cause truncated protein expression, which has been associated with more severe manifestations of NF2.[33] Missense or in-frame deletions have been associated with milder manifestations of the disease. Large deletions of *NF2* have been associated with a mild phenotype.

Selvanathan (2010) reported on genotype-phenotype correlations in 268 patients with an *NF2* variant.[34] Variants that resulted in a truncated protein were associated with statistically significant younger age at diagnosis, higher prevalence and proportion of meningiomas, spinal tumors and tumors of cranial nerves other than VIII, vestibular schwannomas at a younger

age, and more cutaneous tumors. Certain variants, particularly those in exons 14 and 15, were associated with milder disease and fewer meningiomas.

Section Summary

Studies conducted among multiple cohorts of patients meeting diagnostic criteria for NF1 reported a high sensitivity of multistep variant testing protocol in identifying pathogenic *NF1* variants. On the other hand, studies conducted among familial and sporadic NF2 cases reported a variant detection rate exceeding 90% for familial cases and more than 70% in simplex cases.

CLINICAL UTILITY

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Individuals with Suspected NF

In many cases of suspected NF1, the diagnosis can be made clinically based on diagnostic criteria, which are both highly sensitive and specific, except in young children. However, there are suspected cases in children and adults that do not meet the diagnostic criteria. Given the well-established clinical management criteria, these patients benefit from genetic testing to confirm the diagnosis and to direct clinical management according to accepted guideline recommendations. Grossen (2022) has reported in their systematic review cases of pediatric NF that have been diagnosed by genetic testing.[35] Finding from 15 papers were included that identified 16 clinics that treated more than 2000 patients worldwide.

For NF2, affected individuals may have little in the way of external manifestations, and the onset of symptoms may be due to tumors other than vestibular schwannomas, particularly in children. Early identification of patients with NF2 can lead to earlier intervention and improved outcomes, and direct clinical management according to accepted guideline recommendations.

Section Summary

Currently, there is no direct evidence from studies demonstrating that genetic testing for NF1 and NF2 results in improved patient outcomes (e.g., survival or quality of life) among suspected cases. Suspected cases of NF1 or NF2 among children and adults who do not meet the diagnostic criteria might benefit from genetic testing to confirm the diagnosis and receive treatment, which might result in improved outcomes.

At-Risk Relatives

Similar to the case for suspected NF1, a clinical diagnosis can usually be made in an at-risk relative of a proband because one of the diagnostic criteria for diagnosis is having a first-degree relative with NF1 and, therefore, only one other clinical sign is necessary to confirm the diagnosis. Cases with at-risk relatives who do not fulfill the diagnostic criteria may benefit from genetic testing to direct clinical management according to accepted guideline recommendations.

Testing for NF2 may be useful to identify at-risk relatives of patients with an established

diagnosis of NF2, allowing for appropriate surveillance, earlier detection, and treatment of disease manifestations, and avoiding unnecessary surveillance in an individual who does not have the family-specific variant. Unlike NF1, the age of symptom onset for NF2 is relatively uniform within families. Therefore, it is usually not necessary to offer testing or surveillance to asymptomatic parents of an index case. However, testing of at-risk asymptomatic individuals younger than 18 years of age may help avoid unnecessary procedures in a child who has not inherited the variant.[8]

Section Summary

Currently, there is no direct evidence from studies demonstrating that genetic testing for NF1 and NF2 result in improved outcomes (e.g., survival or quality of life) among asymptomatic individuals with a close relative(s) with an NF diagnosis. However, genetic testing of at-risk asymptomatic individuals not fulfilling clinical diagnostic criteria might benefit through diagnosis, clinical management if needed and in avoiding unnecessary procedures in case of individuals who have not inherited the variant.

SUMMARY OF EVIDENCE

For individuals who have suspected NF who receive genetic testing for NF, the evidence includes clinical validation studies of a multistep diagnostic protocol and genotype-phenotype correlation studies. Relevant outcomes are test accuracy and validity, symptoms, morbid events, and functional outcomes. A multistep variant testing protocol identifies more than 95% of pathogenic variants in *NF1*; for NF2, the variant detection rate approaches more than 70% in simplex cases and exceeds 90% for familial cases. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic, with a close relative(s) with an NF diagnosis, who receive genetic testing for NF, there is no direct evidence. Relevant outcomes are test accuracy and validity, symptoms, morbid events, and functional outcomes. For individuals with a known pathogenic variant in the family, testing of at-risk relatives will confirm or exclude the variant with high certainty. While direct evidence on the clinical utility of genetic testing for NF is lacking, a definitive diagnosis resulting from genetic testing can direct patient care according to established clinical management guidelines, including referrals to the proper specialists, treatment of manifestations, and surveillance. Testing of at-risk relatives will lead to initiation or avoidance of management and/or surveillance. Early surveillance may be particularly important for patients with NF2 because early identification of internal lesions by imaging is expected to improve outcomes. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

PRACTICE GUIDELINE SUMMARY

AMERICAN ACADEMY OF PEDIATRICS

The American Academy of Pediatrics (2019) published diagnostic and health supervision guidelines for children with neurofibromatosis type 1.[36] The guidance makes the following statements related to genetic testing:

> *NF1* genetic testing may be performed for purposes of diagnosis or to assist in genetic counseling and family planning. If a child fulfills diagnostic criteria for NF1, molecular genetic confirmation is usually unnecessary. For a young child who presents only with

cafe-au-lait macules, NF1 genetic testing can confirm a suspected diagnosis before a second feature, such as skinfold freckling, appears. Some families may wish to establish a definitive diagnosis as soon as possible and not wait for this second feature, and genetic testing can usually resolve the issue.

Knowledge of the *NF1* [pathogenic sequence variant] can enable testing of other family members and prenatal diagnostic testing.

The guidance includes the following summary regarding 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.

NATIONAL COMPREHENSIVE CANCER NETWORK

The National Comprehensive Cancer Network's consensus guidelines for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic (v.1.2025) addressed the association between NF1 and risk of breast and other cancers.[37] According to the guidelines, there is evidence that individuals with a pathogenic variant in *NF1* have an increased risk of breast cancer, malignant peripheral nerve sheath tumors, and gastrointestinal stromal tumors (GIST). The guidelines recommend annual screening mammogram beginning at age 30 years in people with *NF1* variants. Additionally, it is recommended to consider screening breast MRI with and without contrast between the ages of 30-50 years, with the caveat that there is no increased breast cancer risk after age 50 years, and neurofibromas may lead to false-positive breast MRI. The guidelines also recommend a referral to a NF1 specialist.

SUMMARY

There is enough research to show that genetic testing for neurofibromatosis (NF) can be useful for confirming the diagnosis in an individual with suspected NF who does not fulfill clinical diagnostic criteria. There are specific surveillance recommendations for individuals with NF, and clinical guidelines recommend genetic testing when there are signs of the NF type 1, but they are not enough to make a clinical diagnosis. Therefore, *NF1*, *NF2*, and *SPRED1* genetic testing for neurofibromatosis may be considered medically necessary when the diagnosis is suspected due to signs of the disease, but a clinical diagnosis has not been made. If a clinical diagnosis has already been made, genetic testing results are not necessary for patient management. Therefore, genetic testing for NF type 1 or 2 is considered not medically necessary for patients that already have a clinical diagnosis of the disorder.

There is enough research to show that testing for NF may be useful to identify asymptomatic at-risk relatives of patients with an established diagnosis of NF, allowing for appropriate surveillance, earlier detection, and treatment of disease manifestations, and avoiding unnecessary surveillance in an individual who does not have a family-specific variant.

Therefore, *NF1, NF2, and SPRED1* genetic testing for neurofibromatosis in at-risk relatives, with no signs of disease, may be considered medically necessary.

There is not enough research to show that genetic testing for neurofibromatosis improves health outcomes for patients who do not meet the policy criteria. Therefore, genetic testing for neurofibromatosis for other indications is considered investigational.

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CODES

Codes	Number	Description
CPT	81405	Molecular pathology procedure, Level 6 – which includes *NF2* (neurofibromin 2 [merlin]) (eg, neurofibromatosis, type 2), duplication/deletion analysis and *SPRED1* (sprouty-related, EVH1 domain containing 1) (eg, Legius syndrome), full gene sequence
	81406	Molecular pathology procedure, Level 7 – which includes *NF2* (neurofibromin 2 [merlin]) (eg, neurofibromatosis, type 2), full gene sequence.
	81408	Molecular pathology procedure, Level 9 – which includes I (neurofibromin 1) (eg, neurofibromatosis, type 1), full gene sequence.
HCPCS	None	

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