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Founder mutations in the lipase H (*LIPH*) gene in families with autosomal recessive woolly hair/hypotrichosis

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Abstract

Autosomal recessive woolly hair (ARWH)/hypotrichosis is a hereditary hair disorder which is characterized by tightly curled hair, and is occasionally associated with sparse hair. ARWH can be caused by mutations in the *P2RY5* or lipase H (*LIPH*) gene. Disruption of both genes results in phenotypes with features of both WH and hypotrichosis. In this study, we identified two Guyanese families with ARWH. Both families are of recent Indian descent. Mutation analysis resulted in the identification of mutations in the *LIPH* gene in both families. Affected individuals in the first family carry compound heterozygous mutations Ex7_8del and 1303_1309dupGAAAACG in the *LIPH* gene, and those in the second family have a homozygous mutation 659_660delTA in *LIPH*. The mutations Ex7_8del and 659_660delTA were previously identified in several Pakistani families with ARWH. Haplotype analysis using microsatellite markers close to the *LIPH* gene defined a founder haplotype shared in families from Pakistan and Guyana. Proteomic analysis of hair shaft samples from one of the families revealed no substantial changes among the proteins identified, indicating that the syndrome does not involve global alterations in protein expression. Our results further suggest a crucial role of lipase H in hair growth.

Keywords

LIPH; woolly hair; hypotrichosis; P2RY5; proteomics

INTRODUCTION

The hair shaft is a highly keratinized structure that is produced by the hair follicle. The growth of the hair shaft originates in the matrix region located in the bulb portion of the HF, which gives rise to a highly cohesive structure with close interactions among hair keratins and their associated proteins (Langbein and Schweizer, 2005). In addition, HF compartments which are

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Conflict of Interest Statement
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composed of several distinct layers surround and support the hair shaft. Recent advances in molecular genetics enabled the identification of numerous genes that are expressed in the HF. Furthermore, mutations in some of these genes have been shown to underlie hereditary hair disorders, such as T cell immunodeficiency, congenital alopecia, and nail dystrophy (OMIM 601705), localized autosomal recessive hypotrichosis (OMIM 607903), and autosomal dominant hypotrichosis simplex of the scalp (OMIM 146520), which are caused by mutations in *FOXN1* (Frank *et al.*, 1999), *DSG4* (Kljuic *et al.*, 2003), and *CDSN* (Levy-Nissenbaum *et al.*, 2003), respectively. In addition, it has been shown that mutations in *CDH3* gene underlie both hypotrichosis with juvenile macular dystrophy (OMIM 601553) (Sprecher *et al.*, 2001; Shimomura *et al.*, 2008a) and ectodermal dysplasia, ectrodactyly, and macular dystrophy (OMIM 225280) (Kjaer *et al.*, 2005; Shimomura *et al.*, 2008a). Most recently, we have identified mutations in the *P2RY5* gene in several consanguineous Pakistani families affected with autosomal recessive woolly hair (ARWH; OMIM 278150) (Shimomura *et al.*, 2008b). The *P2RY5* gene encodes a G protein-coupled receptor P2Y5 which is expressed abundantly in the inner root sheath of the hair follicle.

WH is a hair shaft anomaly characterized by tightly curled hair (Chien *et al.*, 2006). WH can appear as a part of some systemic diseases, such as Naxos disease (OMIM 601214) (McKoy *et al.*, 2000) and Carvajal syndrome (OMIM 605676) (Norgett *et al.*, 2000). In addition, an isolated form of WH without associated findings also exists and can display either autosomal dominant (ADWH; OMIM 194300) (Hutchinson *et al.*, 1974) or recessive inheritance (ARWH) (Salamon, 1963; Hutchinson *et al.*, 1974). Before our recent findings of *P2RY5* mutations, only two families with ARWH had been reported in the literature (Salamon, 1963; Hutchinson *et al.*, 1974). Importantly, affected individuals in both families showed not only WH, but also sparse and depigmented hairs (Salamon, 1963; Hutchinson *et al.*, 1974). Consistent with these reports, affected individuals in Pakistani families with *P2RY5* mutations also exhibited various degrees of sparse hair, even though WH was the only common trait among all affected individuals (Shimomura *et al.*, 2008b). In addition, mutations in the *P2RY5* gene were recently reported in Saudi Arabian families with autosomal recessive hypotrichosis simplex (OMIM 146520) which is characterized by early-onset paucity of scalp and body hair (Pasternack *et al.*, 2008).

More recently, we analyzed additional Pakistani families with ARWH, and identified mutations in the lipase H (*LIPH*) gene (Shimomura *et al.*, 2008c). It was originally reported that a common founder mutation in the *LIPH* gene caused an autosomal recessively inherited hypotrichosis (OMIM 604379) in isolated Russian populations (Kazantseva *et al.*, 2006). Later, additional mutations in *LIPH* were identified in families affected with an autosomal recessive hypotrichosis (Ali *et al.*, 2007; Jelani *et al.*, 2008; Kamran-Ul-Hassan Naqvi *et al.*, 2009; Nahum *et al.*, 2009). Notably, the WH phenotype was not mentioned in these reports. Although some of the Pakistani families with *LIPH* mutations showed hypotrichosis, all affected individuals had WH during their early childhood (Shimomura *et al.*, 2008c). Collectively, we have shown that, similar to *P2RY5* mutations, individuals with *LIPH* mutations can also show overlapping phenotypes between ARWH and hypotrichosis.

In this report, we studied two families of Guyanese origin with ARWH, and identified three distinct pathogenic mutations in the *LIPH* gene in both families. In addition, we performed proteomic analysis with hairs from members of one family to analyze how *LIPH* mutations affect the expression of proteins in the hair shaft. Since two of three mutations were previously identified in Pakistani families with ARWH, we performed haplotype analysis with microsatellite markers close to the *LIPH* gene, and defined common founder alleles among these geographically diverse populations.

RESULTS

Clinical features

Clinical features of both families were examined and evaluated by dermatologists who are experts in hair diseases (Y.S. and A.Z.). Family A is a two-generation pedigree with two affected siblings (Figure 1a). There is no consanguinity between the parents, who are unaffected and have straight scalp hair with normal hair density. Both affected individuals had tightly curled hair during their childhood (Figure 1b). Their hair grew slowly and stopped growing after a few inches. Individual II-1 has gradually lost her hair, and at present, she has sparse, thin, and short hair on the scalp (Figure 1c). Under light microscopy, some of her hair shafts are twisted (Figure 1d), and the distal ends are tapered (Figure 1e), suggesting a certain hair growth defect. Her eyebrows, as well as her body hairs, were also sparse, whereas her eyelashes were not affected (not shown). By contrast, individual II-2 has shown a different clinical course with aging. At present, the hair density on his scalp is relatively normal. In addition, his hair shows a mildly wavy or nearly straight appearance, and grows relatively well (not shown). His facial and body hairs are not affected (not shown).

Family B is a two-generation pedigree with two affected and one unaffected siblings (Figure 1f). The parents are first cousins, and neither is affected. Both affected individuals have had sparse hair and different degrees of WH since birth. Individual II-1 has shown tightly curled hair (Figure 1g), while the younger affected individual has showed slightly wavy hair (Figure 1h). Their eyebrows, eyelashes, and body hairs are also sparse. In addition, it is noteworthy that both affected individuals have showed diffuse keratotic follicular papules on their arms, legs, and abdomen since the age of one year (Figure 1i). Affected individuals in both families show normal facial features, teeth, nails, and sweating, and do not show palmoplantar hyperkeratosis. There was no family history of either heart disease, sudden death or neurologic abnormalities.

Identification of mutations in the *LIPH* gene

Since the clinical features of both families are consistent with ARWH, we first performed direct sequencing analysis of exons and exon-intron boundaries of the *P2RY5* gene which is a causative gene for ARWH (Shimomura *et al.*, 2008b), but did not find any sequence variants in *P2RY5* in either family (data not shown). Next, we searched for mutations in the *LIPH* gene that we recently reported as a second causative gene for ARWH (Shimomura *et al.*, 2008c). We sequenced all exons and exon-intron boundary sequences of the *LIPH* gene and identified pathogenic mutations in the *LIPH* in both families.

First, both affected individuals in Family A (II-1 and II-2) are heterozygous for a 7-nucleotide insertion at position 1309 in exon 10 of the *LIPH* gene, which is a tandem repeat of the sequences between positions 1303 and 1309, thus designated 1303_1309dupGAAAACG (Figure 2a). The mutation was not reported previously, and is predicted to cause a frameshift and a premature termination codon (PTC) (Val437GlyfsX4) (Figure 2a). Direct sequencing analysis showed that the mutation was inherited from their mother (I-2) (Figure 2a). Although we postulated the existence of another heterozygous mutation in the *LIPH* of this family, the initial direct sequencing analysis did not detect any other sequence variants. However, a large deletion mutation would not be detected by PCR from heterozygous individuals. We next performed PCR using primer pairs designed at intron 6 and intron 8 of the *LIPH* (E7F2 and E8R2; Figure 2b) to screen for the mutation Ex7_8del that we recently identified in several Pakistani families with ARWH (Shimomura *et al.*, 2008c). A 183 bp fragment was amplified by this PCR from DNA of both affected individuals (II-1 and II-2) and their father (I-1), which was the same size as the fragment amplified from DNA of a Pakistani individual who is homozygous for the mutation Ex7_8del (lane PC, Figure 2b). Direct sequencing of the PCR

product confirmed that Family A carries the identical deletion mutation that we identified in Pakistani families (Figure 2b) (Shimomura et al., 2009). Thus, affected individuals in Family A have compound heterozygous mutations 1303_1309dupGAAAACG and Ex7_8del, on the maternal and paternal alleles of the *LIPH* gene, respectively.

In Family B, both affected individuals (II-1 and II-3) are homozygous for a 2-nucleotide deletion at positions 659-660 in exon 5 of the *LIPH* gene, designated 659-660delTA (Figure 2c), while the unaffected sibling (II-2) is homozygous for the wild type sequence (not shown). This mutation was previously identified in several Pakistani families with ARWH/hypotrichosis (Jelani *et al.*, 2008; Shimomura *et al.*, 2008c), and is predicted to result in a frameshift and a PTC at 25 amino acid residues downstream of the mutation (Ile220ArgfsX25). Screening assays showed that none of these mutations were found in 100 Pakistani control individuals (data not shown).

Evidence for founder mutations

To determine whether the mutations Ex7_8del and 659-660delTA were shared founder mutations between Pakistani and Guyanese families, we performed haplotype analysis using microsatellite markers within and around *LIPH* gene. The haplotype for the mutation Ex7_8del between LIPH-MS1 and LIPH-MS4 was the same between Family A and Pakistani families with this mutation (Figure 3; Shimomura *et al.*, 2008c). In addition, affected individuals in Family B and Pakistani families with the mutation 659-660delTA shared the same haplotype between LIPH-MS1 and D3S1602 (Figure 3; Shimomura *et al.*, 2008c).

Proteomic analysis

Hair samples were analyzed from the unaffected parents (M, mother; F, father) and two affected offspring (D, daughter; S, son) of Family A. From the unfractionated hair shaft, 41 proteins were identified in samples from both parents with no substantive differences between them being observed. The large majority (>95%) were keratins and keratin associated proteins (Figure S1). Since the high keratin content masks less abundant proteins in such analyses, isopeptide cross-linked protein (10-15% of the total hair shaft) was isolated and analyzed. Although major constituents of the cross-linked material were also keratins and keratin associated proteins (≈ 70%), the additional constituents included junctional and other membrane proteins (≈ 5%), histones (≈ 10%) and various proteins ordinarily found in the cytoplasm (≈ 10%), as previously observed (Lee *et al.*, 2006). From this fraction, 53 proteins were identified, 29 of which were also detected in the unfractionated hair (Figure S2).

The 20 most abundant proteins for the total hair shaft and for the cross-linked fractions are shown in Table 1. The numbers of unique peptides attributed to each protein in the parental analyses were compared to those from the afflicted offspring. Because peptide identification by mass spectrometry is a stochastic process, numbers of unique peptides have some variability related to the complexity of the sample. Nevertheless, no large differences were evident between the parental and offspring samples. Table S1 gives the full listing of identified proteins, including those barely detectable. The present approach is not suited for determining the significance of the small differences observed in the latter.

DISCUSSION

In this study, we analyzed two Guyanese families with ARWH and identified mutations in the *LIPH* gene in both families. Affected individuals in Family A carry compound heterozygous mutations Ex7_8del and 1303_1309dupGAAAACG in the *LIPH* gene, which were inherited on their paternal and maternal alleles, respectively (Figure 2a, b). These are the first compound heterozygous mutations identified in the *LIPH* gene. Secondly, affected individuals in Family

B are homozygous for the mutation 659_660delTA in the *LIPH* gene. All three mutations result in a frameshift and downstream PTC. Most likely, aberrant transcripts from both the Ex7_8del and 659_660delTA alleles would be largely degraded due to nonsense-mediated mRNA decay (Maquat, 1996; Frischmeyer and Dietz, 1999), leading to loss of expression of LIPH protein. By contrast, because the mutation 1303_1309dupGAAAACG exists in the last exon of the *LIPH* gene, the mutant allele with this mutation is likely to generate a truncated LIPH protein which would lack only 15 amino acid residues in its C-terminus as compared with the wild type LIPH protein (Figure 2a). Nevertheless, the aberrant protein would not possess a cysteine residue at amino acid 446 which is considered to be important for the formation of a disulfide bond (Figure 2a) (Jin *et al.*, 2002), and thus is predicted to severely affect the structure of LIPH protein.

The mutations Ex7_8del and 659-660delTA were previously identified in several Pakistani families with ARWH (Jelani *et al.*, 2008; Shimomura *et al.*, 2008c). Haplotype analysis suggests a common founder for these mutations between the Pakistani and Guyanese families, living in geographically distinct regions (Figure 3a). Interestingly, history reveals that more than 200,000 people emmigrated from India to Guyana between 1838 and 1917 (Bisnauth, 2000), and since Pakistan was separated from India in the 1940s, it is plausible that India is the common source for these chromosomes. Indeed, it is noteworthy that both families emigrated from India to Guyana about 100 years ago, and all members of the extended pedigrees of both families are of Indian descent.

Mutations in the *LIPH* gene were originally reported to underlie an autosomal recessive form of hypotrichosis (Kazantseva *et al.*, 2006; Ali *et al.*, 2007). Recently, we identified several pathogenic mutations in Pakistani families affected with ARWH (Shimomura *et al.*, 2008c). During early childhood, all affected individuals in our families showed mainly WH, but then exhibited wide variability in the hypotrichosis phenotype with aging. While some affected individuals continued to show only WH, others suffered hair loss, leading not only to WH, but also hypotrichosis. In the most severe cases, the hypotrichosis became the only phenotype, leading even to complete lack of scalp hair. Furthermore, the severity of WH phenotype was also variable among individuals. Such variations in phenotype were detected even within a single family (Shimomura *et al.*, 2008c). Similarly, although all affected individuals in the Guyanese families commonly had WH at birth, they show variations in severity with aging. In family A, the elder affected individual shows a severe hypotrichosis (Figure 1c), while the younger affected individual exhibits a relatively mild phenotype. In Family B, both affected individuals showed overlapping phenotypes between WH and hypotrichosis (Figure 1g, h), but the degrees of WH are different between them. Interestingly, both affected individuals in Family B also have keratosis pilaris-like eruption on their extremities and abdomen (Figure 1i), which may be a non-specific sign seen in many forms of hypotrichosis, atrichia, and fragile hair disorders (Zlotogorski *et al.*, 2002; Weiss *et al.*, 2004; Zlotogorski *et al.*, 2006).

Inherited disorders of lipid metabolism leading to permeability barrier abnormalities of the skin drive pathophysiology of scaling disorders with effects on proliferation and inflammation (Elias *et al.*, 2008). Whether lipid processing defects affect hair structure by altering protein expression is less well studied. The unusual physical properties of WH, including a reported characteristic shape, could plausibly reflect altered protein composition or structural organization. However, present data indicate that predominant proteins detected were affected little, if at all, by the *LIPH* gene defect. Thus, the unusual properties of the hair are likely due simply to defects in the lipid component.

Quantitating relative protein amounts in cross-linked complexes is an incompletely resolved challenge. Nevertheless, limited quantitative comparisons of given proteins among different samples appear feasible on the basis of normalized spectral abundance, where the numbers of

peptides detected are anticipated to be proportional to protein length (Zybailov *et al.*, 2006). While only a rough approximation, the exponentially modified protein abundance index (emPAI) approach used presently has an empirical basis (Ishihama *et al.*, 2005; Ishihama *et al.*, 2008). Such estimates are likely to improve as more targeted measurements of select "proteotypic" peptides of specific proteins are developed (Deutsch *et al.*, 2008). While a more detailed study would be required to detect subtle differences in protein content or to identify changes among less prevalent proteins, our results suffice to rule out major changes in protein expression as a characteristic feature of the WH syndrome. By contrast, subtypes of WH connected with more serious defects could display such changes that reflect downstream effects of the genetic lesion. In that case, proteomic analysis could help in their classification.

We and others have recently shown that mutations in the *P2RY5* gene underlie ARWH/hypotrichosis (Pasternack *et al.*, 2008; Shimomura *et al.*, 2008b). The clinical features of affected individuals with *P2RY5* mutations are indistinguishable from those with *LIPH* mutations. The *LIPH* gene encodes a phospholipase A1 family member and is a key enzyme in the synthesis of lysophosphatidic acid (LPA) (Sonoda *et al.*, 2002), which is an extracellular mediator of many biological functions and is known to promote hair growth in vivo (Takahashi *et al.*, 2003). P2Y5 has recently been shown to be a LPA receptor (Pasternack *et al.*, 2008), and furthermore, we have demonstrated that the expression of P2Y5 partially overlaps with that of LIPH in HFs in vivo (Shimomura *et al.*, 2008c). These data underscore a crucial role of the LIPH/LPA/P2Y5 signaling pathway in hair growth in humans.

MATERIALS AND METHODS

Mutation analysis of the *LIPH* gene

Peripheral blood samples from the family members and 100 unrelated healthy control individuals of Pakistani origin were collected in EDTA-containing tubes following informed consent under institutional approval (IRB-AAAB4246) and in adherence to the Declaration of Helsinki Principles. We also collected blood samples from 100 unrelated healthy Pakistani individuals as controls because both Guyanese families are of Indian descent and Pakistani populations share the same genetic background with Indian populations. Genomic DNA was isolated from these samples using the PUREGENE DNA isolation kit (Gentra System, Minneapolis, MN). All exons and exon-intron boundaries of the *P2RY5* and the *LIPH* genes were amplified by PCR using gene-specific primers and PCR conditions as described previously (Shimomura *et al.*, 2008b; Shimomura *et al.*, 2009). The amplified PCR products were directly sequenced 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).

For screening of the mutation 1303-1309dupGAAAACG, a part of exon 10 and 3′-noncoding sequence of the *LIPH* gene was PCR-amplified using a forward primer (5′-TGTCGGTATGATCTTGTCCTGAT -3′) and a reverse primer (5′-CCTGTGGTTGTAGCTTTCTTTCTA-3′). The amplification conditions were 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 57°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 7 min. The amplified PCR products were run on 8% polyacrylamide gels. Screening assays for the mutations Ex7_8del and 659-660delTA were performed as described previously (Shimomura *et al.*, 2009).

Genotyping and Haplotype Analysis

In order to analyze whether the mutations Ex7_8del and 659_660delTA are common founder mutations between Pakistan and Guyana, genomic DNA from members of families from both populations were amplified by PCR using primers for two microsatellite markers, D3S3592

and D3S1602, close to *LIPH* gene, as well as four additional markers (LIPH-MS1-4) around or within *LIPH* (Shimomura *et al.*, 2008c). PCR products were run on 8% polyacrylamide gels and genotypes were assigned by visual inspection.

Proteomics analysis

Samples of hair shafts (6-8 mg) were rinsed briefly in 2% SDS to remove loosely adhering contaminants, incubated overnight at 70°C in 5 ml of 2% SDS − 0.1 M sodium phosphate (pH 7.8) − 20 mM DTE and pulverized by stirring for several hours with a small magnetic stirring bar. Insoluble material was recovered from parallel samples (15-30 mg of hair) by centrifugation, extracted 4 more times, with the protein content of the extract being monitored to ensure complete extraction. The unfractionated hair and the insoluble fractions were alkylated with iodoacetamide, digested for three days at room temperature with reductively methylated trypsin in fresh 0.1 M ammonium bicarbonate 10% acetonitrile. Our previous hair analysis subjected the digest to ion exchange and reverse phase column chromatography prior to mass spectrometric protein identification (Lee *et al.*, 2006). For the present purpose, a streamlined approach using only the reverse phase separation has proven sufficient to identify numerous prominent constituents and to permit semi-quantiative comparison among the samples. Mass spectrometry of 15 μg of each digested sample was performed using an LTQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) as described in Supplementary Methods. Database searching was performed on MASCOT against the IPI human database. Scaffold (Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Protein identifications were accepted if they could be established at greater than 99.0% probability and contained at least 2 identified peptides. Numbers of unique peptides were tabulated as a basis for selecting prominent proteins and compiling emPAI values from Mascot database reports (cut-off score 35). Estimates of relative molar amount were calculated by normalizing to the total emPAI values for the identified proteins based on the mean emPAI values for the parental samples (Ishihama *et al.*, 2005).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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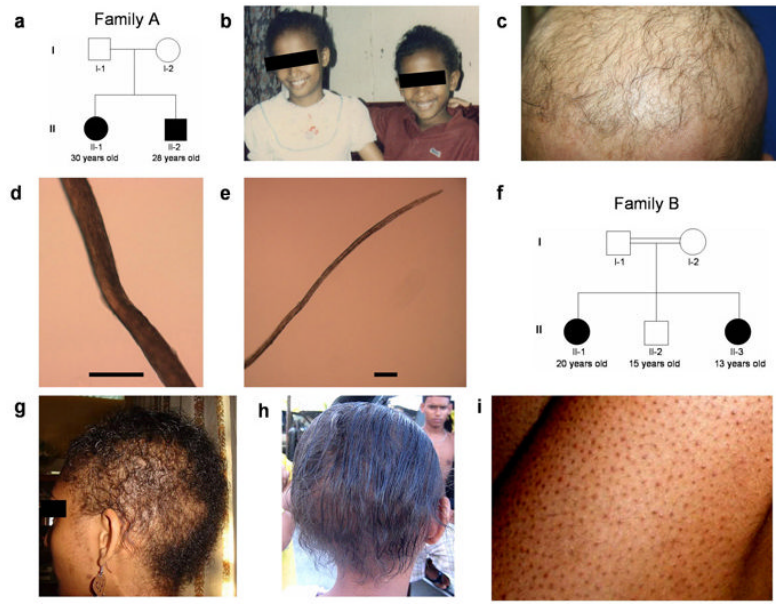

Figure 1. Pedigrees and clinical features of two Guyanese families with ARWH

(a) Pedigree of Family A. (b) Clinical features of the affected individuals II-1 (left) and II-2 (right) in Family A when they were 12 and 10 years old, respectively. Note that both showed an obvious woolly hair phenotype. (c) Present clinical features of the affected individual II-1 in Family A. (d, e) Under light microscopy, hair shafts of the affected individual II-1 in Family A show twisting (d) and a tapered end (e). Scale bars: 100 μm. (f) Pedigree of Family B. (g, h) Hair phenotypes of the affected individuals in Family B. The affected individual II-1 show sparse and tightly curled hair (g), while the affected individual II-3 shows sparse and mildly wavy hair (h). (i) Diffuse keratotic follicular papules on legs of the affected individual II-1 in Family B.

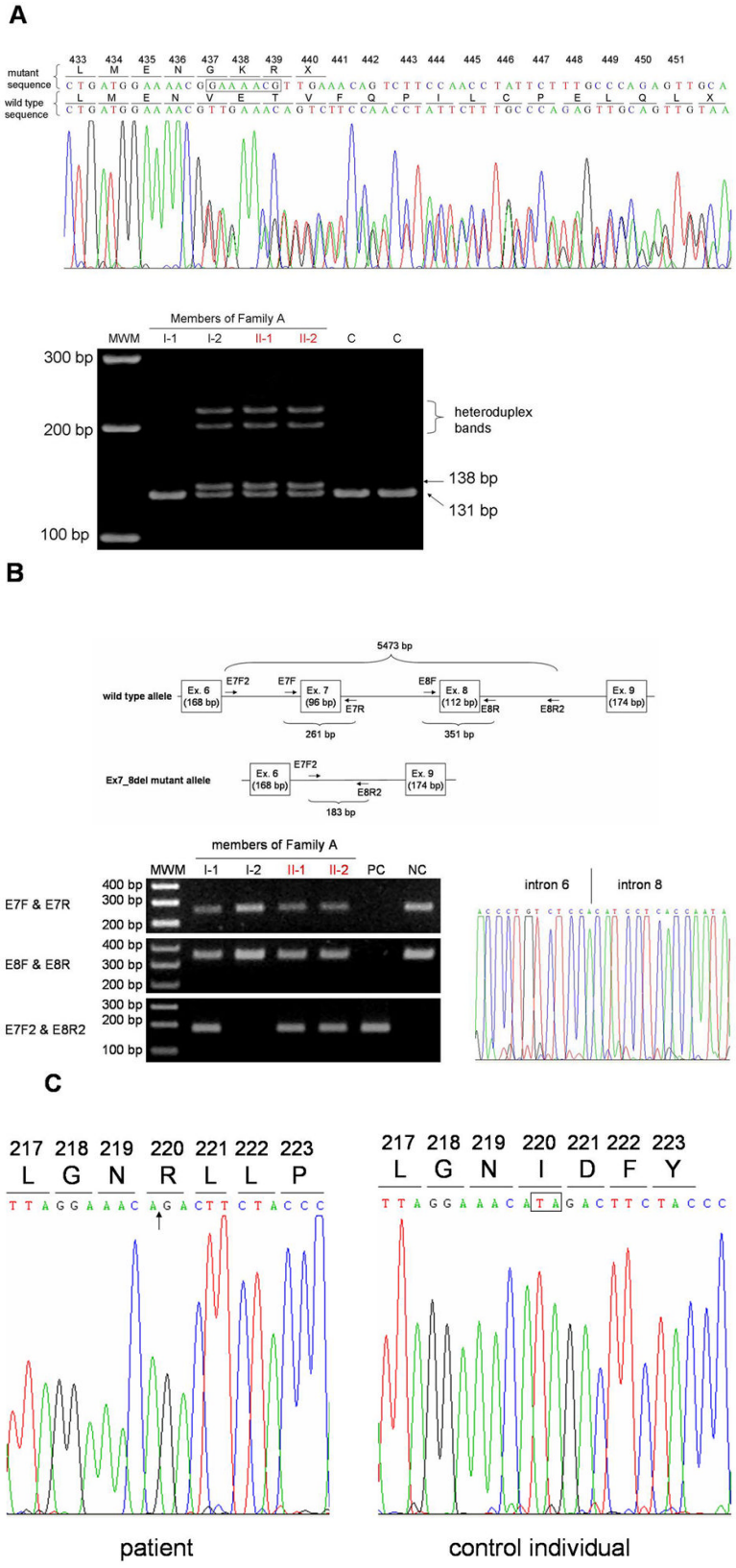

Figure 2. Identification of mutations in the *LIPH* gene

(a) Heterozygous mutation 1303_1309dupGAAAACG in the *LIPH* of Family A (top). The inserted nucleotides are boxed. Screening assay for the mutation 1303_1309dupGAAAACG (bottom) shows that the mutation was inherited from the mother (I-2) of the affected individuals (II-1 and II-2). C, control individuals. (b) Heterozygous mutation Ex7_8del in the *LIPH* of Family A. PCR with E7F2 and E8R2 primers amplified the 183 bp fragment from DNA of both affected individuals (II-1 and II-2) and their father (I-1) in Family A, as well as a Pakistani individual who is homozygous for the mutation Ex7_8del (PC), whereas this fragment was not amplified from DNA of either their mother (I-2) or a normal control individual (NC). Direct sequencing of this product clearly shows that Family A carries exactly the same deletion mutation Ex7_8del that was previously found in Pakistani families with ARWH. Note that the product from the wild type allele, 5473 bp in size, could not be amplified even from DNA of control individuals due to a short extension time of the PCR. Affected individuals are colored in red (a, b). MWM, molecular weight markers (a, b). (c) Homozygous mutation 659-660delTA in the *LIPH* gene of Family B. The position of the deletion is indicated by an arrow (left), and the deleted nucleotides are boxed (right).

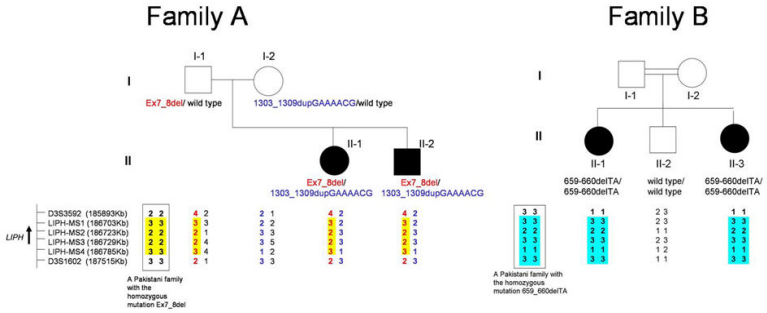

Figure 3. Haplotype of members of Families A and B in the *LIPH* locus

The position and the direction of transcription of the *LIPH* gene are indicated by an arrow. In Family A, the haplotypes for the mutations Ex7_8del and 1303_1309dupGAAAACG are colored in red and blue, respectively. The common haplotype for the mutation Ex7_8del between Family A and Pakistani families is highlighted in yellow, and that for the mutation 659-660delTA between Family B and Pakistani families is highlighted in blue. The haplotypes of a Pakistani family that is homozygous for either Ex7_8del or 659_660delTA are boxed.

Table 1

Number of unique peptides assigned to 20 most prevalent identified proteins among samples from the parents (M, mother; F, father) and afflicted offspring (D, daughter; S, son) in Family A

Total Hair Shaft						Cross-Linked Fraction					
Protein	**D**	**S**	**M**	**F**	**emPAI**	**Protein**	**D**	**S**	**M**	**F**	**emPAI**
KRT86	86	86	90	87	36.2	KRT32	18	15	15	13	14.8
KRT81	5	5	6	7	21.5	KRT82	31	22	27	32	10.4
KRT83	9	5	2	11	9.0	KRT35	22	16	20	22	10.1
KRT85	42	45	38	45	8.2	KRT85	16	13	18	16	7.2
KRT33B	48	43	56	39	7.9	KRT86	27	31	36	26	6.3
KRT31	15	15	19	16	6.0	HIST1H4	9	6	7	5	5.5
KRT33A	3	3	3	3	4.5	SFN	10	11	10	11	4.7
KRT34	15	19	15	13	2.3	H2AFX	5	6	6	7	4.3
KRTAP2-2	13	13	11	12	0.9	KRT34	4	6	9	5	3.7
KRT35	19	13	13	15	0.6	KRT33B	17	23	24	14	3.3
KRT32	10	9	10	10	0.4	KRT31	5	9	8	6	2.9
S100A3	5	4	4	3	0.3	HIST1H2BL	8	6	7	4	2.7
KRT82	21	15	11	17	0.3	S100A3	3	4	4	3	2.6
KRTAP3-1	4	5	6	4	0.3	KRTAP11-1	13	13	10	3	2.5
H2AFX	6	6	6	5	0.2	VSIG8	16	10	10	18	2.3
HIST1H2BL	5	3	4	4	0.2	CALML3	5	4	5	4	2.0
KRTAP3-2	2	3	3	2	0.2	KRT36	5	4	3	3	1.1
HIST1H4	6	5	3	4	0.1	PKP1	10	12	12	10	1.0
KRT36	2	2	2	2	0.1	KRT40	6	5	6	7	0.9
KRTAP11-1	4	4	3	5	0.1	PRDX6	2	5	4	6	0.8

Data include emPAI semi-quantitative estimates of relative molar amounts normalized to 100 (Ishihama *et al.*, 2005).