















SHORT REPORT

Biallelic *KITLG* variants lead to a distinct spectrum of hypomelanosis and sensorineural hearing loss

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Abstract

Background Pathogenic variants in *KITLG*, a crucial protein involved in pigmentation and neural crest cell migration, cause non-syndromic hearing loss, Waardenburg syndrome type 2, familial progressive hyperpigmentation and familial progressive hyper- and hypopigmentation, all of which are inherited in an autosomal dominant manner.

Objectives To describe the genotypic and clinical spectrum of biallelic *KITLG*-variants.

Methods We used a genotype-first approach through the GeneMatcher data sharing platform to collect individuals with biallelic *KITLG* variants and reviewed the literature for overlapping reports.

Results We describe the first case series with biallelic *KITLG* variants; we expand the known hypomelanosis spectrum to include a 'sock-and-glove-like', symmetric distribution, progressive repigmentation and generalized hypomelanosis. We speculate that *KITLG* biallelic loss-of-function variants cause generalized hypomelanosis, whilst variants with residual function lead to a variable auditory-pigmentary disorder mostly reminiscent of Waardenburg syndrome type 2 or piebaldism.

Conclusions We provide consolidating evidence that biallelic *KITLG* variants cause a distinct auditory-pigmentary disorder. We evidence a significant clinical variability, similar to the one previously observed in *KIT*-related piebaldism.

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Conflicts of Interest

None declared.

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Introduction

KITLG/c-Kit and Ras/MAPK pathways are crucial for controlling pigmentation.¹ *KITLG* variants are a rare cause of autosomal dominant (AD), sensorineural hearing loss (HL) and Waardenburg syndrome type 2 (WS2).^{2,3} Gain-of-function variants in *KITLG* are also associated with familial progressive hyperpigmentation (FPH)⁴ and hyper- and hypopigmentation (FPHH).⁵ Variants in *KIT* [OMIM:*164920], the *KITLG* receptor, cause AD piebaldism characterized by symmetric, (mainly) limb hypomelanosis, occasional repigmentation and rare HL.^{6,7}

Auditory-pigmentary disorders are attributed to pathogenic variants in essential genes for differentiation and migration of melanocytes (Table S1).⁸ The most common syndrome, Waardenburg syndrome (WS), is a neurocristopathy caused by migration defects of neural crest cells. WS is genetically and clinically heterogeneous. Type 1 (WS1, *PAX3* [OMIM:*606597]) is distinguished by type 2 (WS2, *MITF* [OMIM:*156845], *SNAIL2* [OMIM:*602150], *SOX10* [OMIM:*602229], *KITLG* [OMIM:*184745]) by the occurrence of dystopia *canthorum*; type 3 (WS3, *PAX3*) usually includes dystopia *canthorum* and upper limb abnormalities; and type 4 (WS4, *EDN3* [OMIM:*131242], *EDNRB* [OMIM:*131244] and *SOX10*), known as Waardenburg-Shah syndrome, includes Hirschsprung disease.⁹ An allelic disorder to WS2 with AD-transmission, Tietz albinism-deafness syndrome, is caused by variants in *MITF* ([OMIM:*156845]), whereby heterochromia or pigmented patches are absent.¹⁰

Here, we describe a case series with biallelic *KITLG* variants causing a variable yet distinct spectrum of hypomelanosis and sensorineural hearing loss.

Materials and methods

We used a genotype-first approach through the GeneMatcher data sharing platform and data mining of aggregated DNA sequences from patients with ultra-rare disorders across multiple research and diagnostic laboratories worldwide to collect individuals with biallelic *KITLG* variants.¹¹ This study was performed according to the Declaration of Helsinki and approved

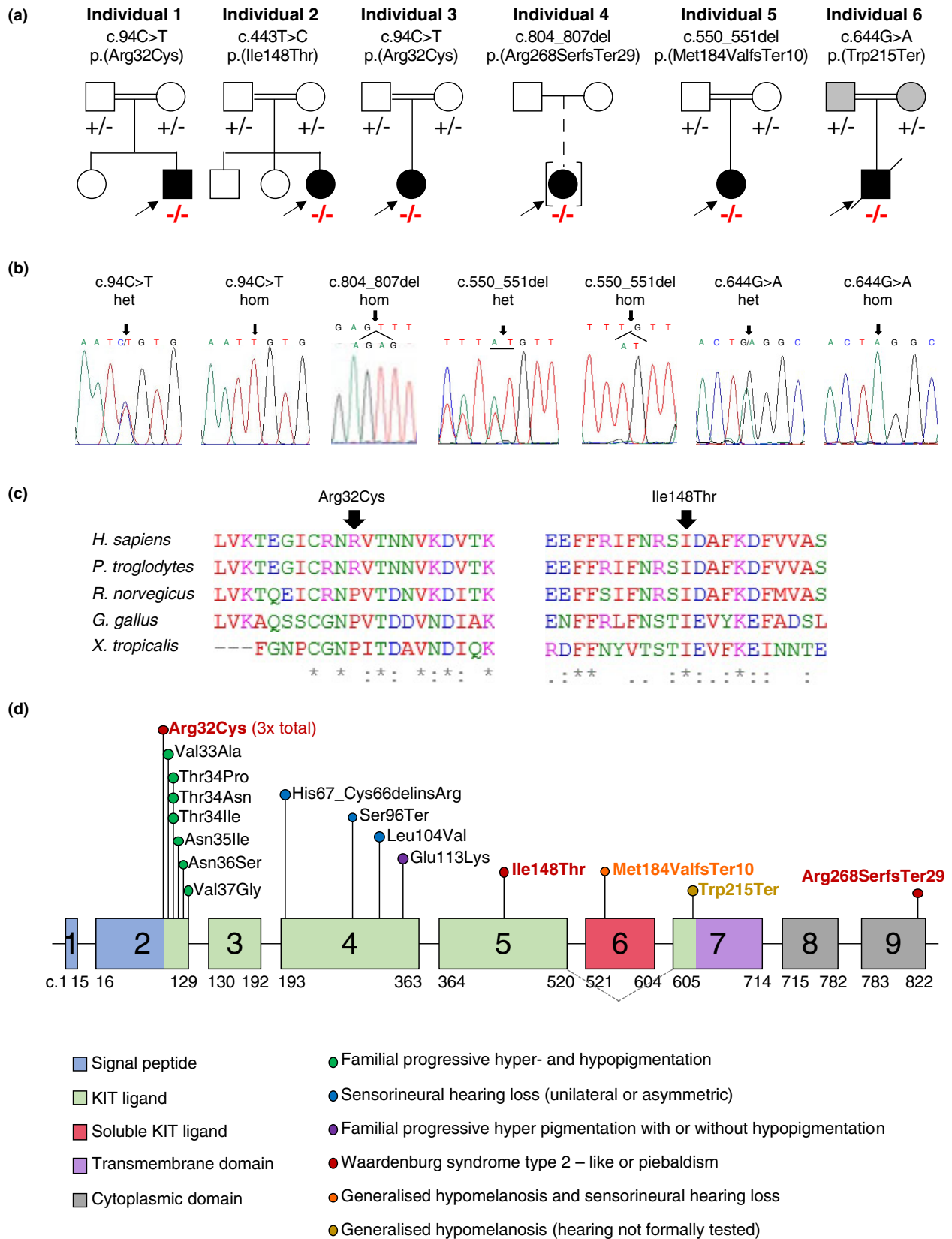
by the Institutional Review Board of University College London. Written informed consent for the publication of photographs and medical information was obtained from parents/guardians. Individuals were clinically characterized by consultants in clinical genetics. The genomic DNA of the proband in each family had been analysed by research exome (Individuals 1,¹² 4¹³ and 6¹⁴), clinical exome (Individual 2¹⁵) or genome (Individuals 3^{16,17} and 5^{16,17}) sequencing, as previously described.^{12–17} A review of previously reported and published pathogenic/likely pathogenic *KITLG* variants used ClinVar, LOVD and the HGMD Professional database version 2021.3. Additionally, querying *KITLG* in PubMed-yielded publications that were reviewed for the reporting of familial data.

Results

We identified six unrelated individuals (two male, four female) with biallelic *KITLG* variants (Figs 1a, 2a–p) and a median age of 5 years at last observation. Individuals 1–4 presented with Waardenburg syndrome type 2-like and individuals 5–6 showed generalized hypomelanosis. Detailed clinical descriptions are presented in Appendix S1 (Clinical Case Reports).

Hypomelanosis was present in all individuals and was either generalized (2/6) or affecting hair, irises and limbs (4/6). Islands of pigmentation within achromic areas on the limbs were present in 4/6 individuals and in two there was progressive repigmentation. Two individuals presented symmetric, lower limb hypomelanosis reminiscent of piebaldism, on one occasion with a ‘sock-and-glove-like’ distribution. Heterochromia irises was present in 4/6 individuals. Hair hypomelanosis includes scattered leucotrichia including eyebrows and eyelashes (5/6), white forelock (3/6) and white-silver hair reminiscent of albinism (2/6). Congenital/neonatal, sensorineural HL was identified in 5/5 individuals and appeared as asymmetric (2/5) (Figure 2r–s), bilateral, symmetric (2/5) or unilateral (1/5). Table S2 compares phenotypes in this case series with previously reported individuals with *KITLG*-related WS2. Table S3 summarizes all individuals reporting *KITLG* variants, including those with FPHH and FPH.

Figure 1 Molecular findings of individuals in our cohort. (a) Pedigree and segregation results (– represents the variant; homozygous variants are marked in red) for the six families with biallelic *KITLG* variants. (b) Available Sanger electropherograms showing heterozygous (het) or homozygous (hom) variants. (c) Interspecies alignment shows conservation of amino acids involved in non-synonymous substitutions. (d) Schematic representation of the *KITLG* gene (NM_000889.4) with c. position, protein domains and features marked. An alternatively spliced isoform skips exon 6 and is represented with grey dotted lines (NM_003994.5). The phenotype of the *KITLG* variants is shown with coloured circles. The variants we describe are marked in red (WS), orange (generalized hypomelanosis and sensorineural hearing loss) or gold (generalized hypomelanosis with hearing not formally tested).



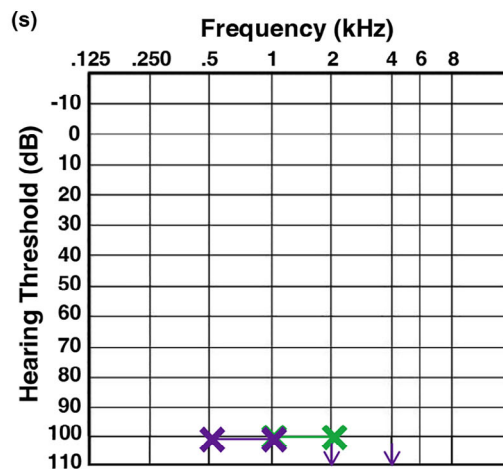
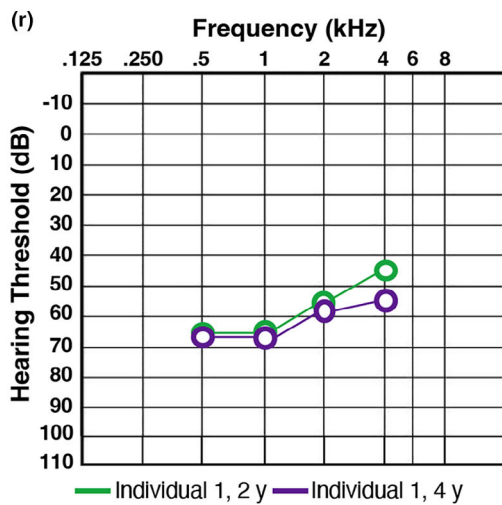


Figure 2 Clinical features of our cohort. (a–f) Individual 1 with partial heterochromia irides, hypomelanosis of hair including eyelashes and eyebrows and of the lower legs with multiple islands of pigmentation. (g–k) Individual 2 with blue irides, sparse white hair and hypopigmentation of the lower legs with a “sock-and-glove-like” distribution and few islands of pigmentation on hands and feet. (l–m) Individual 3 with heterochromia irides, sparse white hair, and a hypopigmented area medially, on the right ankle with small islands of pigmentation. (n) Individual 4 with a hypopigmented area medially above the right ankle. (o) Individual 5 with generalized hypopigmentation of skin and hair. (p) Individual 6 with generalized hypopigmentation of skin and hair. (r, s) Auditory steady state responses from the right (r) and left (s) ears of Individual 1 at age 2 and 4 years of age showing asymmetric hearing loss.

The *KITLG* variants identified (GenBank accession: NM_000889.4) are summarized in Figure 1a and b. Table S4 shows evidence used to support variant interpretation. All variants are ultra rare or novel (Table S4).

Individuals 1 and 3 each presented with a homozygous c.94C>T, p.(Arg32Cys) variant that was previously reported in a 16-year-old Filipino WS2-individual who had more extensive pigmentation anomalies compared to Individuals 1 and 3 (Figure 2a–f, l–m).³ He had a reported, truncal, *café-au-lait* macule and a large well-demarcated area of lower abdominal hyperpigmentation. Consistent with our individuals, he reported congenital, sensorineural HL and hypomelanotic areas with dappled pigmentation around the achromic patches. Melanotic skin was present on his back and buttocks, reportedly as post-inflammatory hyperpigmentation due to psoriasis vulgaris. The authors noted that although the individual fulfilled criteria for WS, they could not exclude a diagnosis of FPHH with concomitant HL derived from a distinct genetic or non-genetic origin.³ These two individuals with identical, biallelic, pathogenic, variants provide strong evidence for c.94C>T, p.(Arg32Cys) as a causal allele for an auditory-pigmentary disorder.

The homozygous likely pathogenic c.443T>C, p.(Ile148Thr) variant in individual 2 impacts a highly conserved amino acid (Figure 1c). The pattern of hypomelanosis affected hair (white forelock and scattered leucotrichia, eyebrows and eyelashes), eyes (blue irides) and limbs (symmetric, “sock-and-glove-like” distribution). Within the depigmented areas on the limbs there were few pigmentation islands on fingers, toes and feet which developed progressively and with occasional symmetric distribution (lateral dorsal area of feet) (Figure 2g–k). Unilateral, sensorineural HL (left) was diagnosed at birth. The “sock-and-glove-like” distribution of hypopigmentation is reminiscent of a peripheral demyelinating neuropathy but neurological examination was normal.

Individual 4 had a homozygous c.804_807delAGAG, p.(Arg268SerfsTer29) variant of uncertain significance that impacts the last six reference amino acids of the protein sequence. She has bilateral sensorineural HL and a hypomelanosis pattern that includes a fair skin complexion, patchy blue irides and two achromic patches over the lower third of the right shin and lateral aspect of right foot (Figure 2n). Due to the variant’s C-terminal location, we suspect an incomplete loss-of-function and preservation of protein expression.

Individual 5, with a homozygous c.550_551delAT, p.(Met184ValfsTer10) likely pathogenic variant, presented generalized

hypomelanosis of the skin and hair (Figure 2o), as well as congenital, asymmetric, sensorineural HL. The frameshift variant introduces a premature stop codon in exon 6. However, alternative splicing of the shorter transcript variant “a”, that is also expressed in cochlear tissue, skips exon 6, leaving the shorter, primarily membrane-bound transcript variant intact.²

Individual 6, with a homozygous c.644G>A, p.(Trp215Ter) likely pathogenic variant, presented generalized hypomelanosis of skin, hair, and eyebrows but was lost to clinical follow-up (Figure 2p). Hearing was not assessed before the individual’s sudden death at 10 months, 22 days. He did not have heterochromia, amblyopia or nystagmus. His parents, who were confirmed carriers of the null variant, also exhibited lighter-coloured skin than expected for their ethnic background.

In individuals with formal hearing assessments (5/5), the HL onset ranged from birth to neonatal period and the severity was moderate-to-profound. Laterality was variable: two individuals each had bilateral and asymmetric HL and one had unilateral HL, resembling the AD *KITLG* family with hereditary HL but no hypomelanosis.²

Conclusions

We present four novel *KITLG* variants leading to a distinct spectrum of hypomelanosis and sensorineural hearing loss. Two out of four variants reported here (c.94C>T, p.(Arg32Cys) and c.443T>C, p.(Ile148Thr) map to the KIT ligand domain, where most FPHH and FPH variants also map (Figure 1d). We present the first variants in the following *KITLG* domains: the c.550_551del, p.(Met184ValfsTer10) frameshift variant affecting the soluble KIT ligand domain; the c.804_807del, p.(Arg268SerfsTer29) frameshift variant maps to the C-terminal cytoplasmic domain, possibly contributing to a non-generalized hypomelanosis; and the c.644G>A, p.(Trp215Ter) nonsense variant located at the beginning of the transmembrane domain in an individual with generalized hypomelanosis.

We expand the clinical spectrum caused by biallelic *KITLG* variants to include (a) “sock-and-glove-like”, symmetric, hypomelanosis, (b) progressive re-pigmentation and (c) generalized hypomelanosis. This significant clinical variability is similar to the one previously observed in individuals with piebaldism, in whom it correlates to the site and type of the *KIT* variant.^{7,18} We hypothesize that (biallelic) loss-of-function variants cause generalized hypomelanosis while variants with residual protein function lead to a distinct spectrum of hypomelanosis and

sensorineural hearing loss mostly reminiscent of WS2 or piebaldism. Functional studies are necessary to confirm this possible genotype–phenotype correlation.

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Author contributions

RMaroofian was involved in conceptualization; BV, SD, DAS, AAR, MBT, PR, NB, RA, MD, RManju, DD, CJS, SN, EGK, HH, GB, MT, KMG and RMaroofian were involved in data curation; SD, DAS, AAR, SSL, PR, CJS and GB were involved in formal analysis; SSL and KMG were involved in funding acquisition; BV, SD, DAS, AAR, SSL, MBT, NB, RA, MD, CJS, SN, EGK, HH, GB, MT, KMG and RMaroofian were involved in investigation; BV, MT and RMaroofian were involved in project administration; MBT, NB, RA, MD, DD, SN, EGK, HH and MT provided key resources; SD and KMG were involved in supervision; SSL was involved in validation; BV, SD, SSL, MT and KMG were involved in visualization; BV, SD, MT, KMG and RMaroofian wrote the manuscript and prepared the original data; BV, SD, DAS, AAR, SSL, MBT, PR, NB, RA, MD, RManju, DD, CJS, SN, EGK, HH, GB, MT, KMG and RMaroofian wrote and edited the manuscript.

Data availability statement

Supporting data are available on request from the corresponding author. The genetic data are not publicly available due to privacy or ethical restrictions. Variants have been submitted to the Leiden Open Variation Database 3 (LOVD3) under variant accession IDs 0000386349–0000396354.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Clinical Case Reports

Table S1. Differential diagnosis with known genetic syndromes causing hypomelanosis

Table S2. Prevalence of clinical features among Individuals with biallelic *KITLG* variants in the current case series and previously reported Individuals with *KITLG*-related syndrome

Table S3. Summary of clinical features of patients with *KITLG* variants

Table S4. Evidence used to support variant interpretation