Desmoglein 4 Mutations Underlie Localized Autosomal Recessive Hypotrichosis in Humans, Mice, and Rats

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A newly defined form of inherited hair loss, named localized autosomal recessive hypotrichosis (LAH, OMIM 607903), was recently described in the literature and shown to be linked to chromosome 18. A large, intragenic deletion in the desmoglein 4 gene (*DSG4*) as the underlying mutation in several unrelated families of Pakistani origin. LAH is an autosomal recessive form of hypotrichosis affecting the scalp, trunk, and extremities, and largely sparing the facial, pubic, and axillary hair. Typical hairs are fragile and break easily, leaving short sparse scalp hairs with a characteristic appearance. Using comparative genomics, we also demonstrated that human LAH is allelic with the *lanceolate hair* (lah) mouse, as well as the *lanceolate hair* (lah) rat phenotype. Together, these models provide new information about the role of desmosomal cadherins in disease, and serve as *in vivo* models for functional and mechanistic studies into the role of desmoglein 4 in the skin and hair follicle.

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Dsg4 is expressed in the inner epithelial layers of the hair follicle, where its function appears to be crucial during differentiation of the hair follicle layers. The significance of properly orchestrated adhesion during hair follicle development is underscored by several human disorders that result from mutations in genes encoding components of adhesion plaques (McKoy *et al*, 2003; Norgett *et al*, 2000, McGrath *et al*, 1997). The desmosomal plaque is composed of proteins from three different protein families, the desmosomal cadherin, plakin, and armadillo families. Mutations in genes encoding proteins in all three families have been shown to result in disorders of skin and hair follicle. For example, mutations in desmoplakin and plakoglobin, members of plakin and armadillo families, respectively, underlie Naxos disease (OMIM 601214, 605676). Naxos disease is an autosomal recessive disorder characterized by wooly, sparse hair, keratoderma, and cardiomyopathy (McKoy *et al*, 2000; Norgett *et al*, 2000). Recessive mutations in plakophillin 1, another armadillo family member, result in ectodermal dysplasia with sparse hair and skin fragility (OMIM 604536) (McGrath *et al*, 1997).

Interestingly, *DSG4* is the only desmosomal cadherin, thus far, which has been associated with a human hair phenotype (Huber, 2003; Kljuic *et al*, 2003a). To date, no diseases have been described resulting from mutations in desmocollins and the dominant mutations identified in *DSG1* result in striate palmoplantar keratoderma (OMIM 148700), characterized by thickening of the skin on palms and soles but no hair involvement (Rickman *et al*, 1999; Kljuic *et al*, 2003c). Furthermore, no human mutations have been found in *DSG2* or *DSG3* genes although mutations in the mouse *Dsg3* result in the balding phenotype, characterized by cyclical hair loss (Koch *et al*, 1997; Pulkkinen *et al*, 2002).

Abbreviations: *DSG4*/DSG4, human desmoglein 4 gene per protein; LAH, localized autosomal recessive hypotrichosis

The Expanding Family of Desmosomal Cadherins

Prior to the discovery of Desmoglein 4, six desmosomal cadherin genes had been identified in the human and mouse genomes: three desmocollins (Dsc1, 2, and 3) and three desmogleins (Dsg1, 2, and 3). The initial identification of these proteins and subsequent cloning of the genes resulted from a series of elegant biochemical experiments that spanned more than a decade of work in the late 1970s and the early 1980s. All six genes were mapped to a single genomic cluster of 700 kb (Hunt *et al*, 1999) and the genomic structure of all six human and mouse genes has been deciphered (Huber, 2003).

Our contribution to gene discovery within this cluster occurred during our attempt to map a mutation responsible for hypotrichosis in the mouse (Kljuic *et al*, 2003a). During our sequence analysis of Dsg1 in this mouse, we noticed an unusually high degree of double and triple sequences corresponding to *Dsg1* in both wild-type and mutant animals. A detailed analysis of these *Dsg1* sequences and BLAT searches at the UCSC Genome Draft site indicated that the locus contained not one, but three, *Dsg1* genes with an exceptionally high overall homology to each other. Together with our collaborators, we cloned the two new genes and designated them *Dsg1*β and *Dsg1*γ (Pulkkinen *et al*, 2003; Kljuic and Christiano, 2003). This designation was chosen since the overall amino acid identity between the three

proteins is 83%, as compared with 32% and 40% identity to Dsg2 and Dsg3, respectively. Both *Dsg1*α (the original Dsg1 gene) and *Dsg1*β were found to be highly expressed in skin. On the other hand, *Dsg1*γ, was found in several additional tissues tested, namely brain, liver, and skeletal muscle. Interestingly, *Dsg1*γ expression was not very high in skin suggesting that although Dsg1α and *Dsg1*β might be *Dsg1* candidate genes for skin related traits, *Dsg1*γ may have more important roles in other desmosome bearing tissues.

We next searched for the human orthologs of these genes, again using the UCSC Genome Draft site. Based on sequence comparisons and gene prediction software, we found only one potential new gene within the human desmosomal cluster. Sequence alignments and homology comparisons with both human and mouse desmoglein genes, however, indicated that the new gene was not the human *Dsg1*β or *Dsg1*γ ortholog, but yet a third novel desmoglein gene, which we designated desmoglein 4 (*DSG4*) (Kljuic *et al*, 2003a; Whittock and Bower, 2003). On the amino acid level, DSG4 shares 38%, 36% and 46% identity and 52%, 51%, and 62% homology to DSG1, DSG2, and DSG3, respectively. This level of homology is comparable with levels among other desmogleins. Once again utilizing *in silico* cloning, we also identified the mouse desmoglein 4 (*Dsg4*) gene. We next tested whether desmoglein 4 was expressed using RT-PCR. We successfully amplified and sequenced *DSG4* from human adult skin RNA. At the amino acid level, the human and mouse desmoglein 4 share 79% identity and 86% homology to each other, as expected for orthologous proteins.

The four new proteins all exhibited the hallmarks of a desmosomal cadherin and from the N-terminus are organized into four extracellular cadherin repeats (EI-EIV), an extracellular anchoring domain, a transmembrane domain, an intracellular anchoring domain, an intracellular cadherin specific sequence, a linker domain, intracellular repeated unit domains (RUD), and in the case of Dsg1b and both desmoglein 4 proteins, a terminal domain (TD) at the C-terminus. The Dsg1γ protein, however, is truncated at the C-terminus lacking 16 of the last amino acids of the RUD domain and the entire TD. The RUD domain is a sequence of unknown function yet it is unique to all desmoglein genes. In both human and mouse, *Dsg1* has five repeats, *Dsg2* has six, and *Dsg3* only two repeats. Consistent with this data, *Dsg1*β and *Dsg1*γ genes have five RUD repeats, and both human and mouse desmoglein 4 genes have three repeats.

The gene order in the human desmosomal cadherin cluster is now: DSC3-DSC2-DSC1-DSG1-**DSG4**-DSG3-DSG2 and in the mouse: Dsc3-Dsc2-Dsc1-**Dsg1**γ-Dsg1α-**Dsg1**β-**Dsg4**-Dsg3-Dsg2. The rat desmosomal cadherin cluster is more similar to that of humans than mice. In this respect, the rat genome has only one desmoglein 1 (*rDsg1*) and the gene order in the cluster is identical to that of humans: Dsc3-Dsc2-Dsc1-Dsg1-Dsg4-Dsg3-Dsg2.

Identification of Dsg4 Mutations in LAH Families from Pakistan

The first evidence for the essential role of desmoglein 4 in skin and hair follicle was the identification of mutations in families with a novel form of hypotrichosis, named localized autosomal recessive hypotrichosis (LAH). We originally described two large consanguineous pedigrees originating from Pakistan in which affected members displayed hypotrichosis restricted to the scalp, chest, arms, and legs (Kljuic *et al*, 2003a). Facial hair, including the eyebrows and beard, was less dense, and axillary, pubic hair, and eyelashes were spared. The patients&aops; skin was normal with the exception of patches of scalp with small papules. Analysis of human desmoglein 4 gene (*DSG4*) revealed an in-frame deletion spanning exons 5–8 in the patients. The deletion begins 35 bp upstream of exon 5 (within intron 4) and ends 289 bp downstream of exon 8 (within intron 8). The mutation was designated EX5_8del and is expected to result in an internally deleted protein missing amino acids 125–335. These amino acids correspond to part of the extracellular domains 1 and 3, and all of extracellular domain 2, which are believed to be critical in cadherin–cadherin interaction and dimerization (Boggon *et al*, 2002).

Recently, our group and others (Rafiq *et al*, 2004; Moss *et al*, 2004) reported the identification of the same deletion mutation in one and three additional LAH Pakistani families, respectively. So far, the EX5_8del mutation has been identified in a total of six unrelated families originating from different regions in Pakistan and currently living in UK, USA, and Pakistan. The dispersion of the identical EX5_8del desmoglein 4 mutation in Pakistani families throughout widespread geographic regions suggests that this allele may represent an ancestral mutation in this population.

Identification of Dsg4 Mutations in the Lanceolate Hair (lah) Mouse

We also cloned the corresponding mouse desmoglein 4 gene and identified two mutant alleles in *lanceolate* mice (Klijuic *et al*, 2003a). Both alleles, *lah*/*lah* and *lah*^{J}/*lah*^{J}, develop sparse and broken hair shortly after birth and their skin histology highly resembles that of affected human subjects. The abnormal hair follicles in these mutant mice develop an amorphous keratinized bleb at the bulb of the hair follicle during the anagen growth stage of the hair follicle. This “bleb” resembles a lance-head tip (hence the *lanceolate* nomenclature), is pushed upward by an abnormal hair shaft until it penetrates the skin and breaks off.

Upon analyzing the *Dsg4* genomic sequences in the *lanceolate* mutant models, we found that *lah*^{J}/*lah*^{J} animals harbor a homozygous single base insertion within exon 7, designated 746insT. This insertion leads to a frame-shift that creates a premature stop codon three codons downstream. Sequence analysis of the *lah*/*lah* mutant revealed a missense homozygous mutation in exon 6 converting a tyrosine to serine, designated Y196S. This mutated residue is highly conserved among the different cadherin family members. RT-PCR analysis revealed that *lah*^{J}/*lah*^{J}, but not *lah*/lah, animals are a null model for *Dsg4*.

Dsg4 Mutations Underlie the Lanceolate Hair Rat Model

In addition to the human and mouse desmoglein 4 genes, we have recently cloned the rat homologue (*rDsg4*)

and subsequently identified a homozygous missense mutation in the *lanceolate* (*lah*/*lah*) rat (Jahoda *et al*, 2004). The *lanceolate* rat phenotype, including skin and hair histology, bears striking similarity to that of *lanceolate* mice, especially the presence of lance-head tips. The identified mutation, an A-to-T transition in exon 6, changes a highly conserved glutamic acid residue at position 228 in extracellular domain 2 (EC2) to a valine residue. Based upon comparative structural studies with the crystal structure of C-cadherin, such a mutation disrupts an essential calcium coordinating residue thus leading to a disruption in side-chain interaction and therefore intermolecular interaction and cadherin–cadherin binding. (Jahoda *et al*, 2004). Recently we have identified two additional *lanceolate* rat models (Bazzi *et al*, 2004; Meyer *et al*, 2004). The Iffa Credo rat (IC) was identified based on phenotypic and histological similarities to *lah*/*lah* animals and harbors a large out of frame intragenic deletion (Ex2 10 del) (Bazzi *et al*, 2004). The other *lanceolate* model arose on the spontaneously hypertensive rat (SHR) background and the nonsense mutation in its Dsg1 gene was mapped using classical linkage analysis (Meyer *et al*, 2004).

Conclusions

Cell adhesion and intercellular communication is essential during the morphogenesis and homeostasis of tissues and organs. The importance of desmosomes in stratified epithelia and their appendages is evident through a variety of human diseases, as well as animal models harboring mutations in desmosomal plaque encoding genes. We have shown that desmoglein 4, a novel desmosomal cadherin, is indispensable for the integrity of human and murine hair shaft differentiation. Desmoglein 4 is expressed in a dynamic region in the hair bulb where proliferation and differentiation intersect, and where cells choose one of at least seven possible fates that eventually make up the hair shaft and the surrounding inner root sheath. We hypothesize that the absence of desmoglein 4 leads to a breakdown in communication thus preventing the cells from conveying the proper signals to each other and hence adopting aberrant fates. The wealth of animal models available allows us to further study how desmoglein 4 functions in the process of hair follicle differentiation.

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