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## A homozygous missense variant in type I keratin *KRT25* causes autosomal recessive woolly hair

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### Abstract

**Background**—Woolly hair (WH) is a hair abnormality that is primarily characterised by tightly curled hair with abnormal growth.

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#### WEB RESOURCES

Clustal Omega, [http://www.ebi.ac.uk/Tools/services/web\\_clustal](http://www.ebi.ac.uk/Tools/services/web_clustal)

dbNSFP, [sites.google.com/site/jpopgen/dbNSFP](https://sites.google.com/site/jpopgen/dbNSFP)

Exome Aggregation Consortium (ExAC), [exac.broadinstitute.org](http://exac.broadinstitute.org)

HomozygosityMapper, [homozygositymapper.org](http://homozygositymapper.org)

InterProScan, <http://www.ebi.ac.uk/Tools/pfa/iprscan>

OMIM, <http://www.omim.org>

Protein BLAST, [blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins)

RCSB Protein Data Bank, <http://www.rcsb.org/pdb>

UCSC Genome Bioinformatics, [genome.ucsc.edu](http://genome.ucsc.edu)

Variant Mendelian Tools, [varianttools.sf.net/VMT](http://varianttools.sf.net/VMT)

**Collaborators** University of Washington Center for Mendelian Genomics.

**Contributors** Conceived and designed the experiments: MA, MJB, DAN, WA and SML. Performed the experiments: MA, SIR, IU, AA, HD, UWCMG and JDS. Analysed the data: MA, KL and RLPS-C. Contributed reagents/materials/analysis tools: SIR, IU, SS, JDS, UWCMG, JS, WA and SML. Contributed to writing: MA, RLPS-C, KL, WA and SML.

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** Institutional review boards of the Quaid-i-Azam University and Baylor College of Medicine and Affiliated Hospitals.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Methods**—In two unrelated consanguineous Pakistani families with non-syndromic autosomal recessive (AR) WH, homozygosity mapping and linkage analysis identified a locus within 17q21.1–q22, which contains the type I keratin gene cluster. A DNA sample from an affected individual from each family underwent exome sequencing.

**Results**—A homozygous missense variant c.950T>C (p.(Leu317Pro)) within *KRT25* segregated with ARWH in both families, and has a combined maximum two-point LOD score of 7.9 at  $\Theta=0$ . The *KRT25* variant is predicted to result in disruption of the second  $\alpha$ -helical rod domain and the entire protein structure, thus possibly interfering with heterodimerisation of K25 with type II keratins within the inner root sheath (IRS) of the hair follicle and the medulla of the hair shaft.

**Conclusions**—Our findings implicate a novel gene involved in human hair abnormality, and are consistent with the curled, fragile hair found in mice with *Krt25* mutations, and further support the role of IRS-specific type I keratins in hair follicle development and maintenance of hair texture.

The structural integrity of several ectodermal appendages, including hair, is in part maintained by keratin proteins. Mutations in several keratin-encoding genes are responsible for a wide spectrum of phenotypes involving hair, skin and nail.<sup>1</sup> Humans have 54  $\alpha$ -keratin genes, which are grouped into type I (n=28) and type II (n=26), and are arranged in two clusters on chromosomes 17q21.2 and 12q13.13, respectively. Of these, 17 keratins (ie, 11 type I and 6 type II) are expressed in the hair follicle, and undergo cyclopolymerisations to form keratin intermediate filaments (KIFs), which in turn interact with keratin-associated proteins to assemble the hair shaft into a tough structure.<sup>2</sup> Four keratin genes from each type, namely *KRT25-KRT28* for type I and *KRT71-KRT74* for type II, are expressed in the medulla, which is the innermost layer of the hair shaft and specifically in the inner root sheath (IRS) of the hair follicle.<sup>3</sup> The IRS is composed of Henle's, Huxley's and cuticle layers, and surrounds the highly keratinised hair shaft. Both the IRS and the hair shaft are important for normal hair growth, especially in the determination and maintenance of hair texture.<sup>4</sup>

Woolly hair (WH) is a hair structural anomaly that is characterised by tightly curled scalp hair.<sup>5–8</sup> It can be observed either as an isolated trait or as a syndromic feature such as: WH and keratoderma with or without heart disease due to mutations in *DSP* (MIM 125647), *DSC2* (MIM 125645), *KANK2* (MIM 616099) or *JUP* (MIM 173325); and trichohepatoenteric syndrome due to *TTC37* (MIM 614589) or *SKIV2L* (MIM 600478) variants.<sup>9</sup> Non-syndromic WH can be inherited either in an autosomal recessive (ARWH, MIM 278150, MIM 604379) or autosomal dominant (ADWH, MIM 194300) manner. To date, mutations in lysophosphatidic acid receptor 6 (*LPAR6/P2RY5*, MIM 609239) and lipase H (*LIPH*, MIM 607365) are known to underlie ARWH with or without hypotrichosis.<sup>5–7</sup> On the other hand, ADWH and hypotrichosis (MIM 613981) are caused by mutations in two type II IRS-specific genes *KRT71* (MIM 608245) and *KRT74* (MIM 608248).<sup>8,10</sup> Additionally, a *KRT74* variant has been identified to cause pure hair and nail ectodermal dysplasia.<sup>11</sup> All four genes are abundantly expressed in the hair follicle. In this study, a novel ARWH locus was mapped to a region containing the type I keratin cluster using data from two families, which segregate ARWH. Using exome sequencing, a rare missense variant *KRT25* c.950T>C (p.(Leu317Pro)) was identified to segregate with ARWH in both families.

Prior to study onset, approval was obtained from the institutional review boards of Quaid-i-Azam University, Islamabad, Pakistan, and Baylor College of Medicine and Affiliated Hospitals, Houston, Texas, USA. Written informed consent was obtained from all study participants from the two families. Family AP188 (figure 1A) was recruited from a remote village in Sindh province, Pakistan, and had four individuals (VI-1, VI-2, VI-4, VI-5) presenting with tightly curled scalp hairs (figure 1C–E). Initially, all four affected individuals possessed soft, short and sparse hairs on the scalp (figure 1D–E), but at around 4 years of age, developed tightly curled hair (figure 1C). Additionally, they had normal eyebrows, but eyelashes were sparse compared with unaffected relatives. Family AP216 was recruited from the Upper Dir district in the Khyber Pakhtunkhwa province of Pakistan, and has three individuals (II-1, II-2, II-4) with WH (figure 1B). As in AP188, the affected individuals from AP216 also presented with tightly curled scalp hair (figure 1F–H). Sparse, WH was more evident in the long-haired female individual II-2 (figure 1G). All individuals from both families showed normal craniofacial features, teeth, nails and sweating, and no evidence of palmoplantar keratoderma, heart disease, chronic diarrhoea, immune deficiencies or intellectual disability.

DNA samples were extracted from venous blood of eight and six individuals from families AP188 and AP216, respectively. DNA samples from selected family members (figure 1A, B) underwent a genome scan using the Infinium HumanCoreExome BeadChip (Illumina, USA), which includes ~550 000 markers. The two families' genotype data were analysed by performing homozygosity mapping and multipoint parametric linkage analysis using the software programmes HomozygosityMapper and Allegro,<sup>12</sup> respectively. In family AP188, a 16.7-Mb homozygous region was identified on chromosome 17q21.1–q22 (figure 1A). For this same region, a maximum multipoint LOD score of 4.14 was observed. For family AP216, a maximum multipoint LOD score of 1.33 was observed at multiple regions of homozygosity, including two 3-Mb regions on chromosomes 2q36.1–q36.3 and 14q24.2–q24.3 and a 20.4-Mb homozygous region on 17q12–q22 (figure 1B). Although the parents (I-1, I-2) from family AP216 did not self-report being related, haplotype analysis showed the presence of the same carrier haplotype between rs9972960 and rs4794665 in both parents (figure 1B). Moreover, the kinship coefficient using KING<sup>13</sup> was estimated to be 0.05, indicating that the parents were third-degree relatives. Comparing the mapped regions from the two families, the homozygous region for family AP188 is completely contained within the homozygous region on chromosome 17 for family AP216. Although the two families are unrelated, and are from different ethnic groups within Pakistan, they segregate the same 15.8-Mb SNP haplotype (figure 1A, B), which encompasses the type I keratin cluster on 17q21.2. Hair expression of type I keratins is known,<sup>3</sup> but no human hair-related traits have been previously associated with IRS-specific type I keratins.

Exome sequencing of DNA samples from affected individuals —VI-2 from family AP188 and II-4 from AP216 (figure 1A, B)—was performed at the University of Washington Center for Mendelian Genomics to average read depth of 36× and 63×, respectively. Sequence alignment, variant detection and calling were performed as previously described.<sup>14</sup> Variant annotation was performed using Variant Mendelian Tools, an in-house pipeline that allows for exome quality control and selection of variants according to mode of inheritance, minor allele frequency (MAF) in the Exome Aggregation Consortium (ExAC) database, variant

type, nucleotide conservation and prediction from multiple bioinformatics tools. Aside from being ruled out by linkage analysis and homozygosity mapping, in the two exomes, we did not identify potentially causal homozygous or compound heterozygous variants in genes that are previously known to be involved in syndromic or non-syndromic WH. Within the mapped interval for AP188, a total of 176 variants that passed quality control were homozygous in both exome sequences. Of 176 homozygous variants, only two variants had MAF <0.01 in South Asian alleles in ExAC: a synonymous *FAM134C* NM\_178126.3:c.906T>C variant that is heterozygous in 89 out of 16 512 South Asian ExAC alleles (MAF=0.005) and in two ExAC alleles of other ethnicity; and a missense variant c.950T>C (p.(Leu317Pro)) within *KRT25* (NM\_18154.3) that is heterozygous in three ExAC South Asian alleles (MAF=0.0002), but was not identified in 104 876 ExAC alleles of other ethnicity. Furthermore, the *KRT25* c.950T>C variant was not found in 154 in-house exome sequences from unrelated Pakistani individuals with non-hair phenotypes, while the *FAM134C* synonymous variant was heterozygous in three in-house Pakistani exomes. Sanger sequencing of the *KRT25* missense variant using DNA samples from the remaining members of AP188 and AP216 confirmed segregation with the ARWH phenotype in both families (figure 1A, B, see online supplementary figure S1A). Additionally, Superlink<sup>15</sup> was used to perform two-point linkage analysis of the *KRT25* c.950T>C variant, and a maximum LOD score of 6.57 and 1.33 ( $\Theta=0$ ) were obtained for AP188 and AP216, respectively. The *KRT25* variant is absent in 462 Pakistani control chromosomes. Comparison of the exome variants within the mapped region narrowed down the haplotype shared by the two exome sequences from the two families to five exome variants that occur within a 48.7-kb region (table 1). Segregation testing of the four variants within *KRT27* and *KRT28* revealed that these four variants did not segregate with fragile WH. Therefore, only the *KRT25* missense variant segregates with ARWH in both families.

The observation of sparse WH in affected individuals who are homozygous for the *KRT25* c.950T>C variant recapitulates the phenotype in mutant mice. Two previously described mutant mouse strains carry *Krt25* missense mutations. *Re/Re* mice who are homozygous for a c.1142T>C (p.(Leu381Pro)) variant within the helix termination motif (HTM) at the end of the rod 2B domain of K25 have strongly curled, extremely fragile fur.<sup>16</sup> On the other hand, *M100573* mice have ragged, fragile vibrissae and carry a dominant c.1135T>A (p.(Tyr379Asn)) variant, which is also within the HTM of mouse K25.<sup>16</sup> At young age, both strains demonstrate bent hair follicles and break points in the Henle's layer of the IRS, but in the *M100573* strain with the dominant variant, the hair becomes less wavy with age.<sup>16</sup> Furthermore, because of heterodimerisation of K25 with type II keratin K71, heterozygous *Re/+* mice have mislocalised K71 protein and irregular Henle's and Huxle's layers within the IRS.<sup>16</sup> In AP188 and AP216 however, family members who are heterozygous for the *KRT25* c.950T>C variant have normal head and facial and body hair throughout life. This may be due to differences in hair structure between mouse and human, and/or the specific K25 motif that is affected by mutation. It should be noted that non-occurrence of the p.(Leu317Pro) variant within either the HTM or helix initiation motif of K25 does not preclude causality for the ARWH phenotype. For example, a p.Phe274Ser variant within the coil 1B domain of K74 is known to cause hair and nail ectodermal dysplasia in humans.<sup>11</sup>

The K25 p.(Leu317Pro) variant is predicted to be damaging or deleterious by multiple bioinformatics tools in the dbNSFP database, including Combined Annotation Dependent Depletion (CADD scaled score=15.0), fathmm, likelihood ratio test, logistic regression, MutationAssessor, MutationTaster, PolyPhen2 (HVAR), PROVEAN, SIFT and support vector machine. Analysis of human K25 with InterProScan showed that the variant occurs within a type I keratin signature at residues 308–323. Alignment of the human sequence with other type I keratins in seven mammals, one marsupial, one bird, one amphibian and two fish shows strong amino acid conservation of Leu317 and several residues, including Arg304, Gln307, Leu309, Ile311, Leu313, Gln314, Ser315, Lys320 and Leu323 (see online supplementary figure S1B). Based on molecular modelling, it is predicted that these conserved residues contribute to the formation of the  $\alpha$ -helix well within the rod 2B domain of K25 (see online supplementary figures S1C and S2A, C). The replacement of leucine at residue 317 with a proline is expected to disrupt the  $\alpha$ -helix, and cause changes to the local domain and the overall protein structure (see online supplementary figure S2B, D).

The *KRT25* transcript and the encoded protein are expressed in all three layers of the follicle IRS and the hair shaft medulla.<sup>3</sup> Likewise, other type I keratins namely *KRT27* and *KRT28* have the same expression pattern, while of the type II keratins, only *KRT71* is expressed in all three IRS layers.<sup>3</sup> The WH phenotype observed in individuals homozygous for the *KRT25* c.950T>C variant implies that heterodimerisation between K25 and K71 is essential for stable hair structure, and is not compensated by interaction of K71 with K27 or K28. This is further supported by the abnormal localisation of K71 and irregularity of Henle's and Huxley's layers of the IRS in *Krt25*-mutant *Re/+* mice.<sup>16</sup> Additionally, *KRT71* mutations independently cause hypotrichosis and WH in humans.<sup>7</sup>

Hair keratins are known to heterodimerise such that a type I keratin is required to partner with a type II keratin to form KIFs that are basic building blocks for hair structure.<sup>17</sup> Interestingly, disulfide bonding between cysteine residues of partnering keratins has been shown as an important mechanism for the assembly of keratins into KIFs.<sup>18</sup> Human K25 has cysteine residues at positions 325 and 335 that are highly conserved in mammalian K25 sequences (see online supplementary figure S1B). Due to the p. (Leu317Pro) variant, the region containing these cysteine residues is predicted to become highly disorganised (see online supplementary figure S2D), which possibly interferes with disulfide bonding with K71 and affects proper heterodimerisation and microfibril structure within the hair follicle. Because K25 is also localised to the medulla, structural defects from improper heterodimerisation with other type II keratins are also expected to affect the hair shaft. For example, type II keratin K75 is also expressed in the medulla.<sup>3</sup> Mutant mice with a dominant *Krt75* mutation have rough hair coat and hair shaft defects,<sup>19</sup> and chickens with a *KRT75* deletion also have frizzled feathers with defects within medulla.<sup>20</sup> Thus, it is postulated that the p. (Leu317Pro) variant also impairs heterodimerisation of K25 with K75 within the medulla of the hair shaft.

In conclusion, we identified a homozygous missense mutation within *KRT25* that causes ARWH in humans, which is consistent with findings in mutant mice. The variant is predicted to disrupt the  $\alpha$ -helical rod 2B domain of K25 and to interfere with heterodimerisation with type II keratins, which are expected to lead to irregular and fragile

hair that is characteristic of the WH phenotype. Taken together, our findings further support the role of type I keratins in hair follicle development and maintenance of hair texture. The identification of a *KRT25* mutation as a cause of WH in humans can help direct efforts in the development of therapies for hair keratin disorders.<sup>1</sup>

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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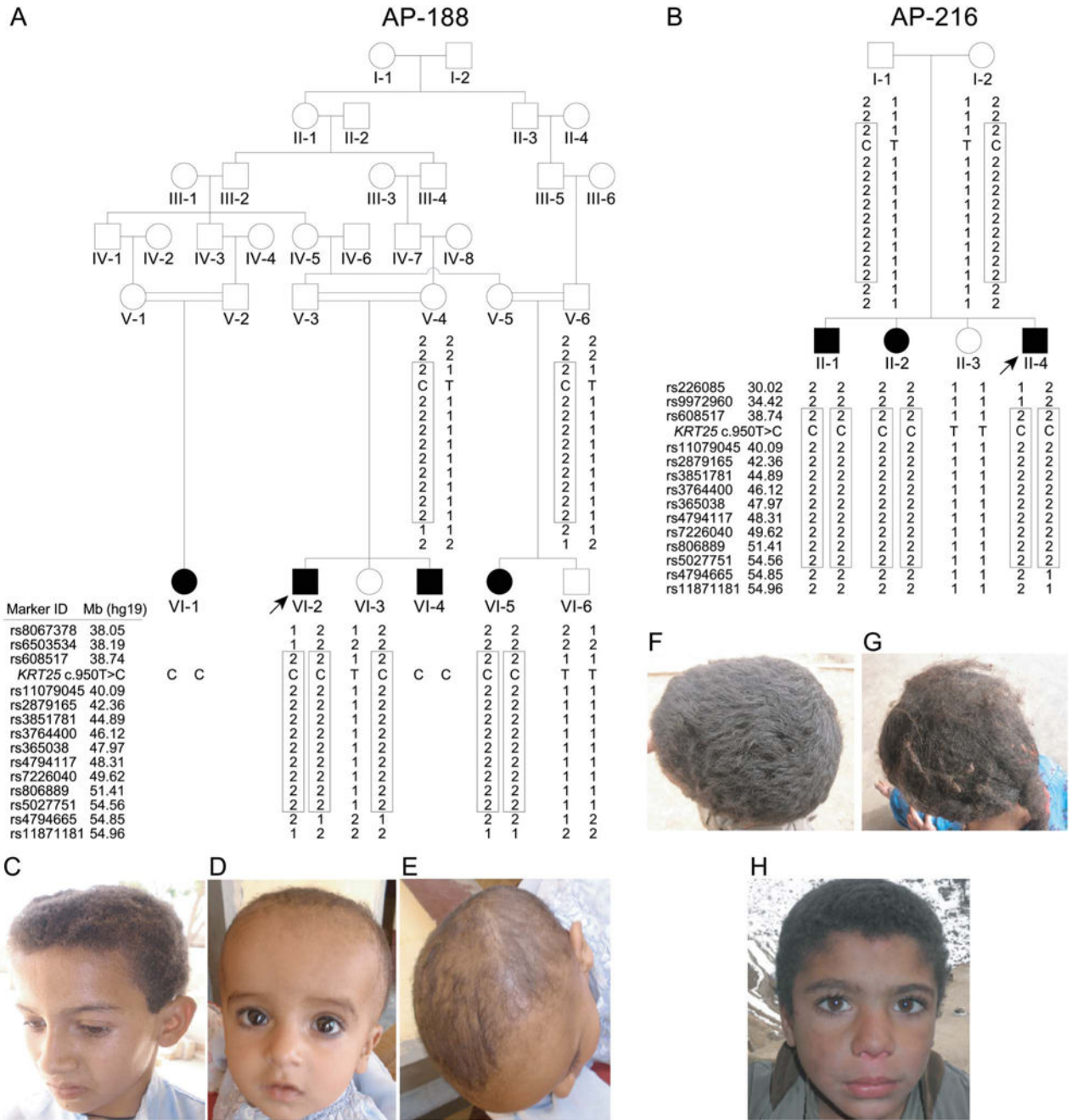
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**Figure 1.** Pedigree drawings, haplotypes and sparse, woolly hair in families AP188 and AP216. (A and B) Drawings of pedigrees AP188 and AP216 displaying segregation of the identical SNP haplotype (*within rectangles*) and the *KRT25* c.950T>C variant with autosomal recessive woolly hair. *Arrows* indicate affected individuals whose DNA samples were submitted for exome sequencing. (C) Individual VI-4 from AP188 had woolly hair at age 8 years. (D and E) Individual VI-5 from AP188 had sparse, soft hairs on scalp at age 1 year. (F and G) Individual II-1 and II-4 from AP216 had woolly hair at age 8 years. (H) Individual II-2 from AP216 had sparse, soft hairs on scalp at age 1 year.



and H) Individual II-4 from AP216 with woolly hair at age 8 years. (G) Sparse, woolly hair is more obvious in 10-year-old female II-2 from AP216 who has long hair.

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Homozygous chromosome 17 variants in AP188 and AP216 individuals with autosomal recessive woolly hair and exome sequence data

**Table 1**

hg19 Position	38 907 213	38 935 876	38 935 880	38 950 272	38 955 961
Reference allele	A	C	C	T	G
Alternate allele	G	T	T	C	A
Gene	<i>KRT25</i>	<i>KRT27</i>	<i>KRT27</i>	<i>KRT28</i>	<i>KRT28</i>
cDNA change	c.950T>C	c.850G>A	c.847-1G>A	c.1005A>G	c.185C>T
Amino acid change	p.(Leu317Pro)	p.(Ala284Thr)	NA	p.(=)	p.(Ala62Val)
Variant type	missense	missense	splice-3'	synonymous	missense
ExAC South Asian MAF	0.0002	0.24	0.06	0.62	0.31
ExAC all MAF	0.00002	0.17	0.008	0.58	0.22
MAF in-house exomes	0	0.22	0.05*	0.69	0.40

mRNA accession numbers: *KRT25*, NM\_18154.3; *KRT27*, NM\_181537.3; *KRT28*, NM\_181535.3.

ExAC, Exome Aggregation Consortium; MAF, minor allele frequency; NA, not available.

\* This *KRT27* splice variant is heterozygous in 12 out of 154 in-house exomes from unrelated Pakistani individuals with normal hair. Additionally, an exome from an individual with microcephaly and intellectual disability, but no hypotrichosis or woolly hair is homozygous for the *KRT27* splice variant. Thus, we do not believe the *KRT27* splice variant is causal of ARWH.