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A Recurrent Intragenic Deletion Mutation in DSG4 Gene in Three Pakistani Families with Autosomal Recessive Hypotrichosis

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To the Editor:

Localized hypotrichosis (MIM 607903) is a rare form of alopecia and is inherited in an autosomal recessive pattern (Kljuic *et al*, 2003; Rafique *et al*, 2003). In individuals affected with this form of hair loss, hypotrichosis is restricted to the scalp, chest, arms, and legs. Facial hair, including the eyebrows and beard, is less dense, and axillary, pubic hair, and eyelashes are sparse. At birth, hairs are present on the scalp, but regrow sparsely after ritual shaving (Fig 1). Affected individuals show no growth or developmental delay, normal hearing, teeth and nails, and no abnormalities in sweating. Histological analysis of scalp skin of the patient's (Kljuic *et al*, 2003) revealed abnormal hair follicles and shafts, which were thin and atrophic and often appeared coiled up within the skin due to their inability to penetrate the epidermis.

We, and others (Kljuic *et al*, 2003; Rafique *et al*, 2003) recently reported a linkage in this form of hypotrichosis to chromo some 18q12. A small region of 700 kb on chromo-some 18q12.1 contains cluster of Desmoglein (DSG1, DSG2, and DSG3) and desmocollin (DSC1, DSC2, and DSC3) genes. Both desmoglein and desmocollins are the glycoproteins of desmosomes, which are the most common type of intercellular junctions mediating cell-to-cell adhesion in vertebrate epithelial cells (Townes and Behringer, 1990). Recently, Kljuic *et al* (2003) and Whittock and Bower (2003) reported the cloning of DSG4, a new member of desmoglein. DSG4 gene is composed of 16 exons spanning approximately 37 kb of genomic DNA and is situated between DSG1 and DSG3. The 3.6-kb human DSG4 cDNA contains an open reading frame of 3120 bp that encodes a precursor protein of 1040 amino acids. The predicted mature protein comprises 991 amino acids with a molecular weight of 107,822 Da. The human DSG4 protein shares 41% identity with human DSG1 protein, 37% with human DSG2 protein, and 50% with human DSG3 protein. The DSG4 syntenic region on mouse chromosome 18 harbors desmosomal cadherin cluster (Montagutelli *et al*, 1996). Recently,

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three additional desmoglein genes (Dsg4, Dsg1b, Dsg1c) have been identified in the cadherin cluster (Kljuic and Christiano, 2003; Pulkkinen *et al*, 2003; Whittock, 2003).

Using RT-PCR on multiple tissue cDNA samples, Whittock and Bower (2003) and Kljuic *et al* (2003) demonstrated that DSG4 has very specific tissue expression in salivary gland, testis, prostate, and skin. Kljuic *et al* (2003) provided evidence that DSG4 is a key mediator of keratinocyte cell adhesion in the hair follicle, where it coordinates the transition from proliferation to differentiation. In affected individuals from two consanguineous Pakistani pedigrees with localized autosomal recessive hypotrichosis (LAH), Kljuic *et al* (2003) identified an identical homozygous deletion mutation (EX5_8del) within the DSG4 gene. The deletion began 35 bp upstream of exon 5 and ended 289 bp downstream of exon 8. This mutation generated an in-frame deletion creating a predicted protein missing amino acids 125–335.

Since the candidate linkage interval of 5.5 Mb identified in three families (LAP1, LAP2, and LAP3) reported by us (Rafique *et al*, 2003) contains all the desmoglein and desmocollin genes, we screened the DSG4 gene for mutations in all three families. Exons and splice junctions were PCR amplified from genomic DNA by primer sets designed from intronic regions of DSG4 and sequenced directly in an ABI Prism 310 Big Dye Terminator Cycle Sequencing reaction Sequencing Kit (PE Applied Biosystems, Foster City, California) following purification in a Centri-Sep Spin Columns (Applied Biosystem). Exons 5–8 failed to get amplified from the genomic DNA of affected individuals in all the three families. Therefore, a new set of primer was designed from introns 4 and 8 to search for the mutation reported earlier by Kljuic *et al* (2003). The primer sequences used were 5′-AAACATGGCAGACTGAACCC-3′ 5′-CAGTATGCAAGGTTCTCAGC-3′

Surprisingly, the same recurrent mutation (EX5_8del) (Fig 1) was identified in all the three families. The identification of a recurrent mutation in DSG4 may suggest that these are hotspot mutations within the DSG4 gene, or may be due to a common ancestor. The fact that all five families including two in Kljuic *et al* (2003) and three in Rafique *et al* (2003) belong to Pakistan, although with different ethnic origin, have the same mutation is highly suggestive of an ancestral allele. Upon examining the haplotypes for LAP1, LAP2, and LAP3 (Rafique *et al*, 2003) it was observed that the disease mutation in the three families appeared on very similar haplotypes. All three families share the same disease haplotype for markers D18S1104 (142), D18S1107 (120), D18S478 (240), D18S847 (220), D18S1133 (192), and D18S67 (121). As members of the cadherin superfamily, the desmoglein and desmocollin genes possess similar structural functional domains, including sites for adhesion recognition, calcium binding, membrane integration, cytoskeletal interactions, and post-translational modifications, such as phosphorylations, glycosylation, and proteolysis (Grunwald, 1993). Therefore, in addition to their functions as static adhesive proteins, they also function as dynamic mediators of morphogenesis during embryonic development and are modulated in response to signals such as calcium concentration, in cell differentiation, motility and are potentially involved in disease phenotypes (Silos *et al*, 1996). Certain members of the desmosomal cadherin family have been implicated previously in a number of acquired and inherited skin diseases (Calvanico *et al*, 1991; Karpati *et al*, 1994; Hashimoto *et al*, 1997).

Kljuic *et al* (2003) determined that mutations in the Dsg4 gene cause the lanceolate hair (lah) phenotype in mice, which maps to chromosome 18. lah/lah pups develop only a few short, fragile hairs on the head and neck that disappear within a few months. The vibrissae are short and abnormal, and the pups have thickened skin. Mutant lah/lah mice do not exhibit any growth retardation relative to their unaffected littermates.

Desmoglein 4 in mouse (Dsg4) and human DSG4 share 79% and 86% amino acid identity and homology, respectively (Whittock and Bower, 2003). The human and mouse mRNA were highly expressed in skin. *In situ* hybridization of mouse skin section and vibrissae follicles revealed that Dsg4 is expressed in anagen stage hair follicles (Kljuic *et al*, 2003; Whittock and Bower, 2003). DSG4 was also detected within anagen follicles where its expression commenced in the matrix and extended throughout precortical cells and IRS. The presence of desmoglein 4 in the inner layer of the hair follicle where DSG1 and DSG3 also show some expression (Wu *et al*, 2003), suggest a critical role for desmoglein 4 in differentiation of ascending HF layers.

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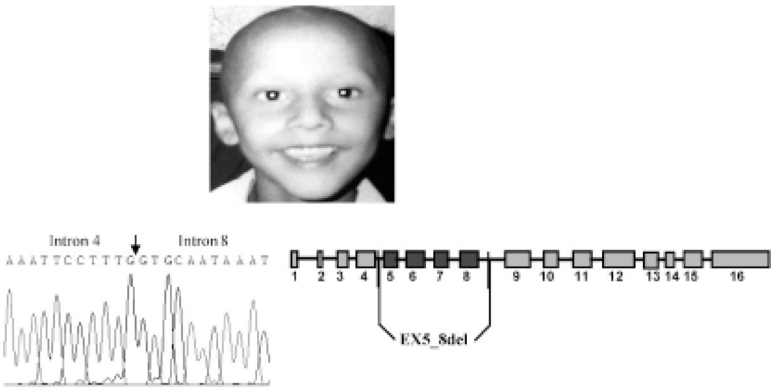

Figure 1. Clinical presentation (above) of the hypotrichosis phenotype from previously reported family LAP3.

DSG4 deletion (below) located in introns 4 and 8. Arrow indicates the breakpoint region.