

2.04.137 Genetic Testing for Neurofibromatosis			
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Policy Statement

Genetic testing for neurofibromatosis may be considered **medically necessary** when the diagnosis is clinically suspected due to signs of disease, but a definitive diagnosis cannot be made without genetic testing.

Genetic testing for neurofibromatosis in at-risk relatives, with no signs of disease, may be considered **medically necessary** when **both** of the following criteria are met:

- A definitive diagnosis cannot be made without genetic testing
- One of the following criteria is met:
 - o A close relative (i.e., first-, second-, or third-degree relative) has a known *NF* variant
 - o A close relative has been diagnosed with neurofibromatosis but whose genetic status is unavailable

Genetic testing for neurofibromatosis for all other situations not meeting the criteria outlined above is considered **investigational**.

Policy Guidelines

Testing Strategy

For evaluation of neurofibromatosis type 1 (NF1), testing for a variety of pathogenic variants of *NF1*, preferably through a multistep variant detection protocol, is indicated. If no *NF1* pathogenic variants are detected in patients with suspected NF1, testing for *SPRED1* variants is reasonable.

Definitions

Mutation Scanning

Mutation scanning is a process by which a particular segment of DNA is screened to identify sequence variants. Variant gene regions are then further analyzed (e.g., by sequencing) to identify the sequence alteration. Mutation scanning allows for screening of large genes and novel sequence variants.

Schwann Cells

Schwann cells cover the nerve fibers in the peripheral nervous system and form the myelin sheath.

Simplex Disease

Simplex disease is a single occurrence of a disease in a family.

Somatic Mosaicism

Somatic mosaicism is the occurrence of 2 genetically distinct populations of cells within an individual, derived from a postzygotic variant. Unlike inherited variants, somatic mosaic variants may affect only a portion of the body and are not transmitted to progeny.

Genetic Counseling

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing.

Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Coding

CPT code 81405 includes the following test:

- *NF2 (neurofibromin 2 [merlin])* (e.g., neurofibromatosis, type 2), duplication/deletion analysis

CPT code 81406 includes the following test:

- *NF2 (neurofibromin 2 [merlin])* (e.g., neurofibromatosis, type 2), full gene sequence

CPT code 81408 includes the following test:

- *NF1 (neurofibromin 1)* (e.g., neurofibromatosis, type 1), full gene sequence

Description

Neurofibromatoses are autosomal dominant genetic disorders associated with tumors of the peripheral and central nervous systems. There are 3 clinically and genetically distinct forms: neurofibromatosis (NF) type 1, NF type 2, and schwannomatosis. 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.

Related Policies

- N/A

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates [e.g., Federal Employee Program (FEP)] prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

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 this test.

Rationale**Background****Neurofibromatosis**

There are 3 major clinically and genetically distinct forms of neurofibromatosis (NF): NF type 1 (NF1; also known as von Recklinghausen disease), NF type 2 (NF2), and schwannomatosis.

Neurofibromatosis Type 1

NF1 is one of the most common dominantly inherited genetic disorders, with an incidence at birth of 1 in 3000 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.¹ 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 6 years of life. Symptomatic optic gliomas usually present before 6 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

The clinical diagnosis of *NF1* should be suspected in individuals with the diagnostic criteria for *NF1* developed by the National Institute of Health (NIH). The criteria are met when an individual has two or more of the following features:

- Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in postpubertal individuals
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axillary or inguinal regions
- Optic glioma
- Two or more Lisch nodules (raised, tan-colored hamartomas of the iris)
- A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudarthrosis
- A first-degree relative with *NF1* as defined by the above criteria.

In adults, the clinical diagnostic criteria are highly specific and sensitive for a diagnosis of *NF1*.¹

Approximately half of the children with *NF1* and no known family history of *NF1* meet NIH criteria for the clinical diagnosis by age 1 year. Almost all do by 8 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 1 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.² A definitive diagnosis of *NF1* can be made in most children by 4 years of age using the NIH 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.^{1,3}

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 for individuals with NF1 aims at early detection and treatment of symptomatic complications.

It is recommended that radiotherapy is avoided, if possible because radiotherapy in individuals with NF1 appears to 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.

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.⁴ 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 (also known as bilateral acoustic neurofibromatosis and central neurofibromatosis) is estimated to occur in 1 in 33,000 individuals.

Clinical Characteristics

NF2 is characterized by bilateral vestibular schwannomas and associated symptoms of tinnitus, hearing loss, and balance dysfunction.⁵ 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.⁶ A significant proportion of cases (20%-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.⁶

Diagnosis

The diagnosis of NF2 is usually based on clinical findings, with diagnosis depending on presence of one of the following modified NIH diagnostic criteria:

- Bilateral vestibular schwannomas
- A first-degree relative with NF2 AND
 - o Unilateral vestibular schwannoma OR
 - o Any two of meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities.
- Multiple meningiomas AND
 - o Unilateral vestibular schwannoma OR
 - o Any two of schwannoma, glioma, neurofibroma, cataract.

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.⁵

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

Management

In an individual diagnosed with NF2, it is recommended that an initial evaluation establish the extent of the disease, typically using cranial MRI, hearing evaluation, and ophthalmologic and cutaneous examinations.

Counseling is recommended for insidious problems with balance and underwater disorientation, which can result in drowning.

Hearing preservation and augmentation are part of the management of NF2, as is early recognition and management of visual impairment from other manifestations of NF2. Therefore, routine hearing and eye examination should be conducted.

Surveillance measures for affected or at-risk individuals include annual MRI beginning at around age 10 and continuing until at least the fourth decade of life.

Treatment of manifestations includes surgical resection of small vestibular schwannomas, which may often be completely resected with preservation of hearing and facial nerve function. Larger tumors are often managed expectantly with debulking or decompression when brain stem compression, deterioration of hearing, and/or facial nerve dysfunction occur.⁵

Radiotherapy should be avoided, because radiotherapy of NF2-associated tumors, especially in childhood, may induce, accelerate, or transform tumors.⁵

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.⁵ Identification of at-risk relatives who do not have the family-specific *NF2* variant eliminates the need for surveillance.

Schwannomatosis

Schwannomatosis is a rare condition defined as multiple schwannomas without vestibular schwannomas that are diagnostic of *NF2*.⁵ 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* variants have been shown to cause 30% to 60% of familial schwannomatosis but only a small number of simplex disease.

Literature Review

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Schwannomatosis is rare and far less well-described than neurofibromatosis type 1 (*NF1*) and neurofibromatosis type 2 (*NF2*); therefore, this review focuses on *NF1* and *NF2*.

Neurofibromatosis**Clinical Context and Test Purpose**

The purpose of genetic testing in patients who have suspected *NF* is to inform a decision to pursue additional surveillance for comorbid conditions as recommended by well-defined management guidelines if a definitive diagnosis can be made.

The question addressed in this evidence review is: For individuals who have suspected *NF* or who are asymptomatic with a close relative(s) with an *NF* diagnosis, does the use of genetic testing result in improved patient outcomes?

The following PICOTS were used to select literature to inform this review.

Patients

The relevant populations of interest are individuals with suspected *NF1* or *NF2*, based on clinical symptoms or because of a family member has been diagnosed with *NF1* or *NF2*.

Interventions

The genetic tests being considered are those for *NF1*, *NF2*, and *SPRED1* variants.

Comparators

The following tool is currently being used to make diagnostic decisions about suspected *NF1* and *NF2*: the National Institutes of Health (NIH) diagnostic criteria.¹

Outcomes

The potential beneficial outcomes of primary interest include earlier intervention and improved outcomes, and direct clinical management according to accepted guideline

recommendations. Harmful outcomes resulting from a false-positive test result include the potential for unneeded additional tests, while false-negative tests could delay care.

Timing

The duration of follow-up is years to assess non-test-related outcomes.

Setting

These tests would typically be ordered by a specialist. Genetic counseling is an important component of care delivery.

Simplifying Test Terms

There are 3 core characteristics for assessing a medical test. Whether imaging, laboratory, or other, all medical tests must be:

- Technically reliable
- Clinically valid
- Clinically useful.

Because different specialties may use different terms for the same concept, we are highlighting the core characteristics. The core characteristics also apply to different uses of tests, such as diagnosis, prognosis, and monitoring treatment.

Diagnostic tests detect presence or absence of a condition. Surveillance and treatment monitoring are essentially diagnostic tests over a time frame. Surveillance to see whether a condition develops or progresses is a type of detection. Treatment monitoring is also a type of detection because the purpose is to see if treatment is associated with the disappearance, regression, or progression of the condition.

Prognostic tests predict the risk of developing a condition in the future. Tests to predict response to therapy are also prognostic. Response to therapy is a type of condition and can be either a beneficial response or adverse response. The term predictive test is often used to refer to response to therapy. To simplify terms, we use prognostic to refer both to predicting a future condition or predicting a response to therapy.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

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 alone⁷ to greater than 95% with sequencing for both cDNA and genomic DNA and testing for whole gene deletions.

Sabbagh et al (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.⁸ The 565 families in this study (N=1697 individuals) were enrolled between 2002 and 2005; 1083 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, 2 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 et al (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.⁹ 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.

Additional studies have described the testing yield in smaller populations; they are summarized in Table 1.

Table 1. Diagnostic Performance of Genetic Testing for Suspected NF1

Study	N	Population	Test Description	Detection Results
Spurlock et al (2009) ¹⁰	85	Patients with NF1-like phenotypes (mild), with negative <i>NF1</i> testing	PCR sequencing of <i>SPRED1</i>	6 <i>SPRED</i> variants
Valero et al (2011) ⁹	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 <i>NF1</i> variant
Sabbagh et al (2013) ⁸	565	Unrelated, well-phenotyped index cases with typical clinical features of NF1	<i>NF1</i> variant screening (sequencing of both cDNA and genomic DNA, as well as large deletion testing by MLPA)	97% (546/565) patients had <i>NF1</i> variant
Zhu et al (2016) ¹¹	32	NF1 patients (plus 120 population match controls)	PCR sequencing of <i>NF1</i> gene, followed by MLPA	93.8% (30/32) patients had <i>NF1</i> variant
Zhang et al (2015) ¹²	109	Patients with NF1-like phenotypes	Sanger sequencing, MLPA, and cDNA of <i>NF1</i> , in sequence; followed by Sanger sequencing and MLPA of <i>SPRED1</i> if all others negative (n=14)	<i>NF1</i> variant in: • 89% (89/100) of NF1 probands • 93% (70/75) of patients met NIH criteria for NF1
Bianchessi et al (2015) ¹³	293	Patients meeting NIH NF1 criteria	MLPA, aCGH, DHPLC, and Sanger sequencing, in sequence, of <i>NF1</i>	70% had <i>NF1</i> variant

Study	N	Population	Test Description	Detection Results
	150	Patients with NF1-like symptoms without meeting NIH criteria	MLPA, aCGH, DHPLC, and Sanger sequencing, in sequence, of <i>NF1</i>	22% had <i>NF1</i> variant
	61	Patients meeting NIH criteria	MLPA followed by RNA sequencing of <i>NF1</i>	87% had <i>NF1</i> variant
	9	Patients with NF1-like symptoms without meeting NIH criteria	MLPA followed by RNA sequencing of <i>NF1</i>	33.3% had <i>NF1</i> variant
Cali et al (2017) ¹⁴	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

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 phenotypes¹:

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.^{1,15,16}
2. A 3-base pair inframe deletion of exon 17 is associated with typical pigmentary features of NF1, but no cutaneous or surface plexiform neurofibromas.¹⁷

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.^{18,19} 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 et al (2013) study (described above), authors evaluated genotype-phenotype correlations for a subset of patients.⁸ This subset, which included 439 patients harboring a truncating (n=368), inframe 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 (e.g., Zhu et al [2016],¹¹ shown in Table 1; Hutter et al [2016]²⁰; Ko et al [2013]²¹) reported no associations between variant type and phenotype.

Legius Syndrome

In 2009, Pasmant et al 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 15q13.2.²² *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.

Also in 2009, Messiaen et al 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.²³ Forty patients were found to be *SPRED1* variant-positive, 20 (50%, 95% 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 1318 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 1086 patients fulfilling NIH criteria for a clinical diagnosis of *NF1*, a *SPRED1* variant was identified in 21 (1.9%; 95% confidence interval, 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.⁵ Wallace et al (2004) conducted *NF2* variant scanning in 271 patient samples (245 lymphocyte DNA, 26 schwannoma DNA).²⁴ 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.²⁵ 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.⁵

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*.²⁶

Frameshift or nonsense variants cause truncated protein expression, which has been associated with more severe manifestations of *NF2*.²⁶ Missense or inframe deletions have been associated with milder manifestations of the disease. Large deletions of *NF2* have been associated with a mild phenotype.

Selvanathan et al (2010) reported on genotype-phenotype correlations in 268 patients with an *NF2* variant.²⁷ 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. Variants found especially exons 14 and 15 were associated with milder disease and fewer meningiomas.

Section Summary: Clinically Valid

Studies conducted among multiple cohorts of patients meeting NIH 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.

Clinically Useful

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.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the

preferred evidence would be from randomized controlled trials. No direct evidence was identified reporting on outcomes for genetic testing of individuals with suspected NF or at-risk relatives with a proband with NF.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. A chain of evidence based on clinical validity may be used to demonstrate clinical utility, if all of the links in the chain are strong.

Individuals with Suspected NF

In many cases of suspected NF1, the diagnosis can be made clinically based on the NIH 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 NIH 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.

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.

Subsection Summary: Individuals with Suspected NF

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 NIH 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, it is most often the case that a clinical diagnosis can be made in an at-risk relative of a proband because one of the NIH criterion 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 NIH 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.⁵

Subsection Summary: At-Risk Relatives

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.

Supplemental Information

Practice Guidelines and Position Statements

In 2008, the American Academy of Pediatrics published diagnostic and health supervision guidelines for children with neurofibromatosis type 1.³

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this policy are listed in Table 2.

Table 2. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT03210285	Whole Exome Sequencing (WES) of NF2-associated in Comparison to Sporadic Vestibular Schwannomas - Correlation With Clinical Data	100	Sep 2021

NCT: national clinical trial.

Appendix

Appendix Table 1. Categories of Genetic Testing Addressed in 2.04.137

Category	Addressed
1. Testing of an affected individual's germline to benefit the individual	
1a. Diagnostic	X
1b. Prognostic	
1c. Therapeutic	
2. Testing cancer cells from an affected individual to benefit the individual	
2a. Diagnostic	
2b. Prognostic	

Category	Addressed
2c. Therapeutic	
3. Testing an asymptomatic individual to determine future risk of disease	
4. Testing of an affected individual's germline to benefit family members	X
5. Reproductive testing	
5a. Carrier testing: preconception	
5b. Carrier testing: prenatal	
5c. In utero testing: aneuploidy	
5d. In utero testing: familial variants	
5e. In utero testing: other	
5f. Preimplantation testing with in vitro fertilization	

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Documentation for Clinical Review

Please provide the following documentation (if/when requested):

- History and physical and/or consultation notes including:
 - o Clinical findings (i.e., pertinent symptoms and duration)
 - o Comorbidities
 - o Activity and functional limitations
 - o Family history if applicable
 - o Reason for procedure/test/device, when applicable
 - o Pertinent past procedural and surgical history
 - o Past and present diagnostic testing and results
 - o Prior conservative treatments, duration, and response
 - o Treatment plan (i.e., surgical intervention)
- Consultation and medical clearance report(s), when applicable
- Radiology report(s) and interpretation (i.e., MRI, CT, discogram)
- Laboratory results
- Other pertinent multidisciplinary notes/reports: (e.g., psychological or psychiatric evaluation, physical therapy, multidisciplinary pain management) when applicable

Post Service

- Results/reports of tests performed
- Procedure report(s)

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

MN/IE

The following services may be considered medically necessary in certain instances and investigational in others. Services may be considered medically necessary when policy criteria are met. Services may be considered investigational when the policy criteria are not met or when the code describes application of a product in the position statement that is investigational.

Type	Code	Description
CPT®	81405	Molecular Pathology Procedure Level 6
	81406	Molecular Pathology Procedure Level 7
	81408	Molecular Pathology Procedure Level 9
HCPCS	None	
ICD-10 Procedure	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action	Reason
04/01/2016	BCBSA Medical Policy adoption	Medical Policy Committee
03/01/2017	Policy revision without position change	Medical Policy Committee
03/01/2018	Policy revision without position change	Medical Policy Committee

Definitions of Decision Determinations

Medically Necessary: A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)
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Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.