Supplementary Notes for

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

**Clinical Case Reports:**

Individual 1: *KITLG* c.94C>T, p.(Arg32Cys) homozygosity

The male proband was the second child born to consanguineous parents of Iranian ethnicity. At last evaluation at 5 years of age, he weighed 15 kg (4th centile), had a height of 108 cm (43rd centile) and an occipital frontal circumference of 48.5 cm (4th centile). Stable, asymmetric hearing loss was diagnosed in the neonatal period that was characterized via auditory steady-state response testing at the age of 2 and 4 years (moderate right and profound left) (Fig. 2r-s). A hypomelanosis pattern affecting hair (white forelock and scattered leucotrichia including eyebrows and eyelashes), eyes (partial left iris heterochromia) and limbs, reminiscent of piebaldism, was present (Fig. 2a-d). Within the depigmented areas on the limbs there were pigmentation islands with occasional hyperpigmented margins (Fig. 2e-f). The islands of repigmentation developed progressively. The boy also had developmental delay, intellectual disability, autism spectrum disorder and hyporeflexia.

Individual 2: *KITLG* c.443T>C p. (IIe148Thr) homozygosity

The proband, a 2-year-old girl, was referred because of a pattern of hypomelanosis and congenital, profound, unilateral (left), sensorineural hearing loss (Fig. 1a). She was the third child of a couple who reported consanguinity (3rd or 4th cousins) which they could not define precisely. At 8 months of age, her growth was normal: weight 8.35 kg (50th centile), height 67 cm (25th centile), head circumference 44.5 cm (50th centile) and presented mild motor delay (not sitting independently). The pattern of hypomelanosis affected hair (white forelock and scattered leucotrichia, eyebrows and eyelashes), eyes (blue irises) and limbs (symmetric, “sock-and-glove-like*”* distribution) (Fig. 2g-h). 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) (Fig. 2i-k). Neurological examination, high-resolution computed tomography scan of the temporal bone and magnetic resonance imaging of the inner ear showed no abnormalities.

Individual 3: *KITLG* c.94C>T, p.(Arg32Cys) homozygosity

The proband is a 5-year-old girl, born at full term without complications to a healthy couple of second cousins. She was diagnosed with bilateral, profound, sensorineural hearing loss by 6 months of age. At age 5 years, her height, weight, and head circumference were 100.5 cm (6th centile), 14.8 kg (5th centile), and 48 cm (5th centile), respectively. She has had normal gross and fine motor development and severely delayed speech, a probable consequence of her hearing loss. She had few mild dysmorphic facial features (synophrys, underdeveloped alae nasi) and a pattern of hypomelanosis which included hair (scattered leucotrichia including the eyebrows), eyes (irises heterocromia) and the right lower limb (achromic patches interspersed with dotted pigmented areas) (Fig 2l-m).

Individual 4: *KITLG* c.804\_807del, p.(Arg268SerfsTer29) homozygosity

The proband is a 34-month-old, adopted girl. Her birth weight was 2,000 g (<3rd centile). She had normal motor development but no meaningful speech at 30 months of age. Brainstem evoked response audiometry at 30 months identified bilateral, sensorineural hearing loss that is currently rehabilitated with hearing aids. Her growth was recorded as height 101 cm (98th centile), weight 13.5 kg (50th centile) and head circumference 46 cm (6th centile). A hypomelanosis pattern included a fair skin complexion, and involved the hair (few white hair over the midline of the anterior hairline), the eyes (patchy blue irises) and the lower limbs (two achromic patches over the lower third of the right shin and lateral aspect of right foot) (Fig 2n). She had mild dysmorphic facial features (telecanthus and a flat nasal bridge).

Individual 5: *KITLG* c.550\_551del, p.(Met184ValfsTer10) homozygosity

The proband is a 7-year-old girl, born at full term with a weight of 3,200 g following an uneventful pregnancy and delivery to second-cousin parents. The proband failed newborn hearing screening (bilateral otoacoustic emissions). Brainstem evoked response audiometry showed bilateral asymmetric, severe to profound, sensorineural hearing loss with >10 decibel difference for at least two frequencies. High-resolution computed tomography scan of the temporal bone and magnetic resonance imaging of the inner ear showed no abnormalities. Gross and fine motor development was normal but speech was severely delayed; she uses lip reading and sign language to communicate. At age 7 years, her height, weight, and head circumference were 108 cm (1st centile), 28 kg (83rd centile), and 51 cm (37th centile), respectively. She has a pattern of hypomelanosis that includes all skin and adnexa (white-silver hair, eyebrows and eyelashes) (Fig. 2o). She presented glove-like, pigmented areas on hands which darken with sun exposure. Ophthalmologic examination showed brown irises with full vision acuity, mild foveal hypoplasia, and mild photophobia; no hypopigmentation or heterochromia of irises or nystagmus was detected.

Individual 6: *KITLG* c.644G>A, p.(Trp215Ter) homozygosity

The proband is the first child of a second-cousin couple. He was born with hypopigmented hair and eyebrows after an uneventful pregnancy at term with normal birth weight at 4,000 g (81st centile). Six days after birth, his weight was 3.4 kg (31st centile), length 50 cm (34th centile), head circumference 36 cm (44th centile). Generalized hypomelanosis of skin, hair, and eyebrows with dark hair in patches on the scalp were noted (Fig 2p). General examination was normal (no cardiac or neurological abnormalities). The Individual was lost to follow-up until the news of sudden infant death at the age of 10 months and 22 days. He had achieved head control and rolled over and had monosyllabic speech by 10 months but was only able to sit with support. Reportedly, both hearing and vision had been normal.

**Supplementary Table 1. Differential diagnosis with known genetic syndromes causing hypomelanosis**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Biallelic *KITLG* variants | Piebaldism (OMIM 172800) | Waardenburg syndrome type 2 (OMIM 193510) and Tietz albinism-deafness syndrome (OMIM 103500) | Waardenburg syndrome type 1 (OMIM 193500) | Waardenburg syndrome type 3 (OMIM 148820) | Shah-Waardenburg syndrome (OMIM 277580) | Hermansky-Pudlak syndrome (OMIM 203300) | Oculocutaneous albinism (OMIM 203100) |
| Hypomelanosis, skin | + | + | + | + | + | + | + | + |
| Hypomelanosis, skin adnexa (hair, eyebrows and eyelashes) | + | + | +  | + | + | + | + | + |
| Hypomelanosis, eyes (heterochromia irises) | + | + | - | + | + | + | + | + |
| Hypopigmented ocular fundi | - | - | + | - | - | - | + | + |
| Nystagmus | - | - | - | - | - | - | + | + |
| Achromic patches/depigmented areas on limbs | + | + | + | + | + | + | - | - |
| Islands of pigmentation | + | - | + | - | - | - | - | - |
| Progressive pigmentation | + | - | + | - | - | + | - | - |
| Sensorineural hearing loss | + | + | + | + | + | + | - | - |
| Bleeding diathesis | - | - | - | - | - | - | + | - |
| Telecanthus/dystopia canthorum | + | - | - | + | - | - | - | - |
| Synophrys | + | - | - | + | + | - | - | - |
| Developmental delay | + | - | - | - | + | - | - | - |
| Limb contractures/abnormalities | - | - | - | - | + | - | - | - |
| Hirschprung disease | - | - | - | - | - | + | - | - |
| Inheritance | AR | AD | AD | AD | AD, AR | AD, AR | AR | AR |

WF: white forelock, NR: no record, UL: unilateral, AR: autosomal recessive, AD: autosomal dominant

**Supplementary Table 2. Prevalence of clinical features among Individuals with biallelic *KITLG* variants in the current case series and previously reported Individuals with *KITLG*-related syndrome**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clinical feature | Individual 1 | Individual 2 | Individual 3 | Individual 4 | Individual 5 | Individual 6 | Zazo Seco et al., 20151 | Ogawa et al., 20172 | Overall (%) |
| Hypomelanosis, skin | + | + | + | + | + | + | + | + | 8/8 (100%) |
| Hypomelanosis, skin adnexa (hair, eyebrows and eyelashes) | + (and WF) | + (and WF) | + | - | + (and WF) | + | - | - | 5/8 (63%); 3/8 and WF (38%) |
| Hypomelanosis, eyes (heterochromia irises) | + | - | + | + | - | + | + | - | 5/8 (63%) |
| Achromic patches/depigmented areas on limbs | + | + | + | + | - | - | + | + | 6/8 (75%) |
| Islands of pigmentation | + | + | - | - | + | + | + | + | 6/8 (75%) |
| Progressive pigmentation | + | + | - | - | - | - | NR | - | 2/7 (29%) |
| Hearing loss | AS | UL | + | + | AS | NR | UL | + | 7/7 /100%) |
| Developmental delay | + | - | - | - | - | + | - | - | 2/8 (25%) |

WF: white forelock, NR: No record, AS: Asymmetric, UL: Unilateral

Supplementary Table 3 is shown in an excel table.

**Supplementary Table 4. Evidence used to support variant interpretation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Tool([Scale]) | c.94C>T, p.(Arg32Cys) | c.443T>C, p.(Ile148Thr) | c.550\_551del, p.(Met184ValfsTer10) | c.644G>A, p.(Tyr215Ter) | c.804\_807del, p.(Arg268SerfsTer29) |
| **Conservation** |
| Grantham dist.([0-215]) | Large(180) | Moderate(89) | NA | NA | NA |
| PhyloP ([-20;10]) | Weakly conserved (3.27) | Moderately conserved(5.63) | NA | NA | NA |
| **Variant Frequency** |
| In-house database (~25,000 exomes) | Absent | Absent | Absent | Absent | Absent |
| UK-Biobank | 4 het/533976 alleles | Absent | Absent | Absent | 2 het/534962 alleles |
| gnomAD v.2.1.1 | Absent | 1 het/249924 alleles | Absent | Absent | 1 het/250484 alleles |
| gnomAD v.3.1.1 | 1 het/152098 alleles | Absent | 1 het/151736 alleles | Absent | Absent |
| Greater Middle Eastern Variome | Absent | Absent | Absent | Absent | Absent |
| Iranome | Absent | Absent | Absent | Absent | Absent |
| **Computational Prediction** |
| CADD | 26.1 | 25.7 | NA | 37 | NA |
| MutationTaster | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing |
| PolyPhen-2 | Probably damaging | Probably damaging | NA | NA | NA |
| SIFT | Deleterious | Deleterious | NA | NA | NA |
| **Clinical Databases** |
| ClinVar | Not present | Not present | Not present | Not present | Not present |
| Deafness Variation Database | Pathogenic | Unknown significance | Not present | Not present | Unknown significance |
| **Variant Interpretation** |
| ACMG/AMP\* Rules Applied | PS1\_Very strong, PM2\_Moderate, PM3\_Supporting, PP4\_Supporting | PM2\_Moderate, PM3\_Supporting, PP3\_Supporting, PP4\_Supporting | PVS1\_Strong, PM2\_Moderate, PM3\_Supporting, PP4\_Supporting | PVS1\_Strong, PM2\_Moderate, PM3\_Supporting | PVS1\_Moderate, PM2\_Moderate |
| Pathogenicity verdict | Pathogenic | Likely pathogenic | Likely pathogenic | Likely pathogenic | Uncertain significance, leaning likely pathogenic |

NA: not available; \*Applying rules from the ACMG/AMP classification rules for hearing loss3.

**References**

1 Zazo Seco, C. *et al.* Allelic Mutations of KITLG, Encoding KIT Ligand, Cause Asymmetric and Unilateral Hearing Loss and Waardenburg Syndrome Type 2. *Am J Hum Genet* **97**, 647-660, doi:10.1016/j.ajhg.2015.09.011 (2015).

2 Ogawa, Y., Kono, M. & Akiyama, M. Pigmented macules in Waardenburg syndrome type 2 due to KITLG mutation. *Pigment Cell Melanoma Res* **30**, 501-504, doi:10.1111/pcmr.12597 (2017).

3 Patel, M. J. *et al.* Disease-specific ACMG/AMP guidelines improve sequence variant interpretation for hearing loss. *Genet Med* **23**, 2208-2212 (2021).