

## Genetic Testing for Neurofibromatosis and Related Disorders

Policy Number: AHS – M2134 – Genetic Testing for Neurofibromatosis and Related Disorders	Policy Revision Date: 10/15/2025 Initial Policy Effective Date: 12/01/2024
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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.<sup>1</sup> 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.<sup>2</sup> Schwannomatosis is caused by inactivating mutations in *SMARCB1* and *LZTR1* and is characterized by multiple schwannomas and pain arising in adulthood.<sup>3</sup>

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 (CMMRD), 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, CMMRD can also result in a variety of different malignancies, including glioblastoma and colorectal cancer.<sup>1</sup>

### II. Related Policies

Policy Number	Policy Title
AHS-M2179	Prenatal Screening (Genetic)

### 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. Specifications pertaining to Medicare and Medicaid can be found in the “Applicable State and Federal Regulations” section of this policy document.

- 1) Prior to genetic testing for neurofibromatosis, *NF2*-, *SMARCB1*-, or *LZTR1*-related schwannomatosis, Legius Syndrome, **or** constitutional mismatch repair deficiency (CMMRD), genetic counseling **IS REQUIRED**.
- 2) For individuals who are clinically suspected of having neurofibromatosis type 1 (NF1), but for whom a definitive diagnosis cannot be made without genetic testing, genetic testing for *NF1* mutations **MEETS COVERAGE CRITERIA** when **one** of the following signs of NF1 is present:

- a) Individual has six or more café-au-lait macules (over 5 mm in greatest diameter in pre-pubertal individuals; over 15 mm in greatest diameter in post-pubertal individuals).
  - b) Individual has two or more neurofibromas of any type or one plexiform neurofibroma.
  - c) Individual has freckling in the axillary or inguinal regions.
  - d) Individual has optic glioma.
  - e) Individual has two or more Lisch nodules (iris hamartomas).
  - f) Individual has a distinctive osseous lesion, such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of the long bone.
  - g) Individual has a first-degree relative (see Note 1) with NF1 as defined by the above criteria.
- 3) For asymptomatic individuals who have a close blood relative (see Note 1) with a deleterious *NF1* or *NF2* gene mutation, the following testing **MEETS COVERAGE CRITERIA**:
- a) Testing restricted to the known familial mutation.
  - b) Comprehensive genetic testing when the specific familial mutation is unknown (i.e., family member is unavailable for testing or testing results are unavailable).
- 4) For individuals who have a clinical diagnosis of neurofibromatosis and who are planning to conceive, preconception screening for *NF1* or *NF2* gene mutations, when the individual has not previously received genetic screening for a pathogenic mutation, **MEETS COVERAGE CRITERIA**.
- 5) For individuals who are clinically suspected of having *NF2*-related schwannomatosis, but for whom a definitive diagnosis and classification cannot be made without genetic testing, genetic testing for *NF2* gene mutations **MEETS COVERAGE CRITERIA** when **one** of the following signs of *NF2*-related schwannomatosis is present:
- a) Individual has bilateral vestibular schwannomas (VS)
  - b) Individual has either two major or one major and two minor criteria:
    - i) Major criteria:
      - (a) Unilateral VS
      - (b) First-degree relative (see Note 1) other than a sibling) with *NF2*-related schwannomatosis
      - (c) Two or more meningiomas (note that a single meningioma qualifies as minor criteria)
    - ii) Minor criteria:
      - (a) Can count >1 of a type (e.g., 2 distinct schwannomas would count as 2 minor criteria): Ependymoma, schwannoma (note that if the major criterion is unilateral VS, at least 1 schwannoma must be dermal in location)
      - (b) Can count only once (e.g., bilateral cortical cataracts count as a single minor criterion): Juvenile subcapsular or cortical cataract, retinal hamartoma, epiretinal membrane in a person aged <40 years, meningioma (because multiple meningiomas qualify as a major criteria).

- 6) For individuals who are negative for *NF2* mutations **and** who have one or more pathologically confirmed schwannoma or hybrid nerve sheath tumor, genetic testing for mutations in *SMARCB1* and *LZTR1* **MEETS COVERAGE CRITERIA**.
- 7) For individuals who are clinically suspected of having Legius Syndrome, genetic testing of *SPRED1* **MEETS COVERAGE CRITERIA** when **one** of the following conditions is met:
- a) The individual has six or more café-au-lait macules (over 5 mm in greatest diameter in pre-pubertal individuals; over 15 mm in greatest diameter in post-pubertal individuals).
  - b) The individual has freckling in the axillary or inguinal regions.
  - c) The individual has symptoms of NF1, but genetic test results for NF1 were negative.
- 8) For individuals who have at least two hyperpigmented skin patches (café-au-lait macules), who have tested negative for *NF1* and *SPRED1* mutations, **and** for whom neither parent has diagnostic signs of NF1 (if known), genetic testing for CMMRD (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) **MEETS COVERAGE CRITERIA** when **one** of the following risk factors is present:
- a) Risk factors in the patient include:
    - i) Atypical café-au-lait macules (irregular borders and/or pigmentation).
    - ii) Hypopigmented skin patches.
    - iii) One or more pilomatricoma(s).
    - iv) Agenesis of the corpus callosum.
    - v) Non-therapy-induced cavernoma.
    - vi) Multiple developmental vascular abnormalities (cerebral venous angiomas) in separate regions of the brain.
  - b) Familial risk factors include:
    - i) Consanguineous parents.
    - ii) A genetic diagnosis of Lynch syndrome in one or both of the parental families.
    - iii) A sibling with diagnostic NF1 sign(s).
    - iv) A sibling, living or deceased, with any type of childhood malignancy.
    - v) A first- or second-degree relative (see Note 1) diagnosed before the age of 60 years with 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.

*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 an individual's illness.*

- 9) For all other situations not meeting the criteria outlined above, genetic testing for neurofibromatosis **DOES NOT MEET COVERAGE CRITERIA**
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**NOTES:**

**Note 1:** Close blood relatives include first-degree relatives (e.g., parents, siblings, and children), second-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and third-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins), all of whom are on the same side of the family.

**Note 2:** For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

**IV. Table of Terminology**

Term	Definition
AACR	American Association for Cancer Research
AAP	American Academy of Pediatrics
ACMG	American College of Medical Genetics and Genomics
BVS	Bilateral vestibular schwannoma
C4CMMRD	Care for constitutional mismatch repair deficiency
CALM	Café-au-lait macule
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMMRD	Constitutional mismatch repair deficiency
CMS	Centers for Medicare and Medicaid Services
EANO	European Association of Neuro-Oncology
<i>EVH1</i>	<i>Enabled/vasodilator-stimulated phosphoprotein homology 1</i>
FDA	Food and Drug Administration
LDT	Laboratory-developed test
<i>LZTR1</i>	<i>Leucin-zipper-like transcriptional regulator 1</i>
MMR	Measles, mumps, and rubella
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NF1	Neurofibromatosis type 1
NF2	Neurofibromatosis type 2
NGS	Next-generation sequencing
NSD	Noonan spectrum disorders
<i>SMARCB1</i>	<i>SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily B, member 1</i>
<i>SPRED1</i>	<i>Sprout-Related EVH1 Domain-Containing Protein 1</i>
sVS	Sporadic vestibular schwannoma
VS	Vestibular schwannoma

## V. Scientific Background

### *Neurofibromatosis type 1*

Neurofibromatosis type 1 is relatively common, affecting approximately one in 3,000 individuals.<sup>1</sup> 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.<sup>4</sup>

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.<sup>1</sup> Ocular, neurologic, musculoskeletal, vascular, cardiac, and malignant manifestations have been reported.<sup>5</sup>

Mutations to *NF1* 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.<sup>6</sup> Despite thousands of *NF1* mutations identified, few genotype/phenotype correlations have been observed.<sup>7</sup> 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.<sup>8</sup>

Neurofibromatosis type 1 is diagnosed clinically using the criteria developed by the National Institutes of Health,<sup>9</sup> 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 one year of age. Nearly all (97%; 95% confidence interval: 94-98) *NF1* patients meet the criteria for diagnosis by eight years old, and all do so by 20 years old.<sup>10</sup>

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. reported identification of the causative DNA mutation in 64 of 67 patients with a clinical diagnosis of *NF1*. Korf, et al. (2025) 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.<sup>1</sup>

Molecular genetic testing is indicated for individuals in whom *NF1* is suspected but who do not fulfill the NIH diagnostic criteria.<sup>6</sup> 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.<sup>1</sup> 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.<sup>6</sup>

Prenatal testing is available through direct mutation testing of fetal DNA taken from CVS or from amniocentesis to diagnose *NF1* pathogenic variants in the fetus. An additional option is assessing DNA markers in families with two or more affected individuals; however, many partners do not perform a

prenatal assessment because of “the inability to determine disease severity” in the fetus.<sup>11</sup> Because the time for prenatal diagnosis is limited, it is common for families to detect pathogenic *NF1* alleles through linkage analysis as a more “rapid and useful” method for diagnosis.<sup>12</sup> Additionally, detection of pathogenic *NF1* mutations can be complex and challenging because of wide phenotypic variability and an absence of genotype-phenotype correlation.<sup>12</sup>

Preimplantation genetic diagnosis is also available to assist individuals who want to avoid a later termination of a pregnancy. Preimplantation genetic diagnosis occurs using cells removed from an embryo (available at the approximately three day mark of embryo development). This helps individuals determine which embryos do not carry the *NF1* mutation in order to transfer unaffected embryos for implantation. Additionally, *NF1* experts recommend that all *NF1* pathogenic mutation affected individuals should receive genetic counseling prior to conception.<sup>11</sup>

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 this case, molecular testing is indicated for at risk relatives.<sup>13</sup>

### *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.<sup>2</sup> 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.<sup>2</sup> The prevalence is about 1:60,000 with a birth incidence of 1:33,000.<sup>14</sup> Skin tumors and ocular findings often are the first manifestations and have been underrecognized in children.<sup>15</sup>

The protein encoded by the *NF2* gene, merlin or schwannomin, is a cell membrane-related tumor suppressor.<sup>2</sup> 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.<sup>16</sup> However, advances in molecular diagnosis, imaging, and treatment of *NF2*-associated tumors have resulted in lower mortality.<sup>17</sup>

Clinical criteria for *NF2* were initially established with those for *NF1*,<sup>9</sup> and they were modified as the Manchester criteria to include molecular diagnostics and increase specificity without affecting sensitivity.<sup>2</sup> 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*,<sup>18</sup> 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*.<sup>2</sup> Early diagnosis of individuals with *NF2* facilitates treatment and reduction of mortality.<sup>17</sup> However, genetic testing and management is complicated by the well-documented risk of mosaicism.<sup>19</sup> More so than with *NF1*, the stronger genotype/phenotype correlations in mutations of *NF2*,<sup>20,21</sup> 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*.

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.<sup>22</sup>

### *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<sup>23</sup> but is thought to be underestimated.<sup>24</sup> 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.<sup>25</sup> *LZTR1* encodes leucin-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.<sup>26</sup>

The median age of symptom onset is 30 years. Pain is the most common presenting symptom in 57% of patients. In others (41%), a mass was the presenting symptom.<sup>27</sup> Other symptoms reported at presentation vary based on the location of the tumors, but they can include focal numbness, weakness, and muscle atrophy.<sup>3</sup> 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.<sup>27</sup>

Diagnostic criteria for schwannomatosis was first set forth by MacCollin, et al. (2005) but has been revised with the addition of molecular diagnostic criteria.<sup>29</sup> More recently combined clinical and molecular criteria from Kehrer-Sawatzki, et al. (2017) have been proposed:<sup>25</sup>

“A combined molecular and clinical diagnosis may be made with  $\geq 2$  tumors with 22q LOH and different somatic *NF2* mutations AND  $\geq 2$  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  $\geq 2$  nonintra-dermal 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.<sup>25</sup>

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 fulfil the diagnostic criteria for schwannomatosis.”<sup>25</sup>

### *Legius Syndrome*

Legius syndrome has similar clinical features to NF1 such as the café-au-lait macules but does not have 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.

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 (CMMRD) syndrome, and Noonan syndrome may present with these indications.<sup>1</sup>

### *CMMRD*

Constitutional mismatch repair deficiency syndrome (CMMRD) has similar clinical symptoms to neurofibromatosis 1 but leads to different malignancies. Hematologic malignancies develop in infancy to early childhood, brain tumors (such as glioblastoma) may present in mid-childhood, and colorectal cancer may show up in adolescence or young adulthood.<sup>1</sup> CMMRD is a childhood cancer predisposition syndrome that is caused by biallelic pathogenic variants in one of four mismatch repair genes.<sup>30</sup> Individuals with this syndrome may develop hematologic or colorectal malignancies in addition to the neurofibromas seen in NF1 patients.<sup>1</sup>

One important characteristic of CMMRD is that it is typically diagnosed in childhood. The Federal Food, Drug, and Cosmetic Act defines pediatric patients as persons aged 21 or younger at the time of their diagnosis or treatment and the *Bright Futures* guidelines from the American Academy of Pediatrics identify adolescence as 11 to 21 years of age, dividing the group into early (ages 11–14 years), middle (ages 15–17 years), and late (ages 18–21 years). CMMRD is most often diagnosed before the age of 18. One collaborative review from the European consortium established the ages of first diagnosis ranging from 0.4 to 39 years. However, the “vast majority” of patients are diagnosed with a first malignancy before the age of 18 (82% diagnosed before age 18).<sup>31</sup>

## ***Clinical Utility and Validity***

### *Neurofibromatosis type 1*

Giugliano, et al. (2019) investigated the clinical and genotypic associations in children with pigmentary features characteristic of a neurocutaneous condition, such as neurofibromatosis type 1. A group of 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 eight 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.”<sup>32</sup>

Castellanos, et al. (2020) developed a custom next-generation sequencing (NGS) panel for testing patients 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 RASopathy, 21 were found to harbor a pathogenic mutation (with *NF1* mutations comprising five 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.”<sup>33</sup>

Witkowski, et al. (2020) studied the benefits of adding *NF1* and *SPRED1* sequencing to the Noonan spectrum/ RASopathy NGS panel. Noonan spectrum disorders (NSD) are a group of disorders caused by problems in the MAPK pathway. NSD's are due to gain of function, while *NF1* is caused by a loss of function. The study included 28 patients with a negative NSD panel that underwent *NF1* and *SPRED1* sequencing, and a validation panel analyzed 14 RASopathy associated genes in 505 patients. In total, 21% of the 28 patients had disease-causing *NF1/SPRED1* variants. In the validation cohort, only two percent of the patients were found to have disease-causing variants in the *NF1/SPRED1* genes. Adding *NF1* and *SPRED1* to the panel increased the diagnostic yield from 23.5% to 25.7%. The authors concluded that “adding the *NF1* and *SPRED1* genes to Noonan spectrum disorder/RASopathy NGS gene panels modestly increases clinical diagnoses.”<sup>34</sup>

In a retrospective study, Elmas (2022) studied the use of artificial intelligence, Face2Gene, to diagnosis neurofibromatosis type 1. Fourteen patients underwent Face2Gene analysis. As a result, the most detected mutation type was nonsense mutation (42.8%) and suggested NF1 diagnosis for 10 of the 14 patients. The authors concluded that Face2Gene will be used a lot in the routines of medical doctors in the next 10 years.<sup>35</sup>

*Neurofibromatosis type 2*

Evans, et al. (2015) 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 1,200 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.<sup>36</sup>

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.”<sup>37</sup>

Castellanos, 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 seven patients 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.”<sup>38</sup>

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.<sup>39</sup>

<b>Genetic Severity</b>	<b>Sub-category</b>	<b>Clinical Characteristics</b>	<b>Definition</b>
<b>1 (Tissue Mosaic)</b>	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

<b>2 (Classic)</b>	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
<b>3 (Severe)</b>	3	Severe NF2	Full <i>NF2</i> truncating mutation exons 2–13

Halliday, et al. (2017) evaluated the validity of this score in 142 patients (63 in group 1, 35 in group 2, and 19 in group 3 with three 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.”<sup>39</sup>

Lu, et al. (2019) examined the efficacy and safety of bevacizumab for 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.<sup>40</sup>

Perez-Becerril, et al. (2024) studied the impact of using additional genetic screening for the detection of pathogenic variants associated with familial *NF2*-related schwannomatosis. The study included 168 individuals, all second-generation *NF2*-related schwannomatosis. Individuals underwent typical clinical screening, including targeted NGS and multiplex ligation-dependent probe amplification analysis. Karyotype and RNA analysis was conducted as additional genetic screening. “Additional genetic analysis resulted in increased sensitivity of detection of pathogenic variants from 87% to 95%.” The authors concluded that “there is added value in performing additional genetic analysis for detection of pathogenic variants that are difficult to identify with current clinical genetic screening methods.”<sup>41</sup>

### *Schwannomatosis*

Hutter, et al. (2014) 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 five *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.<sup>42</sup>

Louvrier, et al. (2018) performed targeted 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.<sup>43</sup>

Sadler, et al. (2021) studied which germline pathogenic variants are associated with sporadic vestibular schwannoma (sVS) through genetic analysis of sVS cases of *NF2*, *LZTR1* and *SMARCB1* genes. *NF2* variants were confirmed in two percent of the patients, *LZTR1* was found in three percent of the patients, and there were no pathogenic *SMARCB1* variants identified in this cohort. Therefore, the authors concluded that “loss of *NF2* function is a common event in sVS tumours and may represent a targetable common pathway in VS tumourigenesis. Earlier identification of patients with these syndromes can facilitate more accurate familial risk prediction and prognosis.”<sup>44</sup>

Piotrowski, et al. (2022) studied the use of targeted massively parallel sequencing to diagnose multiple schwannomas. Thirty-five patients were enrolled in the study and massive parallel sequencing of *LZTR1*, *SMARCB1*, and *NF2* genomic loci was conducted. The study verified whether any other *LZTR1*/*SMARCB1*/*NF2* pathogenic variants could be found in 16 cases carrying a *SMARCB1* constitutional variant in the 3'-untranslated region. “The 3'-UTR variants c.\*17C>T and c.\*82C>T showed pathogenicity. Two novel deep intronic *SMARCB1* variants, c.500+883T>G and c.500+887G>A were identified in two individuals. Further resequencing of chromosome 22q in individuals negative for PVs in the *SMARCB1*/*LZTR1*/*NF2* demonstrated five potential schwannomatosis-predisposing candidate genes (*MYO18B*, *NEFH*, *SGSM1*, *SGSM3*, and *SBF1*.” The authors conclude that noncoding *SMARCB1*/*LZTR1* variants are a molecular cause of schwannomatosis, hence it is essential to include them into the molecular diagnostic panel.<sup>45</sup>

## VI. Guidelines and Recommendations

### **American Academy of Pediatrics (AAP)**

In 2008, the AAP committee on genetics published guidelines on health supervision in children with *NF1*.<sup>46</sup> 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.”<sup>47</sup>

### **American College of Medical Genetics and Genomics (ACMG)**

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.<sup>48</sup>

However, in the ACMG’s guidelines for reporting of secondary findings in exome or genome sequencing (V3.2), mutations in the *NF2* gene were recommended for return as secondary findings related to cancer phenotypes for both pathogenic and likely pathogenic variants.<sup>49</sup>

### **European Association of Neuro-Oncology (EANO)**

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.<sup>50</sup>

### **American Association for Cancer Research (AACR) Childhood Cancer Predisposition Workshop**

The following recommendations were made 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 five mm in prepubertal patients and more than 15 mm in post-pubertal 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 contrasts with 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 pigimentary 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)."<sup>51</sup>

## 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."<sup>52</sup>

## European Consortium 'Care for CMMRD' (C4CMMRD)

The C4CMMRD recommends further testing for patients reaching three points on the clinical 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 scale is as follows:<sup>53</sup>

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  $\geq 2$  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.<sup>53</sup>

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.”<sup>54</sup>

### **National Comprehensive Cancer Network (NCCN)**

The NCCN recommends general testing considerations for cancer risk assessment and counseling. “In children <18 y, genetic testing is generally not recommended unless results would impact medical management, such as initiation of early colonoscopy surveillance. Clear exceptions include when familial adenomatous polyposis (FAP), juvenile polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), or constitutional mismatch repair deficiency (CMMRD) syndrome are suspected or known to be present in a family, in which case testing prior to age 18 is recommended to guide medical management.” The NCCN further states that “Biallelic P/LP variants in some genes, included on gene panels, may be associated with rare autosomal recessive conditions, such as Fanconi anemia (FA) or CMMRD. Thus, for these genes, consideration should be given to carrier testing the partner for P/LP variants in the same gene if it would inform reproductive decision-making and/or risk assessment and management.”<sup>55</sup>

Within the Lynch syndrome guidelines, the NCCN states, “for patients of reproductive age, advise about the risk of a rare recessive syndrome called CMMRD syndrome... If both partners are a carrier of a pathogenic variant(s) in the same MMR gene, then their future offspring will be at risk of having CMMRD syndrome.” The NCCN also states “absence of MMR protein expression in both cancer and normal tissue may be suggestive of CMMRD.”<sup>55</sup>

### **International Consensus Group Recommendation on Neurofibromatosis Type 1**

An international consensus group revised diagnostic criteria for neurofibromatosis type 1 as well as sought to establish diagnostic criteria for Legius syndrome.<sup>56</sup> The group involved global experts, advocacy groups, and patient input in a multistep process to establish criteria.

Diagnostic criteria for neurofibromatosis type 1:

“A: The diagnostic criteria for NF1 are met in an individual who does not have a parent diagnosed with NF1 if two or more of the following are present:

- 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 region
- 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 (CAs)—defined as bright, patchy nodules imaged by optical coherence tomography (OCT)/near-infrared reflectance (NIR) 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 cells.

B: A child of a parent who meets the diagnostic criteria specified in A merits a diagnosis of NF1 if one or more of the criteria in A are present.”<sup>56</sup>

Diagnostic criteria for Legius syndrome:

“A: The diagnostic criteria for Legius syndrome are met in an individual who does not have a parent diagnosed with Legius syndrome if the following CRITERIA are present:

- 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

B: A child of a parent who meets the diagnostic criteria specified in A merits a diagnosis of Legius syndrome if one or more of the criteria in A are present.”<sup>56</sup>

“The diagnostic criteria for mosaic NF1 are met in an individual if any of the following is present:

1. 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)
2. An identical pathogenic heterozygous *NF1* variant in two anatomically independent affected tissues (in the absence of a pathogenic *NF1* variant in unaffected tissue)
3. A clearly segmental distribution of café-au-lait macules or cutaneous neurofibromas AND
  - a. Another NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for *NF1*)
  - or
  - b. Child fulfilling diagnostic criteria for NF1
4. 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 one plexiform neurofibroma AND a child fulfilling the criteria for NF1.”<sup>56</sup>

“The diagnostic criteria for mosaic Legius syndrome are met in an individual if any of the following is present:

1. 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
2. An identical pathogenic heterozygous *SPRED1* variant in two independent affected tissues (in the absence of a pathogenic *SPRED1* variant in unaffected tissue)
3. A clearly segmental distribution of café-au-lait macules AND a child fulfilling the criteria for Legius syndrome.”<sup>56</sup>

### **International Consensus Group Recommendation on Neurofibromatosis Type 2 and Schwannomatosis**

The international consensus group also provided new recommendations on the nomenclature of NF2 and schwannomatosis. Traditionally, NF2 and SWN were identified based on primarily clinical features; however, the group’s consensus is that the “phenotype of these diseases spans a continuum without absolute delineation of subtypes phenotypically” leading to the need for an umbrella.<sup>57</sup> The group chose

the term “schwannomatosis” (i.e. as the umbrella term) to showcase the overlapping clinical phenotype of related conditions. Additionally, the group recommended that the type of SWN be further classified based on the gene that harbors a PV (identified through molecular analysis). According to this nomenclature, NF2 would be renamed “NF2-related schwannomatosis” and SWN would fall as either “SMARCB1-related schwannomatosis,” “LZTR1-related schwannomatosis,” or “22q-related schwannomatosis,” depending on the location of the inherited pathogenic. For patients who have clinical features of NF2/SWN but have not had molecular analysis, the group recommends “schwannomatosis-not otherwise specified” as the type categorization or “schwannomatosis-not elsewhere classified” for patients in whom molecular analysis did not successfully detect a PV variant.<sup>57</sup>

Updated diagnostic criteria for NF2-related schwannomatosis:

“A diagnosis of NF2-related schwannomatosis (previously termed neurofibromatosis 2, NF2) can be made when an individual has one of the following:

1. Bilateral vestibular schwannomas (VS)
2. An identical *NF2* pathogenic variant in at least 2 anatomically distinct NF2-related tumors (schwannoma, meningioma, and/or ependymoma). (Note: if the variant allele fraction (VAF) in unaffected tissues such as blood is clearly <50%, the diagnosis is mosaic NF2-related schwannomatosis)
3. Either 2 major or 1 major and 2 minor criteria as described in the following:
  - Major criteria:
    - Unilateral VS
    - First-degree relative other than sibling with NF2-related schwannomatosis
    - 2 or more meningiomas (Note: single meningioma qualifies as minor criteria)
    - *NF2* pathogenic variant in an unaffected tissue such as blood (Note: if the VAF is clearly <50%, the diagnosis is mosaic NF2-related schwannomatosis)”
  - Minor criteria:
    - Can count >1 of a type (e.g., 2 distinct schwannomas would count as 2 minor criteria)
      - Ependymoma, meningioma (Note: multiple meningiomas qualify as a major criteria), schwannoma (Note: if the major criterion is unilateral VS, at least 1 schwannoma must be dermal in location)
    - Can count only once (e.g., bilateral cortical cataracts count as a single minor criterion)
      - Juvenile subcapsular or cortical cataract, retinal hamartoma, epiretinal membrane in a person aged <40 years, meningioma.”<sup>57</sup>

“Pattern of genetic changes in unaffected and tumor tissue in *NF2*-related schwannomatosis”

Gene locus	Unaffected tissue	Tumor 1	Tumor 2	Comment
<i>NF2</i>				
Allele 1	PV1	PV1	PV1	Shared <i>NF2</i> pathogenic variant
Allele 2	WT	LOH or <i>NF2</i> or PV2	LOH or <i>NF2</i> or PV3	Tumor-specific partial loss of 22q in trans position or a different <i>NF2</i> somatic second PV in every anatomically unrelated tumor” <sup>57</sup>

Diagnostic criteria for *SMARCB1*- and *LZTR1*-related schwannomatosis

“A diagnosis of *SMARCB1*- or *LZTR1*-related schwannomatosis can be made when an individual meets 1 of the following criteria:

- At least 1 pathologically confirmed schwannoma or hybrid nerve sheath tumor and a *SMARCB1* (or *LZTR1*) pathogenic variant in an unaffected tissue such as blood
- A shared *SMARCB1* or *LZTR1* pathogenic variant in 2 schwannomas or hybrid nerve sheath tumors”<sup>57</sup>

“Pattern of genetic changes in unaffected and tumor tissue in *SMARCB1*- and *LZTR1*-related schwannomatosis”

Gene locus	Unaffected tissue	Tumor 1	Tumor 2	Comment
<b><i>SMARCB1/LZTR1</i></b>				
<b>Allele 1</b>	PV1	PV1	PV1	Shared <i>SMARCB1</i> or <i>LZTR1</i> pathogenic variant
<b>Allele 2</b>	WT	LOH	LOH	Tumor-specific partial loss of 22q in trans position, LOH typically entails deletion of 22q region encompassing <i>LZTR1/SMARCB1/NF2</i> .” <sup>57</sup>

Gene locus	Unaffected tissue	Tumor 1	Tumor 2	Comment
<b><i>NF2</i></b>				
<b>Allele 1</b>	WT	PV2	PV3	Tumor-specific pathogenic <i>NF2</i> variant in cis to pathogenic <i>SMARCB1</i> variant
<b>Allele 2</b>	WT	LOH	LOH	Tumor-specific partial loss of 22q in trans position, LOH typically entails deletion of 22q region encompassing <i>LZTR1/SMARCB1/NF2</i> .” <sup>57</sup>

#### Diagnostic criteria for 22q-related schwannomatosis

“A diagnosis of 22q-related schwannomatosis can be made when an individual does not meet criteria for *NF2*-related schwannomatosis, *SMARCB1*-related schwannomatosis, or *LZTR1*-related schwannomatosis, does not have a germline *DGCR8* pathogenic variant, and has both of the following molecular features:

- LOH of the same chromosome 22q markers in 2 anatomically distinct schwannomas or hybrid nerve sheath tumors and
- A different *NF2* pathogenic variant in each tumor, which cannot be detected in unaffected tissue.”<sup>57</sup>

“Pattern of genetic changes in unaffected and tumor tissue in 22q-related schwannomatosis”

Gene locus	Unaffected tissue	Tumor 1	Tumor 2	Comment
<i>SMARCB1/LZTR1</i>				
Allele 1	WT	None found	None found	No shared pathogenic <i>LZTR1</i> or <i>SMARCB1</i> variant
Allele 2	WT	LOH	LOH	Tumor-specific partial loss of the same chromosome 22q, LOH typically entails deletion of 22q region encompassing <i>LZTR1/SMARCB1/NF2</i> <sup>57</sup>

Gene locus	Unaffected tissue	Tumor 1	Tumor 2	Comment
<i>NF2</i>				
Allele 1	WT	PV1	PV2	Tumor-specific pathogenic <i>NF2</i> variant trans to the 22q deletion
Allele 2	WT	LOH	LOH	Tumor-specific partial loss of the same chromosome 22q, LOH typically entails deletion of 22q region encompassing <i>LZTR1/SMARCB1/NF2</i> <sup>57</sup>

## VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: <https://www.cms.gov/medicare-coverage-database/search.aspx>. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

### Food and Drug Administration (FDA)

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). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

## VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
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

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
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning, or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning, or duplication/deletion variants of 26-50 exons)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81479	Unlisted molecular pathology procedure

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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.*

## IX. Evidence-based Scientific References

1. Korf BR, Lobbous M, Metrock LK. Neurofibromatosis type 1 (NF1): Pathogenesis, clinical features, and diagnosis. Updated May 2, 2025. <https://www.uptodate.com/contents/neurofibromatosis-type-1-nf1-pathogenesis-clinical-features-and-diagnosis>
2. Evans DG. NF2-related schwannomatosis (NF2-SWN; formerly neurofibromatosis type 2). Updated April 3, 2025. <https://www.uptodate.com/contents/nf2-related-schwannomatosis-nf2-swn-formerly-neurofibromatosis-type-2>
3. Bergner A, Yohay K. Schwannomatosis. Updated October 30, 2024. <https://www.uptodate.com/contents/schwannomatosis-related-to-genetic-variants-other-than-nf2>

4. Stephens K, Kayes L, Riccardi VM, Rising M, Sybert VP, Pagon RA. Preferential mutation of the neurofibromatosis type 1 gene in paternally derived chromosomes. *Human genetics*. Jan 1992;88(3):279-82.
5. Hirbe AC, Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. *The Lancet Neurology*. Aug 2014;13(8):834-43. doi:10.1016/s1474-4422(14)70063-8
6. Friedman JM. Neurofibromatosis 1. *GeneReviews*. 2025. <https://www.ncbi.nlm.nih.gov/books/NBK1109/>
7. Shofty B, Constantini S, Ben-Shachar S. Advances in Molecular Diagnosis of Neurofibromatosis Type 1. *Seminars in pediatric neurology*. Dec 2015;22(4):234-9. doi:10.1016/j.spen.2015.10.007
8. Fisher MJ, Belzberg AJ, de Blank P, et al. 2016 Children's Tumor Foundation conference on neurofibromatosis type 1, neurofibromatosis type 2, and schwannomatosis. *American journal of medical genetics Part A*. May 2018;176(5):1258-1269. doi:10.1002/ajmg.a.38675
9. NIH. National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, Md., USA, July 13-15, 1987. *Neurofibromatosis*. 1988;1(3):172-8.
10. DeBella K, Szudek J, Friedman JM. Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. *Pediatrics*. Mar 2000;105(3 Pt 1):608-14.
11. Ferner RE, Huson SM, Thomas N, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet*. Feb 2007;44(2):81-8. doi:10.1136/jmg.2006.045906
12. Terzi YK, Oguzkan-Balci S, Anlar B, Aysun S, Guran S, Ayter S. Reproductive decisions after prenatal diagnosis in neurofibromatosis type 1: importance of genetic counseling. *Genet Couns*. 2009;20(2):195-202.
13. Burkitt-Wright EMM, Sach E, Sharif S, et al. Can the diagnosis of NF1 be excluded clinically? A lack of pigmentary findings in families with spinal neurofibromatosis demonstrates a limitation of clinical diagnosis. *J Med Genet*. Sep 2013;50(9):606-13. doi:10.1136/jmedgenet-2013-101648
14. Evans DG, Howard E, Giblin C, et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. *American journal of medical genetics Part A*. Feb 2010;152a(2):327-32. doi:10.1002/ajmg.a.33139
15. Ruggieri M, Iannetti P, Polizzi A, et al. Earliest clinical manifestations and natural history of neurofibromatosis type 2 (NF2) in childhood: a study of 24 patients. *Neuropediatrics*. Feb 2005;36(1):21-34. doi:10.1055/s-2005-837581
16. Baser ME, Friedman JM, Aeschliman D, et al. Predictors of the risk of mortality in neurofibromatosis 2. *American journal of human genetics*. Oct 2002;71(4):715-23. doi:10.1086/342716
17. Hexter A, Jones A, Joe H, et al. Clinical and molecular predictors of mortality in neurofibromatosis 2: a UK national analysis of 1192 patients. *J Med Genet*. Oct 2015;52(10):699-705. doi:10.1136/jmedgenet-2015-103290
18. Smith MJ, Bowers NL, Bulman M, et al. Revisiting neurofibromatosis type 2 diagnostic criteria to exclude LZTR1-related schwannomatosis. *Neurology*. Jan 3 2017;88(1):87-92. doi:10.1212/wnl.0000000000003418
19. Evans DG, Raymond FL, Barwell JG, Halliday D. Genetic testing and screening of individuals at risk of NF2. *Clinical genetics*. Nov 2012;82(5):416-24. doi:10.1111/j.1399-0004.2011.01816.x
20. Baser ME, Kuramoto L, Joe H, et al. Genotype-phenotype correlations for nervous system tumors in neurofibromatosis 2: a population-based study. *American journal of human genetics*. Aug 2004;75(2):231-9. doi:10.1086/422700
21. Baser ME, Kuramoto L, Woods R, et al. The location of constitutional neurofibromatosis 2 (NF2) splice site mutations is associated with the severity of NF2. *J Med Genet*. Jul 2005;42(7):540-6. doi:10.1136/jmg.2004.029504

22. Evans DG. Neurofibromatosis 2. *GeneReviews*. 2023. <https://www.ncbi.nlm.nih.gov/books/NBK1201/>
23. Dhamija R, Plotkin S, Ashok A, Messiaen L, Babovic-Vuksanovic D. Schwannomatosis. *GeneReviews*. University of Washington, Seattle; 2018. <https://www.ncbi.nlm.nih.gov/books/NBK487394/>
24. Koontz NA, Wiens AL, Agarwal A, Hingtgen CM, Emerson RE, Mosier KM. Schwannomatosis: the overlooked neurofibromatosis? *AJR American journal of roentgenology*. Jun 2013;200(6):W646-53. doi:10.2214/ajr.12.8577
25. Kehrer-Sawatzki H, Farschtschi S, Mautner VF, Cooper DN. The molecular pathogenesis of schwannomatosis, a paradigm for the co-involvement of multiple tumour suppressor genes in tumorigenesis. *Human genetics*. Feb 2017;136(2):129-148. doi:10.1007/s00439-016-1753-8
26. Dhamija R, Plotkin S, Asthagiri A, Messiaen L, Babovic-Vuksanovic D. Schwannomatosis. *GeneReviews*. 2023. <https://www.ncbi.nlm.nih.gov/books/NBK487394/>
27. Merker VL, Esparza S, Smith MJ, Stemmer-Rachamimov A, Plotkin SR. Clinical features of schwannomatosis: a retrospective analysis of 87 patients. *The oncologist*. 2012;17(10):1317-22. doi:10.1634/theoncologist.2012-0162
28. MacCollin M, Chiocca EA, Evans DG, et al. Diagnostic criteria for schwannomatosis. *Neurology*. Jun 14 2005;64(11):1838-45. doi:10.1212/01.wnl.0000163982.78900.ad
29. Plotkin SR, Blakeley JO, Evans DG, et al. Update From the 2011 International Schwannomatosis Workshop: From Genetics to Diagnostic Criteria. *American journal of medical genetics Part A*. Mar 2013;0(3):405-16. doi:10.1002/ajmg.a.35760
30. Hizuka K, Hagiwara SI, Maeyama T, et al. Constitutional mismatch repair deficiency in childhood colorectal cancer harboring a de novo variant in the MSH6 gene: a case report. *BMC Gastroenterol*. Feb 10 2021;21(1):60. doi:10.1186/s12876-021-01646-3
31. Wimmer, Kratz CP, Vasen HF, et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). *J Med Genet*. Jun 2014;51(6):355-65. doi:10.1136/jmedgenet-2014-102284
32. Giugliano T, Santoro C, Torella A, et al. Clinical and Genetic Findings in Children with Neurofibromatosis Type 1, Legius Syndrome, and Other Related Neurocutaneous Disorders. *Genes (Basel)*. 2019;10(8):580. doi:10.3390/genes10080580
33. Castellanos E, Rosas I, Negro A, et al. Mutational spectrum by phenotype: panel-based NGS testing of patients with clinical suspicion of RASopathy and children with multiple cafe-au-lait macules. *Clinical genetics*. Feb 2020;97(2):264-275. doi:10.1111/cge.13649
34. Witkowski L, Dillon MW, Murphy E, S Lebo M, Mason-Suares H. Expanding the Noonan spectrum/RASopathy NGS panel: Benefits of adding NF1 and SPRED1. *Molecular Genetics & Genomic Medicine*. 2020;8(4):e1180. doi:10.1002/mgg3.1180
35. Elmas M. The road from mutation to next generation phenotyping: contribution of deep learning technology (Face2Gene) to diagnosis neurofibromatosis type 1. 2022;doi:10.18621/eurj.894631
36. Evans DG, Freeman S, Gokhale C, et al. Bilateral vestibular schwannomas in older patients: NF2 or chance? *J Med Genet*. Jun 2015;52(6):422-4. doi:10.1136/jmedgenet-2014-102973
37. Pathmanaban ON, Sadler KV, Kamaly-Asl ID, et al. Association of Genetic Predisposition With Solitary Schwannoma or Meningioma in Children and Young Adults. *JAMA neurology*. Sep 1 2017;74(9):1123-1129. doi:10.1001/jamaneurol.2017.1406
38. Castellanos E, Plana A, Carrato C, et al. Early Genetic Diagnosis of Neurofibromatosis Type 2 From Skin Plaque Plexiform Schwannomas in Childhood. *JAMA dermatology*. Mar 1 2018;154(3):341-346. doi:10.1001/jamadermatol.2017.5464
39. Halliday D, Emmanouil B, Pretorius P, et al. Genetic Severity Score predicts clinical phenotype in NF2. *J Med Genet*. Oct 2017;54(10):657-664. doi:10.1136/jmedgenet-2017-104519

40. Lu VM, Ravindran K, Graffeo CS, et al. Efficacy and safety of bevacizumab for vestibular schwannoma in neurofibromatosis type 2: a systematic review and meta-analysis of treatment outcomes. *J Neurooncol*. Sep 2019;144(2):239-248. doi:10.1007/s11060-019-03234-8
41. Perez-Becerril C, Burghel GJ, Hartley C, Rowlands CF, Evans DG, Smith MJ. Improved sensitivity for detection of pathogenic variants in familial NF2-related schwannomatosis. *J Med Genet*. Apr 19 2024;61(5):452-458. doi:10.1136/jmg-2023-109586
42. Hutter S, Piro RM, Reuss DE, et al. Whole exome sequencing reveals that the majority of schwannomatosis cases remain unexplained after excluding SMARCB1 and LZTR1 germline variants. *Acta neuropathologica*. Sep 2014;128(3):449-52. doi:10.1007/s00401-014-1311-1
43. Louvrier C, Pasmant E, Briand-Suleau A, et al. Targeted next-generation sequencing for differential diagnosis of neurofibromatosis type 2, schwannomatosis, and meningiomatosis. *Neuro-oncology*. Jun 18 2018;20(7):917-929. doi:10.1093/neuonc/noy009
44. Sadler KV, Bowers NL, Hartley C, et al. Sporadic vestibular schwannoma: a molecular testing summary. *Journal of Medical Genetics*. 2021;58(4):227-233. doi:10.1136/jmedgenet-2020-107022
45. Piotrowski A, Koczkowska M, Poplawski AB, et al. Targeted massively parallel sequencing of candidate regions on chromosome 22q predisposing to multiple schwannomas: An analysis of 51 individuals in a single-center experience. *Human Mutation*. 2022;43(1):74-84. doi:10.1002/humu.24294
46. Hersh JH. Health supervision for children with neurofibromatosis. *Pediatrics*. Mar 2008;121(3):633-42. doi:10.1542/peds.2007-3364
47. Miller DT, Freedenberg D, Schorry E, Ullrich NJ, Viskochil D, Korf BR. Health Supervision for Children With Neurofibromatosis Type 1. *Pediatrics*. 2019;143(5):e20190660. doi:10.1542/peds.2019-0660
48. Stewart DR, Korf BR, Nathanson KL, Stevenson DA, Yohay K. Care of adults with neurofibromatosis type 1: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. Jul 2018;20(7):671-682. doi:10.1038/gim.2018.28
49. Miller DT, Lee K, Abul-Husn NS, et al. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. Aug 2023;25(8):100866. doi:10.1016/j.gim.2023.100866
50. Goldbrunner R, Weller M, Regis J, et al. EANO guideline on the diagnosis and treatment of vestibular schwannoma. *Neuro-oncology*. Jan 11 2020;22(1):31-45. doi:10.1093/neuonc/noz153
51. Evans DGR, Salvador H, Chang VY, et al. Cancer and Central Nervous System Tumor Surveillance in Pediatric Neurofibromatosis 1. *Clinical Cancer Research*. 2017;23(12):e46. doi:10.1158/1078-0432.CCR-17-0589
52. Evans DGR, Salvador H, Chang VY, et al. Cancer and Central Nervous System Tumor Surveillance in Pediatric Neurofibromatosis 2 and Related Disorders. *Clinical cancer research : an official journal of the American Association for Cancer Research*. Jun 15 2017;23(12):e54-e61. doi:10.1158/1078-0432.Ccr-17-0590
53. Wimmer K, Kratz CP, Vasen HFA, et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'Care for CMMRD' (C4CMMRD). *Journal of Medical Genetics*. 2014;51(6):355. doi:10.1136/jmedgenet-2014-102284
54. Suerink M, Ripperger T, Messiaen L, et al. Constitutional mismatch repair deficiency as a differential diagnosis of neurofibromatosis type 1: consensus guidelines for testing a child without malignancy. *Journal of Medical Genetics*. 2019;56(2):53. doi:10.1136/jmedgenet-2018-105664
55. NCCN. Genetic/Familial High-Risk Assessment: Colorectal Version 4.2024 — April 2, 2025. [https://www.nccn.org/professionals/physician\\_gls/pdf/genetics\\_colon.pdf](https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf)

56. Legius E, Messiaen L, Wolkenstein P, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genetics in Medicine*. 2021/08/01 2021;23(8):1506-1513. doi:10.1038/s41436-021-01170-5
57. Plotkin SR, Messiaen L, Legius E, et al. Updated diagnostic criteria and nomenclature for neurofibromatosis type 2 and schwannomatosis: An international consensus recommendation. *Genetics in Medicine*. 2022/09/01/ 2022;24(9):1967-1977. doi:10.1016/j.gim.2022.05.007

**X. Review/Revision History**

Effective Date	Summary
10/15/2025	<p>Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following changes were made for clarity and consistency:</p> <p>Removed age restriction from CC8 to allow for testing in those who may not present in clinic until after age 25.</p> <p>Note 1, changed “1<sup>st</sup>”, “2<sup>nd</sup>”, and “3<sup>rd</sup>” degree to “first”, “second”, and “third” degree for consistency</p> <p>Note 2, changed “2” to “two” for consistency</p> <p>Updated code description for CPT code 81405, 81406</p>
12/01/2024	<p>Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following changes were made for clarity and consistency:</p> <p>New Note 2 was added to reflect changes to Avalon’s definition of a genetic panel within R2162: “Note 2: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.”</p>
12/01/2024	Initial Policy Implementation