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Mutations in *LPAR6/P2RY5* and *LIPH* are associated with woolly hair and/or hypotrichosis

Mazen Kurban[1], **Muhammad Wajid**[1], **Yutaka Shimomura**[1], and **Angela M. Christiano**[1,2]

[1]Department of Dermatology, Columbia University, New York, New York

[2]Department of Genetics & Development, Columbia University, New York, New York

Abstract

Background—Woolly hair (WH) belongs to a family of disorders characterized by hair shaft anomalies that clinically presents with tightly curled hair, which can be divided into syndromic and non syndromic forms of WH. We have recently identified mutations in both *LPAR6/P2RY5* and *LIPH* that are associated with autosomal recessive woolly hair (ARWH).

Objective—To study the underlying genetic causes of autosomal woolly hair in Pakistani population.

Methods—We studied ten Pakistani families with ARWH for mutations in *LPAR6/P2RY5* and *LIPH* and then performed haplotype analysis to confirm their segregation in the families.

Results—We identified five mutations in *LPAR6/P2RY5*, among which three were recurrent and two were novel in eight Pakistani families. We then showed that two of the mutations in *LPAR6/P2RY5* are founder mutations in Pakistani families. Moreover, we identified two recurrent mutations in the *LIPH* gene in two Pakistani families.

Conclusion—Our study extends the spectrum of mutations in *LPAR6/P2RY5* gene and underscores that mutations in *LPAR6/P2RY5* and *LIPH* result in similar phenotypes.

Keywords

Woolly hair; hypotrichosis; LIPH; P2RY5; LPAR6

Introduction

Woolly hair (WH) belongs to a group of disorders characterized by hair shaft anomalies that clinically presents with tightly curled hair.[1] WH is distinct from the tightly curly hair in African populations in that WH shows hair shaft anomalies which can lead to hair loss and hair depigmentation.[1] Woolly hair can be divided into two main categories. The first is syndromic WH, in which WH occurs in the setting of associated cutaneous and/or systemic

Address for Correspondence: Angela M. Christiano, PhD., Columbia University, Departments of Dermatology and Genetics & Development, Russ Berrie Medical Sciences, 1150 St. Nicholas Avenue third floor room 307, New York, NY 10032, Tel. 212-851-4850, Fax. 212-851-4810, amc65@columbia.edu.

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anomalies. The second is non syndromic WH, that can be inherited in an autosomal dominant (ADWH [MIM 194300]) or autosomal recessive (ARWH [MIM 278150]) pattern.[2] The distinction between the two categories is very critical because woolly hair can occur in the setting of syndromes that can be lethal at early ages due to cardiac disease. Naxos (OMIM 601214) and Carvajal syndromes (OMIM 605676) are two conditions that present with woolly hair, palmoplantar keratoderma and ventricular arrhythmias.[3,4]

Until recently, genes associated with non syndromic woolly hair were unknown. We and others have recently reported that mutations in the *LIPH* (MIM 607365) and *LPAR6/P2RY5* (MIM 609239) genes underlie ARWH and/or localized autosomal recessive hypotrichosis (LAH [MIM 604379 and 611452]).[5,6,7] Mutations in both genes, *LPAR6* and *LIPH* act in the same signaling pathway and result in a clinically similar phenotype which can range from woolly hair to sparse hair and complete loss of hair.[5,6,8] More recently, we have shown that mutations in *keratin 74* are associated with ADWH.[9] Here, we studied ten Pakistani families with ARWH/hypotrichosis and identified several mutations in *LPAR6/P2RY5* and *LIPH*.

Materials and Methods

Patients

After obtaining informed consent, we collected peripheral blood samples from the family members and 100 unrelated healthy control individuals in EDTA-containing tubes (under institutional approval and in adherence to the Declaration of Helsinki Principles). Genomic DNA was isolated from these samples according to standard techniques.

Mutation Analysis

All exons and exon-intron boundaries of the *LPAR6*/*P2RY5* and *LIPH* gene were amplified by PCR with primers and conditions described previously.[5,10] 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).

Genotyping and haplotype analysis

To analyze whether the mutations c.69insCATGfsX29 (p.24insH52) and c.562A>T (p.I188F) are common founder mutations in Pakistani population, genomic DNA from members of families affected with either mutation were amplified by PCR using primers for four microsatellite markers, D13S168, D13S153, D13S1307 and D13S165 close to *LPAR6* gene.[5] PCR products were run on 8% polyacrylamide gels and genotypes were assigned by visual inspection.

Screening Assays

We performed screening assays for the novel mutations c.409T>C; c.410-426del17 and c. 734A>G (p.Y245C) in the *LPAR6* gene. For the mutation c.409T>C; c.410-426del17, we amplified DNA from affected individuals and 100 Pakistani controls using primers for exon 3 after which the products were run on 8% polyacrylamide gel and inspected visually. The wild type allele was 301bp while the mutant allele was 284bp. For the mutation p.Y245C we sequenced 100 Pakistani controls.

Results

Clinical features

We studied 10 consanguineous Pakistani families (Family A, B, C, D, E, F, G, H, I and J) (Fig. 1) that had multiple affected individuals showing features consistent with recessively inherited woolly hair that were present since birth. All the families shared similar phenotypes that at times were variable within the same family. The hair over the entire scalp region was coarse, lusterless, dry and tightly curled, leading to a diffuse woolly hair phenotype with varying degrees of hypotrichosis or sparse hair. Additionally several patients showed hair depigmentation (Fig. 2). Eyebrow, eyelash and beard hairs appeared normal. Affected individuals in all families showed normal teeth, nails and sweating and did not show palmoplantar hyperkeratosis or keratosis pilaris. There was no familial history of cardiac disease.

Mutation Analysis and Haplotype Analysis

We identified five mutations in the *LPAR6/P2RY5* gene among which three were recurrent and two novel mutations. Moreover, we identified two recurrent mutations in the *LIPH* gene. Families A and B had a recurrent mutation, designated c.69insCATGfsX29, in the *LPAR6* gene (Fig. 3a). Families C, D and E had a recurrent mutation designated, p.I188F in the *LPAR6* gene (Fig. 3b). Family F had a recurrent mutation, designated c.188A>T (p.D63V), in the *LPAR6* gene (Fig. 3c). Family G had a novel mutation designated c. 409T>C, c.410-426del17 in the *LPAR6* gene (Fig. 3d). This mutation was not present in 100 Pakistani control individuals. Family H had a novel mutation, designated p.Y245C, in the *LPAR6* gene (Fig. 3e). This mutation was not present in 100 Pakistani control individuals. Family I had a recurrent mutation designated c.659_660delTA in the *LIPH* gene (Fig. 3f). Family J had a recurrent mutation that consisted of deletion of exons 7 and 8 in the *LIPH* gene (Fig. 3g). Haplotype analysis showed that the mutations c.69insCATG and p.I188F are founder mutations in the Pakistani population (Fig. 4a).

Discussion

We and others have identified pathogenic mutations in the *LPAR6/P2RY5* gene in several families with ARWH or hypotrichosis.[5,6] Similarly, we have shown that mutations in *LIPH* gene lead to an identical phenotype.[10] *P2RY5* encodes for a seven transmembrane G protein coupled receptor (GPCR)[1] (Fig. 4b) and is located within intron 17 of the retinoblastoma 1 (RB1) gene.[5] *LIPH* encodes for a member of the phospholipase A1 family and is required for the synthesis of lysophosphatidic acid (LPA).[11] LPA plays a critical role in promoting hair growth.[12,13] LPA is a ligand for the receptor, P2Y5,[6] which explains the similar phenotypes in patients with either *LPAR6* or *LIPH* gene mutations. *LPAR6/LIPH* have overlapping expression in the inner root hair sheath of the hair follicle which arise from the hair matrix and differentiate before the keratinocytes of the central hair matrix thus forming a cylinder like structure providing a support for the normal development of the hair shaft[14] which might explain why disruption in the LPA/P2Y5 signaling pathway results in a woolly hair.

We did not find evidence of phenotypic variability within the families we studied, which is in support of no genotype-phenotype correlations and the clinical variation can occur even within individuals of the same family.[5,15] This suggests that other gene modifiers might play a role in phenotypic variability. There are no criteria to predict what patients will progress to develop hair loss and the severity of hair loss.

Here, we identified three recurrent and two novel mutations in the *LPAR6* gene and two recurrent mutations in the *LIPH* gene. The mutation c.409T>C; c.410-426del17 occurs in the fourth transmembrane region (Fig. 4b) of LPAR6 resulting in premature termination codon. The mutation Y245C occurs in a highly conserved region in transmembrane 6 (Fig. 4b) and similarly to other mutations occurring in transmembrane regions is expected to destabilize the tertiary structure of the protein leading to its dysfunction. Moreover, we have shown that mutations c.60insCATGfsX29 and p.I188F are founder mutations in the Pakistani population.

In conclusion, our study increases the spectrum of mutations in *LPAR6*, provides more evidence for the lack of genotype-phenotype correlation and clinical variability in *LPAR6 and LIPH* and underscores the role of this G protein-coupled receptor, together with LIPH and lysophosphatidic acid (LPA), in determination of hair texture.

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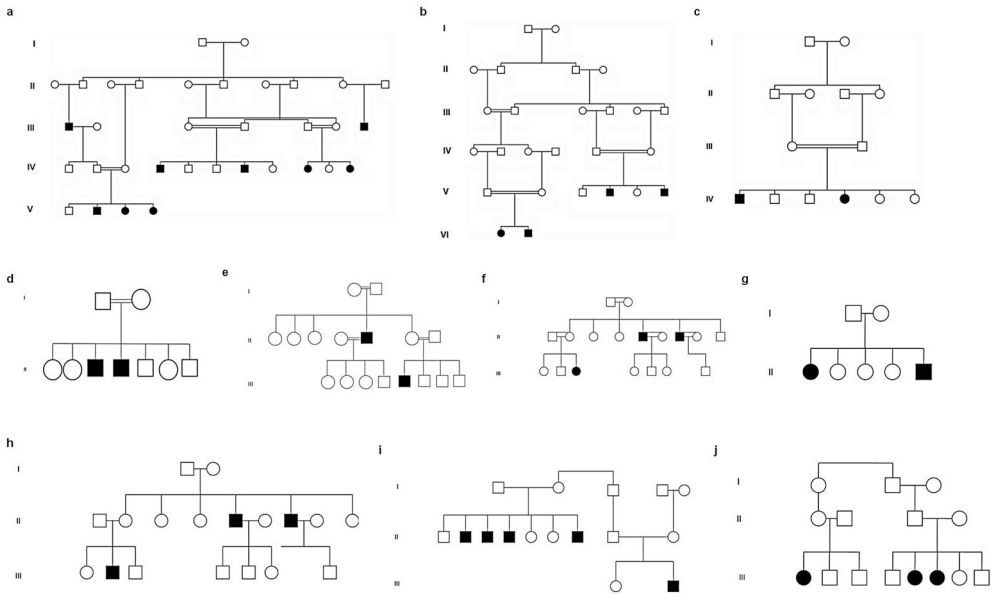

Figure 1.
We studied ten consanguineous Pakistani families (families A to J) with autosomal recessive woolly hair and/or hypotrichosis.

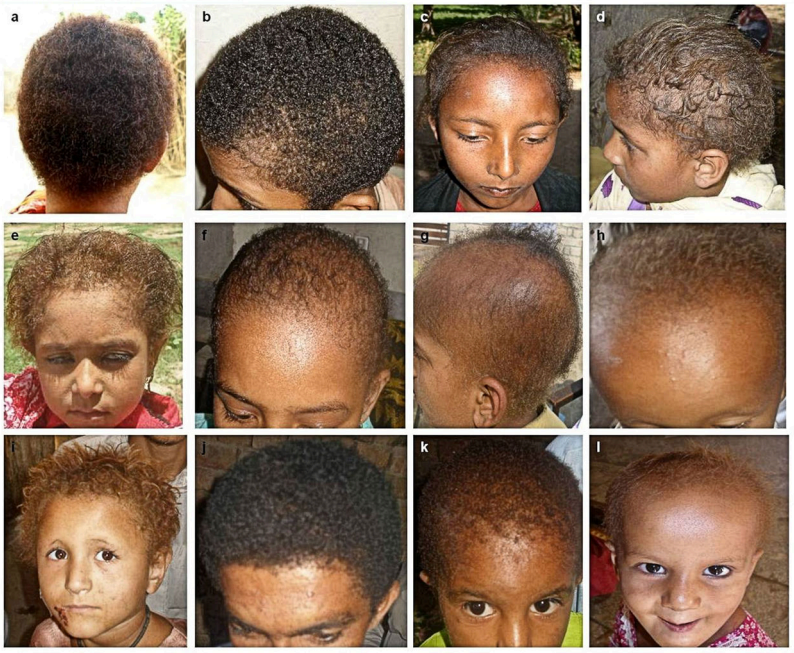

Figure 2.

Affected members from the ten families showed similar phenotypes that varied from persistent woolly hair, to variable degrees of hypotrichosis and hair depigmentation. (a and h) are affected individuals from family A, (b and F) are affected individuals from family B, (c) is an affected individual from family C, (d) is an affected individual from family D, (e) is an affected individual from family E, (g) is an affected individual from family F, (i) is an affected individual from family G, (j) is an affected individual from family H, (k) is an affected individual from family I, (l) is an affected individual from family J.

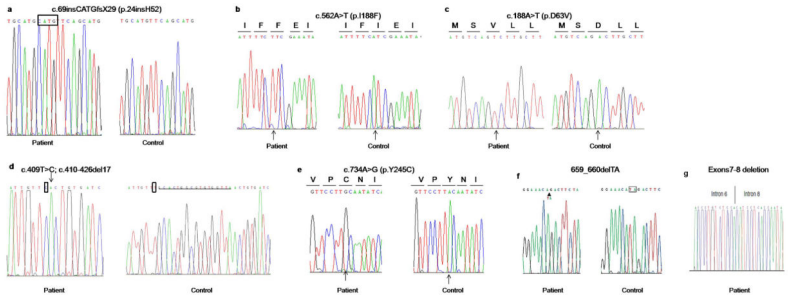

Figure 3.

a) Affected individuals in families A and B had a common homozygous mutation designated, c.69insCATGfsX29 in the *LPAR6* gene. **b)** Affected individuals in families C, D and E had a common homozygous mutation designated, p.I188F in the *LPAR6* gene. **c)** Affected individuals in family F had a homozygous mutation designated, p.D63V in the *LPAR6* gene. **d)** Affected individuals in family G had a homozygous mutation designated c. 409T>C; c.410-426del17 in the *LPAR6* gene. **e)** Affected individuals in family H had the homozygous mutation p.Y245C in the *LPAR6* gene. **f)** Affected individuals in family I had the homozygous mutation c.659_660delTA in the *LIPH* gene. **g)** Affected individuals in family J had homozygous deletion of exons 7 and 8 in *LIPH* gene.

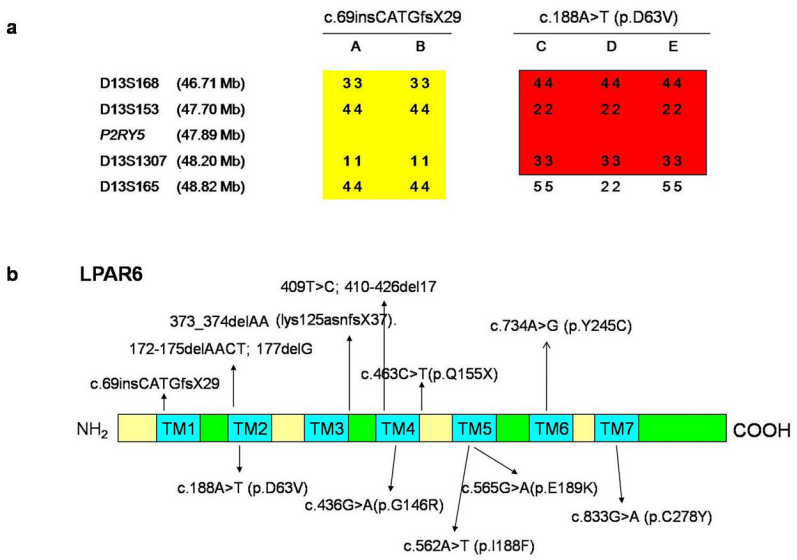

Figure 4.

a) Haplotype analysis showed that c.69insCATGfsX29 and p.I188F are founder mutations in the Pakistani population. **b)** *LPAR6* encodes for a 7 transmembrane G protein coupled-receptor. Mutations occurring in any of the transmembrane (TM) regions are expected to be detrimental. The mutation c.69insCATGfsX29 occurs in TM1, p.D63V occurs in TM2, c. 409T>C; c.410-426del17 occurs in TM4, p.I188F occurs in TM5 and p.Y245C occurs in TM6