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Next-Generation Sequencing of Nevus Spilus–Type Congenital Melanocytic Nevus: Exquisite Genotype–Phenotype Correlation in Mosaic RASopathies

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TO THE EDITOR

Nevus spilus is a descriptive term used to denote any cutaneous lesion with a café-au-lait macular background and superimposed on more pigmented areas. Small single nevus spilus are relatively common, and they have recently been described to be due to somatic activating *HRAS* mutations (Sarin *et al.*, 2014). Larger superficial lesions with small superimposed junctional nevi in association with phakomatosis pigmentokeratotica have also been found to contain *HRAS* mutations (Groesser *et al.*, 2013). However, another nevus spilus–type phenotype has also been well described in which large café-au-lait macules have superimposed lesions that are indistinguishable both clinically and histopathologically from medium/large congenital melanocytic nevi (CMN), exhibit a wide variety of colors and sizes (Schaffer *et al.*, 2001a, b), and continue to develop postnatally in many cases. This is termed nevus spilus–type CMN. In our experience, there may be delayed appearance of the café-au-lait background after birth, but the lesion is still usually predictable on the basis of clustering of many CMN in one anatomical area. Smaller separate lesions in the same individual are often indistinguishable clinically from café-au-lait macules, and are so faint that they can be easily missed (Figure 1). Our primary aim in this study was to look for the genetic basis of this defined phenotypic subset of CMN, in the context of the recent discovery that *NRAS* Q61K and Q61R mutations are the cause of most causes of multiple CMN (Kinsler *et al.*, 2013b). In particular, we were interested in determining the mutation in

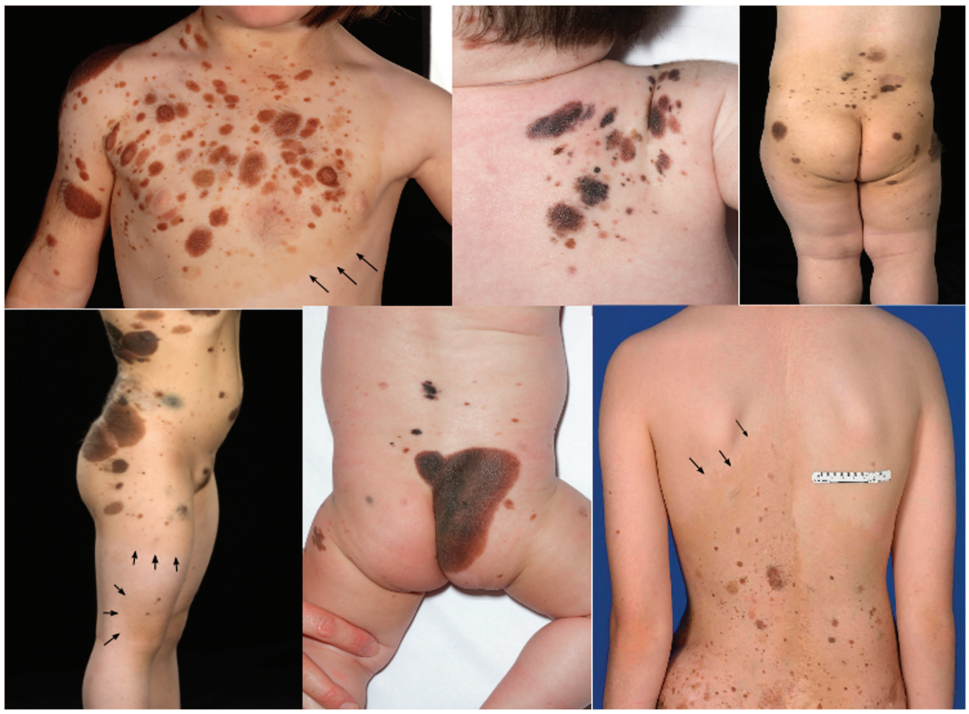

Figure 1. Clinical images of nevus spilus–type CMN in six different patients. The café-au-lait macule background is often invisible at birth. Two separate lesions are indicated in one patient. CMN, congenital melanocytic nevi. Written consent was obtained for publication of all photographs.

Abbreviation: CMN, congenital melanocytic nevus or nevi

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the background macular portion of the nevus spilus–type CMN, working on the hypothesis that this could be the ''first hit'' in a two-hit model of nevogenesis.

These studies were approved by the appropriate Research Ethics Committee, written consent was taken from participants, and the Declaration of Helsinki Principles protocols were followed. A blood sample and skin biopsies of both the café-au-lait macule background and a banal superimposed CMN were taken from three children with large nevus spilus–type CMN, from a total of 17 patients from our combined practices (patients 5, 12, and 13 in Supplementary Table S1 online), and DNA was extracted directly by standard methods. Whole-exome sequencing using Nextera library preparation and an ABI Hi-Seq bioanalyser was undertaken on all three samples from two patients, and data were analyzed using an in-house pipeline designed for somatic mosaicism. The principal governing analysis was to look for a mutation present in the café-au-lait that was not present in the blood, and a further mutation in the CMN not present in the café-au-lait or the blood.

In all, 1,991,478 variants were called automatically in the overlying CMN, affecting 20,693 genes. After filtering, the sequencing files of 133 variants in 39 genes were reviewed manually. A single mutation was found in the café-au-lait macule and the superimposed CMN, with no further mutation confirmed despite extensive analysis. These mutations were missense activating mutations in *NRAS* in the skin, absent from the blood, and this pattern of somatic mosaicism was confirmed in the third patient by Sanger sequencing. The mutations, however, are undescribed so far in typical CMN, being 1:115256528 c.183A>C p.Q61H (two patients, Figure 2) and 1:115258745 c.37G>C p.G13R. Both mutations have

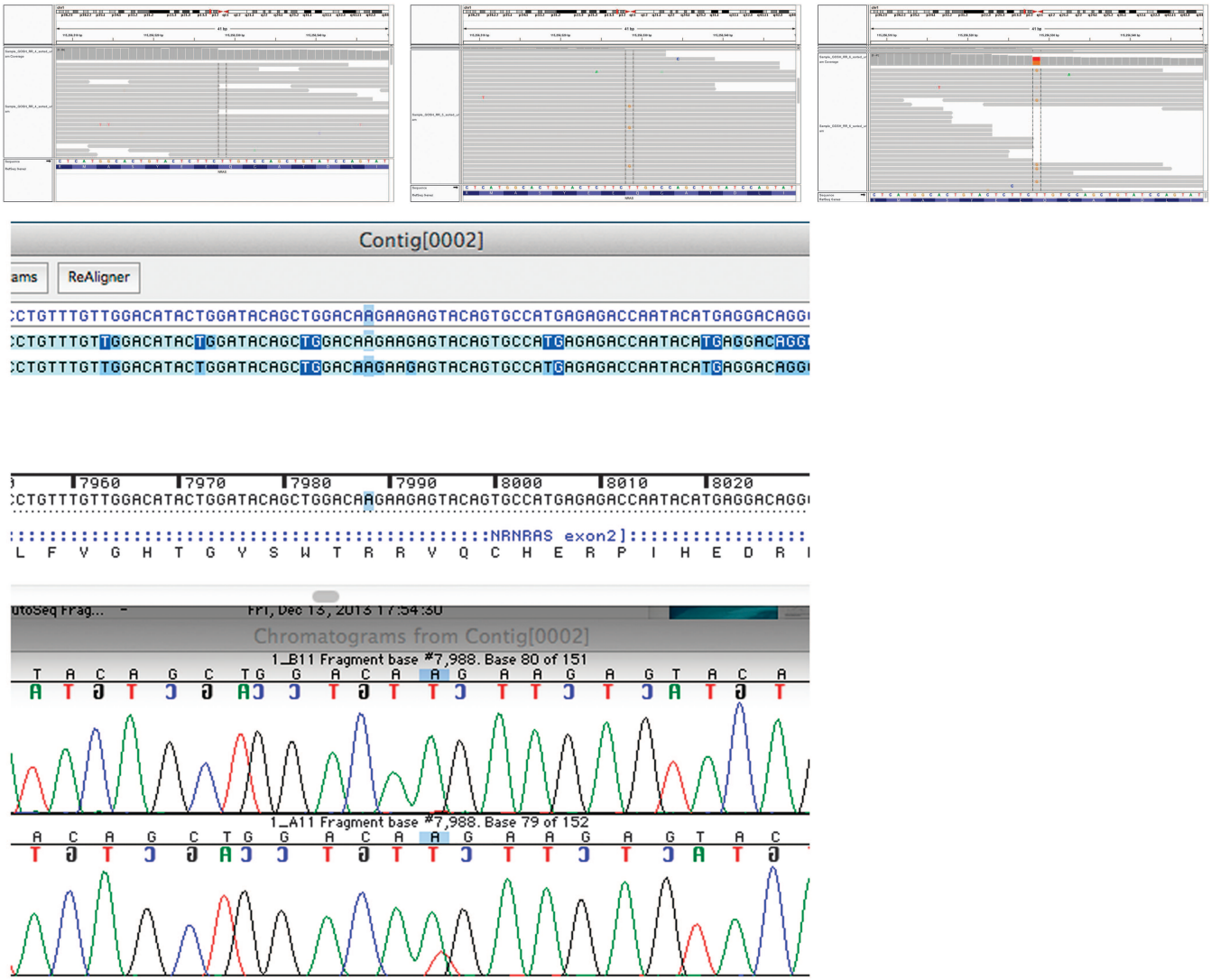

Figure 2. Sequencing results showing *NRAS* mutation. Next-generation sequencing reads of blood (upper left), café-au-lait macule (upper centre), and overlying CMN (upper right) from the same patient showing mutation *NRAS* c.183A>C p.Q61H. Note the absence of mutation in the blood, and the much lower percentage of mosaicism in the café-au-lait than the CMN, as would be expected from the number of affected cells in a biopsy sample. Sanger sequencing (below) confirmation of the heterozygous pQ61H mutation in CMN. CMN, congenital melanocytic nevi.

been described at a somatic level in non-CMN-related malignant melanoma (Forbes *et al.*, 2008). Independently, a fourth patient (patient 17, Supplementary Table S1 online) had targeted exon capture of two skin samples, and analysis also revealed only the same *NRAS* p.Q61H mutation in both the café-au-lait macule and the superimposed CMN.

In conclusion, nevus spilus–type CMN are a phenotypically and genotypically distinct variant of CMN, and are phenotypically and genotypically distinct from nevus spilus maculosus and papulosus due to *HRAS* mutations (Groesser *et al.*, 2013; Sarin *et al.*, 2013). This further extends the exquisite mutation specificity of the newly characterized mosaic RASopathies. We found no evidence of a second mutation to explain the superimposed nevi on the macular background. It is, however, possible that there could be a mutation that does not appear pathogenic to us and to the analysis pipelines at the present time. Alternatively, there could be either a translocation that does not disrupt exonic DNA sequence, or a growth-promoting copy number change, although CMN have previously been documented as having few such changes on array comparative genomic hybridization (Bastian *et al.*, 2002). The data at the moment suggest that only one sequence-level mutation occurs, and that these specific *NRAS* mutations are sufficient to cause café-au-lait macular pigmentation, which can lead to macroscopic nevus formation over time. Other experimental evidence supports that nevi can evolve in this way, from a cell or collection of cells in the skin not visible to the naked eye, as nevus cells have been found in normal skin in patients with acquired melanocytic nevi and typical CMN (Dadzie *et al.*, 2008; Kinsler *et al.*, 2013a).

It is important to note for clinical management that malignant melanoma has been described in patients with nevus spilus–type CMN (Kinsler *et al.*, 2009), and on current knowledge we would consider nevus spilus–type CMN patients at the same risk of malignancy as other genotypes. Similarly, although interestingly none of the 17 patients described here have neurological abnormalities on magnetic resonance imaging scan (Supplementary Table S1 online), these numbers are too small to draw any conclusions about a possible low risk of neurological phenotype. Management of nevus spilus–type CMN should therefore be the same as for other CMN.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Veronica A. Kinsler[1,2,9], Sven Krengel[3,9], Jean-Baptiste Riviere[4], Regula Waelchli[2], Carolina Chapusot[5], Lara Al-Olabi[1], Laurence Faivre[6], Holger A. Haenssle[7], Lisa Weibel[8], Géraldine Jeudy[6] and Pierre Vabres[6]

[1]Clinical and Molecular Genetics Unit, UCL Institute of Child Health, London, UK; [2]Paediatric Dermatology, Great Ormond St Hospital for Children, London, UK; [3]Department of Dermatology, University of Lübeck, Lübeck, Germany; [4]Department of Molecular Genetics, Dijon University Hospital, Dijon, France; [5]Department of Clinical Genetics, Dijon University Hospital, Dijon, France; [6]Department of Dermatology, Dijon University Hospital, Dijon, France; [7]Department of Dermatology, University Medical Centre, Goettingen, Germany and [8]Department of Dermatology, University Children's Hospital, Zurich, Switzerland
E-mail: v.kinsler@ucl.ac.uk or pierre.vabres@chu-dijon.fr
[9]These authors contributed equally to this work.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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