Mosaicism for a KITLG Mutation in Linear and Whorled Neviod Hypermelanosis


TO THE EDITOR

Linear and whorled nevoid hypermelanosis (LWNH) has hitherto been considered a nonspecific manifestation of mosaicism. We performed deep-exome sequencing on skin from a patient with LWNH and identified a postzygotic mutation in KITLG, associated with increased KITLG and c-KIT epidermal expression. Because germline KITLG mutations have previously been described in a Mendelian disorder, familial progressive hyper- and hypopigmentation (FPHH), LWNH can be considered a mosaic presentation of FPHH.

Linear and whorled nevoid hypermelanosis was initially described as a macular hyperpigmentation following Blaschko’s lines (Kalter et al., 1988). Differential diagnosis includes other linear pigmentary anomalies, such as the pigmentary stage of incontinentia pigmenti, the early stage of keratinocytic epidermal nevi, or hypomelanosis of Ito, the hypopigmented counterpart of LWNH, which can be associated with streaks of hyperpigmentation (Cohen et al., 2014). As for hypomelanosis of Ito, LWNH has been considered a nonspecific pigmentary manifestation of mosaicism (Happle, 2014) rather than a specific nosological entity. Indeed, various cytogenetic anomalies have been reported in association with hypomelanosis of Ito (Sybert, 1994) or LWNH, either in a mosaic or nonmosaic state. However, the molecular basis of LWNH has remained unknown. Here, we report identification of a postzygotic KITLG mutation in a sporadic case of LWNH.

A 6-year-old boy previously reported in infancy (Maruani et al., 2012) had congenital linear and mottled hyperpigmentation on his trunk and limbs, without previous rash or additional cutaneous features. He had normal psychomotor, neurosensory, and general development after a 6-year follow-up. Hyperpigmentation increased in the first days of life and then remained stable. No hypopigmentation was found. Results of an audiogram performed because of chronic otitis were normal. Complete blood count and full clinical examination results were otherwise normal. Skin biopsy showed increased keratinocytic melanin content in the lower epidermis, without pigmentary incontinence (Figure 1). We performed deep whole-exome sequencing with a mean coverage of 150.64-fold on hyperpigmented skin from the patient and blood from his unaffected parents (see Supplementary Materials and Methods online). We identified a de novo postzygotic KITLG variant (NM_000899.3:c.329A>G; encoding p.Asp110Gly) in 41 out of 145 reads (Figure 2), which we confirmed by targeted next-generation sequencing using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego). The KITLG c.329A>G variant was found in 28% of reads in affected skin (mean coverage = 2279.8-fold) and in 18% of reads in blood (mean coverage = 927.2-fold). The identified substitution was absent from the COSMIC (i.e., Catalogue of Somatic Mutations in Cancer) and ExAC (i.e., Exome Aggregation Consortium) databases. It alters an evolutionarily conserved nucleotide (Genomic Evolutionary Rate Profiling score = 5.950) and is predicted to be deleterious by in silico algorithms (Combined Annotation Dependent Depletion score = 17.270, Grantham score = 94, and PolyPhen score = 1). Paraffin-embedded skin section immunostaining using anti-KITLG polyclonal antibody (anti-SCF, C19H6; Abcam, Cambridge, MA) showed intense nuclear and cytoplasmic staining of basal and spinous layer keratinocytes. In addition, c-KIT staining showed increased epidermal expression in basal keratinocytes, and HMB45 staining showed an increase in melanocytes in the basal epidermal layer (Figure 1).

Germline missense KITLG mutations in exon 2 have previously been found in patients with familial progressive hyper- and hypopigmentation (FPHH, OMIM 145250), who exhibit progressive early-onset diffuse hyperpigmentation with lentigines, hypopigmented macules, and larger café-au-lait macules (Amyere et al., 2011; Cuell et al., 2015; Wang et al., 2009; Zanardo et al., 2004; Zhang et al., 2016). LWNH is, therefore, a mosaic presentation of FPHH, as previously described for other Mendelian disorders. KITLG, also known as SCF, encodes the sole activating ligand for the tyrosine kinase c-KIT receptor, which acts exclusively in a melanocyte-autonomous manner (Aoki et al., 2015). KITLG controls melanocyte migration, proliferation, and survival, as well as melanin synthesis (Wehrle-Haller, 2003). All known KITLG mutations, including the one we identified, are predicted to affect both its soluble and transmembrane isoforms. They are located near KIT binding sites and may alter binding with c-KIT (Figure 2) (Yuzawa et al., 2007). Previously reported KITLG mutations in FPHH are clustered in exon 2 on binding site III, which interacts with the c-KIT D3 domain. These mutations are presumed to result in a gain of function, because injection of the mutant recombiant protein increases tyrosinase activity and melanin content in melanoma cell lines (Wang et al., 2009). Although no functional analyses were performed on keratinocytes from our patient, immunohistochemistry suggests that the variant encoding p.Asp110Gly in exon 4 results in increased epidermal expression of...
KITLG and an increased number of epidermal melanocytes (Figure 1). This is consistent with previous findings on epidermal KITLG expression in FPHH (Zhang et al., 2016), solar lentigo (Hattori et al., 2004), or UV-induced hyperpigmentation (Hachiya et al., 2001). Hence, hyperpigmentation associated with KITLG mutations appears to result from primary functional alteration of keratinocytes rather than melanocytes. Associated increased epidermal expression of c-KIT had not been reported in FPHH. This suggests that expression of the p.Asp110Gly variant in keratinocytes causes autocrine and paracrine up-regulation of c-KIT, resulting in increased melanogenesis in melanocytes (Wehrle-Haller, 2003). Previously described KITLG mutations in exon 4 (near the KIT D2 binding site) have been reported in patients with non-syndromic unilateral and asymmetric hearing loss or Waardenburg syndrome type 2, where hypopigmentation without hyperpigmentation is present.

Figure 1. Clinical presentation and immunostaining studies. (a–c) Proband aged 6 years: mottled and linear hyperpigmentation on the limbs and abdomen. (d–f) Immunostaining of skin sections from the proband at age 1 month, showing (d) increased epidermal nuclear and cytoplasmic expression of KITLG (SCF) in basal and spinous keratinocytes (anti-SCF C19H6 polyclonal antibody specific for the liaison domain, dilution 1/800), (e) increased epidermal cytoplasmic C-KIT (CD117) expression in basal and suprabasal keratinocytes (dilution 1/500), and (f) increase in melanocytes in the basal epidermal layer (HMB45 staining, dilution 1/500). (g–h) Control immunostaining from a control with cutaneous mastocytosis aged 2 years: (g) KITLG expression in dermal mast cell infiltrate with faint cytoplasmic expression in basal keratinocytes (dilution 1/400), (h) C-KIT expression in dermal mast cells and epidermal dendritic melanocytes; absent expression in keratinocytes (dilution 1/500), and (i) HMB45 staining in control sample. Scale bar = 100 μm.
Our patient had normal audition and no hypopigmentation.

To conclude, this report of a molecular basis for LWNH as a mosaic presentation of FPHH due to $\text{KITLG}$ mutations expands the spectrum of nonlethal gene mutations involved in both constitutive and mosaic disorders. However, because we have not yet studied additional patients with LWNH, we cannot exclude involvement of other genes in this pigmented phenotype. Nevertheless, identification of a previously unreported $\text{KITLG}$ mutation in a mosaic state in LWNH extends the clinical spectrum of $\text{KITLG}$-related pigmentation anomalies and may

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**Figure 2.** Integrative Genomics Viewer (Broad Institute, Cambridge, MA) screenshot of whole-exome sequencing for $\text{KITLG}$ variant and summary of all known $\text{KITLG}$ mutations in human disorders. (a) $\text{KITLG}\ c.329A>G$ (p.Asp110Gly) variant was present in 28% of reads in the proband’s skin and absent in parents’ blood. (b) Genomic structure of $\text{KITLG}$. Membrane-bound and soluble $\text{KITLG}$ isoforms result from alternative splicing of exon 6, which contains a cleavage site (red line). Protein domains: AA 1–25 (black), signal peptide; 26–214 (grey), extracellular domain; 215–237 (black), transmembrane domain; 238–273 (grey), cytoplasmic domain. (c) $\text{KITLG}\ p\.\text{Asp110Gly}$ variant (red) and s$\text{KITLG}$ domain mutations identified in FPHH (top) and nonsyndromic unilateral and asymmetric hearing loss/Waardenburg Syndrome type 2 (bottom), localized near KIT-binding sites (black) (Yuzawa et al., 2007). FPHH, familial progressive hyper- and hypopigmentation.
ultimately provide new insight into pigmentation regulation. Written informed consent for genomic analysis and authorization for publication of photographs were obtained from parents. The study was approved by our regional institutional review board (CPP Est I) and registered as NCT01950975.

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**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 www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2017.01.035.

**REFERENCES**


Sensitization to Protease Allergen via SDS-Treated Skin

Skin Treatment with Detergent Promotes Protease Allergen-Dependent Epicutaneous Sensitization in a Manner Different from Tape Stripping in Mice

**TO THE EDITOR**

Skin is considered to be a major route for allergen sensitization in not only atopic dermatitis but also atopic