### Mutagenetix Phenotypic Mutation 'Plush'

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<th>Allele</th>
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<td>Xin Du, Xiao-hong Li, Bruce Beutler</td>
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<tr>
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Phenotypic Description

The strictly dominant *Plush* mutation was identified in N-ethyl-N-nitrosourea (ENU)-induced G1 mutant mice. The first coat of *Plush* mice is wavy, and strikingly resembles that of *Velvet* mice (which have a dominant mutation in *Egfr*). In older mice, the coat of affected animals looks “plush” instead of creating the disoriented pelage hair seen in *Velvet mice*. Other than hair abnormalities, *Plush* animals are phenotypically normal, and unlike *Velvet* mice, have eyes that are closed at birth.
**Nature of Mutation**

The *plush* mutation was mapped to Chromosome 11, and corresponds to a T to A transversion at position 307 of the *Krt25* transcript, in exon 1 of 8 total exons.

291 AACCTTAATGACCGCTTGGCCTCCTACCTGGAC  
81   -N--L--N--D--R--L--A--S--Y--L--D--

The mutated nucleotide is indicated in red lettering, and causes a leucine to glutamine substitution at residue 86 of the Keratin 25 protein.

**Protein Prediction**

The mouse Keratin 25 (K25) protein has 446 residues, and is 91% identical to the 450 residue human protein. K25 is a cytokeratin belonging to the type I (acidic) keratin family. K25 belongs to a subgroup of the type I keratins known as the type I inner root sheath (IRS) keratins ([1]).

Figure 2. Keratin domain structure showing the ?-helical domain, linker regions and head/tail domains. The *Plush* mutation causes a leucine to glutamine substitution at residue 86 of the Keratin 25 protein. This image is interactive. Click on the image to view other mutations found in Krt25 (red). Click on the mutations for more specific information.
Keratins are intermediate filament (IF) proteins that are primarily involved in the mechanical and structural functions of epithelial tissue. Keratin proteins are composed of an \(\alpha\)-helical rod domain divided into four subdomains, 1A (residues 80-114 for K25), 1B (residues 130-230 for K25), 2A (residues 247-265 for K25), and 2B (residues 274-394 for K25), which are interrupted by a non-helical linker and flanked by non-helical head and tail domains (1). The N- and C-termini of the \(\alpha\)-helical rod domain are highly conserved and known as the helix initiation motif (HIM) and the helix termination motif (HTM), respectively. The HTM motif of most keratins contains the consensus sequence of EIATYRXLLEGEE (2), but the IRS keratins display deviations from this sequence. In type I IRS keratins, this sequence is...
EIETYC(X)L(XXXXX) (3). The ?-helical rod domains, particularly the HIM and HTM motifs, are necessary for interactions with other keratin molecules in order to assemble into IFs (4). The variant HTM sequences in IRS keratins do not seem to affect this property (3). The head and tail domains are sometimes classified into subdomains known as the high homology or H subdomains, regions with sequence variation or V subdomains, and highly charged ends or E subdomains (Figure 2).

In order to form intermediate filaments, the ?-helical chains of two keratin molecules dimerize to form a coiled-coil structure. Keratin dimers then associate via their HIM and HTM motifs in a head-to-tail fashion to form linear arrays, four of which associate in an antiparallel, half-staggered manner to produce protofibrils. Three to four protofibrils intertwine to produce an apolar intermediate filament 10 nm in diameter (Figure 3). The assembly equilibrium is heavily in favor of polymer formation (5). Keratins contain a high proportion of the small amino acids glycine and alanine, which facilitates the assembly of individual keratin molecules into IFs and allows sterically-unhindered hydrogen bonding between the amino and carboxyl groups of peptide bonds on adjacent protein chains, facilitating their close alignment and strong cohesion (4;6).

The Plush mutation results in the substitution of a leucine for a glutamine at amino acid 86 of keratin 25. This residue is located in the HIM located in the 1A ?-helical rod domain.

Expression/Localization

K25 protein and mRNA in humans is localized to the IRS of the hair follicle, and is present in all three layers, the Henle layer, the Huxley layer and the IRS cuticle (please see Background). In the lower portion of the follicle, K25 is present in all three layers. Higher up, K25 expression ceases abruptly in the Henle layer, but continues in the Huxley layer where it disappears slightly below the IRS cuticle. K25 is also expressed in the medulla of human hairs (7). The mouse K25 protein is also likely to be expressed in all three IRS layers as are the mouse homologues of K27 and K28. However, this has not been confirmed due to the lack of an appropriate antibody (3).

In the IRS, keratin IFs are aligned along the longitudinal axis of the cells with trichohyalin, a highly charged keratin-associated protein (KAP) believed to be involved in this alignment (8).

Background

Keratins are cytoplasmic proteins that form the intermediate filaments (IFs) of epithelia including cells of the skin, lung, esophagus, gut and hair. IFs are intermediate in diameter between actin
(microfilaments) and microtubules. Keratins are expressed in a cell type and differentiation specific manner, and are subdivided into the type I (acidic) and type II (basic) keratins. The genes of the type I and type II keratin subfamilies are clustered in the genomes of mice and humans with the type I keratins localized to mouse Chromosome 11 and Chromosome 17q21.2 in humans, and the type II keratins localized to mouse Chromosome 15 and Chromosome 12q13.13 in humans. In humans, there are 54 keratin proteins, 28 type I and 26 type II (3;9). Keratins form obligate heterodimers between one type I and one type II keratin (4). Although all type I keratins can pair with all type II keratins in vitro, in vivo this pairing is much more selective, even when multiple keratins are expressed in the same tissue.

The primary function of keratins is to form a resilient yet adaptable scaffold allowing epithelial cells to sustain mechanical stresses. However, increasing evidence suggests they may be involved in nonmechanical roles such as apoptosis and protein targeting. In order to achieve both mechanical and nonmechanical functions, keratins associate with keratin-associated proteins (KAPs) that result in keratin phosphorylation, glycosylation, transglutamination, proteolytic cleavage, ubiquitination, or association with other cytoplasmic or cytoskeletal elements. KAPs have many functions and include the linker proteins plectin, plakophilin 1 and desmoplakin that connect keratin IFs to cytoskeletal elements, the bundling proteins filaggrin and trichohyalin, adaptor/signaling molecules including 14-3-3 proteins and TNF (tumor necrosis factor) receptor type 2, as well as various kinases, chaperones, and enzymes (10).
Figure 4. Keratin expression in the hair follicle. A, Fully-developed hair follicle. B, Cross-section of hair follicle. Keratins expressed in the layers of the (human) follicle are indicated. Type I keratins are shown in purple, and type II keratins are shown in green. K25 is expressed in all three layers of the IRS as well as the medulla of the hair shaft.

The hair follicle has eight functionally and structurally different epithelial layers: the outer root sheath (ORS) that is continuous with the epidermis, the companion layer (CL), the inner root sheath (IRS) consisting of three layers (Henle’s, Huxley’s and cuticle) and the hair shaft, which also consists of three layers (cuticle, cortex and medulla) (Figure 4). Unlike mouse hair, not all human hair has a medulla (11;12). While the ORS is derived from proliferating cells in the outermost layer, the other layers of the hair follicle are derived from progenitor cells in the matrix of the hair bulb. The morphology of the hair follicle varies with periods of growth (anagen), regression (catagen) and rest (telogen) (13). During anagen, the ORS grows downwards as the CL, IRS, and hair shaft grow upwards. The signaling pathways controlling the differentiation of each layer is poorly understood.

Numerous keratins are expressed in the hair follicle with specific expression patterns in the various epithelial layers. In the IRS of human hair, four type I keratins (K25-K28) and four type II keratins (K71-K74) are expressed. K25, K27, K28 and K71 are expressed in all three layers of the IRS as well as the medulla of the hair shaft. K26, K72, K73 are expressed only in the IRS cuticle, while K74 is expressed in the Huxley layer (9). For the most part, the expression of the IRS keratins is very similar.
in humans and mice. One notable difference is that mouse K71 is not expressed in the IRS cuticle of mouse hairs (14).

Recently, the classical mouse hair/coat phenovariant Rex (Re) was found to have a defect in the Krt25 gene. Re is a semidominant mutation, with both heterozygotes and homozygotes exhibiting curly hair and vibrissae, as well as a growth defect. Both phenotypes are stronger in homozygotes. The diameter of the hair shaft in Re mice is irregular due to morphological abnormalities in all three layers of the IRS, Henle's layer, Huxley's layer, and the cuticle. Re mice also displayed an enlargement of the sebaceous glands and shorter hair follicles, but had normal dermal papillae relative to wild type animals. This allele contains a nucleotide substitution that results in an amino acid substitution of proline for leucine at position 381. An N-ethyl-N-nitrosourea-induced mutation, M100573, also carries a mutation in K25 resulting in an amino acid substitution of asparagine for tyrosine at position 379. Both mutations are located in the helix termination motif of the 2B alpha-helical rod domain of a type I IRS keratin protein. Immunohistological analysis revealed abnormal foam-like immunoreactivity with an antibody raised to type II IRS keratin K71 in the IRS of Re/+ mice. These results suggest that the helix termination motif is essential for the proper assembly of types I and II IRS keratin protein complexes and the formation of keratin intermediate filaments (15).

There are no known disease-causing human mutations in K25 or in any other human IRS keratin. Human mutations in keratins expressed in the other epithelial layers of the hair do cause disease. Mutations in the KRT81, KRT83 and KRT86 genes, which are expressed in the mid-cortex of the hair shaft, cause monilethrix (OMIM #158000) whose hallmark is an unusually deformed hair shaft. A mutation in KRT85, which is expressed very early in the lower most matrix and cuticle of the hair forming compartment of the hair follicle, causes ectodermal dysplasia of hair and nail type (OMIM #602032) (9).
Putative Mechanism

The Plush mutation alters a highly conserved amino acid in the HIM of the K25 protein. As this domain is critical for heterodimerization of keratin molecules, it is likely that the Plush mutation disrupts the proper formation of IF assembly resulting in the hair phenotypes seen in mutant animals.

A large number of autosomal dominantly transmitted diseases of the skin, hair, and various internal epithelia have been found to be caused by mutations in keratin genes. The resulting autosomal dominant pathologies are primarily due to the inability of the mutated keratin protein to form stable IFs with its intact endogenous partner of the opposite type. This leads to an accumulation of disorganized IF bundles that eventually result in failure of tissue integrity, in particular on exposure to mechanical stress. In the majority of cases, the mutations result in inappropriate amino acid substitutions at the beginning of subdomain 1A or at the end of subdomain 2B of the ?-helical rod of either type I or type II keratins (9). In humans, K25 is expressed in all three layers of the inner root sheath. Although no known disease-causing human mutations exist in this gene, it is likely that mutations in K25 will affect the architecture of the IRS as well as cuticle formation. There are numerous other type I keratins expressed in the three layers of the IRS, and likely some redundancy occurs amongst the keratins in the hair follicle. However, mutations in keratins often cause dominant disorders of the skin and hair, because the mutant proteins are expressed and disrupt the natural formation of the intermediate filaments. Two other type I IRS keratins are expressed in all three layers of the IRS, K27 and K28, but only one type II IRS keratin, K71, is expressed in all three layers (in humans) and is the only type II keratin expressed in the Henle layer of the IRS (16). K71 is thus the only available partner for K25, K27 and K28 in the Henle layer. Not surprisingly, mutations in K71 cause hair disorders in mice including the classical wavy coat mutation, caracul (Ca). Mice carrying Ca alleles strongly resemble the K25 mouse mutant, Re, and many of these mutations occur in the HIM and HTM domains of the K71 gene (9).

Genotyping

Plush genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change.

Primers for PCR amplification

Plush(F): 5’- CATCCAATTACGTTAACCTTGCTGAGGC -3’
Plush(R): 5’- CAGCCAAGGGCATGTTTAACATGAAG -3’

PCR program

1) 94°C 2:00
2) 94°C 0:30  
3) 56°C 0:30  
4) 72°C 1:00  
5) repeat steps (2-4) 29X 
6) 72°C 7:00  
7) 4°C ?

**Primers for sequencing**
Plush_seq(F): 5’- TTCGGAGCTGGAATGCATG -3'  
Plush_seq(R): 5’- CTACTGTAGTCATGATCGAGACC -3’

The following sequence of 1044 nucleotides (from Genbank genomic region NC_000077 for linear DNA sequence of Krt25 plus 127 additional nucleotides taken from NCBI m37 mouse assembly Chromosome 11: 99177158:99184400) is amplified:

-127 catccaa
-120 ttacgttaac ttgctgaggg tggcaagccca ggctttaccc tatgtaggca gaatttcata 
-60 gacaaccttt gagaatcgtc tataaaaggg caaacaacaac catcggggttt agaaggcact 
1 ctagtctgac tctcaagaac acagttcagc gacagcgttg ccctgagact atgtctcttc 
61 gcctttccag tggatccagg aggtcttatg ctcgccccag cacagggtcg ctcaggggag 
121 ccagcttcg tggcagggagacatgggaat gcatgtggcc tggcagggct tagaagtggc ggctctctggg 
181 ccttcgccccca gacgcctttg gggggttggc caacctccgctt gctgggcttc 
241 cttcccctttg ctcctcggc agctcccaat atttttttttattttttttt gataaagatg gacatcagc gacactacag ttaggtaata atggagatg clear

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Mutagenetix Phenotypic Mutation 'Plush'

agaaagtata
481 tttttgcacg tcacatgtat gtagtttact atctaaatgt cctttttact
gaaaaaaaaaa
541 aaaagcatgc tacatgatta ctagtggtat ttttaagggc atttaagggc
attggaaata
601 atcatgaagt agattcaagt atttaacttc atgttaaaca tgcccttgqc tq

PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is shown in red text.

References