Original Article

Identification of LIPH gene mutation in a consanguineous family segregating the woolly hair/hypotrichosis phenotype

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Abstract

Objective: To identify the disease causing gene in a four generation consanguineous family in which eleven family members were suffering from Woolly hair/hypotrichosis phenotype.

Methods: Linkage analysis was carried out to identify the disease-causing gene in this family. Genomic DNA of all the available family members was genotyped for the microsatellite markers for all the known woolly hair/hypotrichosis loci. Automated DNA sequencing of the candidate gene was performed to identify the disease-causing mutation.

Results: By using homozygosity linkage analysis we have mapped the family on chromosome 3q27.3 with a two point LOD score of 4.04. Mutation screening of the LIPH gene revealed a homozygous c.659_660delTA deletion mutation segregating with the disease phenotype.

Conclusion: The results indicate that the c.659_660delTA mutation in the LIPH gene cause autosomal recessive WH/hypotrichosis phenotype in this family. This mutation has been reported in several Pakistani and Guyanese families suggesting a founder mutation in the LIPH gene in Indo-Pak sub-continent.

Keywords: Woolly, Hair, Hypotrichosis, LIPH, Pakistan (JPMA 61: 1060; 2011).

Introduction

Woolly hair (WH) is a rare hereditary hair shaft anomaly, characterized by tightly curled hair.[1] WH is occasionally found to be associated with paucity of scalp and body hair. It may be partial or generalized, which often exhibits light pigmentation and increased hair shaft fragility.[1] WH is a heterogeneous trait that can be manifested as part of a genetic syndrome, such as Naxos disease (MIM 601214) or Carvajal syndrome (MIM 605676) that are characterized by cardiomyopathy with palmoplantar keratoderma and WH. Mutations in the plakoglobin (PKG; MIM 173325) and desmoplakin (DSP; MIM 125647) genes have been reported to cause the Naxos disease and the Carvajal syndrome, respectively.[2,3] Furthermore, a homozygous deletion mutation (c.1841delG) in desmocollin-2 (DSC2; MIM 125645) gene has been reported to be associated with cardiomyopathy and mild palmoplantar keratoderma in association with WH phenotype.[4] The plakoglobin, desmoplakin and desmocollin-2 are family members of desmosomal proteins that function as cell-cell adhesion junction, and are expressed in skin, heart and hair.[5]

Nonsyndromic WH is inherited in either autosomal dominant (ADWH; MIM 194300) or autosomal recessive (ARWH; MIM 278150) manner. Hypotrichosis is characterized by sparse hair on scalp and body, sparse or no hair on eyebrow and eyelashes. Mutations in the LPAR6 (MIM 609239) and LIPH (MIM 607365) genes have been reported to cause autosomal recessive WH/hypotrichosis phenotype in various families.[6,7] The LPAR6 and LIPH genes have been shown to have a common signalling pathway and are involved in hair growth in humans.[8] The LPAR6 gene encodes lysophosphatidic acid receptor 6 (LPAR6), whereas, the LIPH codes for lipase H (or a membrane-associated phosphatidic acid-selective phospholipase A1α [mPA-PLA1α]) that produce 2-acyl lysophosphatidic acid (LPA).[8,9] LPA plays an important role in various biological functions and is a ligand of LPAR6.[8,9] The protein products of LPAR6 and LIPH gene are abundantly expressed in the inner root sheath (IRS) of the hair follicle. The LAH2 locus for autosomal recessive hypotrichosis harbouring the LIPH gene has been mapped on chromosome 3q27.3.[10] This locus overlaps with an alopecia mental retardation syndrome (APMR1) locus on chromosome 3q26.33-q27.3.[11]

Here we report a large consanguineous Pakistani family suffering from generalized woolly hair with varying degrees of progressive hypotrichosis. The family was collected from Larkana, Sindh. The family contained multiple consanguineous marriages and disease was segregating in an autosomal recessive manner. Pakistan is inhabited by more than 12 linguistically and genetically diverse ethnic groups with unique socio-cultural norms.[12] In Pakistani population ~60% of marriages are described to be

intra-familial, of which majority (>80%) are among first cousins.[13] Due to the consanguinity, most of the families reported from Pakistan for various genetic diseases are recessive.

The objective of the current study was to identify and localize the disease causing gene segregating in this family. For this purpose, linkage analysis and mutation screening of the family members were carried out. Locus-specific microsatellite markers flanking the known woolly hair/ hypotrichosis loci were used to perform linkage analysis. DNA sequencing was then carried out for the identification of the disease-causing mutation in the candidate gene.

Methods

The study was a cross-sectional family-based genetic study. A four generation consanguineous family in which eleven members were affected was included in this study (Figure-1A). A total of 16 family members, nine males and seven females were included while two affected children (less than 15 years of age) were excluded from the study on the bases of confirmed history of woolly hair/hypotrichosis. Blood samples from two minor children (aged 5years and 7 years) were not collected because their parents were not willing to give consent. Available subjects underwent a detailed clinical examination at Chandka Medical College Hospital, Larkana, Sindh-Pakistan.

DNA extraction and genotyping:

The study was conducted in adherence to the Declaration of Helsinki Principles and was approved by the Institutional Review Board. After obtaining the written informed consent, venous blood samples from affected and unaffected family members were collected. The genomic DNA was extracted from whole blood using standard phenol-chloroform method. To identify the causative gene locus, cosegregation and homozygosity mapping with markers that are known to be linked with WH/hypotrichosis phenotype was carried out. Several polymorphic microsatellite markers specific for the plausible candidate gene loci were genotyped i.e., 13q14.11-q21.32 (LPAR6) and 3q27.3 (LIPH). Following microsatellite markers were analyzed D13S263, D13S1312, D13S153, D13S165, and D3S2427, D3S2314, D3S3578, D3S3592, D3S1617, D3S1530, D3S1262. Sixteen family members were genotyped for these markers and the results were successful for all the cases.

Polymerase chain reactions (PCR) were carried out in a 10 µl total reaction volume, containing 1 U of Taq DNA polymerase (Bio-Line, London, UK) in a PCR buffer with 1.5 mM MgCl2, 200 µM dNTPs (Applied Biosystems), 600 nM microsatellite markers sequence specific forward and reverse primers and 40 ng (2 µl) of genomic DNA. Amplified products were electrophoresed on 10% polyacrylamide gels, and visualized under UV after staining with ethidium bromide.

Linkage analysis:

Two-point linkage analysis was performed using MLINK software programme (Cherwell Scientific Publishing Ltd, Oxford, UK). An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 was used for the analysis. Equal allele frequencies were used for the microsatellite markers in the analysis.

Sequencing of the LIPH gene:

To identify the disease causing mutation, DNA samples of affected and clinically normal subjects were analyzed for LIPH gene mutation. The primers sets were designed using PRIMER3 programme.[14] All exons, and splice junctions of LIPH gene were amplified from genomic DNA by PCR using gene-specific primers. The PCR amplification conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55-60°C for 1 min, 72°C for 1 min; with a final extension at 72°C for 10 min. Both, the forward and reverse strands of the amplified PCR products were sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Perkin Elmer-ABI, UK) on an automated DNA sequencer, (3100 genetic analyzer Applied Biosystems). The sequences of chromatograms were aligned and compared with reference sequence (LIPH, NM_139248).

Results

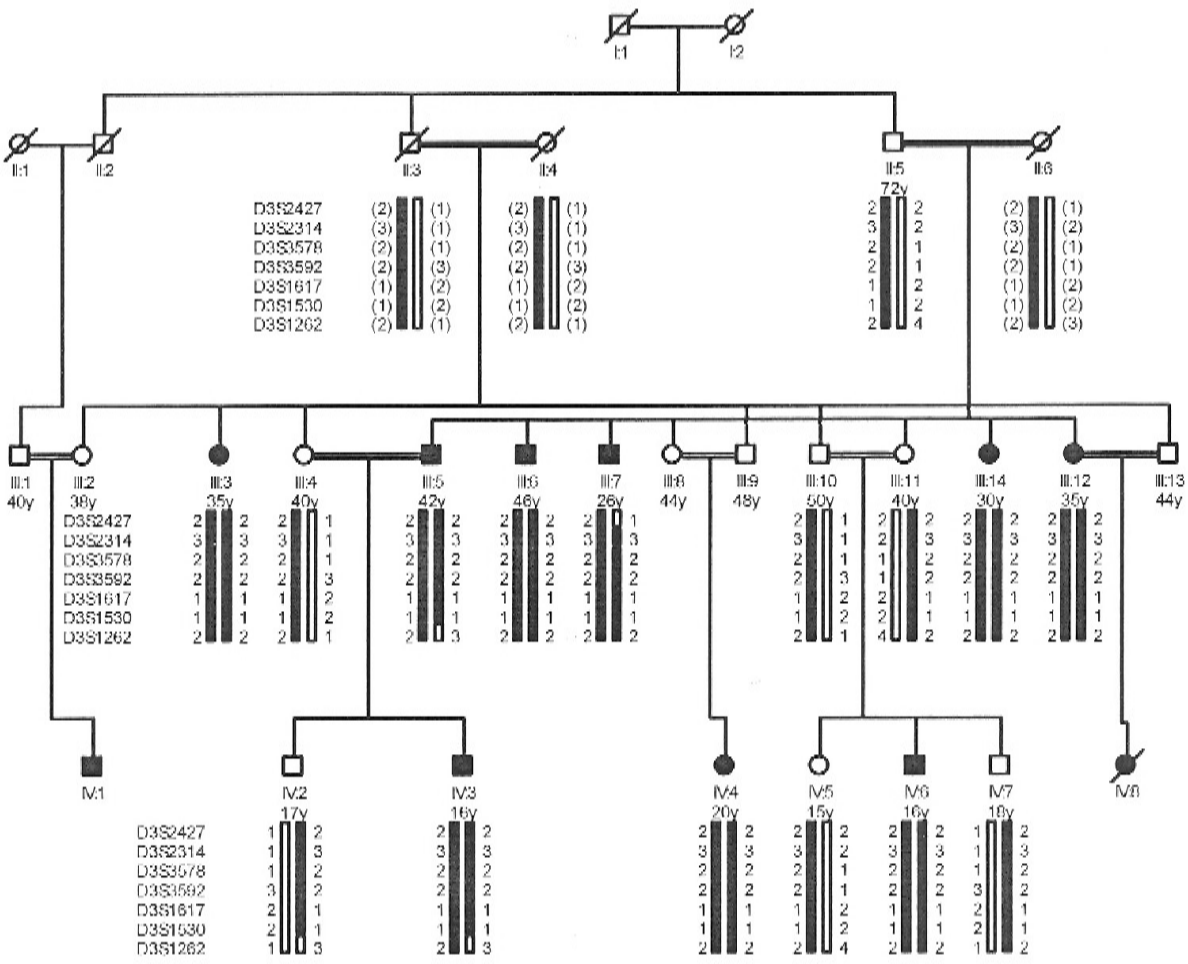
Clinical findings:

All affected individuals had generalized tightly curled hair since birth. Clinically, affected subjects exhibited sparse, rough, dry and very short hair (Figure-2a,b,c). Their hair growth was exceptionally slow, not more than a few centimeters. Phenotypic variability of scalp hair from normal to reduced was noticed among the patients. Skin, nails and teeth were normal. All individuals had usual sweating and did not show hypohidrosis, and had no complaint of heat intolerance. All affected adult males had normal beard and moustache hair; however, they were found to have sparse chest hair as shown in Figure-2 (c). Axillary and pubic hairs were sparse in all male and female patients.

Hair microscopy:

More than 20 hair samples were obtained from the

Figure 1 (A)



(B)

LOD Scores at θ =

Markers	Position (cM)*	0.0	0.01	0.05	0.1	0.2	0.3	0.4	Z max	θ max
D3S2427	188.29	∞	−3.87	−1.35	−0.49	0.03	0.09	0.03	0.09	0.3
D3S2314	193.75	**4.04**	3.95	3.57	3.09	2.13	1.18	0.36	4.04	0.0
D3S3578	195.60	3.99	3.90	3.53	3.07	2.12	1.18	0.36	3.99	0.0
D3S3592	198.68	**4.04**	3.95	3.57	3.09	2.13	1.18	0.36	4.04	0.0
D3S1617	198.68	3.99	3.90	3.53	3.07	2.12	1.18	0.36	3.99	0.0
D3S1530	201.14	3.99	3.90	3.53	3.07	2.12	1.18	0.36	3.99	0.0
D3S1262	201.14	∞	−1.6	−0.4	−0.07	−0.04	−0.00	−0.0	−1.61	0.01

LOD, logarithm of the odds ratio; cM, centimorgans.

* Taken from the Centre for Medical Genetics, Marshfield Medical Research Foundation sex-averaged linkage map

Figure-1: (A) Pedigree of an arWH/hypotrichosis affected family with genotypic data for microsatellite markers analyzed. circles; females, squares; males, filled symbols; affected individuals, open symbols; unaffected individuals, double line between individuals; consanguinity. (B) Two-point LOD scores calculated between the disease locus and chromosome 3 microsatellite markers.

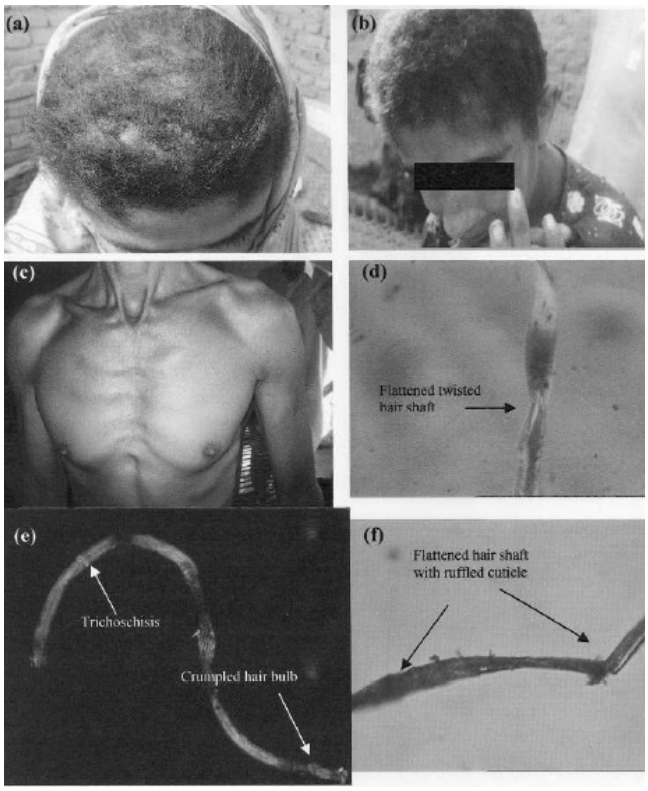


Figure-2: Clinical manifestations and microscopic features of the hair shaft associated with mutation in LIPH. (A) Affected individual III:3 displays rough, dry, sparse and tightly curled hair. (B) 22-year-old patient (IV:4) exhibits uncombable, lusterless, dry, hypopigmnted tightly curled hair with normal hair density on scalp. (C) Affected male (III:6) displays sparse body and scalp hair in association with woolly hair. (D, E, and F) Microscopic features of hair shafts.

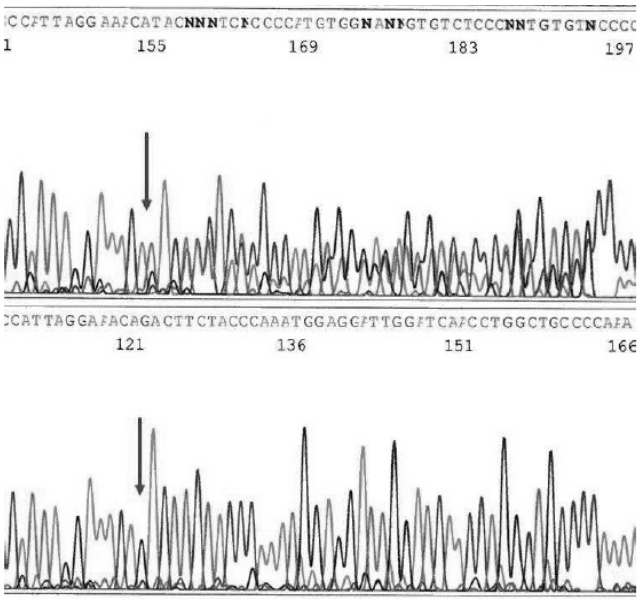


Figure-3: Electropherogram of a heterozygous carrier (top) and a homozyzous affected individual (bottom) for c.659_660delTA deletion mutation in the LIPH gene indicated by arrows.

scalp of five affected individuals (III:3, III:5, III:6, III:7, IV:4), that were easily and painlessly plucked off. Under polarized light microscope hair shaft appeared to be dystrophic and dysplastic. High magnification showed flattened and twisted hair shaft at irregular intervals, ruffled cuticle, and trichoschisis, which is prone to breakage (Figure-2 d,e,f). The hair bulb appeared to be crumpled.

Genetic analysis:

Known loci for autosomal recessive WH were first examined by linkage analysis using locus specific microsatellite markers. Linkage was obtained for markers located on chromosome 3q27.3. Haplotypes for these markers are shown in Figure-1A. Several markers were found to be homozygous in all the patients. Two-point LOD scores between the disease phenotype and markers in this region are summarized in Figure-1B. The maximum LOD score of 4.04 was obtained for the markers D3S2314 and D3S3592 with no crossover. Proximal and distal crossovers were obtained with markers D3S2427 and D3S1262 in individual III:7 and III:5 respectively.

Chromosome 3q27.3 is a locus for LIPH gene. Mutation screening of the candidate gene, LIPH, revealed a homozygous 2-bp deletion (TAdel) mutation in exon 5 in all the affected individuals (Figure-3). To check the segregation of mutation in the family, sixteen members of the family were sequenced and the mutation was found to be segregating in the family. All the obligate carriers, parents and unaffected siblings were heterozygous for the TA deletion (c.659_660delTA). This mutation resulted in a premature termination codon leading to a truncated protein.

Discussion

LIPH gene spans at 45-kb of human genomic DNA. It consists of 10 exons that encode a 451 amino acid protein lipase H that produces LPA.[9] LPA is involved in many cellular processes that are important in brain development and smooth muscle contraction. The expression of LIPH have been described in several tissues including brain, heart, liver, spleen, lungs, kidneys, pancreas, small and large intestine, prostate, male and female gonads.[9,15] Strong expression of the gene has been described in hair follicle and hair shaft.[15] The LIPH protein has an N-terminal signal domain followed by a catalytic domain. The catalytic domain contains three putative catalytic residues ser154, asp178 and his248 encoded by exons 3, 4 and 6 respectively that are important for its catalytic activity. The protein also contains two surface loops, a lid domain (7-12 amino acids) and β9 loop (12-13 amino acids) and four potential N-linked glycosylation sites. The lid domain and β9 loop, covering the active site, are crucial for the substrate recognition.[9,16]

We mapped a family with ARWH/hypotrichosis at

chromosome 3q27.3 that harbours the LIPH gene. The two-point LOD score 4.04 was achieved with two markers D3S2314 and D3S3592. This genetic interval overlaps with an alopecia mental retardation syndrome (APMR1) locus.[11] All affected family members showed tightly curled hairs since birth that lead to variable degrees of hypotrichosis with aging. The direct DNA sequencing analysis of LIPH gene revealed a c.659_660delTA mutation in exon 5 of the gene. The obligate carriers segregated this mutation in a heterozygous state. The mutation shifted the translational reading frame immediately after the β9 loop. Earlier, this mutation has been reported in several families from Pakistan and Guayana to underlie nonsyndromic autosomal recessive hypotrichosis with or without WH phenotype. The c.659_660delTA mutation has been suggested as a common founder mutation between the Pakistani and the Guyanese families that are living in geographically distinct region. The families reported from Guyana had migrated from India to Guyana about 100 years ago and all the affected family members of the extended pedigrees are of Indian descent.[17] The identification of this mutation in another family from Pakistan also confers it as a common mutation in this part of the world.

Acknowledgements

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