Neurofibromatosis Panel
NF1, SPRED1, NF2, and *SMARCB1* Gene Analysis

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.[1–3] Additionally, learning disabilities and behavioral problems of variable severity are present in at least 50% of individuals with NF1 syndrome.[4] Clinical features associated with NF1 syndrome are highly variable, even within the same family.[1] Individuals with NF1 syndrome are often diagnosed clinically in childhood; however as it is a progressive disorder, additional clinical features can present later in life.[1,5]

A diagnosis of NF1 syndrome can be made clinically by meeting two or more of the following features:[6,7]

- 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 first-degree relative (parent, sibling, or offspring) with NF1 by the above criteria

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 syndrome. Additional reported features of Legius syndrome include macrocephaly, lipomas, learning disabilities, ADHD, developmental delays, and facial features similar to Noonan syndrome.[8–11] As the diagnosis of Legius syndrome is difficult to make clinically, molecular testing is necessary to confirm the diagnosis.[12]

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 syndrome, and schwannomas of other cranial nerves are reported in 24-51% of individuals with NF2 syndrome. Additionally, spinal tumors and meningiomas develop in approximately 60-90% and 50-80% of patients with NF2 syndrome, respectively.[13–17] 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 syndrome.[14,16,18] Mononeuropathy of childhood and progressive polyneuropathy of adulthood are also

recognized features of the condition.[13,14,18] 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.[16,19]

A diagnosis of NF2 syndrome can be made clinically by meeting one of the following criteria:[6,20]

- Bilateral vestibular schwannomas (masses of the 8th cranial nerve seen with appropriate imaging techniques (e.g., CT or MRI) OR
- A first-degree relative with NF2 AND either:
  - Unilateral vestibular schwannoma OR
  - Any two of: neurofibroma, meningioma, glioma, schwannoma, or juvenile posterior subcapsular lenticular opacities/cortical cataract
- Unilateral vestibular schwannoma AND any two of: meningioma, schwannoma, glioma, neurofibroma, juvenile posterior subcapsular lenticular opacities/cortical cataract
- Multiple meningiomas AND either:
  - Unilateral vestibular schwannoma OR
  - Any two of: schwannoma, glioma, neurofibroma, juvenile posterior subcapsular lenticular opacities/cortical cataract

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.[21] 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).[22–24] 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 nerve do not have an underlying NF2 disorder but have schwannomatosis.[25] Schwannomas in schwannomatosis are pathologically indistinct from those that develop in individuals with NF2.[26] Most individuals with schwannomatosis present in adulthood, and approximately 10-20% of cases are familial.[21,27,28] Segmental schwannomatosis has also been observed, where the schwannomas are limited to one limb or 5 or less contiguous spine segments.[21,27,28]

There are proposed clinical diagnostic criteria in the literature for schwannomatosis for individuals in whom a diagnosis of NF2 syndrome has been excluded:[25]

- Two or more non-intradermal schwannomas (at least one biopsy-confirmed) AND no evidence of bilateral vestibular schwannomas by high-quality MRI (Note: Presence of a unilateral vestibular schwannoma or meningioma(s) does not exclude the diagnosis.)
- One pathologically confirmed schwannoma, unilateral vestibular schwannoma, or intracranial meningioma AND an affected first-degree relative with confirmed schwannomatosis

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*.[29] Approximately 50-60% of *NF2* pathogenic variants are *de novo*, with one-third of *de novo* cases being mosaic.[20,29–31]
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.[8]
Schwannomatosis is an autosomal dominant disorder with incomplete penetrance and variable expressivity.[27,32] The majority of cases of schwannomatosis are sporadic, and approximately 10% of simplex schwannomatosis cases are attributed to *de novo* pathogenic variants in *SMARCB1*.[32,33] *SMARCB1*-related schwannomatosis is caused by pathogenic variants in the *SMARCB1* gene. 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.[25] More recently, another gene, *LZTR1,* has been associated with familial and sporadic schwannomatosis.[34]

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. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by next generation sequencing (NGS). Reported clinically significant variants are confirmed by an appropriate method. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. 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.

Test Sensitivity:
The clinical sensitivity of sequencing and deletion/duplication analysis of the 4 genes included in the Neurofibromatosis Panel depends in part on the patient's clinical phenotype and family history. 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.[35–37] **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.[19,31] Large rearrangements account for 10-20% of identified variants in *NF2.*[31,38–40] Rarely, affected individuals have a chromosome abnormality that disrupts the *NF2* gene.[19,40] **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.*[8,10,41,42] 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.[41] Large deletions account for approximately 10% of all pathogenic variants identified in the *SPRED1* gene.[9] **Schwannomatosis:** Approximately 40-50% of patients with familial schwannomatosis and less than 10% of patients with sporadic schwannomatosis will have a germline pathogenic variant in the *SMARCB1* gene.[32] *SMARCB1* large rearrangements in association with schwannomatosis are rare.[25,43]

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. Normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test. The methods used cannot reliably detect deletions of 20bp to 250bp in size, or insertions of 10bp to 250 bp in size. Sequencing cannot detect low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Rarely, incidental findings of large chromosomal rearrangements outside the gene of interest may be identified. 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. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active or chronic hematologic neoplasms or conditions, there is a possibility that testing may detect an acquired somatic variant, resulting in a false positive result. As the ability to detect genetic variants and naming conventions can differ among laboratories, rare false negative results may occur when no positive control is provided for testing of a specific variant identified at another laboratory. The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. Consultation with a genetics professional is recommended for interpretation of results.

Gene	Protein	Inheritance	Disease Associations
NF1	NEUROFIBROMIN	AD	Neurofibromatosis type 1 (NF1) syndrome
NF2	MERLIN	AD	Neurofibromatosis type 2 (NF2) syndrome
SMARCB1	SWI/SNF-RELATED MATRIX-ASSOCIATED ACTIN-DEPENDENT REGULATOR OF CHROMATIN SUBFAMILY B MEMBER 1	AD	Schwannomatosis, rhabdoid tumor predisposition syndrome-1 (RTPS1), Coffin-Siris

			syndrome
SPRED1	SPROUTY-RELATED, EVH1 DOMAIN-CONTAINING PROTEIN 1	AD	Legius syndrome (Neurofibromatosis Type 1-like Syndrome)

Abbreviations:
AD – Autosomal dominant
MPNST - Malignant peripheral nerve sheath tumors

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