

NEUROFIBROMATOSIS Type 1-*like* or Legius Syndrome – *SPRED1* testing

Comprehensive Test - **Test 1**

- updated 08-10-09-

DESCRIPTION

Mendelian Inheritance in Man number: [611431](#)

Click here for [Gene Reviews](#) Clinical Summary.

Recently, a new autosomal dominant condition resembling NF1 was described, consisting of mainly multiple CAL-spots, freckling and relative macrocephaly. Some patients also have learning disabilities or hyperactivity. In none of the adult patients identified so far, neurofibromas or central nervous system tumors were observed (Brems et al, 2007). A Noonan-like dysmorphology was observed in some individuals. In the five multi-generation families initially ascertained, several affected individuals fulfilled the NIH diagnostic criteria for NF1. The disorder is caused by germline loss-of-function mutations in *SPRED1*. Analysis of 86 unrelated individuals who presented with multiple CAL-spots only with or without a family history of CAL-spots, revealed a mutation in 7 of them (~8%). All were minor-lesion mutations (nonsense, frameshift and 1 missense mutation); no dosage alterations (total gene deletion or one/multi-exon copy number change) were found in Brems et al, but we have since identified them in our cohort of patients (Messiaen L, unpublished results). At the recent European NF meeting in Killarney (Ireland), it was decided by the community to refer to this condition as “Legius syndrome”.

SPRED1 is a member of the *SPROUTY/SPRED* family of proteins that act as negative regulators of RAS-RAF interaction and mitogen-activated protein kinase (MAPK) signaling.

INDICATIONS FOR DIRECT TESTING

- Individuals with CAL-spots with or without skinfold freckling and no *NF1* mutation after comprehensive *NF1* mutation analysis
- Individuals who seek confirmation of a clinical diagnosis

TESTING METHODOLOGY

We offer a **direct test** based on a RNA core assay resulting in the **full characterization of the *SPRED1* mutation (Test 1-cDNA based)**. From a fresh EDTA blood sample, a lymphocyte culture is made, RNA is extracted, and the complete *SPRED1* coding region is analyzed by **sequencing** exons 1 to 7. In addition, DNA **copy number analysis** by MLPA and quantitative real-time PCR is performed. Mutations screened for include truncating mutations (nonsense, frameshift, splicing mutations), missense mutations, multi-exon deletions or duplications and total gene deletions.

Direct **sequencing test** starting from DNA (**Test 1-gDNA based**), complemented with MLPA and quantitative real-time PCR to detect copy number changes, is available as well, although RNA-based testing is the preferred method.

Copy number analysis is included as part of the comprehensive test 1, but the analysis can be requested separately if sequencing is not needed.

SPECIMEN REQUIREMENTS

We require 10 milliliters of whole blood. Blood samples must be collected in EDTA (purple topped) tubes. For patients for whom venipuncture is very difficult, a minimum of 3 ml in EDTA suffices.

If *NF1* testing was previously performed, but over one year ago, please contact us at mgl@genetics.uab.edu to verify whether residual sample is still available to perform *SPRED1* testing.

TRANSPORT

If specimen is from clinics within UAB or Kirklin Clinic, please call (205)-934-5562 for pick-up. If specimens are being sent from some other location, please ship via UPS or Federal Express.

IMPORTANT!

Blood specimens must be kept at room temperature and received within 60 hours of collection.

1. DO NOT SHIP ON ICE.
2. Be sure that the shipping air bill is marked “**Priority**”, either Domestic or International.
3. Specimens must be packaged to prevent breakage and absorbent material must be included in the package to absorb liquids in the event that breakage occurs. Also, the package must be shipped in double watertight containers (e.g. a specimen pouch + the shipping companies Diagnostic Envelope). **You can use our collection kits, which we will send to physicians directly upon request.**
4. Please contact us (Email – mgl@genetics.uab.edu, Phone – 205-934-5562) prior to sample shipment and provide us with the **date of shipment** and the **tracking number** of the package, so that we can better ensure receipt of the samples within the 60-hour window. Please include the form for customs. This is especially important for samples sent from outside the US.

TURN AROUND TIME

2 weeks if *SPRED1* is requested after *NF1* testing (starting from the available sample)
3-4 weeks if *SPRED1* is requested first (starting from a new blood sample).

CPT CODES AND PRICES

Please note that prices listed correspond to institutional rates; please contact the lab for insurance rates.

Comprehensive Analysis (Test 1 RNA-based) including complete sequencing and copy number analysis, following negative *NF1* testing:

\$500, - USD ([currency converter](#))

83891 (x2), 83913 (x1), 83894 (x1), 83896 (x3), 83898 (x4), 83904 (x4), 83909 (x3), 88230 (x1), 83912 (x1)

OR

Comprehensive Analysis (Test 1 DNA-based) including complete sequencing and copy number analysis; no *NF1* testing previously done:

\$600,- USD ([currency converter](#))

83891 (x1), 83896 (x3), 83898 (x11), 83904 (x16), 83909 (x3), 83912 (x1)

Copy Number Analysis by MLPA and Quantitative PCR only:

\$300, - USD ([currency converter](#))

83891 (x1), 83896 (x3), 83898 (x3), 83909 (x3), 83912 (x1)

*Please note that MLPA is part of the *SPRED1* Comprehensive Analysis Test 1, but can be requested as a stand-alone test at \$300,-USD

REQUIRED FORMS

[SPRED1 Test Requisition including the phenotypic data form.](#)

[Form for customs \(International shipment\)](#)

Note: Requests for Molecular Genetic testing for *SPRED1* will **not** be accepted for the following reasons:

- No label (patient's full name and date of collection) on the specimens
- No referring physician's or genetic counselor's names and addresses
- No billing information
- No informed consent
- **No phenotypic checklist**

For more information, test requisition forms, or sample collection and mailing kits, please call: 205-934-5562.

REFERENCES

Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, Somers R, Messiaen L, De Schepper S, Fryns J-P, Cools J, Marynen P, Thomas G, Yoshimura A, Legius E – Germline loss-of function mutations in *SPRED1* cause a neurofibromatosis 1-like phenotype. Nat Genet 39: 1120-1126, 2007 ([pubmed](#))