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Mosaic activating *RAS* mutations in nevus sebaceus and nevus sebaceus syndrome

Bryan K. Sun[1], **Andrea Saggini**[2,3], **Kavita Y. Sarin**[1], **Jinah Kim**[1], **Latanya Benjamin**[1], **Philip E. LeBoit**[2], and **Paul A. Khavari**[1]

[1]Department of Dermatology, Stanford University School of Medicine, Stanford, California, United States

[2]Department of Dermatology, University of California-San Francisco, San Francisco, California, United States

[3]Department of Dermatology, University of Rome Tor Vergata, Rome, Italy

To the editor:

Nevus sebaceus is a common congenital skin hamartoma, classically appearing as a yellow-hued plaque on the scalp, face, or neck. It is the hallmark lesion of Schimmelpenning/nevus sebaceus syndrome (MIM: 163200), a multisystem disorder that includes a spectrum of central nervous system, ocular, skeletal, and cardiovascular defects. Secondary neoplasms arise within nevus sebaceus at a modest but elevated rate (Moody *et al*, 2012), prompting disagreement about whether they should be routinely excised (Shwayder, 2011). Determining the pathogenesis of nevus sebaceus would provide a framework to better understand this lesion and its associated syndrome.

The appearance of nevus sebaceus along Blaschko's lines suggests that a mosaic genetic mutation causes the lesion, with more extensive multisystem involvement potentially underlying the syndromic form (Happle, 1993). Here, we report a case of an individual with nevus sebaceus syndrome. As individuals with this syndrome are uncommon, we sought to identify associated mutations by comparing the exome sequence of the nevus sebaceus from our patient with those of sporadic nevus sebaceus.

Our index patient is a 38-year old female who was born with Chiari malformation, myelomeningocele, and resultant paraplegia. Due to hydrocephalus and marked ventricomegaly, she required ventriculo-peritoneal shunt placement. Imaging studies demonstrated rotoscoliosis. She has had cognitive developmental delay and suffered from generalized seizures during childhood. In her early thirties, she experienced a middle right cerebral artery stroke. Despite her condition, she remains high functioning and lives in an

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Corresponding Author: Bryan K. Sun, Department of Dermatology, 269 Campus Drive – CCSR 2155, Stanford University School of Medicine, Stanford, California, USA 94305, bryansun@stanford.edu, 650.725.2926 / Office, 650.723.8762 / Fax.

Conflict of Interest
The authors state no conflict of interest.

assisted care facility. No other family members are affected by similar medical conditions, and no cause has been attributed to her findings.

In the past year, the patient became bothered by growths on her forehead and presented to our clinic. On examination, she exhibited frontal bossing and a ~4 cm × ~3 cm yellow-tan, papillomatous plaque on the paramidline forehead that had been present since birth (Figure 1a). Several pedunculated papules were noted within the lesion. No other significant cutaneous findings were appreciated.

Per patient request, the lesion was excised and a portion of the excision specimen was collected with her written informed consent. Our study complied with the Declaration of Helsinki Principles and was approved by the Stanford IRB. Histologic evaluation confirmed features of nevus sebaceus with no secondary neoplasms (Figure 1b). Accordingly, in light of the extensive neurological and skeletal involvement, a diagnosis of Schimmelpenning/nevus sebaceus syndrome was made. In efforts to determine an underlying genetic mutation, four additional, independent nevus sebaceus were collected from elective excisions along with adjacent normal skin controls. The five samples were subjected to exome sequencing and analyzed for mutations using Seqgene (Deng, 2011) and DNAnexus (www.dnanexus.com) as described in the Supplemental Text.

Analysis of recurrent lesion-specific variants identified an *HRAS* point mutation (c.37G>C, p.Gly13Arg) in the index case and 2 of 4 isolated nevus sebaceus, with a variant allele frequency ranging from 17–43%. Sanger sequencing confirmed the *HRAS* mutation in all five lesional samples and its absence in all matched controls (Figure 2a). Examination of the two *HRAS* mutation-negative exomes showed low sequence coverage (<20 reads) at the mutation site, which may account for the false-negative calls.

Lesions arising along Blaschko’s lines are hypothesized to stem from a mosaic mutation affecting a specific cell lineage during development. To evaluate whether the candidate mutation fit this criterion, we used laser capture microdissection to isolate DNA from the lesional epidermis and dermis from the index case and one of the sporadic nevus sebaceus. In both cases, the mutation was limited to the epidermis, supporting the hypothesis of an acquired mutation affecting ectodermal precursors (Figure 2b). Both alleles were represented in approximately equal intensities, indicating that the mutation is likely heterozygous.

We next performed targeted Sanger sequencing on a validation set of 31 independent nevus sebaceus from archived tissues, and identified the *HRAS* p.Gly13Arg mutation in 24/31 samples and p.Gly12Asp in one sample. Remaining mutation-negative cases were evaluated for *KRAS* and *NRAS* hotspot mutations, identifying two samples carrying *KRAS* p.Gly12Asp mutations. Six validation samples had patient-matched normal skin tissue available, and the corresponding *RAS* mutations were absent in all six control samples. In total, 32 of 36 samples (89%) demonstrated *HRAS* or *KRAS* mutations, confirming a strong correlation between activating *RAS* mutations and nevus sebaceus (Figure 2c). We suspect that the remaining negative cases may be due to genetic heterogeneity, or to a low mutant allele frequency secondary to admixture with normal tissue.

RAS promotes cell growth through activation of multiple pathways, chief among them the mitogen-activated protein kinase (MAPK) signal transduction pathway. Activating mutations in this gene family have well-established links to cancer (Schubbert *et al*, 2007). Germline activating *HRAS* mutations cause Costello syndrome, which features predisposition to neoplasia and development of cutaneous papillomas (Gripp and Lin, 2012). Taken together, the known biologic features of activated *RAS* genes are consistent with the hamartomatous overgrowth and elevated neoplasia risk observed in nevus sebaceus.

To evaluate *RAS*-MAPK signaling, we performed phosphorylated ERK (pERK) staining on a set of nevi with confirmed *HRAS* mutations. Immunohistochemistry revealed increased pERK staining in lesional vs. normal epidermis, consistent with *RAS*-MAPK hyperactivation (Figure 2d). In one sample, a squamous cell carcinoma was identified arising from nevus sebaceus, highlighted by elevated p16 staining (Hodges and Smoller, 2002). The pattern of neoplasia arising from a background of upregulated pERK supports the hypothesis that *RAS*-MAPK hyperactivation may predispose towards development of secondary neoplasms in nevus sebaceus.

Basal cell carcinomas were once thought to arise commonly from nevus sebaceus, but others have subsequently contended that the majority of these tumors are actually trichoblastomas (Cribier *et al*, 2000). Our data provide genetic support for the latter opinion, as most basal cell carcinomas arise from Hedgehog pathway dysregulation and lack *RAS* mutations (Reifenberger *et al*, 2005). Our findings also raise the possibility that tumors arising from nevus sebaceus, such as syringocystadenoma papilliferum and trichoblastomas, may be associated with *RAS* mutations as well.

Using targeted sequencing and SNaPshot assays, Hafner and colleagues have recently profiled oncogenic hotspot mutations in epidermal and sebaceous nevi (Groesser *et al*, 2012; Hafner *et al*, 2012). Together with the results presented here and by others in this issue (Levinsohn *et al*, 2012), the cumulative data demonstrate that keratinocytic epidermal nevi and sebaceous nevi are both associated with activating *HRAS* p.Gly13Arg and *KRAS* p.Gly12Asp mutations, supporting the belief held by some clinicians they represent a spectrum of the same entity (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 embryologic patterns and environment. The knowledge of the genetic basis of nevus sebaceus and its associated syndrome represents a further step towards understanding genotype-phenotype correlations arising from genetic mosaicism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations

MAPK	mitogen-activated protein kinase
pERK	phosphorylated ERK
SCC	squamous cell carcinoma

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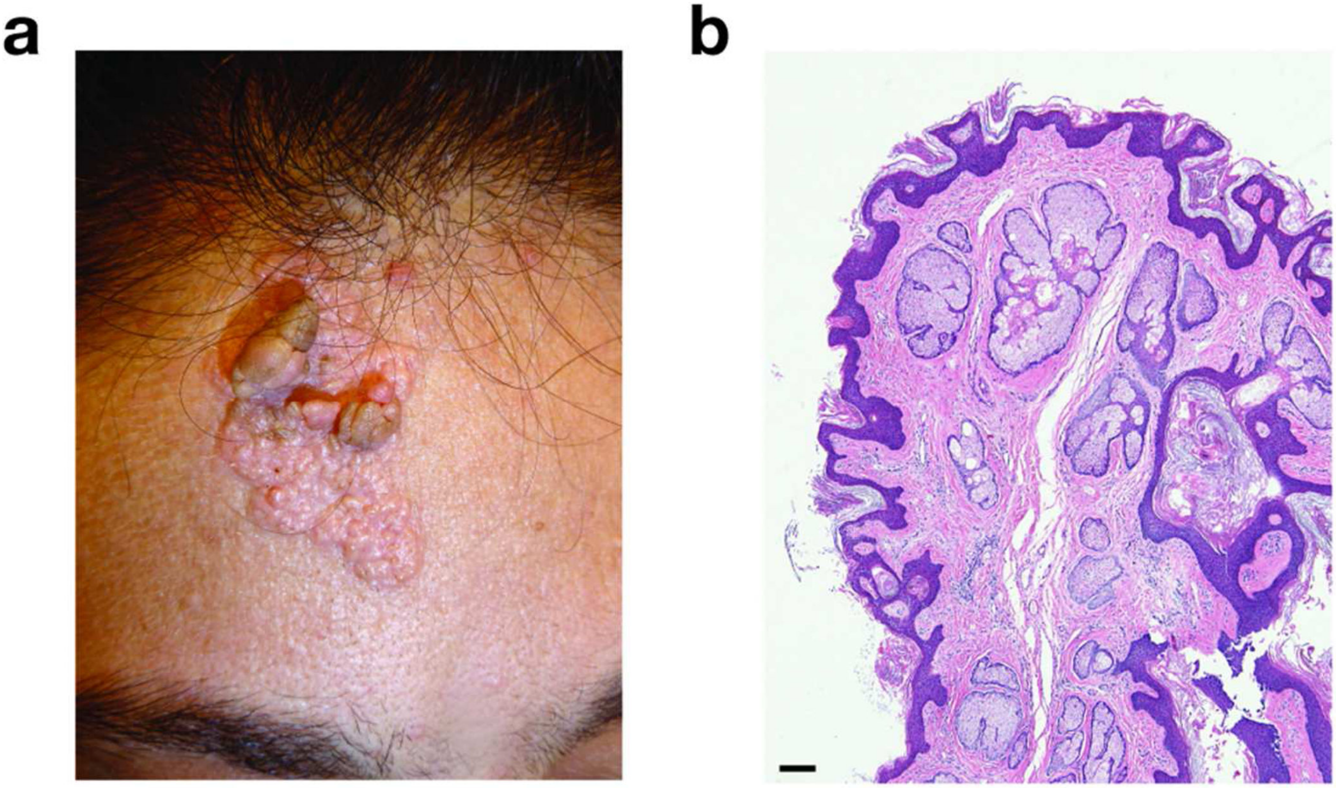
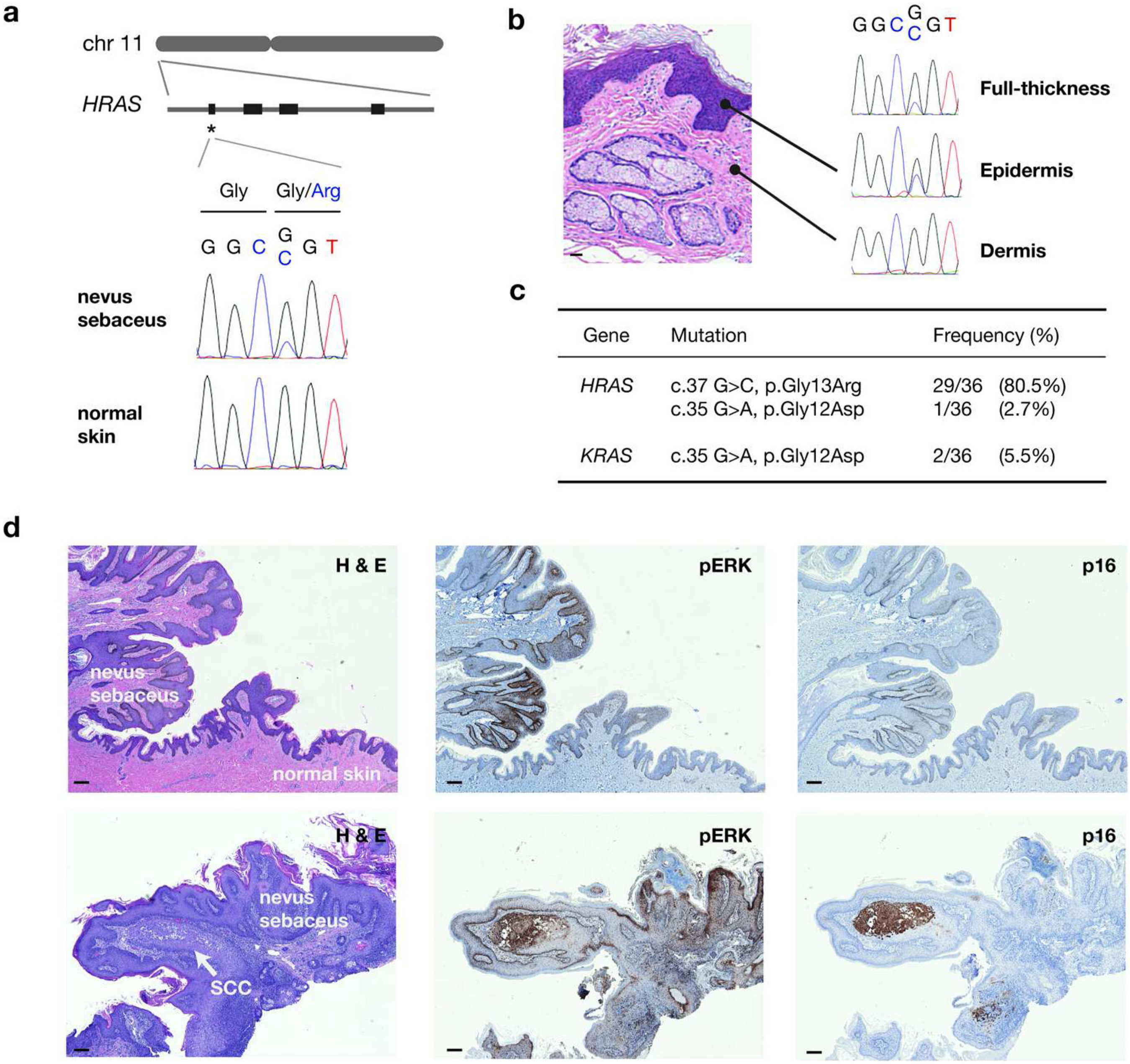


Figure 1. Clinical and histologic features of a patient with nevus sebaceus syndrome

(a) A yellow-hued, papillomatous, oblong plaque on the paramidline forehead of the index patient. (b) Hematoxylin and eosin stained section (40× magnification) of a pedunculated papule from the patient's lesion showing epidermal acanthosis, papillomatosis, absence of hair follicles, and ectopic sebaceous glands opening directly to the epidermal surface. Bar = 100 μm.



Gene	Mutation	Frequency (%)
HRAS	c.37 G>C, p.Gly13Arg c.35 G>A, p.Gly12Asp	29/36 (80.5%) 1/36 (2.7%)
KRAS	c.35 G>A, p.Gly12Asp	2/36 (5.5%)

Figure 2. Activating mosaic *RAS* mutations in nevus sebaceus

(a) Genomic localization of *HRAS* to the short arm of chromosome 11 and schematic of its gene structure. A prominent mutational hotspot in exon 1 (codons 12–13) is marked with an asterisk. Sanger sequencing confirms a c.37G>C, p.Gly13Arg mutation specific to lesional tissue. (b) Laser capture microdissection of the epidermis and dermis of nevus sebaceus demonstrates presence of the *HRAS* mutation exclusively in the epidermis. (c) Summary of *RAS* mutations identified in nevus sebaceus. (d) Activated *RAS*/MAPK pathway in nevus sebaceus. Upper row: Nevus sebaceus transitioning into normal skin. Immunohistochemical staining for phosphorylated ERK (pERK), a downstream effector of the *RAS* pathway, is increased in nevus sebaceus compared to adjacent normal skin. Staining for p16 is homogenously negative. Lower row: An early focus of SCC arising within a nevus sebaceus. This area is characterized by stronger pERK signal and distinct p16 enrichment. All magnifications are at 40×. Bar = 100 μm.