Autosomal Recessive Hypotrichosis with Woolly Hair Caused by a Mutation in the *Keratin 25* Gene Expressed in Hair Follicles

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Hypotrichosis is an abnormal condition characterized by decreased hair density and various defects in hair structure and growth patterns. In particular, in woolly hair, hypotrichosis is characterized by a tightly curled structure and abnormal growth. In this study, we present a detailed comparative examination of individuals affected by autosomal-recessive hypotrichosis (ARH), which distinguishes two types of ARH. Earlier, we demonstrated that exon 4 deletion in the lipase H gene caused an ARH (hypotrichosis 7; MIM: 604379) in populations of the Volga-Ural region of Russia. Screening for this mutation in all affected individuals revealed its presence only in the group with the hypotrichosis 7 phenotype. Other patients formed a separate group of woolly hair-associated ARH, with a homozygous missense mutation c.712G>T (p.Val238Leu) in a highly conserved position of type I keratin *KRT25* (K25). Haplotype analysis indicated a founder effect. An expression study in the HaCaT cell line demonstrated a deleterious effect of the p.Val238Leu mutation on the formation of keratin intermediate filaments. Hence, we have identified a previously unreported missense mutation in the *KRT25* gene causing ARH with woolly hair.

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INTRODUCTION

Isolated congenital hypotrichosis is a group of genetic hair loss disorders characterized by either hypoplastic or aplastic hair, sparse scalp hair, eyebrows, eyelashes, and body hair, as well as abnormal structure and growth of hair in affected individuals. The phenotypic manifestations vary from negligible to complete alopecia. To date, 15 different types of isolated congenital hair loss disorders have been described in the OMIM database (http://www.omim.org), 8 of which are with autosomal dominant inheritance: hypotrichosis 1 (MIM: 605389), hypotrichosis 2 (MIM: 146520), hypotrichosis 3 (MIM: 613981), hypotrichosis 4 (MIM: 146550), hypotrichosis 5 (MIM: 612841), hypotrichosis 11 (MIM: 615059), hypotrichosis 12 (MIM: 615885), and hypotrichosis 13 (MIM: 615896); and 7 with autosomal recessive inheritance: hypotrichosis 6 (MIM: 607903), hypotrichosis 7, woolly hair (WH) autosomal recessive 2 with or without hypotrichosis (MIM: 604379), hypotrichosis 8, WH autosomal recessive 1 with or without hypotrichosis (MIM: 278150), hypotrichosis 9 (MIM: 614237), hypotrichosis 10 (MIM: 614238), atrichia with papular lesions (MIM: 209500), and alopecia universalis (MIM: 203655). For a majority of these disorders, the molecular genetic basis is already known. Several molecular mechanisms were shown to be involved in various forms of congenital hypotrichosis, including the disruption of signaling pathways (*APCDD1*, Shimomura et al., 2010a; lipase H gene *LIPH*, Kazantseva et al., 2006; *LPAR6*, Pasternack et al., 2008; Shimomura et al., 2008), the formation of keratin intermediate filaments (*KRT74*, Shimomura et al., 2010b; *KRT71*, Fujimoto et al., 2012), cell junctions and adhesion (*CDSN*, Levy-Nissenbaum et al., 2003; *DSG4*, Kljuic et al., 2003), the regulation of transcription (*HR*, Liu et al., 2014), splicing (*SNRPE*, Pasternack et al., 2013), and the ribosome complex formation (*RPL21*, Zhou et al., 2011).

However, novel genes for hair growth and structure disorders are still being identified as causative (Skoblov et al., 2008). Our previous study demonstrated that the mutations in the *LIPH* gene are the culprit behind autosomal-recessive hypotrichosis (ARH) 7 (Kazantseva et al., 2006).

One of the most interesting group of genes that is actively studied in hypotrichosis is the keratins, which encode cytoskeletal components known as intermediate filaments and are involved in a variety of processes such as movement, division, intracellular transport, regulation of metabolism, translation, immune response, and other types of signaling (Kim and Coulombe, 2007; Kim et al., 2006; Ku et al., 2010; Long et al., 2006; Magin et al., 2007;

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Abbreviations: ARH, autosomal-recessive hypotrichosis; HF, hair follicle; HS, hair shaft; IRS, inner root sheath; KIF, keratin intermediate filaments; KRT25, keratin 25 gene; KRT71, keratin 71 gene; KRT74, keratin 74 gene; LIPH, lipase H gene; Mut, p.Val238Leu mutant; Wt, wild-type; WH, woolly hair

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Vijayaraj et al., 2009; Windoffer et al., 2011). An interesting characteristic of keratins that distinguishes them from other intermediate filament proteins is the heterodimerization between type I and type II keratin molecules that leads to the formation of keratin intermediate filaments (KIF) (Lu and Lane, 1990). The subdivision of keratins into two types is based on their molecular properties and includes acidic (type I) or basic to neutral (type II) subtypes. Genes encoding these two types of keratins are clustered on chromosomes 17q21.2 and 12q13.13, respectively. According to their expression patterns, keratin genes are subdivided into epithelial keratins, including hair follicle (HF)-specific ones and hair keratins. In total, 54 keratin genes (Langbein and Schweizer, 2005; Moll et al., 2008). Type I *KRT25–KRT28* (K25–K28) and type II *KRT71–KRT74* (K71–K74) keratin genes are expressed in HF, more precisely in the hair medulla and in the inner root sheath (IRS)—a complex formed by three distinct layers: the IRS cuticle, the Huxley layer, and the Henley layer (Langbein et al., 2006). The IRS plays a role in protecting, supporting, and molding of hair shaft (HS). Both *KRT74* and *KRT71* belong to HF-specific keratin genes expressed in the IRS.

In all keratin proteins, an α-helical rod domain is flanked by nonhelical head and tail domains. Four coiled subdomains of the α-helical rod domain, 1A, 1B, 2A, and 2B, are divided by nonhelical linkers L1, L12, and L2. In the intermediate filament formation process, keratins are organized into heterodimers by pairing up with an appropriate partner, and then higher order polymers are formed. The majority of the reported autosomal dominant mutations are localized within subdomains 1A or 2B of either type I or type II keratins (Schweizer et al., 2007). The mutations in these genes were previously observed in individuals with hypotrichosis with the WH phenotype. Further functional analysis of cell lines validated these mutations as pathogenic, as they disrupted the formation of heterodimers between the two types of keratins and led to cytoskeletal damage (Fujimoto et al., 2012; Rasool et al., 2010; Raykova et al., 2014; Shimomura et al., 2010b; Wasif et al., 2011).

The causal impact of other mutations in the IRS expressed keratin genes on the formation of KIF is still unknown. Here, we describe a previously unreported type of ARH with WH and the causative missense mutation in the type I *KRT25* gene that defines this phenotype.

RESULTS

Identification of individuals affected by ARH and WH

The material for this study was collected during a field expedition to the Volga-Ural region of Russia. In total, we observed 119 affected individuals with signs of hypotrichosis. All affected individuals belong to either the Chuvash ethnic group (of the Turkic linguistic group) from Chuvash Republic or the Mari group (Finno-Ugric group) from Mari El Republic. All ascertained individuals underwent a detailed clinical examination including a physical examination, light microscopy of hairs, trichogram, and hair density evaluation. Among affected individuals, high interfamily and intrafamily polymorphism was observed. Nevertheless, based on the clinical examination, two groups of affected individuals were clearly evident.

The majority of affected individuals (116) had a typical phenotype of hypotrichosis 7 (MIM: 604379) (Figure 1). The other group included an affected individual from a nonfamilial case #041 and a family #002 with two affected individuals and their healthy parents. In spite of the phenotypic similarity with the hypotrichosis 7 group, several differences were noted.

Affected individuals (Figures 2 and 3) demonstrated the following:

- Isolated congenital WH.
- Scalp hair length of approximately 5–15 cm.
- Decreasing trend in follicle density from the occipital to frontotemporal scalp region from 210 to 90 cm^{-2}, respectively (with a norm being in the range of 175–300 follicles cm^{-2} (Barman et al., 1965)).
- Frontotemporal hairline close to normal.
- Uniform hair rigidity in different scalp regions; the rate of hair growth was higher than in group 1 (individuals had a haircut every 3–4 years);
- Body hair distribution was similar to that in the hypotrichosis 7 group;

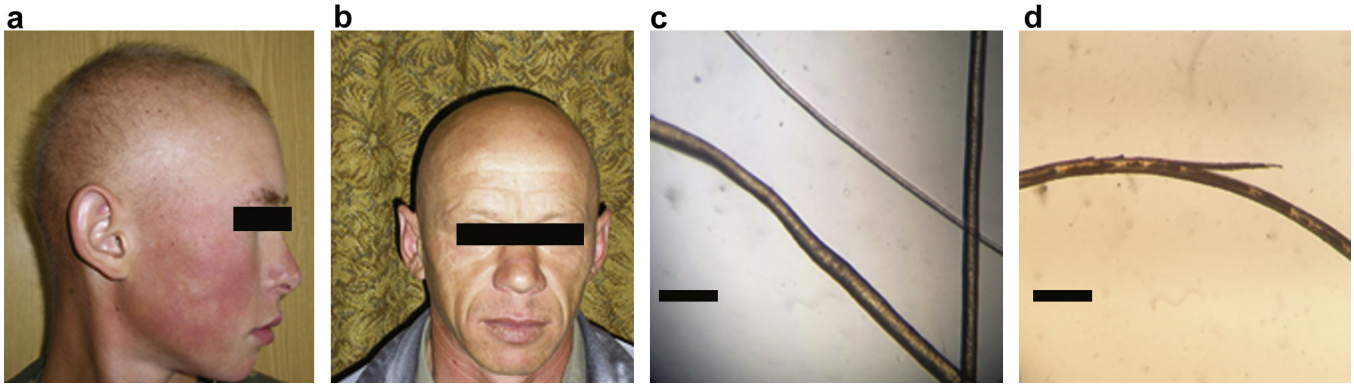

Figure 1. Phenotypic features of individuals from the first group affected by hypotrichosis 7. (**a**, **b**) Two affected individuals demonstrating low hair density varying from (**a**) hypotrichosis to (**b**) alopecia. (**a**) Short, sparse, deformed hair, highly placed frontotemporal hairline. (**c**, **d**) Hair light microscopy (LM). (**c**) LM showing thin hair (approximately 25 μm in diameter), high differences in diameters, and local variations in diameter of hair shaft (HS), (**d**) trichoptilosis. Scale bar = 100 μM. Written permission to publish photographs was obtained from all study participants.