# Research letter

## Mosaicism for a *SPRED1* deletion revealed in a patient with clinically suspected mosaic neurofibromatosis

DOI: 10.1111/bjd.14873

Dear Editor, Inactivating *SPRED1* mutations cause Legius syndrome, an autosomal dominant RASopathy described in 2007.[1] Like neurofibromatosis type 1 (NF1), features include café-au-lait macules (CALMs) and skin-fold freckling. Although neurofibromas, optic gliomas, Lisch nodules, tibial pseudarthrosis and nerve-sheath tumours are not seen in Legius syndrome, other symptoms and signs common to the RASopathies, such as Noonan-like facial features, pulmonary stenosis, pectus deformity and learning disabilities, are reported.[2,3]

A 13-year-old boy presented to our centre with a large hyperpigmented patch over the right trunk (Fig. 1) The patch was present from birth and grew in proportion to his age. It demonstrated sharp midline demarcation. Within the patch were three CALMs. There were no palpable lumps or nodules within the patch. No other pigmentary findings were present. Segmental NF1 was clinically suspected. His growth parameters were appropriate for his age. He did not have macrocephaly or facial features consistent with a RASopathy. He was developmentally normal. Family history was negative for pigmentary abnormalities, genetic or neurodevelopmental disorders.

A 2-mm skin punch biopsy was taken from each CALM at sites (i), (ii) and (iii) (see Fig. 1a). DNA and RNA were extracted from phytohaemagglutinin-stimulated short-term lymphocyte cultures and from melanocytes cultured from the CALMs, as previously described.[4–8] It was previously reported that melanocytes, not the keratinocytes or fibroblasts, from CALMs carry a second-hit mutation in *NF1* or *SPRED1* and therefore biallelic inactivation in melanocytes seems to trigger

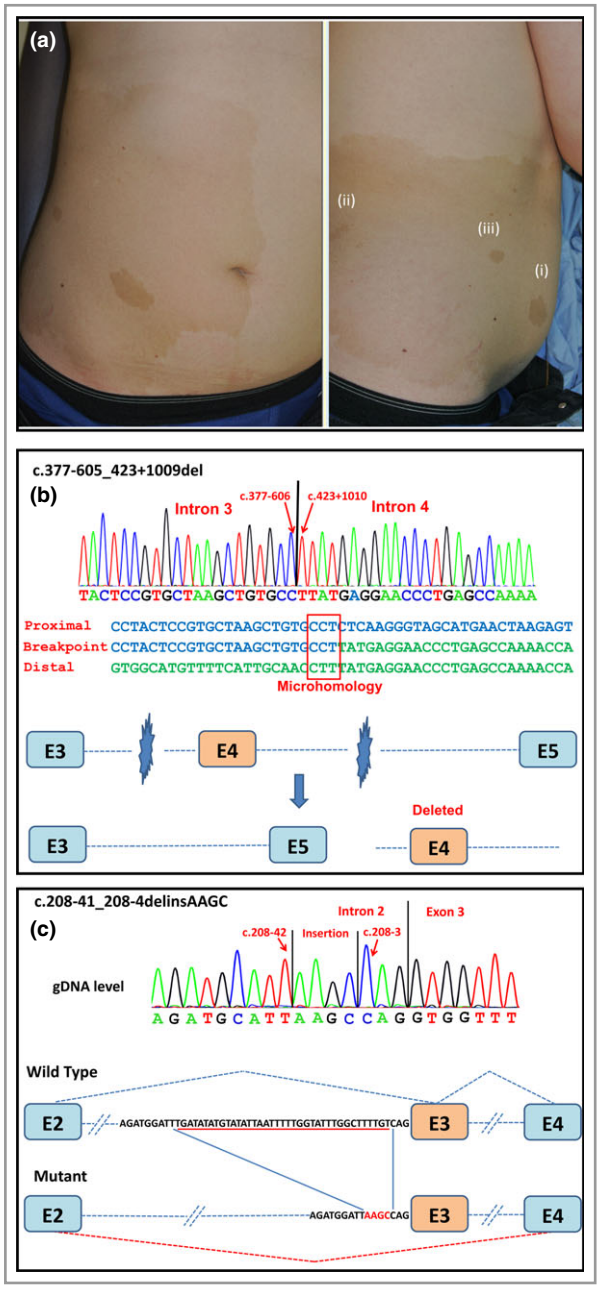


**Fig 1.** (a) Hyperpigmented patch showing sharp midline demarcation and three café-au-lait macules contained within the larger hyperpigmented patch, (i), (ii) and (iii). (b) Genomic rearrangement results in *SPRED1* exon 4 deletion. The upper panel shows the sequencing traces of the mutation c.377-606_423+101del, the middle panel represents the context of the flanking sequence at the junction site (the nucleotide sequences of intron 3 and 4 are represented in blue and green, respectively). The bottom panel represents, schematically, the genomic rearrangement resulting in the deletion of exon 4. As short microhomology was present at the junction site, this rearrangement is most likely mediated by nonhomologous end joining or replication-based mechanism, fork stalling and template switching/microhomology-mediated break-induced replication. (c) Deletion of 38 nucleotides from the polypyrimidine tract of the splice acceptor site of exon 3 reduces the strength of the splice acceptor site and results in exon 3 skipping. The upper panel shows the sequencing trace of the mutation at the DNA level, the middle panel shows the observed exon 3 skipping at the cDNA level and the bottom panel schematically illustrates the exon 3 splice mutation.

*NF1* or *SPRED1*-related CALM development.[1,4,5] *NF1* and *SPRED1* follow the Knudson two-hit model for CALM formation, with a first hit (either germline or somatic) and an additional somatic inactivation of the remaining wild-type allele.[1,4,5] Comprehensive *NF1* and *SPRED1* clinical mutational analysis (sequencing and dosage analysis) was performed at the Certified Authorization Professional-certified University of Alabama at Birmingham Medical Genomics Laboratory, with analytical sensitivity to detect heterozygous *NF1*/*SPRED1* mutations in DNA derived from blood being > 99%.[6–8] Deletion/duplication analysis was performed using multiple ligation-dependent probe amplification [MLPA; *NF1* P081C1; P082C1; P122-C2; and *SPRED1* P295-B1 (MRC-Holland, Amsterdam, the Netherlands)]. Breakpoints of the intragenic *SPRED1* deletion were identified using breakpoint-spanning polymerase chain reaction, as described previously.[6] Mutations are described following recommendations of the Human Genome Variation Society using NM_ 152594·2 as the reference sequence and position 1 being the first nucleotide of the ATG start codon.

Melanocytes from all three CALMs were heterozygous for a *SPRED1* total gene deletion by MLPA. This deletion was not detectable in DNA extracted from peripheral blood lymphocytes, indicating a postzygotic origin and that the *SPRED1* total gene deletion is the common first hit in all three CALMs. Melanocytes from CALMs labelled (i) and (iii) were found to carry second-hit *SPRED1* mutations. Sample (i) contained a 1663-base pair *SPRED1* deletion encompassing the exon 4, c.377-605_423+1009. At the RNA level, this mutation results in skipping of exon 4, r.377_423del (Fig. 1b). Sample (iii) contained an indel mutation, c.208-41_208-4delinsAAGC, destroying the splice acceptor site of exon 3. At the RNA level, this mutation results in an in-frame splice defect, r.208_423del (Fig. 1c). These mutations were also absent in blood, as expected. No mutations or deletions/duplications of *NF1* were detected.

To our knowledge, this is the first reported patient with a documented postzygotic *SPRED1* deletion causing mosaic Legius syndrome. We identified a common heterozygous *SPRED1* deletion in all three CALMs and propose this heterozygous change is responsible for the larger segmental area of hyperpigmentation, similar to segmental NF1, where a heterozygous somatic *NF1* deletion was detected in melanocytes cultured from a hyperpigmented skin area in a patient with no systemic features of NF1 and a second-hit *NF1* mutation was detected only in the melanocytes from a CALM within the area.[7] Our study also reaffirms that CALMs seen in Legius syndrome are the result of biallelic inactivating mutations in *SPRED1*.[1] We did not pursue analysis for a second hit in the CALM labelled (ii).

The mechanism by which *SPRED1* loss of function causes hyperpigmentation is not fully understood. *SPRED1* is a negative regulator of mitogen-activated protein kinase-kinase (MEK) and extracellular-related signal kinase (ERK) phosphorylation. Brems *et al.*[1] analysed levels of activated MEK and ERK in melanocytes from control skin (*SPRED1*$^{+/+}$), unaffected skin of a patient with a germline mutation (*SPRED1*$^{+/-}$) and a CALM with biallelic mutations (*SPRED1*$^{-/-}$). They demonstrated stepwise increase in activated MEK and ERK levels between *SPRED1*$^{+/+}$, *SPRED1*$^{+/-}$ and *SPRED1*$^{-/-}$ melanocytes.[1] Thus, melanocytes haploinsufficient for *SPRED1* are characterized by abnormal RAS–MAPK signalling, even in the absence of a second hit. Hence, it is possible the large hyperpigmented area in our patient is secondary to abnormal MEK and ERK levels.

While our findings are novel, there are likely many more individuals remaining undiagnosed. Mosaic *SPRED1* should be considered as a differential diagnosis to mosaic NF1. Differentiating between the two diagnoses is important, as those with mosaic *SPRED1* are not at risk of the tumours associated with NF1. Genetic counselling regarding the risk of gonadal mosaicism resulting in transmission of a full blown *SPRED1* phenotype should also be discussed.

[1]*Division of Clinical and Metabolic Genetics, and* [2]*Section of Dermatology, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada*
[3]*Department of Genetics, Medical Genomics Laboratory, University of Alabama at Birmingham, Birmingham, AL, U.S.A.*
*Correspondence: Peter Kannu and Ludwine Messiaen*
*E-mails: peter.kannu@sickkids.ca; lmessiaen@uabmc.edu*

R.K. Jobling[1]
I. Lara-Corrales[2]
M.-C. Hsiao[3]
A. Shugar[1]
S. Hedges[1]
L. Messiaen[3]
P. Kannu[1]

L.M. and P.K. contributed equally to this work.

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Funding sources: This work was supported through the Children's Tumor Foundation by the Isaac and Sadie Fuchs Genotype–Phenotype study (to L.M.), and internal funds from the Medical Genomics Laboratory, University of Alabama at Birmingham.

Conflicts of interest: none declared.