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

Hilde Brems and Eric Legius

Department of Human Genetics, KU Leuven, Leuven, Belgium

(Received for publication on April 13, 2013)
(Revised for publication on July 30, 2013)
(Accepted for publication on September 19, 2013)
(Published online in advance on December 10, 2013)

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. 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.

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. 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. Initially the syndrome was named Neurofibromatosis type 1-like...
syndrome, but later it was renamed Legius syndrome.\textsuperscript{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.\textsuperscript{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,\textsuperscript{11}\textsuperscript{12}\textsuperscript{7–10} and unilateral postaxial polydactyly (3/159).\textsuperscript{7,9} Lipomas are present in some individuals with Legius syndrome.\textsuperscript{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,\textsuperscript{11,12} giant cell tumor, dermoid tumor of the ovary, vestibular schwannoma, and desmoid tumor.\textsuperscript{7} No second hit was found in SPRED1 in monoblastic acute leukemia,\textsuperscript{12} and no mutations in SPRED1 were identified in a set of juvenile myeloblastic leukemia cases.\textsuperscript{13}

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

---

**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.
in 15 individuals with Legius syndrome. 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 relatively 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. 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.

**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. 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. Molecular genetic testing for Legius syndrome can be performed on DNA or RNA extracted from periph-
eral white blood cells.\textsuperscript{4,9} Copy number changes such as multi-exon deletions and whole \textit{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.\textsuperscript{10}

All identified mutations and polymorphisms in \textit{SPRED1} are summarized in the Leiden Open Variation Database, which is accessible online at http://www.lovd.nl/SPRED1.\textsuperscript{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.\textsuperscript{15} Familial as well as sporadic cases of Legius syndrome have been reported; the largest study described 13/33 (39\%) sporadic cases in the \textit{SPRED1}-positive group,\textsuperscript{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.\textsuperscript{4}

In that study by Brems et al., melanocytes were cultured from a CALM from an individual with Legius syndrome. A somatic frameshift \textit{SPRED1} mutation was identified in addition to the known germline \textit{SPRED1} mutation. This shows that CALMs in individuals with Legius syndrome are caused by biallelic inactivation of the \textit{SPRED1} gene, a mechanism similar to the biallelic inactivation of the \textit{NF1} gene found in melanocytes derived from CALMs of individuals with NF1.\textsuperscript{17} An overview of the mutations reported in the \textit{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\textsuperscript{18}). Spred1 is a negative regulator of the Ras-MAPK signaling pathway.\textsuperscript{16} Spred1 is enriched in the
central nervous system germinal zones, it downregulates neural stem cell proliferation and maintains ventricular zone structure. Human SPREDI 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. 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. Spred1 inhibits the activation of Raf by active Ras (Ras-GTP).

Several Spred1 interaction partners have been identified, i.e., microtubule-associated protein/microtubule affinity-regulating kinase-activating kinase (MARKK), testis-specific protein kinase (TESK1), fibroblast growth factor receptor like-1 (FGFR1L), DYRK1A, and SHP2. 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. 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. 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. 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. These findings are comparable with the learning and synaptic plasticity defects observed in Nf1+/- mice, 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. 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.

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.

References

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]