Autosomal recessive woolly hair with hypotrichosis caused by a novel homozygous mutation in the *P2RY5* gene

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Abstract

During the last decade, several causative genes for hereditary hair diseases have been identified, which have disclosed the molecular mechanisms involved in hair follicle morphogenesis and cycling. We and others recently reported that mutations in the *P2RY5* gene, encoding an orphan G protein-coupled receptor, underlie autosomal recessive woolly hair and/or hypotrichosis. Although these findings clearly reveal the involvement of *P2RY5* mutations in hereditary hair diseases, the clinical manifestations of *P2RY5* mutations have not completely been elucidated due to limited information to date. In this study, we ascertained a consanguineous family of Iranian origin with an affected girl showing sparse and hypopigmented scalp hair. She had woolly hair phenotype with normal hair density at birth, but progressed with age to have hypotrichosis. Direct sequencing analysis resulted in the identification of a novel homozygous mutation in the *P2RY5* gene of the patient, which results in a non-conservative amino acid change, G146R, at the protein level. Our findings extend the mutation spectrum of *P2RY5* mutations, and further support a crucial role of P2Y5 in hair growth in humans.

Keywords

P2RY5; lipase H; woolly hair; hypotrichosis

INTRODUCTION

The mammalian hair follicle (HF) is a complex multicellular organ, which undergoes dynamic cell kinetics and rearrangements throughout life in the form of a periodic hair cycle (1). Recent advances in molecular genetics have enabled the identification of numerous genes that are expressed in the HF during development and cycling (2). There are several types of hereditary hypotrichosis for which causative genes have been identified. For example, atrichia with papular lesions (OMIM 209500) is caused by mutations in hairless gene (*HR*) (3–5). Mutations in desmoglein 4 gene (*DSG4*) cause localized autosomal recessive hypotrichosis/monilethrix (OMIM 607903/252200) (6–9). Furthermore, it has recently been shown that mutations in the lipase H gene (*LIPH*) underlie an autosomal recessive form of hypotrichosis (OMIM 604379) (10–12). These findings provide insight into mechanisms of hair cycle regulation, hair shaft differentiation, and hair growth. However, mechanisms involved in determining the hair texture, which is variable among individuals and different populations, remain largely unknown.

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Woolly hair (WH) is a group of hair disorders characterized by tightly curled hair (13). There are some syndromes that show WH as a part of the disease, such as Naxos disease (MIM 601214) (14) and Carvajal syndrome (MIM 605676) (15). In addition, isolated WH without systemic symptoms also exists and can show either an autosomal dominant (ADWH; OMIM 194300) (16) or recessive (ARWH; OMIM 278150) (16,17) inheritance pattern. Importantly, it has been suggested that hypotrichosis can be an associated feature of ARWH (16,17). We and others recently performed linkage studies (18–20) and identified pathogenic mutations in *P2RY5* gene in several families with ARWH or hypotrichosis (19,20). The *P2RY5* gene encodes a G protein-coupled receptor P2Y5, which is abundantly expressed in the inner root sheath of the HF, and is likely to be involved in the maintenance of the structural integrity of the hair shaft (19).

In this study, we ascertained a consanguineous family of Iranian origin with an affected girl showing sparse and hypopigmented hairs. Direct sequencing analysis led to the identification of a novel homozygous mutation G146R in the *P2RY5* gene of the affected individual. These data enable a better understanding of the clinical features resulting from *P2RY5* mutations, and underscore the crucial role of P2Y5 in HF development and hair growth in humans.

MATERIALS AND METHODS

Mutation analysis of *P2RY5* gene

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. The *P2RY5* gene was screened for mutations as described previously (19). The mutation 436G>A generates a *Bcl*I restriction enzyme site, which was used to screen parents and control individuals. A part of the coding sequence of the *P2RY5* gene was amplified by PCR using a forward primer (5′-CACGGAATTGGCCATTTGGA-3′) and a reverse primer (5′-GTTTTCCATGTGGCTTCTGG-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, 301 bp in size, were digested with the *Bcl*I at 50°C overnight, and run on 1.5% agarose gels.

RESULTS

Clinical features

The 8-year-old proband is the child of first cousin parents of Iranian origin (Fig. 1A). At birth, she had tightly curled hair on her scalp which was of normal density (Fig. 1B). Since the age of 1 year, her hair loss has gradually progressed. Upon examination, she exhibited sparse, thin, short, and hypopigmented scalp hairs (Fig. 1C, D). Perifollicular papules were detected only in the occipital region (Fig. 1D). The eyebrows were also sparse, whereas the eyelashes were normal. Under light microscopy, the hair shafts showed dystrophic features (Fig. 1E), and the distal portion of the hair shafts were frequently broken (Fig. 1F). Teeth, nails, and sweating were normal, and palmoplantar hyperkeratosis was absent. There was no family history of either heart diseases or neurologic abnormalities.

Identification of a novel mutation in *P2RY5* gene

We first checked for mutations in *DSG4* and *LIPH* genes which are known to underlie autosomal recessive forms of hypotrichosis (6,10), but found no sequence variants in either gene. Based on the recent findings of *P2RY5* mutations in ARWH or hypotrichosis (19,20),

we performed direct sequencing analysis of *P2RY5* gene, and identified a novel mutation in the *P2RY5* in the family. The affected individual is homozygous for a G- to -A transition at position 436 of the *P2RY5* gene, designated 436G>A, which results in a substitution of glycine by an arginine residue at amino acid 146 (G146R) (Fig. 2A). Both parents are heterozygous for this nucleotide change (Fig. 2A). Screening assays using the *Bcl*I restriction enzyme excluded the existence of this mutation in 100 unrelated healthy control individuals (200 chromosomes) (Fig. 2B; data not shown).

Discussion

In this study, we studied a consanguineous Iranian family with an affected girl showing sparse, thin and depigmented scalp hairs, and identified a novel homozygous mutation 436G>A (G146R) in the *P2RY5* of the patient (Fig. 2A). The *P2RY5* gene is a nested gene residing within intron 17 of the retinoblastoma 1 (*RB1*) gene on human chromosome 13q14, and encodes P2Y5 protein which is a heptaspan transmembrane G protein-coupled receptor (GPCR) (21). The glycine residue at codon 146 is located within the fourth transmembrane domain in P2Y5 protein and is highly conserved between different species (Fig. 3). The non-conservative amino acid change G146R is most likely to severely affect the structure of P2Y5 protein.

We previously identified five distinct pathogenic mutations in the *P2RY5* genes in several consanguineous Pakistani families with affected individuals showing tightly curled hair (19) (Fig. 4). Although WH is a unifying feature among all affected individuals, hair density is variable from normal to sparse, and such variations in severity are detected even within a single family (19). In addition, we recently identified a homozygous mutation C278Y in the *P2RY5* in a consanguineous Brazilian family with several affected individuals with a severe hypotrichosis (Fig. 4) (22). It is noteworthy that all affected individuals in the Brazilian family showed WH phenotype at birth and lost their hair during the first decade of life. In addition to our findings, Pasternack et al. recently identified two distinct mutations in the *P2RY5* gene in Saudi Arabian families with affected individuals characterized by progressive hair loss that were diagnosed as autosomal recessive hypotrichosis simplex (MIM 146520), but display abnormal hair texture (Fig. 4) (20). Collectively, it is evident that the phenotypes resulted from mutations in the *P2RY5* gene are highly variable (23). A similar phenomenon has been noted with mutations in the *DSG4* gene, which display an overlap in phenotype between hypotrichosis and monilethrix (7–9,24).

Mutations in *LIPH* gene were recently shown to cause an autosomal recessive form of hypotrichosis (10–12), as well as ARWH (Shimomura *et al.*, submitted). Interestingly, clinical features of affected individuals with *LIPH* mutations are indistinguishable from those with *P2RY5* mutations. The *LIPH* gene encodes a phospholipase A1 family member and is known to produce lysophosphatidic acid (LPA) (25). LPA is an extracellular mediator of many biological functions and has been reported to promote hair growth in vivo (26), and P2Y5 is a receptor of LPA (20). Our work further suggests that the LIPH/LPA/P2Y5 signaling pathway plays a crucial role in the hair structure in humans, and also provides a new therapeutic target for hair disorders.

Acknowledgments

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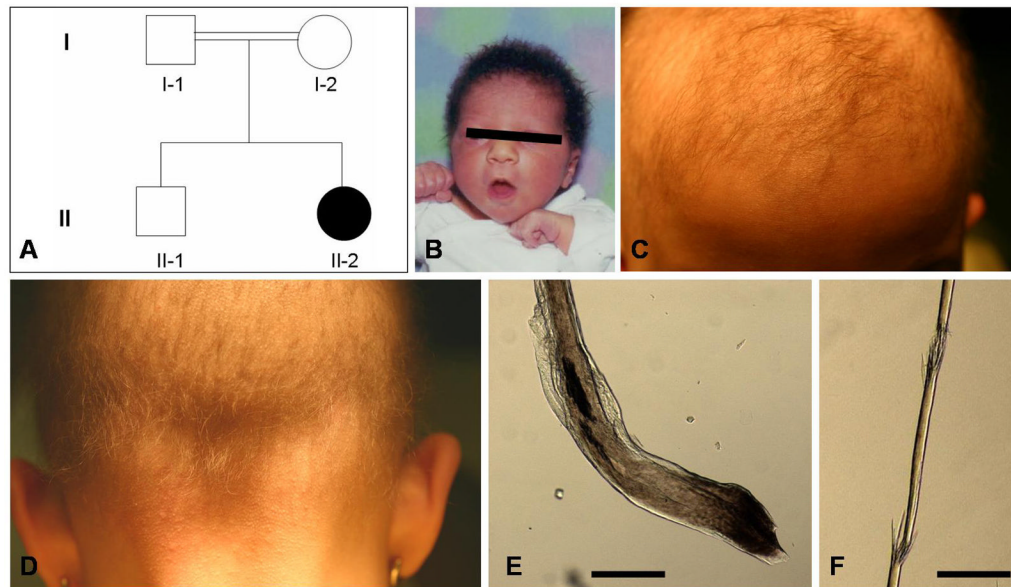
Figure 1. Clinical features of the proband with ARWH

(A) Pedigree of the family. Clinical appearance at the ages of 1 (B) and 8 (C, D). (E, F) Appearance of the hair shafts under light microscopy. Scale bars: 100 μm.
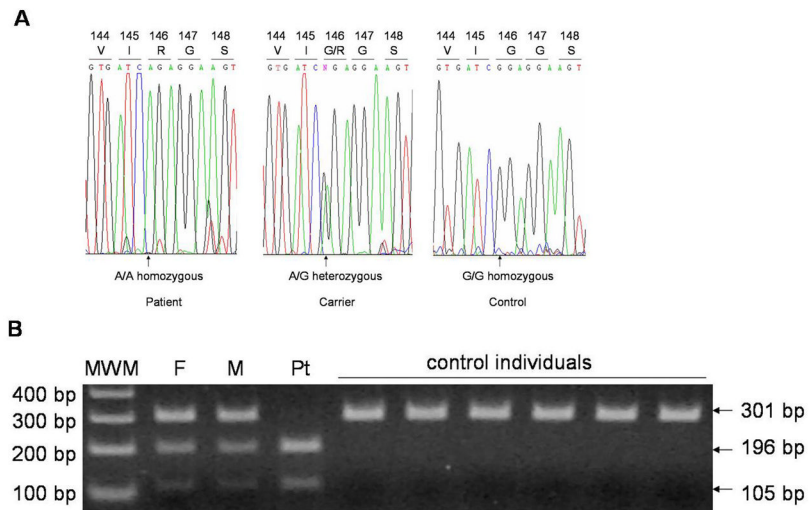
Figure 2. Identification of a mutation in the *P2RY5* gene
(A) Homozygous mutation 436G>A (G146R) in the *P2RY5* gene of the patient. (B) Screening assays for the mutation 436G>A (G146R). PCR products from the mutant allele were digested into 196 bp and 105 bp fragments with the *Bcl*I restriction enzyme. MWM, molecular weight marker; F, father; M, mother; Pt, patient.
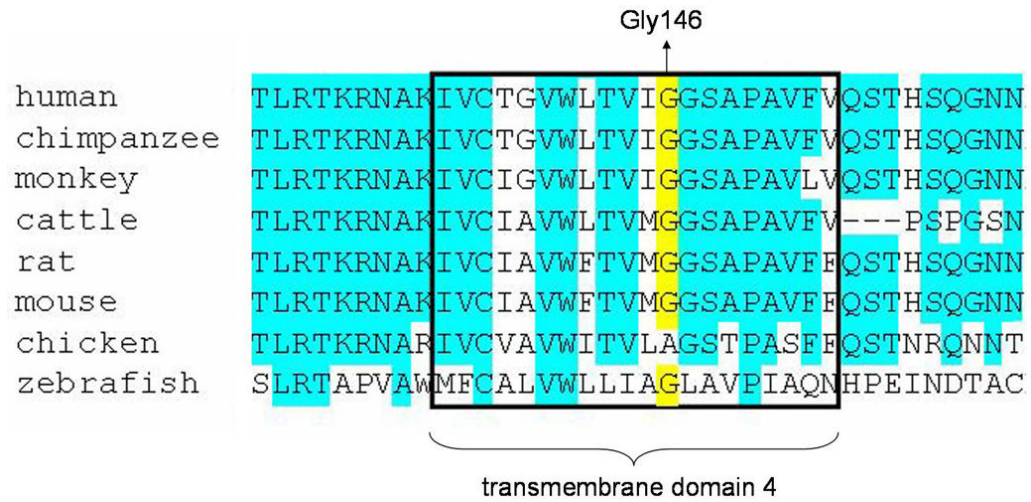
Figure 3. Multiple amino acid sequence alignment of P2Y5 between different species
Residue Gly146 is indicated in yellow. Residues that are conserved among at least six species are colored blue. The fourth transmembrane domain is boxed.
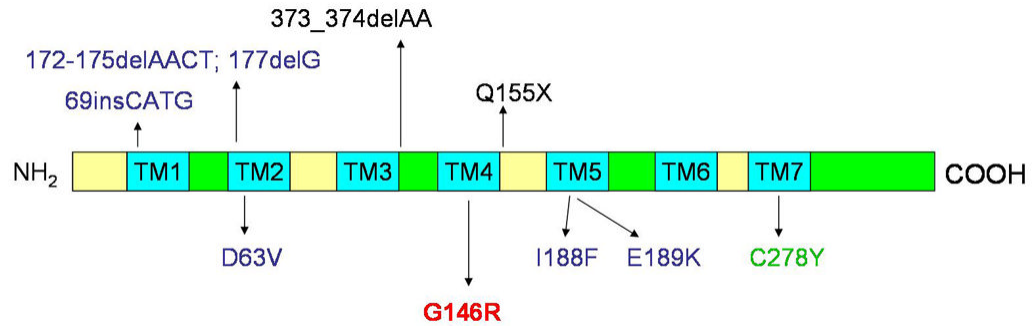
Figure 4. Schematic representation of P2Y5 protein and the *P2RY5* mutations
Premature termination codon mutations are shown above the schematic, and missense
mutations are shown below. The mutation G146R reported in this study is indicated in red.
Mutations identified in Pakistani families and Saudi Arabian families are colored in blue and
black, respectively. The mutation C278Y found in a Brazilian family is indicated in green.
Transmembrane domains (TM1-7) are colored in blue.