Mutations in Lipase H Gene Underlie Autosomal Recessive Hypotrichosis in Five Pakistani Families

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Sir,

Autosomal recessive hypotrichosis is a form of hereditary alopecia that affects men and women equally. This form of hair loss usually begins in early childhood. Affected individuals have sparse hair on the scalp, sparse-to-absent eyebrows and eyelashes, and sparse body hair. Affected male individuals have normal beard hair (1–3). Causative genes for three similar forms of autosomal recessive hypotrichosis, desmoglein 4 (*DSG4*) for Localized Autosomal Recessive Hypotrichosis (LAH)1 (MIM 607903), *LIPH* for LAH2 (MIM 604379), and *P2RY5* for LAH3 (MIM 611452), have been identified. To date, nine mutations in the *LIPH* gene have been reported in several families with autosomal recessive hypotrichosis and woolly hair from Russia, Pakistan and Guyana (4–11). We studied five consanguineous Pakistani families with autosomal recessive hypotrichosis and identified a novel splice site and two previously reported mutations in the *LIPH* gene.

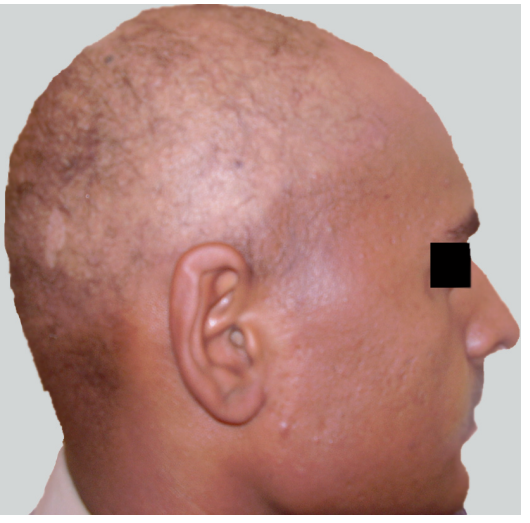

Fig. 2. Phenotypical appearance of an affected individual (V-4) of family A, showing sparse hair on the scalp.

RESULTS

The present study involves five unrelated consanguineous Pakistani families (A–E) (for pedigrees see electronic Fig. 1; http://adv.medicaljournals.se/article/abstract/10.2340.00015555-0766/fig1), demonstrating autosomal recessive form of hypotrichosis, from small villages in the Punjab and Sind provinces of Pakistan. Approval for the study was obtained from the Quaid-i-Azam University Institutional Review Board. Informed consent was obtained from all subjects participating in the study. Affected individuals in four families (A– D) exhibited typical features of hypotrichosis, as described previously (5–8). Scalp hairs were fragile, slow-growing, and sparse-to-absent in the affected individuals. Eyebrows, eyelashes, axillary hair, pubic hair and body hair were sparse-to-absent in the affected individuals (Fig. 2). Affected males had normal beard and moustache hairs. In the fifth family (E), affected individuals had a light-coloured woolly hair phenotype similar to that reported recently by Shimomura et al. (9). Affected individuals in all five families had normal teeth, nails, and sweating. Obligate heterozygous carrier individuals in each family had normal scalp and body hair and were clinically indistinguishable from genotypically normal individuals. Genotyping was performed with microsatellite markers flanking *DSG4* gene on chromosome18q12.1, *LIPH* gene on chromosome 3q26.33, and *P2RY5* gene on chromosome 13q14.2. The markers were fully informative and the affected members of all five families were homozygous for markers linked to *LIPH* locus on chromosome 3q26.33. The *LIPH* gene was sequenced in all affected and unaffected individuals in the families for whom DNA samples were available. The primer sequences used to amplify exons and flanking splice junctions of the *LIPH* gene were the same as described previously (5). Sequence analysis of the *LIPH* gene in affected individuals in the five families revealed one novel and two previously described mutations. In family A, sequence analysis detected a novel homozygous G→C transversion in the splice acceptor site of intron 4 (IVS4-1G→C) of the *LIPH* gene (Fig. 3). Two previously described deletion mutations Ex7-8del (11) and c.659-660delTA (8) were identified in three (B, C, D) and one (E) family, respectively.

All the pathogenic sequence variants reported here were found in the heterozygous state in the obligate carriers and segregated with the disease in the respective families. To exclude the possibility that the novel and other recurrent mutations do not represent non-pathogenic polymorphisms, a panel of 40 unaffected unrelated ethnically control individuals was screened and these mutations were not identified outside the respective families.

DISCUSSION

The human *LIPH* gene is composed of 10 exons separated by 9 introns in an approximately 45-kb region. All the exon-intron boundaries comply with the GT/AG rule (12) and it is strongly expressed in the hair shaft, Huxley's layer of the inner root sheath, and the outer root sheath of the hair follicle (9). The LIPH protein has an N-terminal catalytic domain, which contains three catalytic residues at positions 154, 178 and 248. In addition, it possesses two surface loops, β9 loop (12–13 amino acids) and a lid domain (7–12 amino acids) and four potential N-linked glycosylation sits. The three amino acids ser154, asp178 and his248 are encoded by exons 3, 4 and 6, respectively. The β9 loop and a lid domain are considered to be crucial structures for substrate recognition and are encoded by exon 5 and 6 of the gene (12). The mutations in the *LIPH* gene, identified in the present study, include a novel

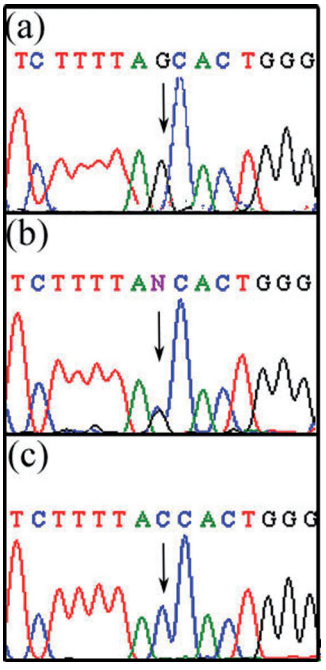

Fig. 3. DNA sequence analysis of a splice site mutation (IVS4-1G→C) in the *LIPH* gene in family A. The sequences in: (a) the control individual; (b) the heterozygous carrier; and (c) the homozygous affected individual.

homozygous splice acceptor site mutation (IVS4-1G→C). Mutations at splice sites make a significant contribution to human genetic diseases, since approximately 15% of disease-causing point mutations affect pre-mRNA splicing (13). Such splice site mutations may result in exon skipping, activation of a cryptic splice site, creation of a pseudo-exon within an intron, and intron retention in ratios of 51%, 32%, 11%, and 6%, respectively (14). Exon skipping is the most frequent phenotype from an exon perspective, because mutation of the splice site at one side of an exon should inhibit pairing of splice site across exons and inhibit recognition of the exon. Rejection of the exon leads directly to exon skipping. The G→C transversion in the splice acceptor site of intron 4 (IVS4-1G→C) of the *LIPH* gene is very likely to result in skipping of exon 5. A possible effect of such skipping would be either loss of *LIPH* expression, possibly due to mRNA decay or synthesis of truncated protein. The truncated protein, if produced, will lack the β9 loop and part of the lid domain, thus eliminating the substrate recognition ability of the LIPH enzyme. The recurrent deletion mutation (Ex7-8del), identified in families (B–D) result in a frameshift and downstream premature termination codon, thereby predicting degradation of non-functional transcripts due to nonsense-mediated mRNA decay and absence of the functional LIPH. The other recurrent mutation (c.659-660delTA), identified in family E, changes the translation reading frame immediately after the β9 loop, thereby eliminating the lid peptide sequence required for substrate recognition in combination with β9 loop. In addition, this mutation also eliminates the critical His248, which is important for the catalytic triad of lipase H (15).

On examining the haplotypes (electronic Fig. 1b), it was observed that deletion mutations Ex7-8del in families B, C, D and c.659-660delTA in family E presented here and reported earlier (6) appeared on very similar haplotypes, suggesting that the mutation in these families was due to the same mutation event.

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