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A Missense Mutation within the Helix Termination Motif of KRT25 Causes Autosomal Dominant Woolly Hair/Hypotrichosis

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Abbreviations used:

WH: woolly hair

AD: autosomal dominant

AR: autosomal recessive

ADWH: autosomal dominant woolly hair

ARWH: autosomal recessive woolly hair

KIF: keratin intermediate filament

IF: intermediate filaments

HS: hair shaft

HIM: helix initiation motif

HTM: helix termination motif

Key Words: autosomal dominant; *KRT25*; whole exome sequencing; ADWH/hypotrichosis

TO THE EDITOR

Woolly hair(WH)/hypotrichosis is an unusual condition characterised by sparse and tightly curled hair (Ramot and Zlotogorski, 2015a). WH may be isolated or be accompanied by additional complications including palmoplantar keratoderma, hypotrichosis, epidermal naevus and cardiomyopathy (Veraitch et al., 2016; Ramot et al., 2014). Isolated WH can manifest with autosomal dominant (AD) or autosomal recessive (AR) trait of inheritance (Shimomura. 2016).

Keratins are scaffolding proteins that form a network of intermediate filaments (IFs). Heterodimerization between type I and II keratin to form keratin intermediate filaments (KIFs) is the basic building block for hair structure (Ramot and Zlotogorski, 2015b). The phenotypic heterogeneity caused by different keratin genes also depends on their location within different hair structures, including the cortex of the hair shaft (HS), the cuticle and the IRS (Naeem et al., 2006).

Variants in keratins K71 and K74 were described in ADWH pedigrees, and polymorphisms in *KRT75* were implicated in the pathogenesis of pseudofolliculitis barbae.(Fujimoto et al., 2012; Wasif et al., 2011; Winter et al., 2004.) Recently, biallelic variants within *KRT25* were also related to ARWH/hypotrichosis pedigrees (Ansar et al., 2015; Zernov et al., 2016).

Here, we describe a monoallelic pathogenic variant in a Chinese ADWH/hypotrichosis family, five-generation pedigree consisting of 23 individuals.(**Figure 1a)**. The proband (IV-3) was a 40-year-old female, born with sparse, soft and curled scalp hairs. Her hairs were less dense and slower to grow compared to age-matched individuals. Her hairs looked wiry, coarse, curled, dry, hypopigmented and fragile. The frontotemporal hairline was normal. There were no other obvious abnormalities. Other patients showed similar manifestation.(**Figure 1b**). Light microscopy (LM) of the patients demonstrated local variations in diameter, showing irregular curled contours and sharp corners (**Figure 1c**). Scanning electron microscopy (SEM) showed the cortex was partially exposed with irregular overlay of the cuticle. Desquamation of lifting cuticle layers was obvious.(**Figure 1d**). More

frequent longitudinal grooves suggested a tendency to break at the delicate location(**Figure 1e-f**). The damage level was ten out of the 12-point scale classification system (Lee et al., 2016),or four out of the 5-grading system (Kim et al., 2010).

The genomic DNA was extracted from four patients (III-1, III-3, IV-3 and V-3) and six unaffected individuals (III-2, IV-1, IV-2, IV-4, V-1 and V-2). We sequenced the exomes of three patients (IV-3, III-1,V-3) and two unaffected individuals(V-2 and IV-2). **(Supplemental Materials 1)**. Sequence variants were initially filtered against dbSNP146, the 1000 Genomes Project, ExAC and our internal database. Assuming AD transmission, the downstream variant filtering strategy was as follows: heterozygous variants present in the patients but not in the unaffected individuals were treated as possible candidates **(Supplemental Materials 1)**. This strategy reduced the number of variants to 8 missense variants. Finally, only 1 out of the 8 SNPs passed through the conservation assay. Sanger sequencing also confirmed that the heterozygous missense mutation in *KRT25* (c.1127T>G, p.Leu376Arg) fully co-segregated with the disease status in this family. No mutation detection in 200 ethnically matched normal controls suggested the likely deleterious variant **(Supplementary Figure S1a-b).**

KRT25 encodes a member of the type I keratin family with a characteristic structure: the N-terminal head domain; the central α-helical rod domain and the C-terminal tail domain. The central α-helical rod domain starts with the helix initiation motif (HIM) and ends with the helix termination motif (HTM). Leu residue at position 376 is

located within the HTM (**Figure 1g**), where it is highly conserved amongst all human type I keratin members **(Supplementary Figure S1c**). Besides, sequence alignment of K25 among various mammals indicates that Leu376 is highly conserved **(Supplementary Figure S1d**). A single missense substitution in the HTM of the type I IRS gene *krt25* was shown to underlie wavy/curly coat phenotype in *M100573* and *Re* mice (**Figure 1g**) (Tanaka et al., 2007).

In order to predict the potential effect of p.Leu376Arg variant on the function of human K25，we integrated multiple bioinformatics tools. The results suggested that p.Leu376Arg variant influenced the structure of K25 and the binding between K25 and its partner K71. Since K25 and K71 belong to the superfamily of intermediate filament proteins which form an integral part of the cytoskeleton, changes in their binding may affect a variety of cellular characteristics. **(Supplementary Materials 1)**. In order to determine the impact at the cellular level, we transfected the plasmids for either wild-type (Wt) or p.Leu376Arg mutant (Mut) K25 into HaCaT cells. (**Supplemental Materials 1)**. The results showed the Wt-K25 protein colocalizing with K5 in KIFs **(Figure 2a-c)**, indicating that Wt-K25 efficiently forms a functional KIF cytoskeleton via a heterodimer with the endogenous K5 protein. Conversely, the Mut-K25 protein showed an aberrant expression around the nucleus with a tendency to form aggregates with the K5 protein **(Figure 2d–f)**. These data strongly suggest the mutation in KRT25 affects the stability of the cytoskeleton.

Previous studies suggested that variants in the α-helical subdomains of keratin genes interfere with keratin heterodimer formation and cause dominant-negative effects or

haploinsufficiency. By contrast, variants in the non-helical linker subdomains interfere with the higher order formation of heterodimers into KIF, manifest in homozygotes and transmit recessively (Zernov et al., 2016). The variant we identified within *KRT25* exhibited moderate phenotypic expression and involved the scalp hairs alone with a clinically similar appearance to homozygous mutations in *KRT25* albeit (Ansar et al., 2015; Zernov et al., 2016). We speculate that the mutation may act as a dominant-negative in this pedigree. In addition, such variable clinical severity might be the coordinated consequence of the nature of the pathogenic variant, modifier genes and/or compensatory pathways.

Further research will be beneficial to fully clarify the genetic and phenotypic heterogeneity of WH/hypotrichosis. Recent research showed that allele-specific silencing of mutant *krt75* could achieve durable correction of hair structural defect (Liu et al., 2016), indicating that the identification of the pathogenic loci could open up new avenues for treatment of hair keratin disorders.

In summary, we report a Chinese ADWH/hypotrichosis pedigree that results from a heterozygous non-synonymous variant in *KRT25*. We conclude that mutation in *KRT25* affects the stability of the cytoskeleton and hypothesize this is how it affects hair in WH syndromes.

This study was approved by the Ethics Committees of Shanghai Jiaotong University School of Medicine and conducted in accordance with the principles of the Declaration of Helsinki. After obtaining written informed consent, we collected peripheral blood samples from the family members. Patients or guardians gave

permission to publish their images and information.

Conflict of interest: The authors have declared that they have no conflict of interest with regard to the publication of this research report.

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REFERENCES

Ansar M, Raza SI, Lee K, Irfanullah, Shahi S, Acharya A, et al. A homozygous missense variant in type I keratin KRT25 causes autosomal recessive woolly hair. J Med Genet 2015;52:676-80.

Fujimoto A, Farooq M, Fujikawa H, Inoue A, Ohyama M, Ehama R,et al. A missense mutation within the helix initiation motif of the keratin K71 gene underlies autosomal dominant woolly hair/hypotrichosis. J Invest Dermatol 2012;132:2342-49.

Kim YD, Jeon SY, Ji JH, Lee WS. Development of a Classification System for Extrinsic Hair Damage: Standard Grading of Electron Microscopic Findings of Damaged Hairs. Am J Dermatopathol 2010;32:432-38.

Lee SY, Choi AR, Baek JH, Kim HO, Shin MK, Koh JS. Twelve-point scale grading system of scanning electron microscopic examination to investigate subtle changes in damaged hair surface. Skin Res and Technol 2016;22:406–11.

Liu Y, Snedecor ER, Zhang X, Xu Y, Huang L, Jones EC et al. Correction of hair shaft defects through allele-specific silencing of mutant Krt75. J Invest Dermatol 2016;136: 45–51.

Naeem M, Wajid M, Lee K, Leal SM, Ahmad W. A variant in the hair matrix and cuticle keratin KRTHB5 gene causes ectodermal dysplasia of hair and nail type. J Med Genet 2006;43:274-9.

Ramot Y, Molho-Pessach V, Meir T, Alper-Pinus R, Siam I, Tams S, et al. Variant in KANK2, encoding a sequestering protein for steroid receptor coactivators, causes keratoderma and woolly hair. J Med Genet 2014;51:388-94.

Ramot Y, Zlotogorski A. The twisting tale of woolly hair: a trait with many causes. J Med Genet 2015a;52:217-23.

Ramot Y, Zlotogorski A. Keratins: the hair shaft's backbone revealed. Exp Dermatol 2015b;24:416-17.

Shimomura Y. Journey toward unraveling the molecular basis of hereditary hair disorders. J Dermatol Sci 2016;84:232-38.

Tanaka S, Miura I, Yoshiki A, Kato Y, Yokoyama H, Shinogi A, et al. Variants in the helix termination motif of mouse type I IRS keratin genes impair the assembly of keratin intermediate filament. Genomics 2007;90:703-11.

Veraitch O, Perez A, Hoque SR, Vizcay-Barrena G, Fleck RA, Fenton DA,et al. Hair

Follicle Miniaturization in a Woolly Hair Nevus: A Novel "Root" Perspective for a Mosaic Hair Disorder. Am J Dermatopathol 2016;38:239-43.

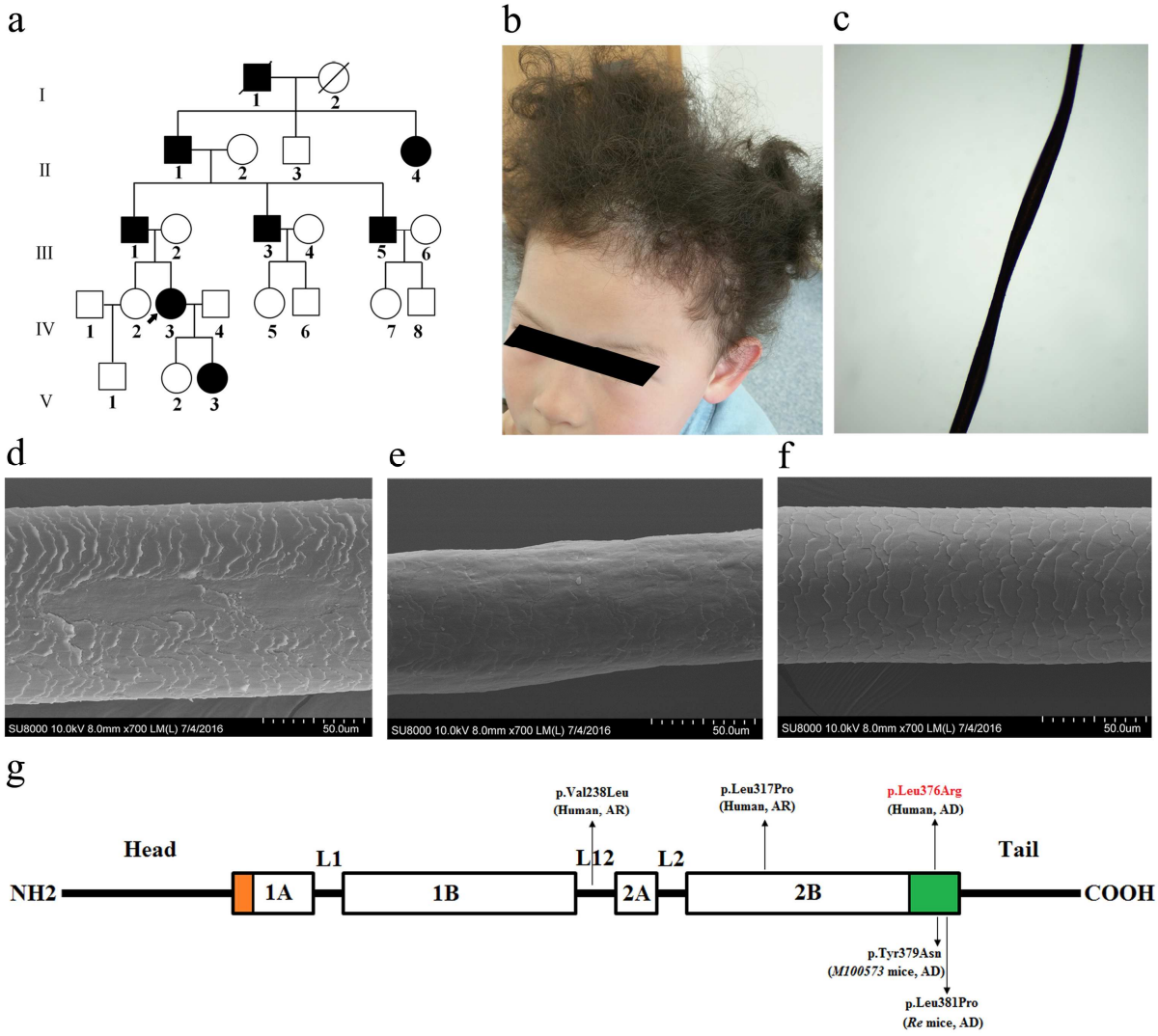
Wasif N, Naqvi SK, Basit S, Ali N, Ansar M, Ahmad W. Novel mutations in the keratin-74 (KRT74) gene underlie autosomal dominant woolly hair/hypotrichosis in Pakistani families. Hum Genet 2011;129:419-24.

Winter H, Schissel D, Parry DA, Smith TA, Liovic M, Birgitte Lane E, et al. An unusual Ala12Thr polymorphism in the 1A alpha-helical segment of the companion layer-specific keratin K6hf: evidence for a risk factor in the etiology of the common hair disorder pseudofolliculitis barbae. J Invest Dermatol 2004;122:652-57.

Zernov NV, Skoblov MY, Marakhonov AV, Shimomura Y, Vasilyeva TA, Konovalov FA, et al. Autosomal Recessive Hypotrichosis with Woolly Hair Caused by a Variant in the Keratin 25 Gene Expressed in Hair Follicles. J Invest Dermatol 2016;136:1097-105.

Figure 1 Family pedigree, clinical features, LM and SEM observation of the HS from the proband's **younger daughter . SEM observation of the HS from the** proband's **elder daughter, and schematic representation of K25 protein. (a)** Family pedigree. **(b)** The proband's younger daughter showed ADWH/hypotrichosis: wiry, coarse, curled, dry, hypopigmented and fragile **(c)** Under LM, the HS of affected individuals characteristically showed local variations in diameter.,irregular curled HS contours, sharp corners. (40×) **(d)** Under SEM, the cortex of affectec HS was partially exposed with irregular overlay of the cuticle. Desquamation of lifting cuticle layers was obvious. **(e)** HS of affected individuals frequently showed longitudinal grooves. **(f)** The proband's elder daughter showed intact hair with regular overlay of the cuticle. Scale bar =50μm. **(g)** Schematic representation of K25 protein. The location of mutation p.Leu376Arg in human K25 is shown above the scheme and is indicated in red. The two variants of K25 identified in the mice are shown below the scheme. Mutations in the *M100573* mice and the *Re* mice are dominantly inherited, whereas the other two variants (Val238Leu and Leu317Pro) in human are recessive variants. The HIM and HTM are colored in orange and green, respectively.

Figure 2 Mutant K25 protein leads to the disruption of endogenous keratin intermediate filament (KIF) formation in HaCaT cells. (a-c) Ectopically expressed wild-type (Wt)-K25 protein formed a KIF network via heterodimerization with endogenous K5 protein. (d-f) The p.Leu376Arg mutant (Mut)-K25 protein formed aggregates with K5 protein around the nuclei and a collapse of the endogenous KIF network around the nucleus. The right hand panels are merged images of a and b and d and e respectively, nuclei are counterstained with 4,6-diamidino-2-phenylindole (DAPI) in blue (c and f). Scale bar =25μm.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p.Leu317Pro
(Human, AR)
p.Leu376Arg
(Human, AD)
Head
L1
L12
L2
Tail
NH2
1A
1B
2A
2B
COOH
p.Tyr379Asn
(M100573 mice, AD)
p.Leu381Pro
(Re mice, AD)