

A Recurrent Intragenic Deletion in the Desmoglein 4 Gene Underlies Localized Autosomal Recessive Hypotrichosis

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

To the Editor:

A newly defined form of inherited hair loss, named localized autosomal recessive hypotrichosis (LAH, OMIM 607903), was recently described in the literature (Kljuic et al. 2003a; Rafique et al. 2003) and shown to be linked to chromosome 18. We identified a large, intragenic deletion in the desmoglein 4 gene (DSG4) as the underlying mutation in two unrelated families of Pakistani origin (Kljuic et al. 2003a). 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 (Kljuic et al. 2003a), as well as the lanceolate hair (lah) rat phenotype (Jahoda et al. 2004). In order to expand the series of allelic mutations in the desmoglein 4 gene underlying LAH in humans, we begin molecular analysis of DSG4 in families from around the world.

Here, we describe the study of a family of Pakistani origin with two siblings affected with LAH (Figure 1). The two affected children, a girl aged 5 y 9 mo and a boy aged 18 mo, have two sisters with normal hair. Their parents, first cousins of Pakistani origin, are unaffected. They are part of a large family with extensive consanguinity but no other affected individuals. Both affected children were born without hair and neither infant was ritually shaved. Subsequently, sparse hair growth was accompanied by itching, redness, and roughness of the scalp. Both children are otherwise healthy and developing normally. The findings on serial examination have been the same in both children. At the age of 2 mo the proband showed complete alopecia with scalp follicular prominence. By 15 mo there was sparse, coarse, brittle hair with follicular hyperkeratosis, erythema, and scaling affecting particularly the scalp, but also eyebrows and eyelashes. Now aged 5, the girl's scalp hair

remains sparse and is clearly brittle, less than 1 cm long at sites of friction and up to 8 cm in other areas. She now has marked follicular hyperkeratosis on the extensor aspects of the limbs. The skin is otherwise normal with no papular lesions on the limbs, and no palmoplantar keratoderma. Sweating, teeth, and nails appear normal. The clinical findings are most consistent with a diagnosis of LAH (OMIM#607903).

We obtained DNA from the two affected individuals and both parents. Genomic DNA was isolated from peripheral blood collected in EDTA-containing tubes according to standard techniques (Sambrook et al. 1989). All samples were collected following informed consent. To screen for a mutation in the human DSG4 gene, all exons and splice junctions were PCR amplified from genomic DNA (Table S1) and sequenced directly in an ABI Prism 310 Automated Sequencer, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, California), following purification in Centriflex Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, Maryland) as we described earlier (Kljuic et al. 2003a). The mutation was identified by visual inspection and comparison with control sequences generated from unrelated, unaffected individuals. The deletion mutation is identified by the failure to PCR amplify exons 5, 6, 7, and 8 from homozygous affected individuals, followed by PCR (refer to Table S1 for PCR primers) and direct sequencing of the breakpoints in the surrounding introns (Figure 2).

The deletion in DSG4 begins 35 bp upstream of exon 5 (within intron 4) and ends 289 bp downstream of exon 8 (within intron 8). This results in an in-frame deletion, leading to an internally truncated protein missing amino acids 125–335. These amino acids correspond to part of the extracellular repeat domain EC1, all of extracellular repeat domain EC2, and the beginning of the extracellular repeat domain EC3. These regions of DSG4 are believed to be critical in cadherin–cadherin interaction and dimerization (Boggon et al. 2002; He et al. 2003) necessary for proper cell–cell adhesion. Missense mutations in this same region have recently been demonstrated in the lah/lah mouse (Kljuic et al. 2003) and the lah/lah rat (Jahoda et al. 2004).

Desmoglein 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 adhesion plaque genes. 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 woolly, sparse hair, keratoderma, and cardiomyopathy (McKoy et al. 2000; Norgett et al. 2000). Recessive mutations in plakophilin 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 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. 2003b). 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).

It is not surprising that mutations in molecules that regulate desmosomal function can also give rise to related skin and hair phenotypes. Hailey–Hailey disease (HHD) (OMIM 604384) and Darier (DD) (OMIM 124200) disease which affect calcium pumps both present with loss of epidermal cell adhesion, acantholysis, and abnormal keratinization (Hu et al. 2000; Sakuntabhai et al. 1999). Furthermore, mutations in the components of the desmosome attached cytoskeleton, such as the IF keratin genes, hHb6 and hHb1, to the hair dystrophy disease, monilethrix (OMIM 158000) (Korge et al. 1998).

Mutations in P-cadherin, a member of the classical cadherin family and a component of adherent junctions, another type of adhesion plaque, have also been shown to result in hypotrichosis with fragile, beaded shafts and macular dystrophy (Sprecher et al. 2001; Indelman et al. 2002). It is interesting to note that one of the mutations described for P-cadherin is a missense mutation of a conserved residue within the fourth extracellular domain. All cadherins share a high level of homology with respect to protein domain organization. Each cadherin consist of five extracellular repeat domains (EC1-5), the transmembrane region, and the intracellular tail. The observation that mutations in the EC domains in both desmosomal and classical cadherins lead to comparable hypotrichosis phenotype underscores the functional similarity of the two proteins as well as the critical role of EC domains in epithelial adhesion.

We have previously identified the same deletion of exons 5–8 in the DSG4 gene in two Pakistani families, one residing in the US (Kljuic et al. 2003a). A recent report of three additional Pakistani families (Rafique et al. 2003) with LAH-like features and linked to chromosome 18 also suggests that DSG4 mutations underlie the disease in these families as well. Here, we report the identification of a LAH pedigree in the United Kingdom. There is a large Pakistani population in

the UK; therefore, this report should raise the awareness of LAH as a differential diagnosis to clinicians in this part of the world. Interestingly, the propagation of the identical EX5_8del desmoglein 4 mutation in Pakistani families throughout widespread geographic regions suggests that this allele could represent an ancestral mutation that has been widely dispersed.

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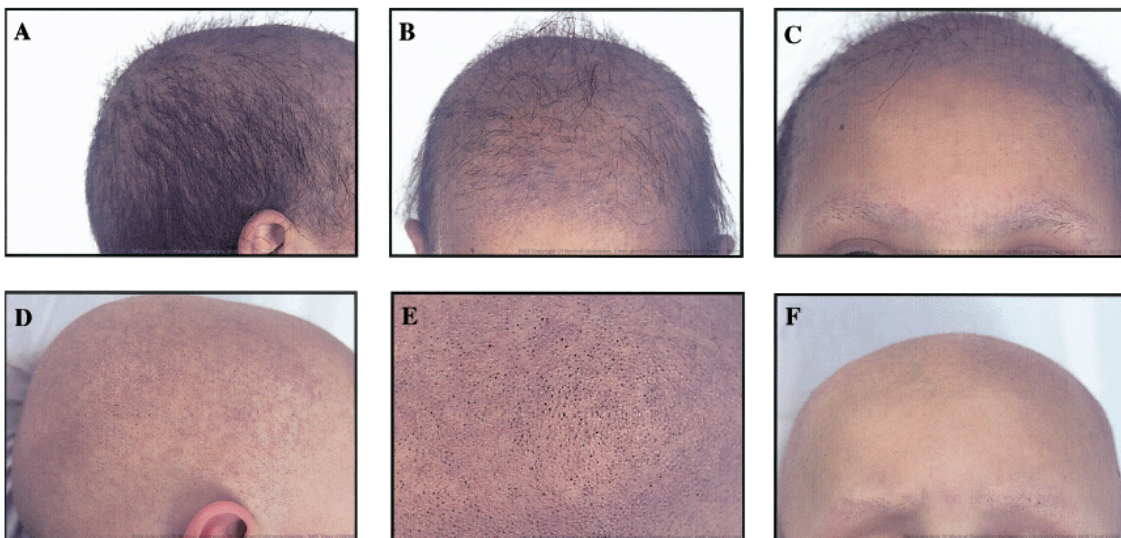
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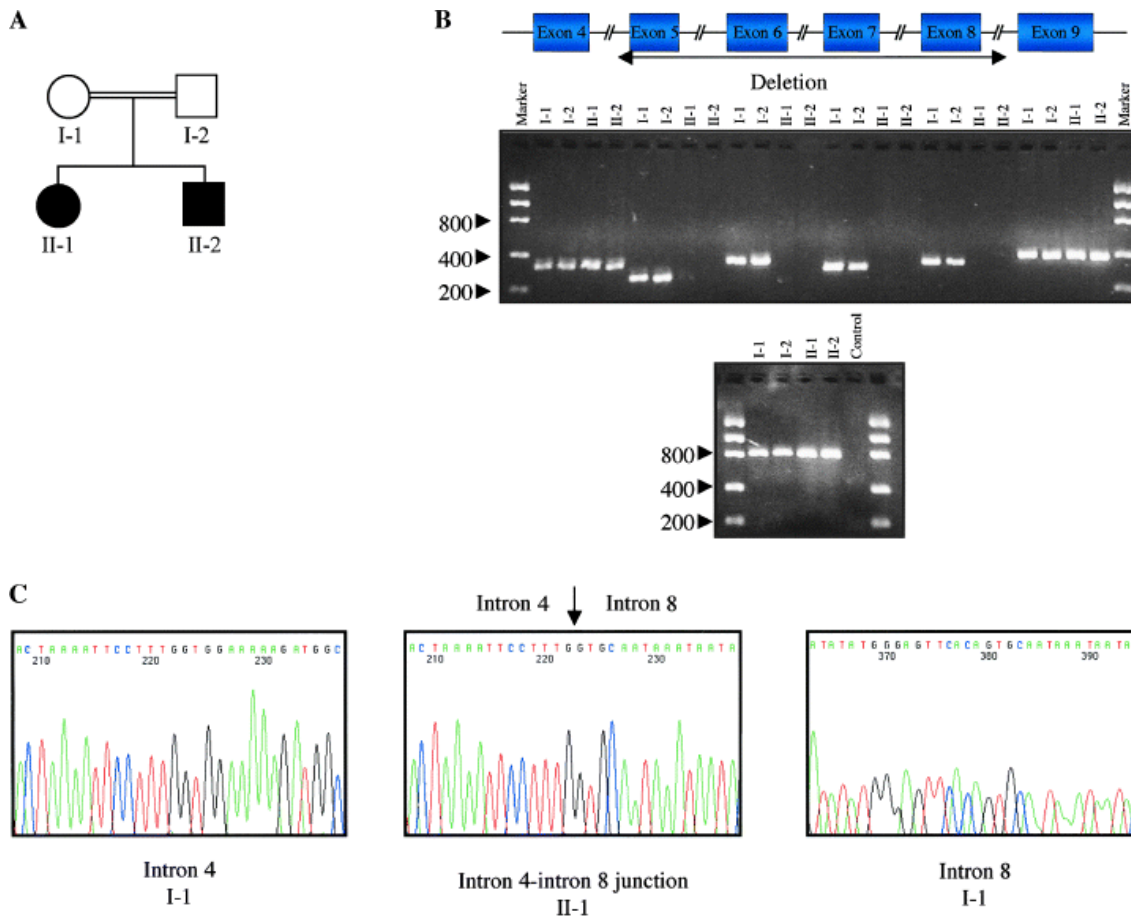
Figures

Figure I



Clinical presentation of LAH in individuals II-1 (A–C) and II-2 (D–E). (A) Note the sparse hair in the older sibling, age 5 y and 9 mo (A–C) and the complete alopecia in the younger sibling, aged 18 mo (C–D). Follicular hyperkeratosis is apparent on the scalp and the eyebrows.

Figure II



Molecular analysis of the DSG4 gene in the family. (A) The two affected siblings belong to a consanguineous pedigree, with first-cousin parents. (B) PCR amplification of exons 4 through 9 (upper panel) revealed absence of amplification of exons 5–8 in the two patients (II-1 and II-2), whereas PCR bands of correct size were obtained from the parents' genomic DNA (I-1 and I-2). When using a forward PCR primer in intron 4 and a reverse primer in intron 8 (lower panel), a novel PCR fragment was obtained in all family members, corresponding to the deletion allele. (C) Sequence analysis of the PCR products revealed a homozygous deletion encompassing exons 5 through 8 in the two affected individuals. Both parents are heterozygous for a wild-type and a deletion allele. The sequence of the mutant allele at the intron 4–intron 8 junction is shown (arrow). Wild-type maternal sequence for introns 4 and 8 are shown for comparison.