Neurofibromatosis Type 1 Sequencing and Deletion/Duplication and Legius Syndrome Sequencing Panel

Neurofibromatosis type 1 (NF1) is one of the most common genetic conditions and has highly variable symptoms, even among family members with the same causative *NF1* gene variant and within an individual at different times in life. A clinical diagnosis can be made in 50% of affected children by 1 year of age and in nearly all by 8 years of age. Molecular testing is recommended for symptomatic individuals who do not fulfill National Institutes of Health (NIH) diagnostic criteria for NF1 (see Disease Overview) and for adults who desire prenatal or preimplantation genetic diagnosis in current or future pregnancies. Life expectancy of affected individuals is 8 years shorter than that of the general population; malignant peripheral nerve sheath tumors (present in 10% of those affected) and vasculopathies are leading causes of early death.

Symptoms of Legius syndrome (LS), such as café au lait macules, overlap with those of NF1. However, LS is not typically associated with neurofibromas, Lisch nodules, or central nervous system (CNS) tumors. An estimated 8% of children with six or more café au lait macules, but no other NF1 clinical features, have LS.

Tests to Consider

Neurofibromatosis Type 1 Sequencing and Deletion/Duplication and Legius Syndrome Sequencing Panel 3003927

Method: Massively Parallel Sequencing/ Multiplex Ligation-dependent Probe Amplification

Use to confirm diagnosis of NF1 or Legius syndrome.

Disease Overview

A diagnosis of NF1 can be made if two or more of the following NIH diagnostic criteria[1] are present:

- Six or more café au lait macules
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Axillary or inguinal freckling
- Optic glioma
- Two or more Lisch nodules
- Sphenoid dysplasia, tibial pseudarthrosis, or other distinctive osseous lesion
- First-degree relative with NF1 who meets the previously listed criteria

A diagnosis of LS can be made if at least two of the three criteria below are met:

- Five or more café au lait macules bilaterally distributed and no other NF1-specific diagnostic criteria except axillary or inguinal freckling
- A parent with a diagnosis of LS
- A known pathogenic *SPRED1* gene variant

Other common symptoms of LS include:

- Intertriginous freckling
- Lipomas
- Macrocephaly
- Learning disabilities

Genetics

NF1

Approximately 90% of pathogenic variants are detectable by sequencing and deletion/duplication analysis.[2]

Only a few genotype/phenotype correlations have been made for *NF1* variants.

- *NF1* whole gene deletions are associated with more severe cognitive issues, somatic overgrowth, large numbers of cutaneous neurofibromas, and dysmorphic facial features.
- A three base pairs (bp) in-frame insertion (c.2970-2972delAAT) leads to typical pigmentary findings of NF1 but is not associated with neurofibromas.
- Missense variants of codon Arg1809 are associated with pulmonic stenosis, café au lait spots, learning disabilities, and short stature but are not associated with neurofibromas.[3]

SPRED1

Approximately 89% of pathogenic variants are sequence variants, whereas 10% are large deletion/duplications.

Etiology

NF1 and LS are caused by pathogenic germline variants in the *NF1* gene and *SPRED1* gene, respectively. Half of disease-causing *NF1* variants are de novo.

Prevalence

NF1: 1 in 3,000[4]

LS: 1 in 46,000-75,000[5]

Inheritance

Autosomal dominant (AD) in NF1 and LS

Penetrance

Complete after childhood in NF1

Test Interpretation

Clinical Sensitivity

Disease (Associated Gene)	Method(s)	Variants Detected	Clinical Sensitivity
Neurofibromatosis type 1 (*NF1*)	NGS and MLPA	Sequence variants and large deletion/duplications	90%
Legius syndrome (*SPRED1*)	NGS	Sequence variants only	89%

MLPA, multiplex ligation-dependent probe amplification; NGS, next generation sequencing

Analytical Sensitivity

For MLPA of *NF1*: 99%

For massively parallel sequencing analytical sensitivity, refer to the following table.

Variant Class	Analytical Sensitivity (PPA) Estimate[a] (%)	Analytical Sensitivity (PPA) 95% Credibility Region[a] (%)
SNVs	99.2	96.9-99.4
Deletions, 1-10 bp	93.8	84.3-98.2
Deletions, 11-44 bp	99.9	87.8-100
Insertions, 1-10 bp	94.8	86.8-98.5
Insertions, 11-23 bp	99.9	62.1-100

[a]Genes included in this test are a subset of a larger methods-based validation from which the PPA values are derived.

bp, base pairs; PPA, positive percent agreement; SNVs, single nucleotide variants

Results

- Identification of one pathogenic or likely pathogenic variant in the *NF1* or *SPRED1* gene is consistent with a diagnosis of NF1 or LS, respectively.
- Detection of a variant of unknown significance in either the *NF1* or *SPRED1* gene does not definitively indicate whether the individual is affected with NF1 or LS.
- If no pathogenic *NF1* or *SPRED1* variants are detected, the risk that the individual is affected is reduced but not eliminated.

Limitations

- A negative result does not exclude a diagnosis of NF1 or LS.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - Single exon deletions/duplications based on the breakpoints of the rearrangement
  - Variants outside the coding regions and intron-exon boundaries of targeted genes
  - Regulatory region and deep intronic variants
  - Noncoding transcripts
  - Breakpoints of large deletions/duplications
  - Large deletions/duplications in the *SPRED1* gene
- The following may not be detected:
  - Deletions/duplications/insertions of any size by massively parallel sequencing
  - Some variants due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions
  - Low-level somatic variants

Genes Tested

Gene	MIM Number	Disorder	Inheritance
NF1	601321	Neurofibromatosis-Noonan syndrome	AD
	162210	Neurofibromatosis, familial spinal	AD
	162200	NF1	AD
	193520	Watson syndrome	AD
SPRED1	611431	LS	AD

References

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