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

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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.

Featured ARUP Testing

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

Method: Massively Parallel Sequencing

Use to confirm diagnosis of NF1 or Legius syndrome.

If a familial sequence variant has been previously identified, targeted sequencing for that variant may be appropriate; refer to the Laboratory Test Directory for additional information.

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

Genes

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

Methodology

This test is performed using the following sequence of steps:

- Selected genomic regions, primarily coding exons and exon-intron boundaries, from the targeted genes are isolated from extracted genomic DNA using a probe-based hybrid capture enrichment workflow.
- Enriched DNA is sequenced by massively parallel sequencing (MPS; also known as next generation sequencing [NGS]) followed by paired-end read alignment and variant calling using a custom bioinformatics pipeline. The pipeline includes an algorithm for detection of large (single exon-level or larger) deletions and duplications.
- Sanger sequencing is performed as necessary to fill in regions of low coverage and in certain situations, to confirm variant calls.
- Large deletion/duplication calls made using MPS are confirmed by an orthogonal exon-level microarray when sample quality and technical conditions allow.

Clinical Sensitivity

Disease (Associated Gene)	Method(s)	Variants Detected	Clinical Sensitivity
Neurofibromatosis type 1 (*NF1*)	NGS	Sequence variants and large deletion/duplications	90%
Legius syndrome (*SPRED1*)	NGS	Sequence variants and large deletion/duplications	99%

Analytic Sensitivity

Variant Class	Analytic Sensitivity (PPA) Estimate[a] (%) and 95% Credibility Region	Analytic Specificity (NPA)
SNVs	>99 (96.9-99.4)	>99.9
Deletions 1-10 bp[b]	93.8 (84.3-98.2)	>99.9
Insertions 1-10 bp[b]	94.8 (86.8-98.5)	>99.9
Exon-level[c] deletions	97.8 (90.3-99.8) [2 exons or larger] 62.5 (38.3-82.6) [single exon]	>99.9
Exon-level[c] duplications	83.3 (56.4-96.4) [3 exons or larger]	>99.9

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

[b]Variants greater than 10 bp may be detected, but the analytic sensitivity may be reduced.

[c]In most cases, a single exon deletion or duplication is less than 450 bp and 3 exons span a genomic region larger than 700 bp.

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

Results

Result	Variant(s) Detected	Clinical Significance
Positive	Pathogenic *NF1* or *SPRED1* variant detected	Confirms diagnosis of diagnosis of NF1 or LS, respectively.
Negative	No known pathogenic *NF1* or *SPRED1* variant detected	Reduces possibility of, but does not exclude, a diagnosis of NF1 or LS, respectively.
Inconclusive	Variant of uncertain clinical significance detected	Unclear if variant is disease-causing or benign

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:
  - Variants outside the coding regions and intron-exon boundaries of targeted genes
  - Regulatory region and deep intronic variants
  - Breakpoints of large deletions/duplications
- The following may not be detected:
  - Deletions/duplications/insertions of any size by MPS
  - Large duplications less than 3 exons in size
  - Noncoding transcripts
  - 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

Gene	MIM Number	Disorder	Inheritance
SPRED1	611431	LS	AD

References

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3. Friedman JM. Neurofibromatosis 1. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. *GeneReviews.* University of Washington, Seattle. Updated Apr 2022; accessed May 2022.

4. Lammert M, Friedman JM, Kluwe L, et al. Prevalence of neurofibromatosis 1 in German children at elementary school enrollment. *Arch Dermatol*. 2005;141(1):71-74.

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