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# Localized Autosomal Recessive Hypotrichosis Due to a Frameshift Mutation in the Desmoglein 4 Gene Exhibits Extensive Phenotypic Variability within a Pakistani Family

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## 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 and shown to be linked to chromosome 18 by us and others (Kljuic *et al.*, 2003a; Rafique *et al.*, 2003). We first 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). This mutation and others have subsequently been found in other Pakistani families around the world (Moss *et al.*, 2004; Rafiq *et al.*, 2004; John *et al.*, 2006). Additionally, other mutations have been identified in patients who have monilethrix hairs as part of their phenotypic presentation (Schaffer *et al.*, 2006; Schweizer, 2006; Shimomura *et al.*, 2006; Zlotogorski *et al.*, 2006) that have begun to broaden our understanding of the genotype–phenotype relationships within LAH. LAH typically affects the scalp, trunk, and extremities, 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 *lah* rat phenotype (Jahoda *et al.*, 2004; Bazzi *et al.*, 2004; Meyer *et al.*, 2004). In order to expand the allelic series of mutations in the *DSG4* underlying LAH in humans, and to gain an appreciation of the variation in clinical phenotype among affected individuals, we are performing molecular analysis of *DSG4* in families from around the world.

Here, we describe a large consanguineous family of Pakistani origin with four males and two females affected with LAH with varying degrees of phenotypic severity (Figure 1a and b, Figure S1a and b). All affected children were born without hair and hairs were ritually shaved at approximately 3 weeks of age. Subsequently, sparse, coarse hair growth occurred and was sometimes accompanied by itching, redness, and roughness of the scalp. Fragile hairs were present in all affected individuals (Figure 1, Figure S1). All children are otherwise healthy and developing normally.

A comparison of the clinical findings of the four male individuals revealed marked variability within the family that is not age-dependent in terms of severity. Individuals V-4, V-1, V-6, and VI-1 were 5, 7, 13, and 17 years of age at the time of examination (Figure 1a,b, Figure S1a and b, Figure 2a). V-4 and V-1 display a relatively mild sparse hair phenotype, with stiff, sparse, coarse, brittle hair with sparing of the eyebrows and lashes (Figure 1a). In contrast, VI-6, 17 years of age, presented with sparse and brittle hair, follicular hyperkeratosis, erythema, and scaling affecting particularly the scalp, as well as the eyebrows and eyelashes (Figure 1b, Figure S1b). Finally, individual V-6, 13 years of age, is almost completely without hair, and exhibits the most extreme phenotype within the family (Figure 1b, Figure S1a). Plucked hair fibers from affected individuals revealed the presence of abnormally broken hairs and tapered ends, as well as occasional swellings in the hair shaft (Figure 1c and d, Figure S1c and d). In all affected individuals, the skin is otherwise normal with no papular lesions on the limbs, and no palmoplantar keratoderma. Sweating, teeth, and nails are normal and no beaded monilethrix hairs were observed in

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

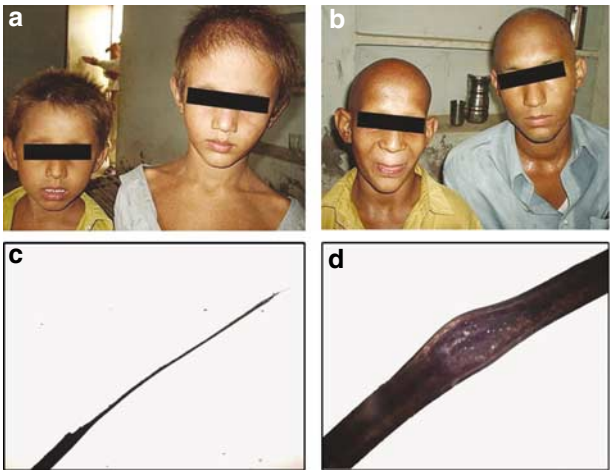


**Figure 1. Variable clinical presentation of LAH in the affected males.** (**a**) individuals V-1 (right, 7 years old) and V-4 (left, 5 years old) are first cousins (see Figure 2a) with sparse abnormal hair that is more evident in V-1. (**b**) Hypotrichosis in individuals V-6 (left, 13 years old) and VI-1 (right, 17 years old) is more severe especially in individual V-6 who completely lacks eyebrows. (**c**, **d**) Hair shafts from the affected males typically have (**c**) tapered broken ends in addition to (**d**) monilethrix-like nodes.

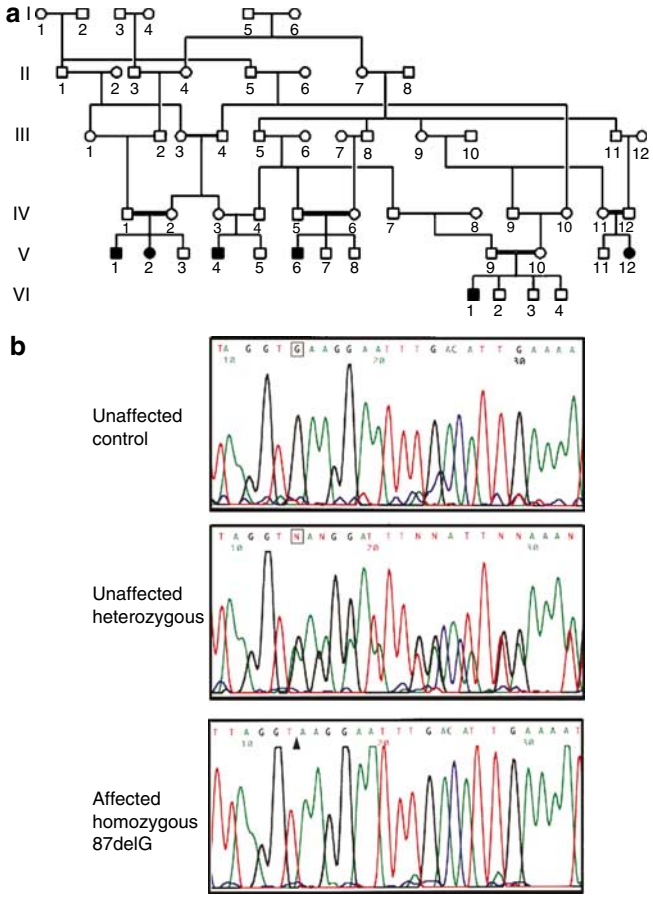


**Figure 2. Pedigree of the large consanguineous family with representative molecular analysis of *DSG4* in affected, carrier, and unaffected individuals.** (**a**) Pedigree of the Pakistani family showing several generations of consanguinity and the affected individuals in generations V and VI. (**b**) Sequence analysis of the *DSG4* gene revealed a single nucleotide deletion in the 5′ splice junction of exon 3 (87delG) in affected individuals (bottom). Unaffected individuals exhibit a wild-type allele (top), whereas heterozygous carriers have both the normal and wild-type alleles (middle).

these individuals. Despite the marked variation in severity, the clinical findings are most consistent with a diagnosis of localized autosomal recessive hypotrichosis.

We obtained DNA from the six affected individuals and 12 unaffected individuals from the family. 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, under institutional approval and in adherence to the Declaration of Helsinki Principles. To screen for a mutation in the human *DSG4* gene, all exons and splice junctions were PCR amplified from genomic DNA 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, CA), following purification in Centriflex™ Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, MD) 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.

We identified a single nucleotide deletion within exon 3, designated 87delG (Figure 2b). This results in a frameshift and premature termination codon 162 bp downstream of the deletion. The resulting nonsense-bearing mRNA transcript will likely be degraded via nonsense-mediated mRNA decay (Khajavi *et al.*, 2006) leading to a presumable absence of functional DSG4 protein in affected individuals. This is predicted to result in a null mutation in DSG4, comparable to the *lah*$^{J}$/*lah*$^{J}$ mouse (Kljuic *et al.*, 2003a) and the Iffa–Credo and SH rats (Bazzi *et al.*, 2004; Meyer *et al.*, 2004), which have an absence of Dsg4 mRNA.

All but one of the previously reported mutations in Pakistani families have involved a recurrent in-frame deletion of exons 5–8, removing amino acids 125–335 (Kljuic *et al.*, 2003a; Moss *et al.*, 2004; Rafiq *et al.*, 2004; John *et al.*, 2006). These amino acids correspond to part of the EC1 domain, all of EC2 and the beginning of the EC3 domain. These regions of DSG4 are

believed to be critical in cadherin–cadherin interaction and dimerization (Boggon *et al.*, 2002), which is necessary for proper cell–cell adhesion. Missense mutations in this same region have been demonstrated in the *lah*/*lah* mouse (Kljuic *et al.*, 2003a) and the *lah*/*lah* rat (Jahoda *et al.*, 2004). Additionally, we have recently identified a missense mutation in the R-A-L cadherin interaction site of DSG4 in a family of Iraqi origin (Messenger *et al.*, 2005).

We and others recently described the presence of monilethrix-like hairs in LAH patients from different ethnic origins (Schaffer *et al.*, 2006; Shimomura *et al.*, 2006; Zlotogorski *et al.*, 2006). Dominantly inherited monilethrix (OMIM 158000) is caused by mutations in hair shaft keratins (reviewed by Schweizer, 2006), and since DSG4 is a desmosomal protein mainly expressed in the cortex of the hair shaft (Bazzi *et al.*, 2006; Mahoney *et al.*, 2006), it is likely that DSG4 is directly associated or linked to hair shaft keratins, although this has yet to be demonstrated experimentally. Despite the distinct patterns of inheritance, it is perhaps not surprising that LAH and monilethrix share the common phenotypic features of a periodically uneven diameter as well as stiffness and brittleness of the hair shaft.

Interestingly, DSG4 is the only desmosomal cadherin to date, which has been associated with a human hair phenotype (Huber, 2003; McGrath and Wessagowit, 2005). No human 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). Recently, mutations in *DSG2* were identified in arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), an autosomal dominant disorder characterized by fibro-fatty replacement of cardiac myocytes in the right ventricle, but without a hair phenotype (Awad *et al.*, 2006; Pilichou *et al.*, 2006). Furthermore, no human mutations have been found in the *DSG3* gene although mutations in the mouse *Dsg3* result in the balding phenotype, characterized by cyclical hair loss (Koch *et al.*, 1997; Pulkkinen *et al.*, 2002).

To our knowledge, the patients described in this report represent the first example of a null mutation of *DSG4* in humans with LAH. The phenotype in this family, albeit variable, is no more severe than individuals with LAH resulting from other types of homozygous mutations, including the recurrent intragenic deletion (Kljuic *et al.*, 2003a; Moss *et al.*, 2004; Rafiq *et al.*, 2004; John *et al.*, 2006), the cadherin-interaction site (Messenger *et al.*, 2005), or compound heterozygous for two different types of mutations (Schaffer *et al.*, 2006; Shimomura *et al.*, 2006; Zlotogorski *et al.*, 2006). The wide variation in phenotype within affected members of the family in this report, who are each homozygous for the same deletion mutation, 87delG, correlates with the variable inter- and intra-familial involvement mentioned by Zlotogorski *et al.* (2006). It is also of interest that moniliform hairs were found in most (but not all) families reported by us (Kljuic *et al.*, 2003a; Moss *et al.*, 2004; Messenger *et al.*, 2005; Schaffer *et al.*, 2006) and described in many (but not all) families reported by others (Rafiq *et al.*, 2004; John *et al.*, 2006; Shimomura *et al.*, 2006; Zlotogorski *et al.*, 2006). Additional hair shaft abnormalities similar to those in monilethrix have been reported in humans and mice carrying mutations in *DSG4* (Sundberg *et al.*, 2000; Zlotogorski *et al.*, 2006). These findings suggest that LAH and recessively inherited monilethrix are related conditions and part of a spectrum with variable expressivity. The variations in inter- and intra-familial findings indicate that mutations in *DSG4* may present with a different clinical picture due to potential modifying genes among familial cases, or different types/combinations of mutations between families.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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## SUPPLEMENTARY MATERIAL

**Figure S1.** Close up of the scalp of affected individuals and the abnormal hair shafts.

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# Rodent *Lce* Gene Clusters; New Nomenclature, Gene Organization, and Divergence of Human and Rodent Genes

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## TO THE EDITOR

Human and rodent *LCE* gene clusters are located on the epidermal differentiation complexes (EDC; 1q21 in human, 3F2.1 in mouse and 2q34 in rat) and encode multiple small genes with similarities, particularly over their N-terminal region, to small proline-rich proteins. Many *LCE* genes contain the glycine–serine–cysteine-rich motif typical of cornified envelope proteins such as loricrin (Zhao and Elder, 1997; Marshall *et al.*, 2001; Wang *et al.*, 2001; Jackson *et al.*, 2005). Their N- and C-termini are similar to known transglutaminase substrates and LCE proteins are demonstrated cornified envelope constituents (Marshall *et al.*, 2001; Steinert *et al.*, 2003). LCE proteins upregulate in the loricrin null mouse, suggesting that they can functionally substitute for loricrin (Koch *et al.*, 2000; Hohl, 2005). We report here major dissimilarity between human and rodent *LCE* gene clusters, with a surprising level of divergence between mouse and rat.

The human 17 gene LCE cluster comprises three major groups in three chromosomal clusters. Group members encode similar proteins with related expression patterns – thus human group 1 and 2 genes express mainly in external epithelia such as skin, whereas group 3 genes express usually in internal stratum corneum-forming epithelia (e.g. tongue surface) (Jackson *et al.*, 2005). Recently, a nomenclature was agreed for the 17 human *LCE* genes (formerly *XP5, EIG, SPRL, SPRRL*, and *LEP* genes) (Jackson *et al.*, 2005). We present here a related agreed nomenclature for mouse and rat *Lce* genes (Table 1 and Figure 1). All work reported here was institutionally approved.

Rodent and human *LCE* gene expansion appears to have been largely independent (i.e. after the ancestral species diverged), and/or there has

*Abbreviations: LCE, late-cornified envelope; Sprr, small proline-rich*