REVIEW

Legius Syndrome, an Update. Molecular Pathology of Mutations in *SPRED1*

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Multiple café-au-lait macules (CALMs) are the hallmark of Von Recklinghausen disease, or neurofibromatosis type 1 (NF1). In 2007 we reported that some individuals with multiple CALMs have a heterozygous mutation in the *SPRED1* gene and have NF1-like syndrome, or Legius syndrome. Individuals with Legius syndrome have multiple CALMs with or without freckling, but they do not show the typical NF1-associated tumors such as neurofibromas or optic pathway gliomas. NF1-associated bone abnormalities and Lisch nodules are also not reported in patients with Legius syndrome. Consequently, individuals with Legius syndrome require less intense medical surveillance than those with NF1. The *SPRED1* gene was identified in 2001 and codes for a protein that downregulates the RAS-mitogen activated protein kinase (RAS-MAPK) pathway; as does neurofibromin, the protein encoded by the *NF1* gene. It is estimated that about 1–4% of individuals with multiple CALMs have a heterozygous *SPRED1* mutation. Mutational and clinical data on 209 patients with Legius syndrome are tabulated in an online database (http://www.lovd.nl/SPRED1). Mice with homozygous knockout of the *Spred1* gene show learning deficits and decreased synaptic plasticity in hippocampal neurons similar to those seen in *Nf1* heterozygous mice, underlining the importance of the RAS-MAPK pathway for learning and memory. Recently, specific binding between neurofibromin and SPRED1 was demonstrated. SPRED1 seems to play an important role in recruiting neurofibromin to the plasma membrane. (doi: 10.2302/kjm.2013-0002-RE; Keio J Med 62 (4) : 107–112, December 2013)

***Keywords*: Legius syndrome, neurofibromatosis type 1, SPRED1, NF1, café-au-lait macule**

Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder with an incidence of 1/3000 births.[1] Typical manifestations include multiple café-au-lait macules (CALMs), freckling, Lisch nodules on the iris, neurofibromas, and optic pathway gliomas. Other symptoms are learning disabilities, macrocephaly, and specific skeletal abnormalities. Individuals with NF1 have an increased risk for specific malignancies.[2]

NF1 is a member of the RASopathy syndromes. RASopathies are caused by germline mutations in genes coding for proteins with an important role in the RAS-mitogen-activated protein kinase (RAS-MAPK) pathway.[3] RASopathies exhibit overactivation of the RAS-MAPK pathway. Examples of such syndromes include Noonan, cardio-facio-cutaneous, LEOPARD, Costello, capillary malformation–arteriovenous malformation, and CBL-mutation associated syndromes. These syndromes have an overlapping phenotype, although specific features can often be distinguished. In 2007 we identified a new syndrome resembling NF1. This syndrome is caused by heterozygous germline mutations in *SPRED1*.[4] Initially the syndrome was named Neurofibromatosis type 1-like

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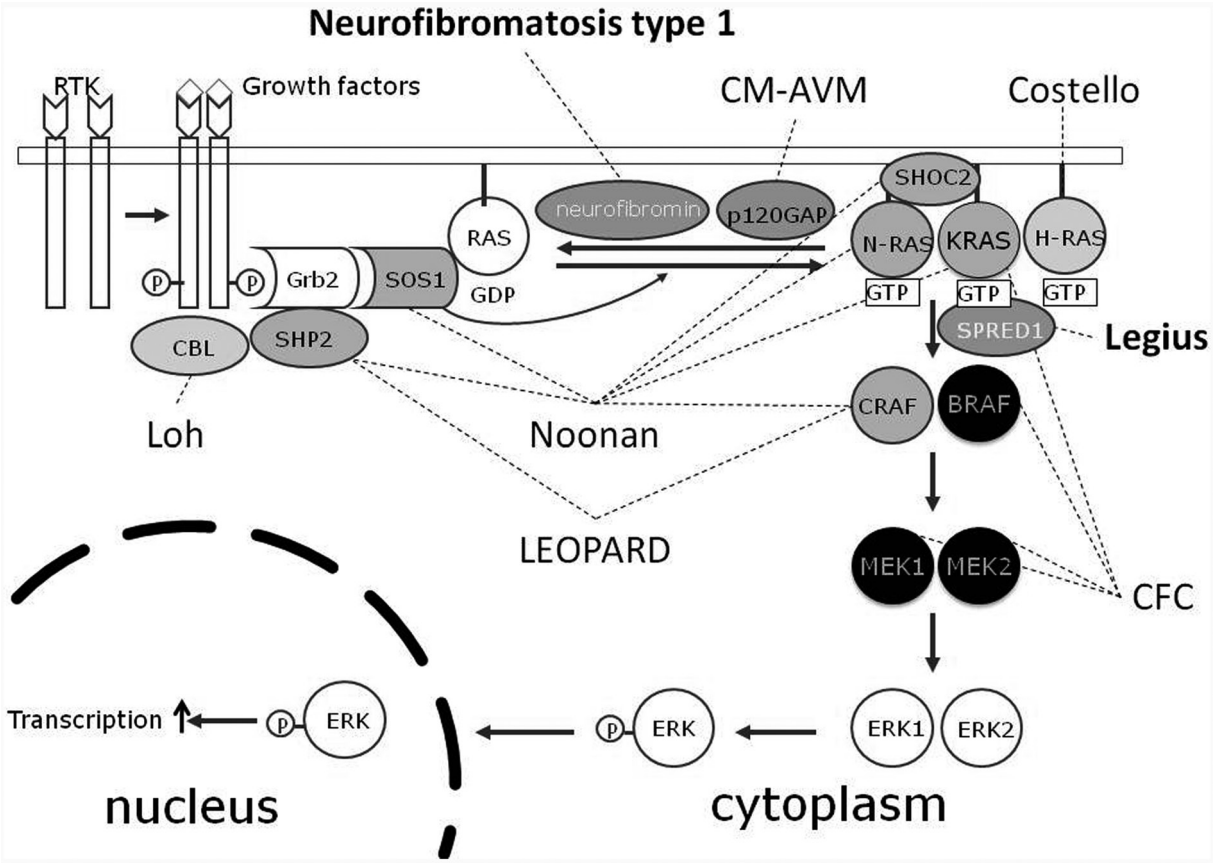

Fig. 1 Schematic of the RAS/mitogen-activated protein kinase pathway.
Associated developmental syndromes (RASopathies) are indicated by dashed lines. RAS is inactive when attached to GDP and is active when attached to GTP. The transition from inactive to active RAS is mediated by SOS1 and the transition from active RAS to inactive RAS is mediated by neurofibromin and p120GAP. SPRED1 is a negative regulator of BRAF and CRAF activation by active RAS. CM-AVM, capillary malformation–arteriovenous malformation syndrome; CFC, cardio-facio-cutaneous syndrome; CBL, Cas-Br-M (murine) ectropic retroviral transforming sequence; Loh syndrome is a Noonan-like syndrome with a high risk of juvenile myelomonocytic leukemia.

syndrome, but later it was renamed Legius syndrome.[5,6] Legius syndrome also belongs to the group of RASopathies (**Fig. 1**).

Clinical Features of Legius Syndrome

NF1-like syndrome (Legius syndrome) was initially reported in five families (37 individuals) and in six unrelated individuals.[4] It is not possible to diagnose Legius syndrome based on clinical features alone because of the important clinical overlap with NF1. Individuals with Legius syndrome show the same multiple CALMs with or without axillary or inguinal freckling as seen in NF1 (**Fig. 2**). However, in Legius syndrome, certain other NF1-associated features such as Lisch nodules, neurofibromas, NF1-specific bone lesions, optic pathway gliomas, and malignant peripheral nerve sheath tumors are systematically absent (**Table 1**). Additional clinical features have been reported in Legius syndrome: pectus excavatum or carinatum 12/87[7–10] and unilateral postaxial polydactyly (3/159).[7,9] Lipomas are present in some individuals with Legius syndrome.[4,7,9–11]

It is not yet clear whether malignancies are associated with Legius syndrome. A small number of neoplastic events have been reported only once in an individual with Legius syndrome, i.e., monoblastic acute leukemia,[11,12] giant cell tumor, dermoid tumor of the ovary, breast cancer,[9] vestibular schwannoma, and desmoid tumor.[7] No second hit was found in *SPRED1* in monoblastic acute leukemia,[12] and no mutations in *SPRED1* were identified in a set of juvenile myeloblastic leukemia cases.[13]

Learning disabilities, developmental delay, Noonan-like characteristics, Attention Deficit (Hyperactivity) Disorde, hyperactivity, autistic behavior, and concentration problems are frequently reported in children with Legius syndrome. A cognitive phenotype was suggested

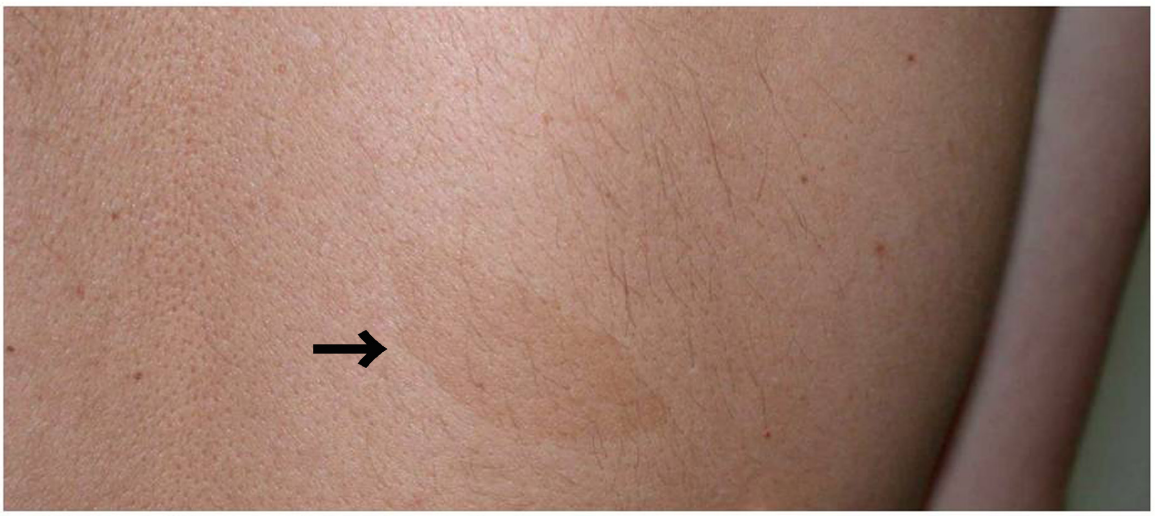

Fig. 2 Café-au-lait macules on the back of an adult with Legius syndrome.

Table 1 Comparison of clinical features of neurofibromatosis type 1 and Legius syndrome

	Neurofibromatosis type 1	Legius syndrome
Café-au-lait macules	++	++
Freckling	++	++
Macrocephaly	+	+
Noonan-like facial dysmorphy	+/–	+/–
Pulmonary valve stenosis	+/–	+/–
Learning disabilities	++	+
Familial occurrence	50%	>60%
Juvenile xanthogranuloma	+/–	–
Lisch nodules	+	–
Neurofibromas	++	–
Malignant peripheral nerve sheath tumor	+/–	–
Optic pathway glioma	+	–
Bone lesions (sphenoid wing dysplasia or pseudarthrosis)	+	–

in 15 individuals with Legius syndrome.[14] These children showed a lower average performance IQ and more learning disabilities compared to unaffected family members. Based on these few observations, we believe that a cognitive phenotype is present in Legius syndrome but it is milder than that in NF1.

Because the total number of reported cases of Legius syndrome is relative small (159, all ages), rare complications could have been missed. To detect a rare complication with a prevalence of 1%, at least 250 adults with Legius syndrome should be investigated.[9] Therefore it is important to keep collecting detailed clinical information from newly diagnosed individuals with Legius syndrome. It is estimated that about 1–4% of individuals with multiple CALMs and a clinical diagnosis of NF1 in fact have Legius syndrome caused by a heterozygous *SPRED1* mutation.[15]

SPRED1 Gene

The *Sprouty-related, EVH1 domain containing 1* (*Spred1*) gene was discovered in 2001 by Yoshimura and colleagues in a mouse cDNA osteoclast library.[16] Human *SPRED1* is located on chromosome band 15q13.2 and spans 104.4 kb of genomic sequence. It consists of seven exons. The transcript is 7255 bp in length with an open reading frame coding for 444 amino acids. In 2007, linkage analysis in two of five families with an NF1-like phenotype mapped the phenotype to a region on chromosome 15 where *SPRED1* had been previously localized. Inactivating germline *SPRED1* mutations were detected in the affected individuals of these five families and in an additional six unrelated patients with a similar phenotype.[4] Molecular genetic testing for Legius syndrome can be performed on DNA or RNA extracted from periph-

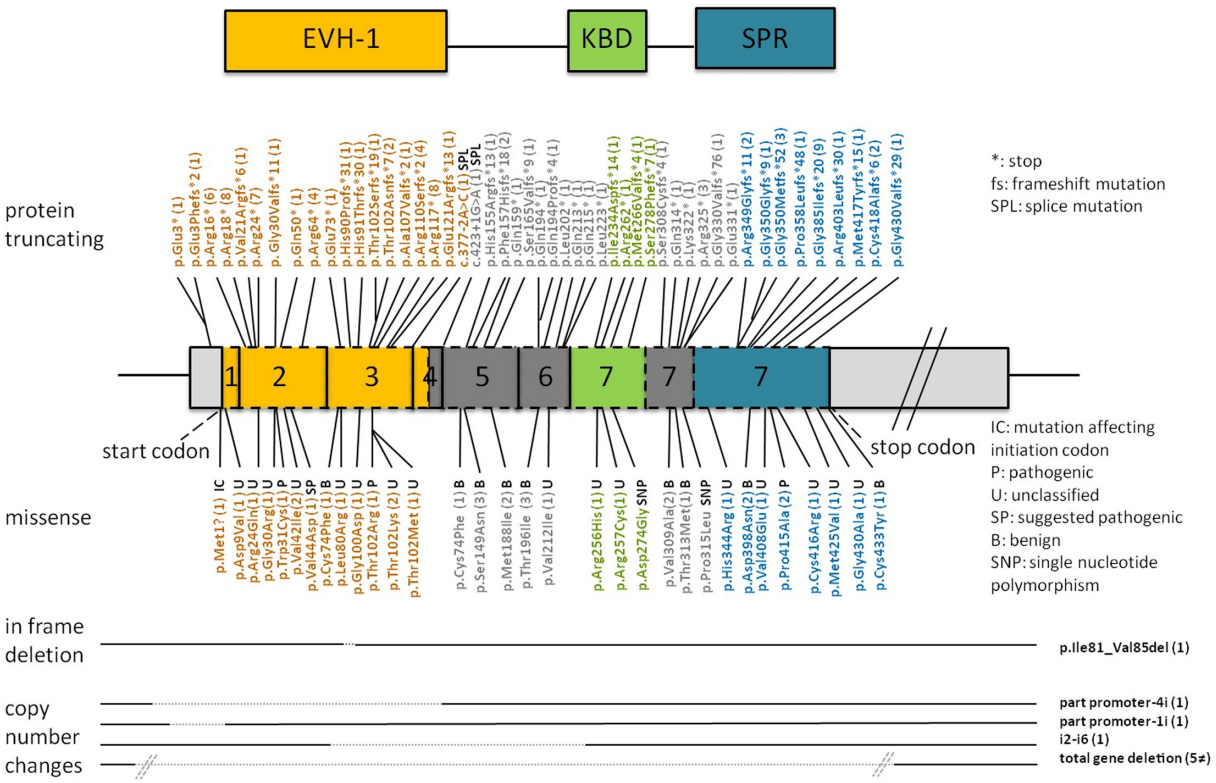

Fig. 3 Schematic of the seven exons of the human *SPRED1* gene and their corresponding functional domains in the protein. EVH-1, EVH-1 domain; KBD, c-KIT-binding domain; SPR, SPROUTY-related domain. Protein truncating mutations are listed above the exons. *, stop codon; fs, frameshift mutation; SPL, splice mutation. Missense mutations and deletions are listed below the exons. IC, missense mutation affecting initiation codon; P, proven pathogenic missense mutation; U, unclassified missense mutation; SP, suggested pathogenic missense mutation; B, benign sequence variant without effect on SPRED1 function; SNP, single nucleotide polymorphism. The numbers in parentheses indicate the number of independent individuals reported with that specific mutation.

eral white blood cells.[4,9] Copy number changes such as multi-exon deletions and whole *SPRED1* gene deletions can be detected by several methods such as multiplex ligation-dependent probe amplification, array comparative genomic hybridization, or other techniques that detect deletions.[10]

All identified mutations and polymorphisms in *SPRED1* are summarized in the Leiden Open Variation Database, which is accessible online at http://www.lovd.nl/SPRED1.[15] Most of these mutations are predicted to be protein truncating. From the 29 missense mutations currently reported, 8 are probably rare benign variants, 3 are clearly pathogenic, and 1 was suggested to be pathogenic (based on amino acid conservation and segregation within a large family). Some missense mutations are still unclassified and need further investigation.[15] Familial as well as sporadic cases of Legius syndrome have been reported; the largest study described 13/33 (39%) sporadic cases in the *SPRED1*-positive group,[9] but it should be taken into account that this study was biased toward sporadic cases. Only one somatic mutation has been published so far.[4] In that study by Brems et al., melanocytes were cultured from a CALM from an individual with Legius syndrome. A somatic frameshift *SPRED1* mutation was identified in addition to the known germline *SPRED1* mutation. This shows that CALMs in individuals with Legius syndrome are caused by biallelic inactivation of the *SPRED1* gene, a mechanism similar to the biallelic inactivation of the *NF1* gene found in melanocytes derived from CALMs of individuals with NF1.[17] An overview of the mutations reported in the *SPRED1* gene can be found in **Figure 3**.

SPRED1 Protein

Human SPRED1 contains 444 amino acids. Three functional domains have been identified: an N-terminal EVH-1 domain, a central c-KIT-binding domain, and a C-terminal SPRY-related domain. The SPRED1 protein is approximately 50 kDa in size and belongs to the SPRED family together with SPRED2 and SPRED3 (reviewed by Yoshimura[18]). Spred1 is a negative regulator of the Ras-MAPK signaling pathway.[16] Spred1 is enriched in the

central nervous system germinal zones, it downregulates neural stem cell proliferation and maintains ventricular zone structure.[19] Human *SPRED1* is highly expressed in lung, brain, spinal cord, and spleen. Lower expression levels are seen in liver, pancreas, prostate, kidney, heart, thymus, muscle, and bone marrow.[20,21] Spred1 is phosphorylated by kinases and regulates activation of the Ras-MAPK cascade in response to several growth factors without affecting the Akt or Rac pathway.[16] Spred1 inhibits the activation of Raf by active Ras (Ras-GTP).[16]

Several Spred1 interaction partners have been identified, i.e., microtubule-associated protein/microtubule affinity-regulating kinase-activating kinase (MARKK), testis-specific protein kinase (TESK1),[22] fibroblast growth factor receptor like-1 (FGFRL1),[23] DYRK1A,[24] and SHP2.[25] Recent research has shown that Spred1 binds to neurofibromin and recruits it to the cell membrane where neurofibromin can function as a negative regulator of Ras-GTP.[26] It has been shown that some SPRED1 missense mutants (e.g., p.Thr102Arg) are unable to bind neurofibromin and are unable to recruit neurofibromin to the cell membrane.

Spred1 knockout mice show spatial learning deficits in the Morris water maze and T-maze tests. In the cognitively more demanding stages of the T-maze, the heterozygous knockout mice performed at an intermediate level between those of the wild type and their homozygous knockout littermates.[27] Both of these spatial learning tasks are hippocampus dependent. Knockout mice showed a decreased synaptic plasticity in the hippocampus as reflected by a diminished long-term potentiation after stimulation of the Schaffer collaterals.[27] The exact pathogenesis of the learning problems in *Spred1*knockout mice is not known, but increased Ras-MAPK signaling in neurons is probably an important factor.[27] These findings are comparable with the learning and synaptic plasticity defects observed in $Nf1^{+/-}$ mice,[28] thus stressing the importance of the RAS-MAPK pathway in learning and memory. The learning and synaptic plasticity defects in adult $Nf1^{+/-}$ mice can be acutely rescued with lovastatin treatment.[28] Similar experiments have not yet been reported in *Spred1* knockout mice.

Medical Management and Surveillance

The phenotype associated with Legius syndrome is milder than the NF1 phenotype, and less stringent medical follow-up seems to be appropriate for those with Legius syndrome. However, at the moment, clinical data are available only from a limited number of individuals with Legius syndrome, and certain rare complications might have been missed. A correct diagnosis of Legius syndrome has important implications for prognosis, counseling, and prenatal diagnosis. The diagnosis of Legius syndrome may relieve the psychological burden in families who would otherwise be expecting more serious NF1-related complications.[9]

Clinical cohorts of patients with multiple CALMs contain individuals negative for a pathogenic *NF1* or *SPRED1* mutation, suggesting that there probably remains a small group of unclassified individuals with mutations in yet unidentified genes. In sporadic cases, mosaicism for an *NF1* (or *SPRED1*?) mutation can be present, explaining the presence of NF1 features in the absence of a detectable *NF1* or *SPRED1* mutation in white blood cells.[17]

References

1. Huson SM, Compston DA, Clark P, Harper PS: A genetic study of von Recklinghausen neurofibromatosis in south east Wales. I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 1989; **26**: 704–711. [Medline] [CrossRef]
2. Brems H, Beert E, de Ravel T, Legius E: Mechanisms in the pathogenesis of malignant tumours in neurofibromatosis type 1. Lancet Oncol 2009; **10**: 508–515. [Medline] [CrossRef]
3. Zenker M: Clinical manifestations of mutations in RAS and related intracellular signal transduction factors. Curr Opin Pediatr 2011; **23**: 443–451. [Medline] [CrossRef]
4. Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, Somers R, Messiaen L, De Schepper S, Fryns JP, 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 2007; **39**: 1120–1126. [Medline] [CrossRef]
5. Stevenson D, Viskochil D: Pigmentary findings in neurofibromatosis type 1-like syndrome (Legius syndrome): potential diagnostic dilemmas. JAMA 2009; **302**: 2150–2151. [Medline] [CrossRef]
6. Spurlock G, Bennett E, Chuzhanova N, Thomas N, Jim HP, Side L, Davies S, Haan E, Kerr B, Huson SM, Upadhyaya M: SPRED1 mutations (Legius syndrome): another clinically useful genotype for dissecting the neurofibromatosis type 1 phenotype. J Med Genet 2009; **46**: 431–437. [Medline] [CrossRef]
7. Denayer E, Chmara M, Brems H, Kievit AM, van Bever Y, Van den Ouweland AM, Van Minkelen R, de Goede-Bolder A, Oostenbrink R, Lakeman P, Beert E, Ishizaki T, Mori T, Keymolen K, Van den Ende J, Mangold E, Peltonen S, Brice G, Rankin J, Van Spaendonck-Zwarts KY, Yoshimura A, Legius E: Legius syndrome in fourteen families. Hum Mutat 2011; **32**: E1985–E1998. [Medline] [CrossRef]
8. Laycock-van Spyk S, Jim HP, Thomas L, Spurlock G, Fares L, Palmer-Smith S, Kini U, Saggar A, Patton M, Mautner V, Pilz DT, Upadhyaya M: Identification of five novel SPRED1 germline mutations in Legius syndrome. Clin Genet 2011; **80**: 93–96. [Medline] [CrossRef]
9. Messiaen L, Yao S, Brems H, Callens T, Sathienkijkanchai A, Denayer E, Spencer E, Arn P, Babovic-Vuksanovic D, Bay C, Bobele G, Cohen BH, Escobar L, Eunpu D, Grebe T, Greenstein R, Hachen R, Irons M, Kronn D, Lemire E, Leppig K, Lim C, McDonald M, Narayanan V, Pearn A, Pedersen R, Powell B, Shapiro LR, Skidmore D, Tegay D, Thiese H, Zackai EH, Vijzelaar R, Taniguchi K, Ayada T, Okamoto F, Yoshimura A, Parret A, Korf B, Legius E: Clinical and mutational spectrum of neurofibromatosis type 1-like syndrome. JAMA 2009; **302**: 2111–2118. [Medline] [CrossRef]
10. Spencer E, Davis J, Mikhail F, Fu C, Vijzelaar R, Zackai EH, Feret H, Meyn MS, Shugar A, Bellus G, Kocsis K, Kivirikko S, Pöyhönen M, Messiaen L: Identification of SPRED1 deletions using RT-PCR, multiplex ligation-dependent probe amplification

and quantitative PCR. Am J Med Genet A 2011; **155**: 1352–1359. [Medline] [CrossRef]
11. Pasmant E, Sabbagh A, Hanna N, Masliah-Planchon J, Jolly E, Goussard P, Ballerini P, Cartault F, Barbarot S, Landman-Parker J, Soufir N, Parfait B, Vidaud M, Wolkenstein P, Vidaud D, France RN: SPRED1 germline mutations caused a neurofibromatosis type 1 overlapping phenotype. J Med Genet 2009; **46**: 425–430. [Medline] [CrossRef]
12. Pasmant E, Ballerini P, Lapillonne H, Perot C, Vidaud D, Leverger G, Landman-Parker J: SPRED1 disorder and predisposition to leukemia in children. Blood 2009; **114**: 1131–04. [Medline] [CrossRef]
13. Batz C, Hasle H, Bergstrasser E, van den Heuvel-Eibrink MM, Zecca M, Niemeyer CM, Flotho C: Does SPRED1 contribute to leukemogenesis in juvenile myelomonocytic leukemia (JMML)? Blood 2010; **115**: 2557–2558. [Medline] [CrossRef]
14. Denayer E, Descheemaeker MJ, Stewart DR, Keymolen K, Plasschaert E, Ruppert SL, Snow J, Thurm AE, Joseph LA, Fryns JP, Legius E: Observations on intelligence and behavior in 15 patients with Legius syndrome. Am J Med Genet C Semin Med Genet 2011; **157C**: 123–128. [Medline] [CrossRef]
15. Brems H, Pasmant E, Van Minkelen R, Wimmer K, Upadhyaya M, Legius E, Messiaen L: Review and update of SPRED1 mutations causing Legius syndrome. Hum Mutat 2012; **33**: 1538–1546. [Medline] [CrossRef]
16. Wakioka T, Sasaki A, Kato R, Shouda T, Matsumoto A, Miyoshi K, Tsuneoka M, Komiya S, Baron R, Yoshimura A: Spred is a Sprouty-related suppressor of Ras signalling. Nature 2001; **412**: 647–651. [Medline] [CrossRef]
17. Maertens O, De Schepper S, Vandesompele J, Brems H, Heyns I, Janssens S, Speleman F, Legius E, Messiaen L: Molecular dissection of isolated disease features in mosaic neurofibromatosis type 1. Am J Hum Genet 2007; **81**: 243–251. [Medline] [CrossRef]
18. Yoshimura A: Regulation of cytokine signaling by the SOCS and Spred family proteins. Keio J Med 2009; **58**: 73–83. [Medline] [CrossRef]
19. Phoenix TN, Temple S: Spred1, a negative regulator of Ras-MAPK-ERK, is enriched in CNS germinal zones, dampens NSC proliferation, and maintains ventricular zone structure. Genes Dev 2010; **24**: 45–56. [Medline] [CrossRef]
20. Shmueli O, Horn-Saban S, Chalifa-Caspi V, Shmoish M, Ophir R, Benjamin-Rodrig H, Safran M, Domany E, Lancet D: GeneNote: whole genome expression profiles in normal human tissues. C R Biol 2003; **326**: 1067–1072. [Medline] [CrossRef]
21. Yanai I, Benjamin H, Shmoish M, Chalifa-Caspi V, Shklar M, Ophir R, Bar-Even A, Horn-Saban S, Safran M, Domany E, Lancet D, Shmueli O: Genome-wide midrange transcription profiles reveal expression level relationships in human tissue specification. Bioinformatics 2005; **21**: 650–659. [Medline] [CrossRef]
22. Johne C, Matenia D, Li XY, Timm T, Balusamy K, Mandelkow EM: Spred1 and TESK1 – two new interaction partners of the kinase MARKK/TAO1 that link the microtubule and actin cytoskeleton. Mol Biol Cell 2008; **19**: 1391–1403. [Medline] [CrossRef]
23. Zhuang L, Villiger P, Trueb B: Interaction of the receptor FGFRL1 with the negative regulator Spred1. Cell Signal 2011; **23**: 1496–1504. [Medline] [CrossRef]
24. Li D, Jackson RA, Yusoff P, Guy GR: Direct association of Sprouty-related protein with an EVH1 domain (SPRED) 1 or SPRED2 with DYRK1A modifies substrate/kinase interactions. J Biol Chem 2010; **285**: 35374–35385. [Medline] [CrossRef]
25. Quintanar-Audelo M, Yusoff P, Sinniah S, Chandramouli S, Guy GR: Sprouty-related Ena/vasodilator-stimulated phosphoprotein homology 1-domain-containing protein (SPRED1), a tyrosine-protein phosphatase non-receptor type 11 (SHP2) substrate in the Ras/extracellular signal-regulated kinase (ERK) pathway. J Biol Chem 2011; **286**: 23102–23112. [Medline] [CrossRef]
26. Stowe IB, Mercado EL, Stowe TR, Bell EL, Oses-Prieto JA, Hernandez H, Burlingame AL, McCormick F: A shared molecular mechanism underlies the human rasopathies Legius syndrome and neurofibromatosis-1. Genes Dev 2012; **26**: 1421–1426. [Medline] [CrossRef]
27. Denayer E, Ahmed T, Brems H, Van Woerden G, Borgesius NZ, Callaerts-Vegh Z, Yoshimura A, Hartmann D, Elgersma Y, D'Hooge R, Legius E, Balschun D: Spred1 is required for synaptic plasticity and hippocampus-dependent learning. J Neurosci 2008; **28**: 14443–14449. [Medline] [CrossRef]
28. Cui Y, Costa RM, Murphy GG, Elgersma Y, Zhu Y, Gutmann DH, Parada LF, Mody I, Silva AJ: Neurofibromin regulation of ERK signaling modulates GABA release and learning. Cell 2008; **135**: 549–560. [Medline] [CrossRef]