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Independent *NF1* mutations underlie café-au-lait macule development in a woman with segmental NF1

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Segmental neurofibromatosis type 1 (NF1) is an under-recognized form of NF1 caused by postzygotic somatic loss-of-function *NF1* gene mutations that affect a subset of cells in the body.[1] This is in contrast to classic or generalized NF1, in which a germline *NF1* gene mutation affects all diploid cells in the body. In the segmental NF1 variant, individuals typically exhibit clinical features characteristic of generalized NF1, such as café-au-lait macules (CALMs), skinfold freckling, and neurofibromas, restricted to one segment of the body. For this reason, establishing the diagnosis can be challenging because the underlying *NF1* gene mutation is often not detected in the blood. Underscoring the challenges of caring for individuals with this variant of NF1, we describe a woman with segmental NF1 referred to us at 22 years of age for evaluation.

The patient first came to medical attention at four months of age when her pediatrician noted that she had more than six CALMs localized to her right abdomen, back, and hip. Over the next five years, she developed additional hyperpigmented macules in this region, followed by freckling in the right inguinal region. Collectively, these findings met the criteria for a diagnosis of segmental NF1. On examination, the CALMs and skinfold freckling in the affected region did not cross either the anterior or posterior midlines, and there were no neurofibromas, other hyperpigmented lesions, or Lisch nodules detected. Because the affected body segment could potentially include her right ovary, at 28 years of age, she underwent genetic testing (direct Sanger sequencing, mixed ligation-dependent probe amplification, and interphase fluorescence in situ hybridization [FISH]), which failed to identify an *NF1* gene mutation in her blood. She subsequently underwent biopsy of three of her CALMs (right lower back, hip, and abdomen), followed by primary culture of these skin melanocytes for genetic testing as above.[2] As shown in the figure, sequencing and FISH revealed that all three CALM-derived melanocyte cultures shared a common 1.4 megabase deletion of the *NF1* gene (type 1 total gene deletion [TGD]). Furthermore, in each of the three CALMs, a different second-hit mutation was identified that affected the remaining (non-deleted) *NF1* gene copy.

This case report is instructive for several reasons. First, it underscores the utility of analyzing the affected tissues (e.g., melanocytes from multiple CALMs), rather than the blood, in establishing a diagnosis of segmental NF1. Although this patient met the diagnostic criteria for segmental NF1 on clinical grounds (regional distribution of CALMs and inguinal freckling), the genetic findings provide a reference for future prenatal genetic counseling and diagnostic testing.[3] Second, genetic testing of affected tissue suggests that this patient sustained a total *NF1* gene deletion during post-zygotic development and thus affected only a subset of her cells. Individuals with the generalized form of NF1 who harbor this type of mutation (type 1 TGD) frequently have a more severe phenotype, with greater numbers of neurofibromas and an increased risk of cancer, compared with individuals with generalized NF1 caused by other *NF1* gene mutations.[4] By contrast, this patient with segmental NF1 has no visible neurofibromas and is otherwise healthy, which is different from a previous report of several patients with segmental

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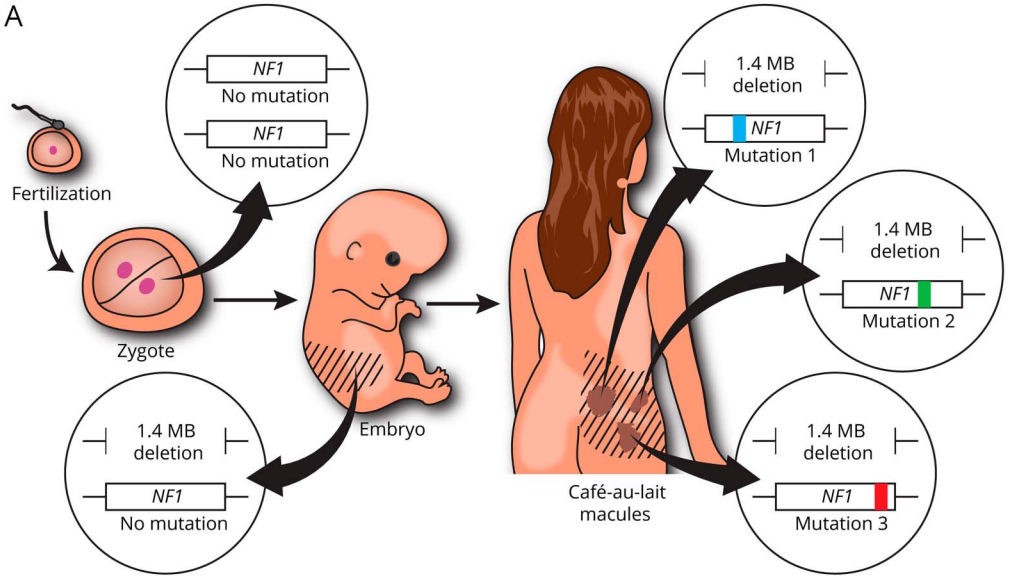
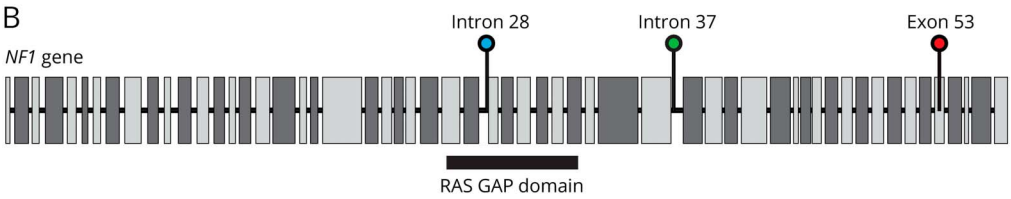
Figure Unique second neurofibromatosis type 1 (*NF1*) gene mutations in multiple café-au-lait macules (CALMs) from an individual with segmental NF1





	NF1 gene mutation	Exon/intron	Mutation type	mRNA/protein change
1	c.3871-2A>G	Intron 28	Splice acceptor variant	Possible exon 29 skipping
2	c.5546+5G>A	Intron 37	Splice donor variant	Possible exon 37 skipping
3	c.7846C>T	Exon 53	Nonsense mutation	Arg2616X

(A) Segmental or mosaic NF1 is caused by de novo *NF1* gene mutations that occur during postzygotic development and affect only a subset of cells in the body. Genetic testing of melanocytes derived from three different CALMs in the patient's affected body segment (denoted by the diagonal stripes) revealed a common 1.4 megabase (MB) deletion, resulting in total loss of one copy of the *NF1* gene, and three unique second-hit mutations affecting the remaining *NF1* allele. The insets depict both *NF1* gene copies in the indicated cell types. (B) Locations (colored pinheads) and predicted messenger RNA (mRNA) and protein changes resulting from the second *NF1* gene mutations in melanocytes derived from three different CALMs. Two intronic mutations were located within splice sites, and one exonic mutation resulted in a premature stop codon (nonsense mutation). Short black boxes denote introns; tall gray boxes denote exons.

NF1 and type 1 TGDs.[5] In that series, four of the five cases had either numerous dermal neurofibromas or a plexiform neurofibroma. The less severe phenotype in the current subject suggests that, compared with previously reported cases, (1) her total *NF1* gene deletion may have arisen at a later developmental stage, (2) it is restricted to a different population of cells, or (3) modifier genes additionally influence clinical expression. Third, each of the CALMs in this patient contained melanocytes harboring a unique second *NF1* gene mutation that presumably resulted in loss of or impaired function of the *NF1*-encoded protein (neurofibromin) in those cells. This finding is consistent with previous reports demonstrating biallelic *NF1* gene inactivation in CALMs,[6,7] similar to that observed in benign tumors from patients with NF1.[2] The finding that the second-hit mutations were distinct in each CALM argues that each macule arose independently, similar to benign neoplasms (i.e., neurofibromas) that arise in this population of tumor-prone individuals.

Author contributions

M.E. Freret contributed to manuscript preparation and made the figure. C. Anastasaki contributed to manuscript preparation. D.H. Gutmann wrote the first draft of the manuscript.

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Disclosure

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