

DATA REPORT

Legius Syndrome: two novel mutations in the *SPRED1* geneMarika Bianchi¹, Veronica Saletti², Roberto Micheli³, Silvia Esposito², Anna Molinaro⁴, Stella Gagliardi¹, Simona Orcesi⁵ and Cristina Ceredà¹

The *SPRED1* gene encodes a protein involved in the Ras/MAPK (mitogen-activated protein kinase) signaling pathway. Mutations in *SPRED1* have been reported to cause Legius Syndrome, a rare developmental disorder that shares some clinical features with Neurofibromatosis-1. Direct sequencing was used to define *SPRED1* mutations. We present two previously undescribed mutations: a frameshift mutation causing a stop codon, which was identified in an Italian family (p.Ile60Tyrf*18) and a missense variation, which was identified in one sporadic Italian case (p.Pro422Arg). Our results led us to hypothesize that these modifications may contribute to the Legius Syndrome phenotype. Further studies will be needed to determine the roles of these mutations in the mechanisms of Legius Syndrome.

Human Genome Variation (2015) 2, 15051; doi:10.1038/hgv.2015.51; published online 3 December 2015

The *SPRED1* gene, located on chromosome 15q13.2, encodes a protein of 444 amino acids that contains an N-terminal Enabled/VASP homology-1 (EVH-1) domain, a central KIT-binding domain (KBD) and a C-terminal SPRY domain.^{1,2} *SPRED1* inhibits the Ras/Raf/MEK/ERK pathway as a negative growth factor, and is regulated by cytokine and chemokine-induced ERK.²⁻⁴

Heterozygous germline loss-of-function *SPRED1* mutations have been described in patients affected by Legius Syndrome,⁵ which is a developmental disorder that shares the pigmentary phenotype of and some additional clinical features with Neurofibromatosis-1 (NF1).⁶ Both of these syndromes belong to the group of rasopathies or neuro-cardio-facial-cutaneous syndromes, which are caused by germline mutations that affect proteins involved in the Ras/MAPK pathway.³

Legius Syndrome presents as an autosomal dominant condition characterized by multiple café-au-lait macules and skin fold freckling, with or without macrocephaly, a Noonan-like appearance, learning difficulties and/or attention deficit in children and by lipomas in adults. Some typical NF1 features, such as Lisch nodules of the iris, neurofibromas and central nervous system tumors are absent. Whether Legius Syndrome is associated with an increased risk for a specific range of malignancies remains unknown. Several *SPRED1* variants, including sequence-based changes and large deletions/duplications, have been linked to Legius Syndrome.^{7,8}

All identified mutations in *SPRED1* are reported in the Leiden Open Variation Database (<http://www.lovd.nl/SPRED1>) and the Disease Databases on the ARUP Scientific Resource for Research and Education webpage (http://www.arup.utah.edu/database/SPRED1/SPRED1_welcome.php).

Here, we describe two novel mutations (p.Ile60Tyrf*18 and p.Pro422Arg) in an Italian family with Legius Syndrome and in a sporadic case of Legius Syndrome.

Case 1: The family has four affected members: two 3-year-old probands who are bichorionic-biamniotic twins, their 35-year-old mother and their 66-year-old grandmother.

All individuals exhibited a similar phenotype: multiple café-au-lait spots and axillary freckling without macrocephaly and Noonan-like traits.

Dermatologic and ophthalmologic evaluations excluded lipomas, cutaneous neurofibromas and Lisch nodules in all of the family members. Brain magnetic resonance imaging (MRI) and cognitive assessments were performed in the twins, revealing normal results and mild language development delay.

Case 2: The proband was a 10-year-old boy who was referred to our department at 1 year of age. He was the first child of non-consanguineous Caucasian parents. His family history was unremarkable. On clinical examination, he exhibited multiple café-au-lait spots and bilateral axillary freckling. Macrocephaly and Noonan-like traits were not observed. Dermatologic and ophthalmologic evaluations, and abdominal sonography and brain MRI did not reveal any abnormal findings. His neuropsychological assessment was normal. The patient had shown vocal and motor tics since the age of 7, without any changes in behavior or scholastic performance and without features of an associated obsessive-compulsive disorder or attention deficit hyperactivity disorder.

Both cases were also tested for the *NF1* gene. The control subjects were recruited at the Transfusional Service and Centre of Transplantation Immunology, Foundation San Matteo, IRCCS, Pavia, Italy.

All patients consented to genetic testing. The study was approved by the 'C. Mondino' Institute Review Boards and was performed in accordance with the Ethical Standards of the Declaration of Helsinki.

Genomic DNA was extracted from peripheral blood using an automated system (Maxwell 16 Blood DNA—Promega, Milan, Italy). Primer sequences are available on request.

All 7 coding exons of *SPRED1* were amplified and screened by direct sequencing using a Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems, Milan, Italy) and an ABI 3130 Genetic Analyzer (Applied Biosystems). Each fragment was sequenced on both strands. The alignment to the reference sequence

¹Laboratory of Experimental Neurobiology, 'C. Mondino' National Neurological Institute, Pavia, Italy; ²Developmental Neurology Unit, 'C. Besta' National Neurological Institute Foundation, IRCCS, Milan, Italy; ³Child Neurology and Psychiatry Unit, Spedali Civili, Brescia, Italy; ⁴School in Reproductive and Developmental Sciences, University of Trieste, Trieste, Italy and ⁵Child Neurology and Psychiatry Unit, 'C. Mondino' National Neurological Institute, Pavia, Italy.

Correspondence: S Gagliardi (stella.gagliardi@mondino.it)

Received 20 May 2015; revised 31 August 2015; accepted 30 September 2015

ACKNOWLEDGEMENTS

We thank 'our' patients who trust our work. We would also like to thank Dr Stefano Bernuzzi (Immunohematological and Transfusional Service and Centre of Transplantation Immunology, IRCCS, Policlinico 'San Matteo' Foundation—Pavia, Italy) for the dispensation of the biological samples from blood donors. Support was also provided by the Italian Ministry of Health (Ricerca Corrente 2009–2011) to the 'C. Mondino' National Institute of Neurology Foundation.

COMPETING INTERESTS

The authors declare no conflict of interest.

REFERENCES

- 1 Miyoshi K, Wakioka T, Nishinakamura H, Kamio M, Yang L, Inoue M *et al*. The Sprouty-related protein, Spred, inhibits cell motility, metastasis, and Rho-mediated actin reorganization. *Oncogene* 2004; **23**: 5567–5576.
- 2 Wakioka T, Sasaki A, Kato R, Shouda T, Matsumoto T, Miyoshi K *et al*. Spred is a Sprouty-related suppressor of Ras signalling. *Nature* 2001; **412**: 647–651.
- 3 Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev* 2009; **19**: 230–236.
- 4 McClatchey AI, Cichowski K. SPRED proteins provide a NF-ty link to Ras suppression. *Genet Dev* 2012; **26**: 1515–1519.
- 5 Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R *et al*. Germline loss-of-function mutations in *SPRED1* cause a neurofibromatosis 1-like phenotype. *Nat Genet* 2007; **39**: 1120–1126.

- 6 Stowe IB, Mercado EL, Stowe TR. A shared molecular mechanism underlies the human rasopathies Legius syndrome and Neurofibromatosis-1. *Genet Dev* 2012; **26**: 1421–1426.
- 7 Brems H, Legius E. Legius syndrome, an update. molecular pathology of mutations in *SPRED1*. *Keio J Med* 2013; **62**: 107–112.
- 8 Brems H, Pasmant E, Van Minkelen R, Wimmer K, Upadhyaya M. Review and update of *SPRED1* mutations causing Legius syndrome. *Hum Mutat* 2012; **33**: 1538–1546.
- 9 Artimo P, Jonnalagedda M, Konstantin A, Baratin D, Csardi G, de Castro E *et al*. ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Res* 2012; **40**: W597–W603.
- 10 Rost B, Yachdav G, Liu J. The PredictProtein server. *Nucleic Acids Res* 2004; **32**: W321–W326.
- 11 Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics* 2010; **11**: 548.



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>