

Genetic Testing for Neurofibromatosis and Related Disorders

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I. Policy Description

Neurofibromatoses are a group of three clinically and genetically distinct disorders that cause tumors to form on nerve tissue. Neurofibromatosis type 1 (NF1) is caused by autosomal dominant mutations in the neurofibromin (*NF1*) gene and is characterized by multiple café-au-lait macules and neurofibromas (Korf, 2018). Neurofibromatosis type 2 (NF2) is caused by autosomal dominant mutations in the merlin, also known as schwannomin, (*NF2*) gene, and is characterized by multiple tumors of the nervous system, including the more common bilateral vestibular schwannomas as well as intracranial and spinal meningiomas, intrinsic ependymomas, and other spine tumors (Evans, 2020). Schwannomatosis is caused by inactivating mutations in *SMARCB1* and *LZTR* and is characterized by multiple schwannomas and pain arising in adulthood (Yohay & Bergner, 2019).

Legius syndrome is an NF1-like disorder caused by autosomal dominant mutations in the sprout-related EVH1 [enabled/vasodilator-stimulated phosphoprotein homology 1] domain-containing protein 1 (*SPRED1*) gene, resulting in café-au-lait macules. Constitutional mismatch repair-deficiency syndrome (CMMR-D), caused by mutations in mismatch repair genes, can also result in café-au-lait macules, axillary freckling, and Lisch nodules similar to NF1; however, unlike NF1, CMMR-D can also result in a variety of different malignancies, including glioblastoma and colorectal cancer (Korf, 2018).

II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website <http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx> or the manual website

1. Genetic counseling for genetic testing for neurofibromatosis, schwannomatosis, Legius Syndrome, and constitutional mismatch repair deficiency (CMMRD) **MEETS COVERAGE CRITERIA.**
2. Genetic testing for neurofibromatosis type 1 (NF1) **MEETS COVERAGE CRITERIA** when the diagnosis is clinically suspected due to signs of disease, but a definitive diagnosis cannot be made without genetic testing. The patient must have one of the following signs of NF1:
 - a. Six or more café-au-lait macules over 5 mm in greatest diameter in pre-pubertal individuals and over 15 mm in greatest diameter in post-pubertal individuals
 - b. Two or more neurofibromas of any type or one plexiform neurofibroma
 - c. Freckling in the axillary or inguinal regions
 - d. Optic glioma
 - e. Two or more Lisch nodules (iris hamartomas)
 - f. A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudarthrosis
 - g. A first-degree relative (parent, sib, or offspring) with NF1 as defined by the above criteria
3. Genetic testing for neurofibromatosis type 1 or type 2 in at-risk relatives with no signs of disease **MEETS COVERAGE CRITERIA** when a definitive diagnosis cannot be made without genetic testing AND at least ONE of the following criteria is met:
 - a. A close relative (i.e. first, second, or third degree relative) has a known NF mutation; OR
 - b. A close relative has been diagnosed with neurofibromatosis but whose genetic status is unavailable.
4. Prenatal testing for diagnosis of neurofibromatosis **MEETS COVERAGE CRITERIA** only if the disease-causing allele of an affected family member has been identified before prenatal testing.
5. Preimplantation genetic diagnosis of neurofibromatosis **MEETS COVERAGE CRITERIA** only if the NF1 or NF2 pathogenic variant has been identified in the family.
6. Genetic testing for diagnosis of NF2 **MEETS COVERAGE CRITERIA** when the diagnosis is clinically suspected due to signs of disease, but a definitive diagnosis cannot be made without genetic testing. The patient must meet one of the following criteria:
 - a. Individuals with a first degree relative with NF2 (ie, affected parent, sibling, or offspring)
 - b. Multiple spinal tumors (schwannomas, meningiomas)
 - c. Cutaneous schwannomas

- d. Apparently sporadic vestibular schwannoma less than 30 years of age, or spinal tumor or meningioma less than 20 years of age
 - e. Unilateral vestibular schwannoma in those less than 20 years of age
7. Genetic testing for mutations in SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 (*SMARCB1*) and *leucine-zipper-like transcriptional regulator 1 (LZTR1)* in individuals with one or more non-intradermal schwannoma, including those with VS (vestibular schwannoma) negative for NF2 **MEETS COVERAGE CRITERIA.**
8. Genetic testing of sprouty-related, EVH1 domain-containing protein 1 (*SPRED1*) for the diagnosis of Legius Syndrome **MEETS COVERAGE CRITERIA** for individuals with the at least one of the following:
- a. Six or more café-au-lait macules over 5 mm in greatest diameter in pre-pubertal individuals and over 15 mm in greatest diameter in post-pubertal individuals
 - b. Freckling in the axillary or inguinal regions
 - c. Symptoms of NF1, but genetic test results for NF1 were negative
9. Genetic testing for CMMRD (the four mismatch repair genes mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH6*), and postmeiotic segregation increased 2 (*PMS2*)) in children and adolescents **MEETS COVERAGE CRITERIA** when the following criteria are met:
- a. All of the following are required, AND
 - i. The presence of at least two hyperpigmented skin patches (café-au-lait macules)
 - ii. No *NF1* and *SPRED1* germline mutations detected
 - iii. Absence of diagnostic NF1 sign(s) in both parents, if known
 - b. At least one of the following is required (either in the family or in the patient)
 - i. In the family
 - (1) Consanguineous parents
 - (2) Genetic diagnosis of Lynch syndrome in one or both of the parental families
 - (3) Sibling with diagnostic NF1 sign(s)
 - (4) Sibling, living or deceased, with any type of childhood malignancy
 - (5) One of the following carcinomas from the Lynch syndrome spectrum in a first- or second-degree relative before the age of 60 years: colorectal cancer, endometrial cancer, ovarian cancer, gastric cancer, small bowel cancer, cancer of the bile duct or gall bladder, pancreatic cancer or urothelial cancer
 - ii. In the patient

- (1) Atypical café-au-lait macules (irregular borders and/or pigmentation)
- (2) Hypopigmented skin patches
- (3) One or more pilomatricoma(s)
- (4) Agenesis of the corpus callosum
- (5) Non-therapy-induced cavernoma
- (6) Multiple developmental vascular abnormalities (cerebral venous angiomas) in separate regions of the brain

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

10. Genetic testing for neurofibromatosis for all other situations not meeting the criteria outlined above **DOES NOT MEET COVERAGE CRITERIA**

IV. Scientific Background

Neurofibromatosis type 1

Neurofibromatosis type 1 is relatively common, affecting approximately 1 in 3,000 individuals (Korf, 2018). Almost half of these cases are *de novo* mutations, resulting from the unusually high (~1:10,000) mutation rate in the *NF1* tumor suppressor gene primarily in paternally derived chromosomes (Stephens et al., 1992).

The GTPase protein product of the *NF1* gene, neurofibromin, is expressed in many tissues, including brain, kidney, spleen, and thymus leading to a wide spectrum of clinical manifestations. *NF1* typically presents as café-au-lait macules, followed by axillary and/or inguinal freckling, and later Lisch nodules (iris hamartomas), and neurofibromas (Korf, 2018). Ocular, neurologic, musculoskeletal, vascular, cardiac, and malignant manifestations have been reported (Hirbe & Gutmann, 2014).

NF1 mutations are highly penetrant and inherited dominantly; however, *NF1* is variably expressed resulting in significant clinical variability, not only between unrelated individuals and among affected individuals within a single family but even within a single person with *NF1* at different times in life (Friedman, 2018). Despite thousands of *NF1* mutations identified, few genotype/phenotype correlations have been observed (Shofty, Constantini, & Ben-Shachar, 2015). Recent reports indicate the growing utility of next generation sequencing to provide solutions for problems like genetic heterogeneity, overlapping clinical manifestations, or the presence of mosaicism, and interactions between *SPRED1* and neurofibromin provide functional insight that will help in the interpretation of pathogenicity of certain missense variants identified in *NF1* and Legius syndrome patients (Fisher et al., 2018).

Conditions similar to neurofibromatosis type 1 exist. Legius syndrome has similar clinical features to *NF1* such as the café-au-lait macules, but does not have the neurofibromas or central nervous system tumors. Furthermore, the primary genetic alteration in Legius syndrome is the sprouty-related *EVH1* [enabled/vasodilator-stimulated phosphoprotein homology 1 gene (*SPRED1*)] compared to *NF1* for neurofibromatosis 1. Another similar condition is constitutional mismatch repair-deficiency syndrome (CMMR-D) which also has similar clinical symptoms, but leads to different malignancies compared to neurofibromatosis 1. CMMR-D patients may develop hematologic or colorectal malignancies in addition to the neurofibromas seen in *NF1* patients (Korf, 2018).

NF1 is diagnosed clinically using the criteria developed by the National Institutes of Health (NIH, 1988), which are both highly specific and sensitive in all but very young children. Approximately 46% of sporadic *NF1* cases fail to meet the NIH Diagnostic Criteria by 1 year of age. Nearly all (97%; 95% confidence interval: 94-98) *NF1* patients meet the criteria for diagnosis by 8 years old, and all do so by 20 years old (DeBella, Szudek, & Friedman, 2000).

Molecular testing for *NF1* includes sequencing of all the coding exons as well as deletions/rearrangements due to the large size of the gene and the heterogeneity of mutations. Messiaen et al (2000) reported identification of the causative DNA mutation in 64 of 67 patients with a clinical diagnosis of *NF1*. Korf (2018) states that molecular testing is reported to identify approximately 95 percent of causative mutations. However, a positive *NF1* mutation test does not predict the severity or complications of the disorder (Korf, 2018).

Molecular genetic testing is indicated for individuals in whom *NF1* is suspected but who do not fulfill the NIH diagnostic criteria (Friedman, 2018). Additionally, there is increasing use of genetic testing in the diagnosis of *NF1* for patients who meet only these two NIH criteria; moreover, individuals with only one NIH criterion as a positive genetic test may shorten the period of diagnostic uncertainty, allowing the initiation of appropriate screening evaluations (Korf, 2018). Further examples of clinical utility that justify molecular testing include: a young child with a serious tumor (e.g., optic glioma) in whom establishing a diagnosis of *NF1* immediately would affect management, an adult with *NF1* if prenatal or preimplantation genetic diagnosis in a current or future pregnancy is anticipated (Friedman, 2018). Lastly, some rare variants of *NF1* including spinal *NF1* are known to produce a phenotype in which affected individuals may not meet the NIH diagnostic criteria in which case molecular testing is indicated for at-risk relatives (Burkitt Wright et al., 2013).

A negative *NF1* mutation test in patients with only café-au-lait macules and axillary freckling should be tested for *SPRED1* mutations followed by the four mismatch repair genes as Legius syndrome, constitutional mismatch repair-deficiency (CMMR-D) syndrome, and Noonan syndrome may present with these indications (Korf, 2018).

Clinical Validity and Utility

Giugliano et al investigated the clinical and genotypic associations in children with pigmentary features characteristic of a neurocutaneous condition, such as neurofibromatosis type 1. 281 patients were included, with 150 definitively diagnosed with *NF1*, 95 presenting with only pigmentary features such as café au lait macules (CALMs), and 36 presenting with a clinical suspicion of another “RASopathy” (a condition caused by mutations in the MAPK pathway) or other neurocutaneous disorder. The authors identified the causative pathogenic variant in 239 of 281 cases (leaving 42 undiagnosed). Of the patients diagnosed with *NF1*, mutations were detected in 98% of cases (147/150) but in patients with only pigmentary features, the detection rate fell to 69.5% (66/95), with *SPRED1* accounting for 8 of those cases. In patients presenting with a separate neurocutaneous condition, mutation detection rate was found to be 72.2% (26/36), with pathogenic variants found in 10 genes such as *PTPN11*. The authors recognized the difficulty of diagnosing these neurocutaneous and concluded that a “combined NGS-based approach can assist clinicians in the diagnosis of *NF1* as well as other neurocutaneous disorders and overlapping conditions (Giugliano et al., 2019)”.

Castellanos et al developed a custom next-generation sequencing (NGS) panel for testing patients with “with a clinical suspicion of a RASopathy (n = 48) and children presenting multiple CALMs [café-au-lait macules] (n = 102)”. The authors stated that phenotypic overlaps may exist in children if

multiple CALMs are the only clinical symptom present and that genetic testing may differentiate between conditions. Of the 48 patients with clinical suspicion of a RASopathy, 21 were found to harbor a pathogenic mutation (with *NF1* mutations comprising 5 of 48 cases). Of the patients with multiple CALMs, both *NF1* and *SPRED1* pathogenic mutations were identified. Overall, the authors concluded that “an NGS panel strategy for the genetic testing of these two phenotype-defined groups outperforms previous strategies (Castellanos et al., 2020)”.

Neurofibromatosis type 2

Neurofibromatosis type 2 refers to what was originally thought to be a rare subtype of neurofibromatosis type 1, but rather is a distinct entity both genetically and clinically (Evans, 2018c, 2020). It is characterized by bilateral vestibular schwannomas with associated symptoms of tinnitus, hearing loss, and balance dysfunction resulting from mutation in the *NF2* gene. Affected individuals may also develop schwannomas of other cranial and peripheral nerves, meningiomas, ependymomas, and, very rarely, astrocytomas. Typical age of onset is 18 to 24 years, with almost all affected individuals developing bilateral schwannomas by the age of 30 (Evans, 2018a). The prevalence is about 1:60,000 with a birth incidence of 1:33,000 (Evans et al., 2010). Skin tumors and ocular findings often are the first manifestations and have been underrecognized in children (Ruggieri et al., 2005).

The protein encoded by the *NF2* gene, merlin or schwannomin, is a cell membrane-related tumor suppressor (Evans, 2020). Inactivation of both alleles is necessary for tumor development. Variable expressivity of *NF2* results in varying size, location, and number of tumors. Despite that these tumors are not malignant, their number and anatomical location contribute significantly to morbidity and mortality with the average age of death being 36 (Baser et al., 2002). However, advances in molecular diagnosis, imaging, and treatment of *NF2*-associated tumors have resulted in lower mortality (Hexter et al., 2015).

Clinical criteria for *NF2* were initially established with those for *NF1* (NIH, 1988), and they were modified as the Manchester criteria to include molecular diagnostics and increase specificity without affecting sensitivity (Evans, 2018c, 2020). Most recently, the identification of *LZTR1* as a cause of schwannomatosis reduces the specificity of these more inclusive criteria and even the presence of bilateral VS is now no longer sufficient to be certain that an individual has *NF2* (Smith et al., 2017), resulting in further modification of the Manchester criteria.

Detailed molecular testing is reported to identify mutations in *NF2* in 93% of families with multiple members affected by *NF2* (Evans, 2018c). Early diagnosis of individuals with *NF2* facilitates treatment and reduction of mortality (Hexter et al., 2015); however, genetic testing and management is complicated by the well-documented risk of mosaicism (Evans, Raymond, Barwell, & Halliday, 2012). More so than with *NF1*, the stronger genotype/phenotype correlations in mutations of *NF2* (Baser et al., 2004; Baser et al., 2005), high frequency of de novo mutations, and presentation of patients before clinical diagnostic criteria are fulfilled have provided a stronger rationale for the clinical utility of molecular testing than for *NF1* (Evans, 2018b).

Molecular testing approaches can differ for *NF2* based on the clinical picture. Patients with the distinctive phenotypic and laboratory findings suggestive of *NF2* are likely to be diagnosed using gene-targeted testing (75%), whereas those where the diagnosis of *NF2* has not been considered or had met the diagnostic criteria (such as children) are diagnosed after exome sequencing (Evans, 2018b).

Clinical Validity and Utility

Evans et al investigated the clinical validity of the primary development of NF2, the bilateral vestibular schwannoma (BVS). The authors observed that out of a database of over 1200 patients, approximately 25% of them over 50 developed a BVS without any other clinical features of NF2. Over 50% of the patients over 70 developed a BVS as well. This lack of other clinical features in addition to the BVS led the authors to suggest that these developments of a BVS were due to chance rather than an NF2 mutation (Evans et al., 2015).

Pathmanaban et al (2017) analyzed the database of the Manchester Centre for Genomic Medicine to determine the frequency of the known heritable meningioma- or schwannoma-predisposing mutations in children and young adults presenting with a solitary meningioma or schwannoma. They found that “A significant proportion of young people with an apparently sporadic solitary meningioma or schwannoma had a causative predisposition mutation. This finding has important clinical implications because of the risk of additional tumors and the possibility of familial disease. Young patients presenting with a solitary meningioma or schwannoma should be referred for genetic testing (Pathmanaban et al., 2017).”

Castillanos et al (2018) recently demonstrated the clinical utility of a careful dermatological inspection and the correct identification of skin plaques in children for an early diagnosis of NF2. Skin plaques from 7 patients (4 male and 3 female) were analyzed and histologically characterized as plexiform schwannomas. Genetic analysis of primary Schwann cell cultures derived from them allowed the identification of a constitutional and a somatic NF2 mutation. Genetic testing allowed the early diagnosis of NF2 in a child only exhibiting the presence of skin plaques. Most of the patients with NF2 analyzed had an early presentation of skin plaques and a severe NF2 phenotype. The authors remarked that “Dermatological identification of skin plaque schwannomas in children would facilitate the early diagnosis and treatment of patients with NF2 before development of severe adverse effects.”

A genetic severity score has recently been developed to draw these factors together to enable genotypic data to be routinely factored into clinical and research use. This UK NF2 Genetic Severity Score classifies patients into three categories, which are tissue mosaic (1), classic (2), and severe (3). Within each category are subcategories, which consists of the following in increasing severity: presumed tissue mosaicism (1A), confirmed tissue mosaicism (1B), mild NF2 (2A), moderate NF2 (2B), and severe NF2 (3). These categories are separated by severity of mutation shown below (Halliday et al., 2017).

Genetic Severity	Subcategory	Clinical Characteristics	Definition
1 (Tissue Mosaic)	1A	Presumed tissue mosaicism	Meets clinical criteria for sporadic NF2 but not confirmed molecularly with identical NF2 mutations detected in two separate tissue samples
	1B	Confirmed tissue mosaicism	Mosaic NF2 confirmed molecularly with identical NF2 mutations detected in two or more separate tissue samples

2 (Classic)	2A	Mild NF2	Full or mosaic <i>NF2</i> mutation identified in blood excluding those found in group 2B or 3: missense mutations; in-frame deletions and duplications; deletions involving the promoter region or exon 1; splice site mutations in exons 8–15; truncating mutations of exon 1; mosaicism in blood for mutations other than truncating mutations in exons 2–13 Inherited NF2 but no <i>NF2</i> , <i>SMARCB1</i> or <i>LZTR1</i> mutation identified in blood
	2B	Moderate NF2	Full or mosaic NF2 mutation identified in blood including: splicing mutation involving exons 1–7; large deletion not including the promoter or exon 1; truncating mutations in exons 14–15; mosaic in blood for a truncating mutation in exons 2–13
3 (Severe)	3	Severe NF2	Full <i>NF2</i> truncating mutation exons 2–13

Halliday et al evaluated the validity of this score in 142 patients (63 in group 1, 35 in group 2, and 19 in group 3 with 3 with no mutation identified) More severe symptoms such as intracranial meningiomas, BVS, and spinal schwannomas, were more likely to be found in group 3 compared to group 1. For example, BVS and intracranial meningiomas were found in 100% and 94.7% of group 3 patients respectively, compared to 54% and 59% in group 1. Spinal meningiomas were found in 36.8% of group 3 patients compared to 15.3% of group 1, and schwannomas were found in 94.7% of group 3 patients compared to 48.3% of group 1. The authors concluded that “The biggest single factor that determines NF2 severity is the type of mutation, its position within the gene and the proportion of cells carrying it (Halliday et al., 2017).”

Lu et al examined the efficacy and safety of bevacizumab for vestibular schwannomas (VS) in neurofibromatosis type 2. The authors included eight articles including 161 patients and 196 VS. The authors identified radiographic response in 41% of cases (termed “partial regression”), no change in 47% of cases, and tumor progression of 7% of cases. Bevacizumab treatment also resulted in hearing improvement in 20% of cases, stability in 69% of cases, and further hearing loss in 6% of cases. Bevacizumab toxicity was observed in 17% of cases, and surgical intervention was needed in 11% of cases. Overall, the authors concluded that bevacizumab may “arrest” tumor progression and hearing loss in NF2 patients presenting with VS lesions, but recommended judicious use of bevacizumab due to serious adverse events (Lu et al., 2019).

Schwannomatosis

Schwannomatosis is an uncommon form of neurofibromatosis characterized by predisposition to develop multiple schwannomas and, less frequently, meningiomas. Its estimated prevalence is 1:70,000 (Dhamija, Plotkin, Asthagiri, Messiaen, & Babovic-Vuksanovic, 2018) but is thought to be

underestimated (Koontz et al., 2013). Although there is clinical overlap with NF2, schwannomatosis is caused by the concomitant mutational inactivation of two or more tumor suppressor genes. Germline mutations of either the *SMARCB1* or *LZTR1* tumor suppressor genes have been identified in 86% of familial and 40% of sporadic schwannomatosis patients (Kehrer-Sawatzki, Farschtschi, Mautner, & Cooper, 2017). *LZTR1* encodes leucine zipper-like transcriptional regulator 1 and *SMARCB1* (also known as INI1) encodes a subunit of the SWI/SNF chromatin remodeling complex, and both act as tumor suppressors. Biallelic inactivation of these tumor suppressor genes leads to schwannomatosis (Radhika Dhamija, 2018).

The median age of symptom onset is 30 years with pain being the most common presenting symptom in 57 percent of patients. In others (41 percent), a mass was the presenting symptom (Merker, Esparza, Smith, Stemmer-Rachamimov, & Plotkin, 2012). Other symptoms reported at presentation vary based on the location of the tumors, but they can include focal numbness, weakness, and muscle atrophy (Bergner & Yohay, 2018; Yohay & Bergner, 2019). Peripheral and spinal schwannomas are common in schwannomatosis patients. Severe pain is difficult to treat in these patients and often associated with anxiety and depression (Merker et al., 2012).

Diagnostic criteria for schwannomatosis was first set forth by MacCollin et al (2005) but has been revised with the addition of molecular diagnostic criteria (Plotkin et al., 2013). More recently combined clinical and molecular criteria from Kehrer-Sawatzki et al, have been proposed (Kehrer-Sawatzki et al., 2017).

“A combined molecular and clinical diagnosis may be made with ≥ 2 tumors with 22q LOH and different somatic *NF2* mutations AND ≥ 2 pathologically confirmed schwannomas or meningiomas

OR

Germline *SMARCB1* or *LZTR1* pathogenic mutation AND one pathologically confirmed schwannoma or meningioma”

“A strictly clinical diagnosis may be made with ≥ 2 nonintradural schwannomas, one pathologically confirmed and no bilateral vestibular schwannoma by high quality MRI (some mosaic *NF2* patients will be included in this diagnosis at a young age and some schwannomatosis patients may have unilateral vestibular schwannomas or meningiomas)

OR

one pathologically confirmed schwannoma or intracranial meningioma AND an affected first degree relative.

Exclusion criteria for schwannomatosis are as follows:

- Germline pathogenic *NF2* mutation
- First degree relative with NF2
- Fulfillment of diagnostic criteria for NF2
- If schwannomas occur exclusively in a region of previous radiation therapy (Kehrer-Sawatzki et al., 2017)

Kehrer-Sawatzki et al (2017) also recommended, “Comprehensive mutation analysis of all three genes, *LZTR1*, *SMARCB1*, and *NF2*, in patients with schwannomatosis should be performed to identify the complete mutational spectra and the number of mutational hits that affect these genes. This comprehensive testing may help to classify the tumors according to their mutation-profile. The mutation analysis should also include methods, such as next-generation sequencing, which are well suited to detect somatic mosaicism with mutant cells present in low proportions. This approach should identify tumor heterogeneity and help to distinguish between mosaic *NF2* and schwannomatosis, since some *NF2* patients with somatic mosaicism for an *NF2* gene mutation fulfill the diagnostic criteria for schwannomatosis (Kehrer-Sawatzki et al., 2017).”

Clinical Validity and Utility

Hutter et al evaluated the proportion of schwannomatosis cases that come from mutations aside from the germline variants in *SMARCB1* and *LZTR1*. The authors performed whole exome sequencing on 23 patients with sporadic schwannomatosis (without *SMARCB1* mutations) and found only 5 *LZTR1* or *NF2* mutations. However, since the authors noted the reported frequency of *SMARCB1* mutations to be only 10% in sporadic schwannomatosis patients, they concluded that approximately 65% (or at least the “majority”) of sporadic schwannomatosis mutations are caused by an unknown gene (Hutter et al., 2014).

Louvrier and colleagues performed targeted next generation sequencing (NGS) to investigate genetic differences between *NF2*, schwannomatosis, and meningiomatosis. The authors sequenced 196 patients (79 with *NF2*, 40 with schwannomatosis, 12 with meningiomatosis, and 65 with no clearly established diagnosis) for *NF2*, *SMARCB1*, *LZTR1*, *SMARCE1*, and *SUFU*. The *NF2* and schwannomatosis results were as follows: “An *NF2* variant was found in 41 of 79 *NF2* patients (52%). *SMARCB1* or *LZTR1* variants were identified in 5/40 (12.5%) and 13/40 (~32%) patients in the schwannomatosis cohort. Potentially pathogenic variants were found in 12/65 (18.5%) patients with no clearly established diagnosis. A *LZTR1* variant was identified in 16/47 (34%) *NF2*/*SMARCB1*-negative schwannomatosis patients.” The authors concluded that targeted NGS was a suitable strategy for identifying *NF2* mosaicism in blood and for investigation of these tumors (Louvrier et al., 2018).

V. Guidelines and Recommendations

American Academy of Pediatrics (AAP) (Hersh, 2008; Miller et al., 2019)

In 2008, the AAP committee on genetics published guidelines on health supervision in children with *NF1* (Hersh, 2008). The committee stated that genetic consultation and genetic testing should be considered to expedite a diagnosis when there is uncertainty regarding a definitive diagnosis of *NF1*. The committee also noted that “molecular testing also may represent an option in those instances when a couple in which one person has *NF1* is seeking prenatal diagnosis.”

This guideline was reaffirmed in 2017.

A Clinical Report from the AAP comments on the role of genetic testing for Neurofibromatosis Type 1. They state that 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.” (Miller et al., 2019)

American College of Medical Genetics and Genomics (ACMG) (ACMG, 2016, 2018)

In their guidelines detailing the care of adults with NF1, the ACMG noted that “In most cases, the diagnosis can be easily made based on a history, physical exam, and pedigree review and no additional imaging or NF1 genetic testing is needed”. Furthermore, the ACMG stated that genetic testing can quickly establish a diagnosis for children thereby relieving anxiety, but this is not as significant an issue for adults (ACMG, 2018).

However, in the ACMG’s guidelines for reporting of secondary findings in exome or genome sequencing, mutations in the NF2 gene were recommended for return (ACMG, 2016).

European Association of Neuro-Oncology (EANO, 2020) (Goldbrunner et al., 2020)

This EANO guideline on “diagnosis and treatment of vestibular schwannoma” comments on neurofibromatosis type 2, stating that NF2 “should be considered when an individual presents with a unilateral vestibular or other sporadic schwannoma at <30 years or meningioma at <25 years. Germline pathogenic variants can be identified in 1-10% of cases. NF2 should also be considered in older patients with two NF2 related tumors. (Goldbrunner et al., 2020)

American Association for Cancer Research (AACR) Childhood Cancer Predisposition Workshop (D. G. R. Evans et al., 2017; D. Gareth R. Evans et al., 2017)

The following recommendations were created based on expert review of the literature and discussion brought to this workshop.

NF1

- “A child who meets one or more clinical criterion should now have NF1 molecular genetic testing (sequencing and deletion/duplication analysis) offered to confirm if NF1 is the correct diagnosis.” Genetic testing is especially recommended in children fulfilling only pigmentary features of the criteria.

The clinical diagnostic criteria are as follows:

- Six or more CAL macules, the greatest diameter of which is more than 5 mm in prepubertal patients and more than 15 mm in postpubertal patients
- Two or more neurofibromas of any type, or one plexiform neurofibroma

- Axillary or inguinal freckling
- Optic glioma
- Two or more Lisch nodules
- A distinctive osseous lesion such as sphenoid dysplasia or pseudarthrosis
- A first-degree relative with NF1 according to the preceding criteria

The guidelines note that according to the NIH, two or more of these criteria must be present. This is in contrast to their own guidelines' statement of only requiring one clinical criterion.

The guidelines summarize their genetic testing recommendations as follows:

- "Children considered at risk of NF1 especially with 6+ CAL macules or diagnosed with NIH criteria should ideally have genetic testing of the NF1 gene with an RNA-based approach and testing of SPRED1 if pigmentary features only".
- "Those testing negative should be considered for a panel of genes including GNAS, MLH1, MSH2, MSH6, NF2, PMS2, PTPN11, SOS1, and SPRED1 (if not already tested)" (D. Gareth R. Evans et al., 2017).

NF2

- "All children presenting with either clear diagnostic criteria for NF2, including combined retinal hamartomas, or those with an NF2 tumor (any schwannoma/meningioma) presenting in childhood should undergo genetic testing of NF2, ideally in both blood and tumor if available in sporadic cases."

Schwannomatosis

- "Test for mutations in SMARCB1 and LZTR1 in children and young adults with one or more non-intradermal schwannoma, including those with VS (vestibular schwannoma) negative for NF2" (D. G. R. Evans et al., 2017).

European consortium 'Care for CMMRD' (C4CMMRD, 2014) (Suerink et al., 2019; Wimmer et al., 2014)

The C4CMMRD recommends further testing for patients reaching three points on the clinical scoring scale. "Further testing" generally follows the protocols for Lynch syndrome, which involves analysis of microsatellite instability or immunohistochemistry staining of the main mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2). The clinical scoring scale is as follows (Wimmer et al., 2014):

Malignancies/premalignancies: one is mandatory; if more than one is present in the patient, add the points.

- Carcinoma from the LS spectrum* at age <25 years 3 points
- Multiple bowel adenomas at age <25 years and absence of APC/MUTYH mutation(s) or a single high-grade dysplasia adenoma at age <25 years 3 points
- WHO grade III or IV glioma at age <25 years 2 points
- NHL (non-Hodgkin's lymphoma) of T-cell lineage or sPNET (supratentorial primitive neuroectodermal tumour) at age <18 years 2 points
- Any malignancy at age <18 years 1 point

Additional features: optional; if more than one of the following is present, add the points

- Clinical sign of NF1 and/or ≥ 2 hyperpigmented and/or hypopigmented skin alterations $\varnothing > 1$ cm in the patient 2 points
- Diagnosis of LS in a first-degree or second-degree relative 2 points
- Carcinoma from LS spectrum* before the age of 60 in first-degree, second-degree, and third-degree relative 1 point
- A sibling with carcinoma from the LS spectrum*, high-grade glioma, sPNET or NHL 2 points
- A sibling with any type of childhood malignancy 1 point
- Multiple pilomatricomas in the patient 2 points
- One pilomatricoma in the patient 1 point
- Agenesis of the corpus callosum or non-therapy-induced cavernoma in the patient 1 point
- Consanguineous parents 1 point
- Deficiency/reduced levels of IgG2/4 and/or IgA 1 point

*Colorectal, endometrial, small bowel, ureter, renal pelvis, biliary tract, stomach, bladder carcinoma (Wimmer et al., 2014).

The consortium in 2018 issued the selection strategy for CMMRD testing as follows:

Prerequisites for testing are...

- “Suspicion of NF1 due to the presence of at least one diagnostic NF1 feature, including at least two hyperpigmented skin patches reminiscent of CALMs [café-au-lait macules]
- No NF1 and SPRED1 germline mutations detected using comprehensive and highly sensitive mutation analysis protocols.
- Absence of diagnostic NF1 sign(s) in both parents
- Additional features, at least one (either in the family or in the patient) is required
 - In the family
 - Consanguineous parents.
 - Genetic diagnosis of Lynch syndrome in one or both of the parental families.
 - Sibling with diagnostic NF1 sign(s).
 - A (deceased) sibling§ with any type of childhood malignancy.
 - One of the following carcinomas from the Lynch syndrome spectrum: colorectal cancer, endometrial cancer, ovarian cancer, gastric cancer, small bowel cancer, cancer of the bile duct or gall bladder, pancreatic cancer or urothelial cancer before the age of 60 years in first-degree or second-degree relative.
 - In the patient
 - Atypical CALMs (irregular borders and/or pigmentation).
 - Hypopigmented skin patches.
 - One or more pilomatricoma(s) in the patient.
 - Agenesis of the corpus callosum.
 - Non-therapy-induced cavernoma.
 - Multiple developmental vascular abnormalities (also known as cerebral venous angiomas) in separate regions of the brain.

§This can be expanded to second-degree and third-degree relatives in populations with a high prevalence of founder mutations (Suerink et al., 2019).”

National Comprehensive Cancer Network (NCCN) (NCCN, 2019)

Within the Lynch Syndrome guidelines, the NCCN states, “For patients of reproductive age, advise about the risk of a rare recessive syndrome called constitutional MMR deficiency (CMMRD) syndrome...if both partners are a carrier of a mutation/s in the same MMR gene or *EPCAM* (for example, if both partners carry a mutation in the *PMS2* gene, then their future offspring will be at risk of having CMMRD syndrome) (NCCN, 2019).”

VI. State and Federal Regulations, as applicable

A. Food and Drug Administration

No FDA-approved tests for neurofibromatosis or schwannomatosis were found as of March 31, 2020. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

B. Centers for Medicare & Medicaid Services (CMS)

L35024 MoIDX: Genetic Testing for Lynch Syndrome - <https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?lcdid=35024&ver=61&bc=0>

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81405	Molecular pathology procedure, Level 6; Includes NF2 ((D. G. R. Evans et al., 2017)2 [merlin]) (e.g., neurofibromatosis, type 2), duplication/deletion analysis
81406	Molecular pathology procedure, Level 7; NF2 (neurofibromin 2 [merlin]) (e.g., neurofibromatosis, type 2), full gene sequence
81408	Molecular pathology procedure, Level 9; NF1 (neurofibromin 1) (e.g., neurofibromatosis, type 1), full gene sequence
81292	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81293	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants

Code Number	Code Description
81294	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81295	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81296	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81297	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81298	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81299	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81300	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81301	Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81317	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81318	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81319	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81479	Unlisted molecular pathology procedure
96040	Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family
S0265	Genetic counseling, under physician supervision, each 15 minutes

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Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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