

Neurofibromatosis Panels

Panel Gene List:

NF1 Panel Genes: NF1, SPRED1

NF2 Panel Genes: LZTR1, NF2, SMARCB1

Any gene not assessed in its entirety by sequencing and copy-number assessment is addressed in the Test Methods section.

Clinical Features

Neurofibromatosis Type 1 (NF1) is characterized by multiple café au lait macules, axillary or inguinal freckling, multiple cutaneous neurofibromas, and iris Lisch nodules (iris hamartomas). Other manifestations include plexiform neurofibromas, optic gliomas and other central nervous system gliomas, malignant peripheral nerve sheath tumors (MPNST), vasculopathy, hypertension, and skeletal complications such as scoliosis, tibial and vertebral dysplasia, pseudoarthritis, osteopenia and osteoporosis (PMID: 12403552; 19764036; 26926675). Additionally, learning disabilities and behavioral problems of variable severity are present in at least 50% of individuals with NF1 (PMID: 22934576). Clinical features associated with NF1 are highly variable, even within the same family (PMID: 12403552). Individuals with NF1 are often diagnosed clinically in childhood; however, as it is a progressive disorder, additional clinical features can present later in life (PMID: 12403552; 19764036; 26926675; 22934576; 10699117).

A diagnosis of NF1 can be made in an individual with two or more of the following criteria (PMID: 34012067; 3128965; 20301288):

- 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
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axillary or inguinal region
- Optic glioma
- Two or more Lisch nodules (iris hamartomas)
- A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex with or without pseudoarthrosis
- A parent who meets diagnostic criteria for NF1
- A heterozygous pathogenic *NF1* variant in apparently normal tissue

Legius syndrome (Neurofibromatosis Type 1-like syndrome) is characterized by multiple café au lait macules, with or without intertriginous freckling, in the absence of neurofibromas or other tumor manifestations seen in NF1. Additional reported features of Legius syndrome include macrocephaly, lipomas, learning disabilities, ADHD, developmental delays, and facial features similar to Noonan syndrome (PMID: 19920235; 21548021; 17704776; 21089071). The diagnostic criteria for Legius syndrome are met if at least two of the following criteria are present (PMID: 34012067; 20945555):

- 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* in apparently normal tissue
- A parent with a diagnosis of Legius syndrome by the above criteria

Neurofibromatosis Type 2 (NF2) is characterized by bilateral vestibular schwannomas with associated symptoms of tinnitus, hearing loss, and balance dysfunction. Vestibular schwannomas develop in over 90% of individuals with NF2, and schwannomas of other cranial nerves are reported in 24–51% of individuals with NF2. Additionally, spinal tumors and meningiomas develop in approximately 60–90% and 50–80% of patients with NF2, respectively (PMID: 10569966; 21278391; 26043141; 27655473). Skin tumors (including skin plaques, subcutaneous tumors and intradermal tumors) and ocular manifestations (including cataracts, epiretinal membranes and retinal hamartomas) are present in the majority of individuals with NF2 (PMID: 26043141; 26564072). Mononeuropathy of childhood and progressive polyneuropathy of adulthood are also recognized features of the condition (PMID: 10569966; 26564072). The average age of onset for NF2 is in the second decade (18–24 yrs of age); however, many individuals present with symptoms in childhood (PMID: 26043141).

A diagnosis of NF2 can be made in an individual with one of the following (PMID: 35674741; 20301380; 3128965):

1. Bilateral vestibular schwannomas
2. An identical *NF2* pathogenic variant in two or more anatomically distinct NF2-related tumors (schwannoma, meningioma, and/or ependymoma)
3. Two major or one major and two minor criteria:

Major Criteria

- Unilateral vestibular schwannoma
- A first-degree relative (other than a sibling) with NF2
- Two or more meningiomas
- NF2 pathogenic variant in an unaffected tissue

Minor Criteria

Can count >1 of a type (e.g., 2 distinct schwannomas would count as 2 minor criteria)

- Ependymoma, meningioma, schwannoma
 - Note: if the major criterion is unilateral vestibular schwannoma, 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

Schwannomatosis is characterized by multiple schwannomas and, less commonly, meningiomas, in the absence of bilateral vestibular schwannomas. Affected individuals may develop schwannomas in the spinal cord and along the peripheral and cranial nerves with the tumors manifesting with pain and/or neurological deficit (PMID: 22927469). Nonvestibular cranial nerve schwannomas are observed in up to 10% of affected individuals and some individuals may develop other tumors, including meningiomas, rhabdoid tumors, and malignant peripheral nerve sheath tumors (MPNSTs) (PMID: 22038540; 23036231; 22082606). Unilateral vestibular schwannoma is reported in association with LZTR1-related schwannomatosis (PMID: 27856782). Some individuals with schwannomatosis and a negative family history are mosaic for a pathogenic variant in the NF2 gene. In contrast, a subgroup of patients in whom schwannomas are largely

confined to the peripheral nerves do not have an underlying NF2 disorder but have schwannomatosis (PMID: 27921248). Schwannomas in schwannomatosis are pathologically indistinct from those that develop in individuals with NF2 (PMID: 26459515). Most individuals with schwannomatosis present in adulthood, and approximately 10–20% of cases are familial (PMID: 22927469; 15955931; 26407091). Segmental schwannomatosis has also been observed, where the schwannomas are limited to one limb or 5 or less contiguous spine segments (PMID: 22927469; 15955931; 26407091). Germline pathogenic variants in *LZTR1* and *SMARCB1* are associated with familial and sporadic schwannomatosis, and consensus diagnostic criteria for *LZTR1*- and *SMARCB1*-related schwannomatosis are available (PMID: 35674741).

Genetics

Neurofibromatosis type 1 (NF1) and Neurofibromatosis type 2 (NF2) are autosomal dominant disorders with variable expressivity, nearly complete penetrance by adulthood, and possible segmental mosaicism. Approximately 50% of *NF1* pathogenic variants are de novo (PMID: 20082463). Approximately 50–60% of *NF2* pathogenic variants are de novo, with one-third of de novo cases being mosaic (PMID: 28620005; 20082463; 1479598; 17307835).

Legius syndrome is an autosomal dominant disorder with possible reduced penetrance. De novo variants accounted for approximately 30% of all *SPRED1* pathogenic variants in a cohort of individuals with Legius syndrome (PMID: 19920235).

Schwannomatosis is an autosomal dominant disorder with incomplete penetrance and variable expressivity (PMID: 15955931; 23401320). The majority of cases of schwannomatosis are sporadic, and approximately 10% and 30% of simplex schwannomatosis cases are attributed to de novo pathogenic variants in *SMARCB1* and *LZTR1*, respectively (PMID: 23401320; 22434358; 27921248). Of note, loss of function pathogenic variants in *SMARCB1* have also been associated with rhabdoid tumor predisposition syndrome type-1, while gain of function pathogenic variants have been observed in individuals with Coffin–Siris syndrome (PMID: 27921248). *LZTR1* pathogenic variants are also associated with both autosomal dominant and autosomal recessive forms of Noonan syndrome (PMID: 30859559).

Clinical Sensitivity

The clinical sensitivity of this test depends in part on the patient's clinical presentation and is expected to be highest for individuals with a clearly defined phenotype and/or family history. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. In general, the sensitivity is highest for individuals with features suggestive of a neurofibromatosis syndrome as outlined above. NF1 Syndrome: Pathogenic variants in *NF1* are identified in greater than 90% of individuals meeting diagnostic criteria for NF1 syndrome, with 5–10% of identified variants being multi-exon rearrangements or whole gene deletions (PMID: 26056819; 26073919; 27322474). NF2 Syndrome: Pathogenic variants in *NF2* are identified in over 90% of individuals meeting clinical diagnostic criteria for NF2 syndrome with a positive family history; the detection rate in simplex cases is lower (approximately 60%) due to somatic mosaicism (PMID: 17307835). Large rearrangements account for 10–20% of identified variants in *NF2* (PMID: 17307835; 26615784; 15645494; 16983642). Rarely, affected individuals have a chromosome abnormality that disrupts the *NF2* gene (PMID: 16983642). Legius Syndrome: Pathogenic variants in *SPRED1* have been reported in approximately 2% of individuals who meet NIH NF1 diagnostic criteria, and in 4–8% with an NF1-like phenotype who do not have a pathogenic variant in *NF1* (PMID: 19920235; 17704776; 20179001; 19443465). The sensitivity of *SPRED1* sequencing increases to 20% in patients without an affected parent,

optic pathway tumor, Lisch nodules, neurofibromas, long bone dysplasia, or sphenoid wing dysplasia (PMID: 20179001). Large deletions account for approximately 10% of all pathogenic variants identified in the SPRED1 gene (PMID: 21548021). Schwannomatosis: Approximately 85% of patients with familial schwannomatosis and 40% of patients with sporadic schwannomatosis will have a germline pathogenic variant in the SMARCB1 or LZTR1 gene (PMID: 23401320; 27921248). *SMARCB1* large rearrangements in association with schwannomatosis are rare, and gross deletions or duplications have not been reported in LZTR1-associated schwannomatosis to date (PMID: 27921248; 26799435).

Test Methods

Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. This DNA is enriched for the complete coding region and splice site junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

Disclaimer

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. However, normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test.

Unless otherwise indicated, the methods used cannot reliably detect deletions of 20bp to 500bp in size, or insertions of 10bp to 500bp in size. Sequencing cannot detect low-level mosaicism. Copy number assessment methods cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results.