Neurofibromatosis Panel
NF1, SPRED1, NF2 and SMARCB1 Gene Analysis

Clinical Features:

Neurofibromatosis Type 1 (NF1) is characterized by multiple café au lait spots, axillary and inguinal (groin) freckling, multiple benign, cutaneous neurofibromas and iris Lisch nodules (iris hamartomas). Other less common features include plexiform neurofibromas, optic gliomas and other central nervous system gliomas, malignant peripheral nerve sheath tumors (PNSTs), vasculopathy and hypertension. Skeletal complications can include scoliosis, tibial and vertebral dysplasia, pseudoarthritis, osteopenia and osteoporosis. Additionally, learning disabilities of variable severity are present in at least 50% of individuals with NF.[1] Clinical features associated with NF1 are highly variable, even within the same family. Many individuals with NF1 are often diagnosed clinically in childhood; however as it is a progressive disorder, additional clinical features can present later in life. Diagnosis of NF1 can be made clinically by meeting two or more of the following features:[15]

1. 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
2. Two or more neurofibromas of any type or one plexiform neurofibroma
3. Freckling in the axillary or inguinal regions (Crowe's sign)
4. Optic glioma
5. Two or more Lisch nodules (iris harmartomas)
6. A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex with or without pseudoarthrosis
7. 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, intertriginous freckling, lipomas, macrocephaly, learning disabilities, ADHD or developmental delays and facial features similar to Noonan syndrome.[3] Legius Syndrome is sometimes clinically diagnosed as NF1, however, after molecular testing, about 5-8% of those negative for a pathogenic variant in the NF1 gene have a pathogenic variant in the SPRED1 gene.[8,18] Unlike NF1, the syndrome does not include cutaneous, ocular or neural fibromas or other tumor manifestations of NF1. As the diagnosis of Legius syndrome is difficult to make clinically, molecular testing is necessary to confirm the diagnosis.[3]

Neurofibromatosis Type 2 (NF2) is characterized by bilateral vestibular schwannomas with associated symptoms of tinnitus, hearing loss, and balance dysfunction. Affected individuals may also develop retinal hamartomas, schwannomas of other cranial and peripheral nerves, spinal tumors and meningiomas, skin tumors, and mono- or polyneuropathy in childhood, which presents as a persistent facial palsy, a squint (third nerve palsy) or hand/foot drop. Rarely, clinical findings of NF2 include astrocytomas and posterior subcapsular lens opacities that may become a visually significant cataract (the first sign of NF2 in some individuals).[2] 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.[9,27] A diagnosis of NF2 can be made clinically by meeting one of the following criteria: [2,15]

Definite NF2:

1. Bilateral masses of the 8th cranial nerve (vestibular schwannoma) seen with appropriate imaging techniques (e.g., CT or MRI) **OR**

2. A first-degree relative with NF2 *and* either: unilateral mass of the 8th cranial nerve (vestibular schwannoma) in an individual under 30y of age, *or* any two of: neurofibromas, meningioma, glioma, schwannoma, or juvenile posterior subcapsular lenticular opacities/cortical cataract

Probable NF2:

1. Unilateral vestibular schwannoma in an individual under 30y of age *and* any two of: meningioma, schwannoma, glioma, neurofibroma, juvenile posterior subcapsular lenticular opacities/cortical cataract

2. Multiple meningiomas *and* either a unilateral vestibular schwannoma in an individual under 30y of age *or* any two of: schwannoma, glioma, neurofibroma, cataract

Schwannomatosis is characterized by multiple schwannomas, without involvement of the vestibular nerve that is diagnostic of NF2. 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.[20] Nonvestibular cranial nerve schwannomas are observed in up to 10% of affected individuals and some individuals may develop other tumors, including meningiomas, rhaboid tumors and malignant PNSTs.[22,23] Schwannomas present in NF2 and Schwannomatosis are pathologically indistinct.[25] 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. Most individuals with schwannomatosis present in adulthood and approximately 20% of cases are familial with family members affected with at least 1 schwannoma.[24] Segmental schwannomatosis has also been observed where the schwannomas are limited to one limb or 5 or less contiguous spine segments.[24] There are proposed criteria in the literature for a diagnosis of schwannomatosis:[24]

Definite schwannomatosis:

1. Individual is greater than 30 years of age with two or more nonintradermal schwannomas (at least 1 histologically confirmed), no evidence of a vestibular schwannoma, does not meet diagnostic criteria for NF2 and no evidence of a constitutional NF2 mutation **OR**

2. One pathologically confirmed nonvestibular schwannoma plus a first degree relative who meets criteria in 1.

Possible schwannomatosis:

1. Individual is less than 30 years of age with two or more nonintradermal schwannomas (at least 1 histologically confirmed), no evidence of a vestibular schwannoma, does not meet diagnostic criteria for NF2 and no evidence of a constitutional NF2 mutation **OR**

2. Individual is greater than 45 years of age and has two or more nonintradermal schwannomas (at least 1 histologically confirmed), no symptoms of 8th cranial nerve dysfunction, no family history of NF2 and no evidence of a constitutional NF2 mutation **OR**

3. Radiographic evidence of a nonvestibular schwannoma plus a first degree relative who meets criteria for a definite schwannoma

Genetics:

Neurofibromatosis type 1 (NF1) and Neurofibromatosis type 2 (NF2) are autosomal dominant disorders with variable expressivity, nearly complete penetrance (by adulthood) and segmental mosaicism. Approximately 50% of NF1 pathogenic variants are *de novo*1 and approximately 50-60% of NF2 pathogenic variants are *de novo.*[2] NF1 is caused by pathogenic variants in the neurofibromin gene, located on chromosome 17q11.2, which encodes a cytoplasmic protein that regulates cell growth in neurons, Schwann cells, oligodendrocytes, astrocytes and leukocytes. As a tumor suppressor gene, neurofibromin acts intracellularly to regulate at least two known cellular pathways, Ras and cyclic AMP (cAMP). NF2 is caused by pathogenic variants in the neurofibromin 2 gene, located on chromosome 22q12.2. The protein produced by neurofibromin 2 is called Merlin (Moesin-Ezrin-Radixin-Like Protein) or schwannomin, and is thought to be a tumor suppressor gene. Merlin regulates cell growth by monitoring cell-to-cell adhesion, transmembrane signaling receptors and actin in the cellular cytoskeleton.

Legius Syndrome is an autosomal dominant disorder with possible reduced penetrance. *De novo* pathogenic variants accounted for approximately 30-40% of all pathogenic variants in two study cohorts among individuals with Legius Syndrome.[3,16] Legius Syndrome is caused by pathogenic variants in the SPRED1 gene (Sprouty-related EVH1 Domain-containing Protein 1), located on chromosome 15q13.2. Few studies have characterized the function of the SPRED1 gene in humans. Mouse models show that the SPRED1 protein is highly expressed during neurogenesis, and in adult mice, the protein is mainly expressed in the brain. Though more research is needed to fully understand the role of SPRED1 in humans, studies indicate that the human SPRED1 protein inhibits the Ras-MAPK pathways, preventing cell growth and division.

Schwannomatosis is an autosomal dominant disorder although a majority of cases are sporadic. Incomplete penetrance and variable expressivity has also been demonstrated.[26] Schwannomatosis is primarily caused by pathogenic variants in the SMARCB1 gene, located on 22q11.23, which is a tumor suppressor gene and encodes a subunit of the SWI/SNF complex, a prototypical ATP-dependent chromatin remodeling complex.

Test Methods:

Using genomic DNA from the submitted specimen, the coding regions and splice junctions of the genes on the NF panels are enriched using a proprietary targeted capture system developed by GeneDx. The targeted regions are sequenced simultaneously by massively

parallel (NextGen) sequencing on an Illumina platform with paired-end reads. The sequence data is aligned to the reference sequences based on human genome build GRCh37/UCSC hg19. Sanger sequencing is used to obtain sequence data for regions of low coverage and refractory amplifications. Concurrently, multiplex ligation-dependent probe amplification (MLPA) of the NF1 gene and targeted array CGH with exon-level resolution (ExonArrayDx) of SPRED1, NF2 and SMARCB1 is performed to evaluate for a deletion or duplication of one or more exons of these genes. If testing for the NF1 gene only is requested, sequence analysis and MLPA of the NF1 gene are performed concurrently. The presence of any potentially disease-associated sequence variant(s) or copy number alteration(s) is confirmed by dideoxy DNA sequence analysis or quantitative PCR, respectively, or by other appropriate methods.

Test Sensitivity:

NF1: The vast majority of pathogenic variants in patients with NF1 are intragenic sequencing variants. A smaller proportion is due to whole gene deletions (4-10%) and partial deletions/duplications (2-7%).[12] In patients with NF1 gene deletions that encompass varying amounts of neighboring genes, there are three recurrent large microdeletions, which lead to a so-called “NF1 microdeletion syndrome.” These deletions occur in sizes of 1.0 Mb, 1.2 Mb and 1.4Mb.[17] These microdeletions may be inherited or *de novo.* There is a weak genotype phenotype correlation with most NF1 pathogenic variants, though individuals with a NF1 microdeletion often have learning disabilities, facial dysmorphic features, childhood overgrowth and cardiovascular malformations.[13, 17] In addition, a 3-bp in-frame deletion is characterized by the *absence* of cutaneous neurofibromas.[13] Approximately 10% of individuals with a NF1 pathogenic variant present with both NF1 and Noonan syndrome features (Neurofibromatosis-Noonan syndrome).[29] Though the overwhelming majority of NF1 pathogenic variants are constitutional, mosaicism has been observed in some individuals who have milder symptoms or have features of NF1 restricted to one area of the body.[1] Sequencing with deletion/duplication analysis is expected to detect a pathogenic variant in over 90% of individuals who fulfill clinical diagnostic criteria for NF1.[1]

Legius Syndrome: The majority of reported pathogenic variants in the SPRED1 gene are truncating variants, which lead to loss-of-function of the protein. Pathogenic variants in SPRED1 have been reported in approximately 2% of individuals who meet NIH NF1 diagnostic criteria, and in 8% who met NF1 criteria and were negative for a pathogenic variant in NF1.[6] The sensitivity of SPRED1 sequencing increased to 20% if patients with an affected parent, optic pathway tumor, Lisch nodules, neurofibromas, long bone dysplasia, or sphenoid wing dysplasia were excluded.[19] Deletions of the SPRED1 gene account for approximately 10% of all pathogenic variants identified in the SPRED1 gene.[8]

NF2: Pathogenic variants in the NF2 gene include missense, nonsense, frameshift and splice-site variants, partial or whole gene deletions/duplications, and, rarely, chromosome

abnormalities that disrupt the NF2 gene. Approximately 25-33% of pathogenic variants are not detected in blood samples due to somatic mosaicism.9 In one study of 529 families with NF2, intragenic pathogenic variants were observed in about 58% (303 of 529) of reported cases.9 Approximately 10-20% of pathogenic variants in the NF2 gene are single or multi-exon deletions/duplications.[2,10,28] Few genotype-phenotype studies have been performed for NF2. However, studies have found a more severe phenotype—with more tumors and a younger age of onset—in NF2 patients with nonsense or frameshift pathogenic variants while patients with missense, large deletions or somatic mosaicism have a milder form of NF2, as characterized by fewer tumors and a later age of onset.[9, 14] For individuals who fulfill clinical diagnostic criteria for NF2, sequencing and deletion/duplication analysis of the NF2 gene is expected to detect as high as 90% of pathogenic variants, with a higher detection for familial case.

Schwannomatosis: Pathogenic variants in SMARCB1 include primarily missense and splice site variants although other loss of function variants, particularly frameshift variants associated with isolated and familial schwannomatosis have been observed. Approximately 40-60% of patients with familial schwannomatosis and 10% of patients with isolated schwannomatosis will have a germline pathogenic variant in the SMARCB1 gene.[21] A four-hit, three-step hypothesis has been proposed as the mechanism leading to disease manifestation: the patient is heterozygous for a germline pathogenic variant in the SMARCB1 gene (hit 1), acquires a somatic loss of the other SMARCB1 allele along with the neighboring NF2 gene on chromosome 22 (hits 2 and 3), and ultimately gains a somatic pathogenic variant in the remaining NF2 allele (hit 4).[21] 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 type. [3] More recently, another gene, LZTR1 was identified in some cases of schwannomatosis (MIM: 600574). As a result, some of the literature may reference pathogenic variants in SMARCB1 to be associated with schwannomatosis-1.

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