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Somatic *HRAS* p.G12S Mutation Causes Woolly Hair and Epidermal Nevi

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TO THE EDITOR

Woolly hair nevus (WHN) is a mosaic disorder characterized by distinct patterns of tightly curled scalp hair which can appear concurrently with epidermal nevi (EN) at other sites (Peteiro *et al.*, 1989; Venugopal *et al.*, 2012). Woolly hair is also found in congenital disorders resulting from mutations affecting diverse cellular components including intermediate filament, adherens junction, and signal transduction proteins (Harel and Christiano, 2012).

Embryonic somatic mutation causes mosaic disorders which appear in patterns of ectodermal progenitor dorsovental migration. Somatic mutations causing mosaic disorders including Proteus syndrome (Lindhurst *et al.*, 2011), port-wine stains (Shirley *et al.*, 2013), and EN (Levinsohn *et al.*, 2013; Sun *et al.*, 2013) have been found using exome sequencing.

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CONFLICT OF INTEREST

The authors claim no conflict of interest

Recognizing that exome sequencing would permit identification of mutations causing WHN, we ascertained two cases. Our first (WHN100, Figure 1a-d) was a 10 year-old girl without history of developmental delay who had regions of slightly curly hair over her occipital scalp from infancy which progressively curled with no scalp surface change and lie alongside areas of straight hair. She has hyperpigmented patches on her neck, trunk, and arms, with more keratotic lesions on her distal extremities, and acanthosis nigricans in both axillae. There was linear palmar keratoderma (PPK) and hyperkeratosis over most metacarpophalangeal and some proximal interphalangeal joints. Given concurrent PPK and woolly hair, clinical concern for Naxos or Carvajal syndromes led to regular cardiology evaluations that found no abnormalities.

Our second case (WHN101, Figure 1e-h) was a 6 year-old girl whose hair developed at age one and consisted of a mixture of poker-straight hair and curly, thin hair. In infancy, she developed linear dyspigmentation on the right arm and trunk, which became more raised and scaly on the distal extremities over time. She had normal development, with no cardiac or ophthalmic abnormalities found on routine physical examination, cardiac MRI and serial electrocardiograms. Clinical suspicion of mosaic Naxos or Caravajal syndrome motivated clinical sequencing of *DSP*, *DSC1*, *DSG1*, *JUP*, *PKP2*, and *TMEM43*; no mutations were found.

To determine the genetic basis of WHN, we performed paired whole exome sequencing of DNA isolated from affected tissue and blood in both cases (Supplementary Figure 2). Data was analyzed to identify somatic single nucleotide variants (SNVs), deletions and insertions (Supplementary Methods). A somatic heterozygous *HRAS* c.34G>A, p.G12S substitution was found in each (Figure 2a). There was no evidence of loss of heterozygosity (LOH) (Supplementary Figure 3) or secondary mutation somatic mutation, suggesting that *HRAS* mutation alone is sufficient to cause WHN. Sanger sequencing confirmed mutation presence in affected tissue (Figure 2b, c). To determine if this mutation causes woolly hair, we prepared DNA from hair bulbs of straight and curly hair obtained from affected individual WHN101, finding the *HRAS* p.G12S mutation in curly hair only (Figure 2d, Supplementary Figure 1).

Consistent with somatic mosaicism in an epidermal progenitor, prior cases of WHN have been reported with concurrent keratinocytic epidermal nevi (KEN). KEN result from somatic mutations in *HRAS*, *KRAS*, *PIK3CA*, *FGFR3*, and *NRAS* (Hafner *et al.*, 2012) including the *HRAS* p.G12S mutation found in WHN (Hafner *et al.*, 2011). Furthermore, Costello syndrome (CS), in which patients present with developmental delay, high birth weight, feeding difficulties, failure to thrive, cardiac anomalies, and curly hair, results from germline heterozygous *HRAS* mutations, including p.G12S (Gripp and Lin, 2012; Siegel *et al.*, 2012). The timing of somatic mutation during embryonic development determines extent of cutaneous involvement and presence of other systemic abnormalities (Moss *et al.*, 1993).

Notably, somatic activating *HRAS* mutations are found in most cases of nevus sebaceus (NS), a mosaic lesion which typically appears on the scalp and features alopecia, papillomatosis, and marked sebaceus hyperplasia (Groesser *et al.*, 2012; Levinsohn *et al.*, 2013; Sun *et al.*, 2013). These features contrast with those of WHN in which hair is present

but curly, and sebaceous hyperplasia is absent. Given that WHN and NS are both caused by somatic *HRAS* mutations, we hypothesize that their phenotypic divergence may derive from relative potency of the mutant allele with respect to MAP kinase activation. *HRAS* mutations in WHN and NS fall within the finger loop of *HRAS*, replacing glycine residues with larger amino acids which prevent GTP hydrolysis (Malumbres and Barbacid, 2003). Though comparison of the WHN p.G12S mutation and the common NS p.G13R mutation has not been performed, *HRAS* codon 12 serine substitutions have been shown to be less activating than arginine, aspartic acid or valine substitutions (Fasano *et al.*, 1984).

To evaluate the frequency of *HRAS* mutation in NS, we screened 116 archival scalp NS lesions for *HRAS* and *KRAS* mutation. We found 88 *HRAS* and 9 *KRAS* mutations. *HRAS* p.G13R was present in 85 NS and p.G12S was not found (Supplementary Table 2). In prior reports, 64 additional samples were screened, and *HRAS* p.G12S mutations were not found (Levinsohn *et al.*, 2013; Sun *et al.*, 2013). In one report, 3 specimens with *HRAS* p.G12S mutations were identified; in 2 there was a concurrent *HRAS* p.G13R mutation, and in one, the lesion was on the ear, a site at which it could be difficult to distinguish EN and NS (Groesser *et al.*, 2012). These data combined with evidence from CS suggest that more strongly activating *RAS* mutations may cause the alopecia and sebaceous hyperplasia found in NS, and the more mildly activating p.G12S mutation causes woolly hair phenotypes.

In summary, we find somatic *HRAS* c.34G>A, p.G12S mutation in affected tissue from two cases with mosaic woolly hair and EN. Consistent with reports of WHN and in KEN, the identified p.G12S mutation causes an EN phenotype on the body, but the finding of curly hair on the scalp suggests that WHN represents a mosaic RASopathy with phenotype determined by location, either due to distinct epidermal progenitor types or site-specific mesenchymal interactions. We hypothesize that in contrast to strongly activating *RAS* mutations found in NS which drive hair follicle progenitors toward sebocyte differentiation, the more weakly activating mutation found in WHN permits an intermediate phenotype with abnormal curly hair growth but without sebaceous hyperplasia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

WHN	woolly hair nevus
KEN	keratinocytic epidermal nevus

NS	nevus sebaceus
CS	Costello syndrome
SNV	single nucleotide variation
LOH	loss of heterozygosity
PPK	palmoplantar keratoderma

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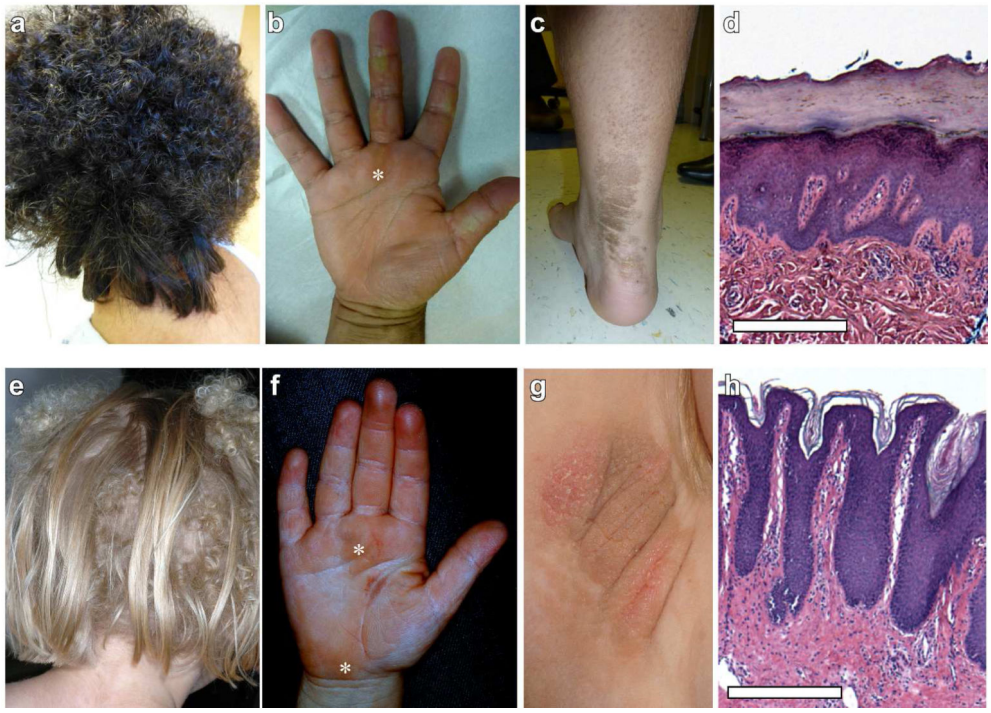

Figure 1.

Clinical features of index cases with woolly hair nevi. On the scalp, woolly hair nevus presents with a portion of the scalp exhibiting patches of curly, thin, hair intermixed with regions of normal, straight hair, as observed in WHN100 and WHN101. On the body, additional findings of linear palmoplantar keratoderma (b, f, asterisks) and epidermal nevi (c, g) are found. In WHN100, histology of linear palmar keratoderma (d) shows papillomatosis, hypergranulosis and compact hyperkeratosis, scale bar = 0.5 mm. In the epidermal nevus of WHN101, histology of the epidermal nevus (h) shows acanthosis, papillomatosis, and mild hyperkeratosis, scale = 0.25 mm.

a

Sample	Position (hg19)	Gene	Base change	Protein Effect	# reads-tissue Ref.	# reads-tissue Non-ref.	# reads-blood Ref.	# reads-blood Non-ref.	P-value
WHN100	Chr11: 534,289	***HRAS***	G>A	**G12S**	65	79	63	5	$2.3x10^{-12}$
WHN101	Chr11: 534,289	***HRAS***	G>A	**G12S**	29	19	85	0	$2.4x10^{-10}$

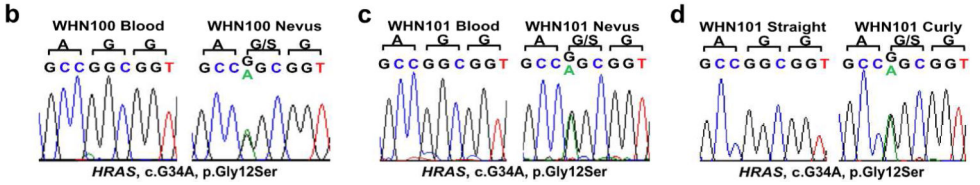

Figure 2.

Somatic HRAS p.G12S mutation causes WHN. (a) In WHN100 and WHN101, exome sequencing of affected tissue and blood was performed. Tissue-specific SNVs are annotated bychromosome, position, base change, protein consequence, and numbers of reference and non-reference reads from affected tissue and genomic (blood) DNA. The p-values denoting the significance of the differences in reference and non-reference reads in tissue versus blood were calculated using a one-tailed Fisher's exact test. After filtering (Supplementary methods), only one SNV surpassed genome-wide significance (1.7×10⁻⁶), HRAS p.G12S in each case. (b, c) Sanger sequencing of blood and tissue of WHN100 and WHN101 confirmed this HRAS p.G12S mutation. (c) In WHN100, there is a small mutant allele fraction in blood demonstrated by exome (8% mutant reads) sequencing, but this mutation is enriched to the expected 50:50 ratio in tissue. (d) Sanger sequencing of HRAS in DNA from plucked straight and curly hair from WHN101 shows that HRAS p.G12S mutation is specific to curly hair.