(Sybert, 2010). We postulate that the phenotypic difference between these nevi may be related to the extent of the mutation, as well as body site–specific embryological patterns and environment. The knowledge of the genetic basis of nevus sebaceus and its associated syndrome represents a further step toward understanding genotype–phenotype correlations arising from genetic mosaicism.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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## SUPPLEMENTARY MATERIAL

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

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*See related commentary on pg 597*

# Whole-Exome Sequencing Reveals Somatic Mutations in *HRAS* and *KRAS*, which Cause Nevus Sebaceus

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## TO THE EDITOR

Epidermal genetic mosaicism is evident as stripes of affected skin that typically appear in S- or V-shaped whorled, streaked, and linear patterns called lines of Blaschko (Blaschko, 1901). These patterns represent dorsoventral migratory pathways of neuroectoderm during embryogenesis (Moss *et al.*, 1993). Mosaic lesions result from somatic mutation during development, with timing of such events determining the extent and distribution of skin involvement. Epidermal nevi (EN) are common cutaneous mosaic disorders seen in 0.1–0.3% of births, and fall into two classes: keratinocytic epidermal nevi (KEN) and organoid epidermal nevi, which includes nevus sebaceus (NS) and follicular nevi (Solomon and Esterly, 1975). NS comprises approximately half of EN, and typically appears on the scalp as a yellowish–orange linear plaque with hyperkeratosis, acanthosis, a markedly increased number of sebaceous lobules, and abortive hair follicles with resulting alopecia (Figure 1a–d). In contrast to KEN, in which neoplasia is rare, tumors develop in nearly 14% of all NS cases, and in more than 23% of affected adults (Cribier *et al.*, 2000), suggesting that the mutation(s) causing NS also increase the risk of tumorigenesis (Figure 1e–g).

Recently, somatic mosaicism has been identified in KEN using SNaPshot assays to identify mutations in mitogen-activated protein kinase (MAPK) pathway genes including *FGFR3*, *HRAS*, *KRAS*, *NRAS*, and *PIK3CA*. Activating Ras mutations, including *HRAS* p.Gly13Arg and *KRAS* p.Gly12Asp, were most common and accounted for 39% of KEN, with *HRAS* mutations predominating (Hafner *et al.*, 2012). Similar approaches have been used in NS, identifying *HRAS* p.Gly13Arg in 91% of lesions and *KRAS* p.Gly12Asp in 5% of lesions (Groesser *et al.*, 2012). We present an independent, complementary approach to genetic pathogenesis in NS, in which we used whole-exome sequencing to characterize the spectrum of *de novo* coding mutations present within NS lesions.

*Abbreviations: EN, epidermal nevi; KEN, keratinocytic epidermal nevi; LOH, loss of heterozygosity; MAPK, mitogen-activated protein kinase; NS, nevus sebaceus*

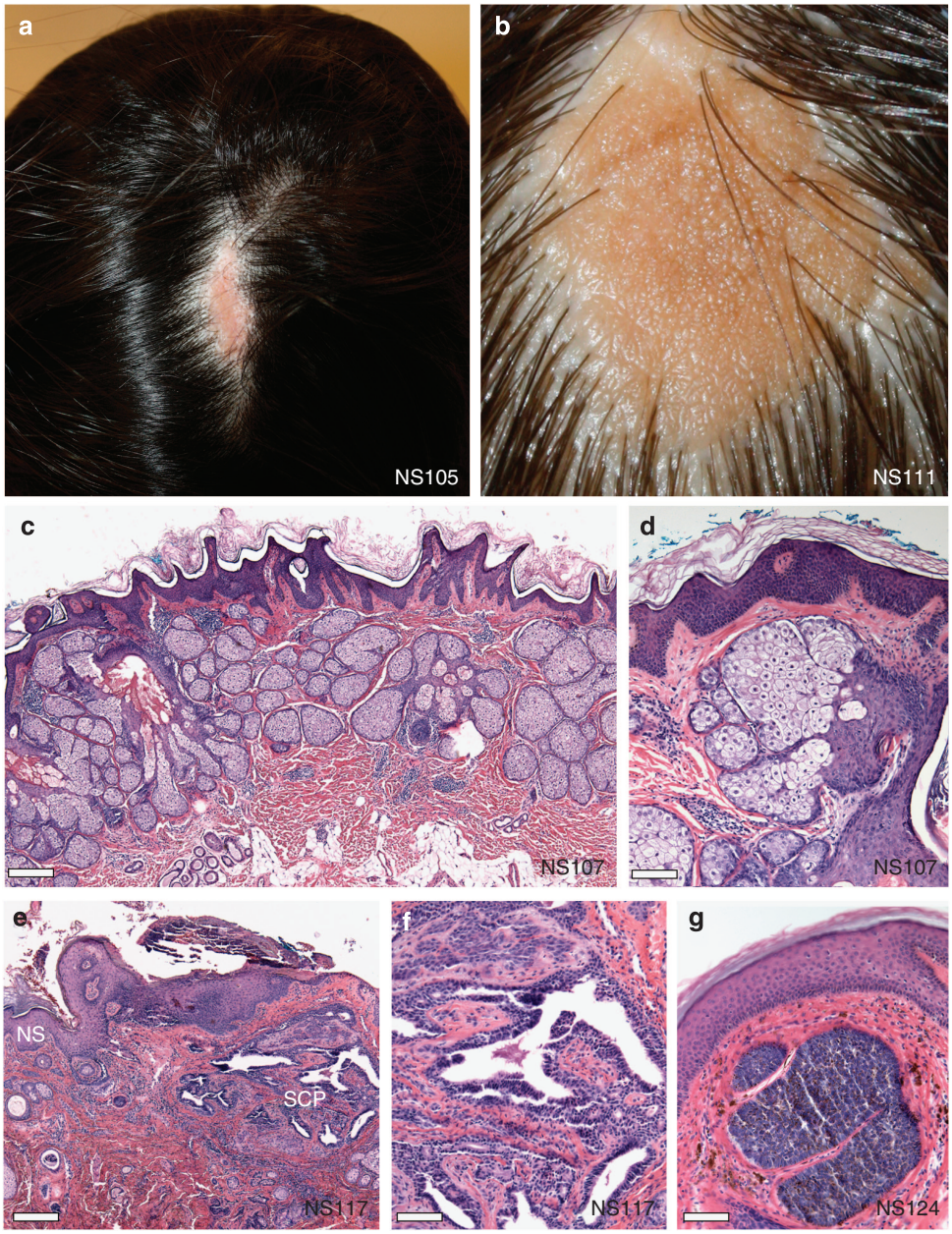


**Figure 1. Clinical and microscopic features of nevus sebaceus.** (**a**, **b**) Solitary, well-demarcated lesions on the scalp of two individuals show alopecia and a yellow–orange waxy appearance. (**c**) On histological examination, there is epidermal acanthosis, papillomatosis, and hyperkeratosis with a marked increase in the number of sebaceous lobules and abortive hair follicles, scale bar = 288 μm, which is more evident at (**d**) higher magnification, scale bar = 85 μm. (**e–g**) Up to 20% of nevus sebaceus (NS) lesions develop tumors, including syringocystadenomas, trichoblastomas, trichilemmomas, and tubular apocrine adenomas. (**e**) Nevus sebaceus with syringocystadenoma papilliferum (SCP) composed of villous structures lined by a columnar epithelium with stromal plasma cells, scale bar = 570 μm, which is most evident at (**f**) higher magnification, scale bar = 92 μm. (**g**) A trichoblastoma arising within a nevus sebaceus shows a well-circumscribed nodule of basaloid cells with a dense fibrocytic stroma, scale bar = 92 μm.

For our study cohort, we identified five individuals with NS and performed exome sequencing of paired DNA from blood and NS tissue in each (Supplementary Table S1 online). Genetic variants were annotated and compared to identify *de novo* somatic mutations present solely within NS tissue and not in germ-line DNA. By using this analysis, we identified two genes with recurrent somatic mutations: three NS samples had an identical somatic mutation in *HRAS* (p.Gly13Arg), and the remaining two had a somatic mutation at the Gly12 residue of *KRAS* (p.Gly12Asp and p.Gly12Val, respectively) (Figure 2, Supplementary Figure S1 online). Furthermore, no genes other than *HRAS* and *KRAS* were found to be mutated in more than one lesion. Notably, one sample showed a concurrent *HRAS* p.Gly13Arg and a *BRAF* p.Arg347Gln mutation, which has not been previously described as a germ-line or somatic mutation. This *BRAF* mutation was the only other somatic mutation found in exome sequences of these five NS samples.

**a**

| Sample | Position (hg18) | Gene | Base change | Protein effect | No. of reads for tissue | | No. of reads for blood | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Ref. | Non-ref. | Ref. | Non-ref. | |
| NS101 | Chr11:524,286 | *HRAS* | G>C | G13R | 96 | 17 | 70 | 1 | $1.2\times10^{-3}$ |
| NS102 | Chr11:524,286 | *HRAS* | G>C | G13R | 212 | 62 | 123 | 0 | $8.9\times10^{-12}$ |
| NS103 | Chr11:524,286 | *HRAS* | G>C | G13R | 135 | 19 | 141 | 0 | $2.5\times10^{-6}$ |
| NS107 | Chr12:25,289,551 | *KRAS* | G>T | G12V | 202 | 62 | 102 | 0 | $1.6\times10^{-10}$ |
| NS109 | Chr12:25,289,551 | *KRAS* | G>A | G12D | 154 | 31 | 77 | 0 | $9.1\times10^{-6}$ |

**b**

HRAS
WT: MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET
NS102: MTEYKLVVVGAGRVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET

KRAS
WT: MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET
NS107: MTEYKLVVVGAVGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET
NS109: MTEYKLVVVGADGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET

**Figure 2. Exome sequencing reveals somatic *HRAS* and *KRAS* mutations in nevus sebaceus tissue.** (**a**) *HRAS* and *KRAS* mutation annotation, including genomic position, nucleotide change, protein consequence, and number of reference and nonreference reads obtained from paired sequencing of tissue and blood in five independent, unrelated nevus sebaceus cases. Significance of the mutant allele frequency difference between tissue and blood DNA was calculated with a one-tailed Fisher's exact test. When corrected for multiple testing, $2.4\times10^{-6}$ is the threshold for genome-wide significance. In each case, *HRAS* and *KRAS* mutations showed the lowest *P*-value. (**b**) Alignment of the N-termini of HRAS and KRAS reveals identical residues through position 94, with an overall 95% identity and 99% similarity. The first 50 amino acids are shown for the wild-type and each mutant protein, with mutant residues indicated in red.

To examine whether these mutations were present uniquely in the epidermis, and not in the underlying dermis, we used laser capture microdissection to prepare DNA independently from the epidermis and dermis of NS lesions and then performed Sanger sequencing with *HRAS*- or *KRAS*-specific primers. We found the mutant allele in an approximately equimolar ratio in the epidermis and sebaceous lobules, but it was entirely absent in the dermis and blood (Supplementary Figures S2 and S3 online). Employing Sanger sequencing, we evaluated 11 additional paired samples for *HRAS* and *KRAS* mutations, and all were found to have the same somatic p.Gly13Arg *HRAS* mutation (Supplementary Table S2 online; Supplementary Figure S3 online).

Prior studies have reported that up to 40% of NS lesions exhibit loss of heterozygosity (LOH) on chromosome 9q, inclusive of *PTCH* (Xin *et al.*, 1999). Examination of exome data from our discovery cohort found no evidence of LOH in NS lesions at this locus or elsewhere (Supplementary Figure S4 online).

Recognizing that *HRAS* and *KRAS* are oncogenes, that observed mutations could serve as an initiating event in multistep carcinogenesis (Knudson, 2001), and that tumors frequently develop in NS, we sought to determine whether tumors arise specifically in *HRAS* or *KRAS* mutation-positive lesions. Neoplasms arising within NS are typically benign and consist primarily of trichoblastomas, syringocystadenoma papilliferum, trichilemmomas, and tubular apocrine adenomas (Cribier *et al.*, 2000), although occasional basal cell carcinomas and rarely more aggressive malignant tumors have been reported (Moody *et al.*, 2012). We identified 11 archival NS specimens containing tumors, isolated DNA from the NS portion of the lesion, and found that all samples had an *HRAS* p.Gly13Arg mutation (Supplementary Table S2 online, Supplementary Figure S5 online). The spectrum of additional genetic events necessary for tumorigenesis in NS lesions remains unknown.

In total, 27 NS samples were evaluated, with 25 harboring an identical *HRAS* mutation (p.Gly13Arg), and two exhibiting a *KRAS* mutation in the adjacent paralogous residue (p.Gly12Asp or p.Gly12Val). The occurrence of multiple, tightly clustered somatic mutations in adjacent residues of these highly homologous proteins is definitive proof of a role for these mutations in NS and suggests a gain-of-function mechanism. Indeed, expression of the *KRAS* p.Gly12Asp allele within murine hair follicles reproduces features of NS including abortive hair follicles and epidermal and sebaceous gland hyperplasia (Lapouge *et al.*, 2011; Mukhopadhyay *et al.*, 2011).

Missense mutations at codons 12 and 13 of *HRAS* and *KRAS*, respectively, are common in malignancies, including squamous cell carcinoma, and lead to constitutive activation of Ras by blocking the activity of GTPase-activating proteins (Grewal *et al.*, 2011). Ras isoforms are central regulators of the MAPK pathway, which controls cell proliferation, differentiation, and survival. Rare inherited disorders caused by germ-line mutations in Ras and other MAPK pathway members, known as ''RASopathies,'' include Costello, Noonan, and cardio-facio-cutaneous syndrome. These show variable cutaneous features, particularly hyperkeratosis, palmoplantar keratoderma, papillomas, and hair abnormalities in addition to craniofacial and systemic findings. KEN and NS have not been reported in affected individuals. Notably, *HRAS* p.Gly13Arg and *KRAS* p.Gly12Asp have not been reported as germ-line mutations in these or other disorders, and *KRAS* p.Gly12Asp leads to embryonic lethality in mice, suggesting that both mutations grossly disrupt embryonic development and are thus likely to be found primarily in mosaic states (Tuveson *et al.*, 2004). Our findings confirm the predominance of *HRAS* mutations in NS, including those with tumors (Groesser *et al.*, 2012), and provide evidence that *HRAS* and *KRAS* mutations are sufficient to cause NS without genome instability, LOH, or secondary mutation.

The marked sebaceous hyperplasia observed in NS, which are found almost exclusively on the scalp and face, is not seen in KEN, which appear primarily on the torso, despite identical underlying somatic *HRAS* and *KRAS* mutations. This suggests that body site determines phenotype and is supported by a report of a contiguous linear nevoid lesion extending from the scalp to the neck with transition in clinical and histologic appearance from KEN on the upper back and neck to NS on the scalp (Waltz *et al.*, 1999). The specific determinants of such site-specific phenotypes are unknown, although distinct epithelial–mesenchymal interactions are a possible cause.

Human subjects gave written, informed consent to the study protocol, which was approved by the Yale Human Investigational Committee, and complies with the Declaration of Helsinki Principles.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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## SUPPLEMENTARY MATERIAL

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# Genetic Profiling of BRAF Inhibitor–Induced Keratoacanthomas Reveals No Induction of MAP Kinase Pathway Expression

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## TO THE EDITOR

The new RAF inhibitor vemurafenib represents one of the first specifically targeted drugs for melanoma (Bollag *et al.*, 2010; Flaherty *et al.*, 2010; Joseph *et al.*, 2010). However, some individuals receiving vemurafenib develop eruptive squamous neoplasms such as keratoacanthomas (KAs) (Chapman *et al.*, 2011). KAs are usually considered low-grade squamous proliferations (Cribier *et al.*, 1999; Clausen *et al.*, 2006), and are often interpreted as a subtype of squamous cell carcinoma (SCC). Their clinical course involves rapid growth followed by spontaneous involution. The mechanism by which KAs develop is still unknown. Improved understanding of the molecular and genetic basis of KA formation would allow their improved management.

To investigate genes involved in the development of KAs in patients receiving vemurafenib, we obtained biopsy specimens and studied gene expression profiles by a microarray approach; full details are available in the Supplementary Material online. These biopsies were originally obtained by the

*Abbreviations: KA, keratoacanthoma; MAP, mitogen-activated protein; SCC, squamous cell carcinoma*