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Nonsense mutations in the hairless gene underlie APL in five families of Pakistani origin

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Abstract

(1) Background—Atrichia with papular lesions (APL) is a rare autosomal recessive form of inherited alopecia. Affected individuals present with a distinct pattern of total hair loss on the scalp, axilla and body shortly after birth and are essentially devoid of eyelashes and eyebrows. This form of hair loss is irreversible and the histology is consistent with an absence of mature hair follicles. In addition to total atrichia, APL patients also present with papules and follicular cysts filled with cornified material. Mutations in the *Hairless* (*HR*) gene have been shown to underlie APL.

(2) Objective—Here, we studied five unrelated large Pakistani families with clinical manifestations of APL.

(3) Methods—Based on previous reports of *HR* mutations in APL, we performed direct DNA sequencing analysis.

(4) Results—DNA sequencing of the *HR* gene in APL patients revealed three novel nonsense mutations in five unrelated families. All affected individuals were homozygous for a nonsense mutation due to C-to-T transitions at different positions in the amino acid sequence. Two families carry the mutation Q323X (CAG-TAG) in exon 3, two families harbor the mutation Q502X (CAG-TAG) in exon 6, and one family had a mutation at R940X (CGA-TGA) in exon 14. Haplotype analysis revealed that all affected individuals of both APL1 and APL16 families were homozygous for the same haplotype, and likewise, the mutation in families APL2 and APL19 was on the the same haplotype.

(5) Conclusions—We report three novel nonsense mutations in the *HR* gene in APL. Two of the newly identified mutations, Q323X and Q502X, were found to be shared between unrelated families and marker analysis confirmed an identical homozygous haplotype for APL1 and APL16, and for APL2 and APL19. These findings suggest that Q323X and Q502X did not arise independently, but instead appear to have been propagated in the population. Collectively, these findings contribute further evidence for the involvement of hairless mutations in papular atrichia.

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Keywords

Nonsense mutation; hairless gene; atrichia with papular lesions (APL); autosomal recessive; alopecia; hairloss

Introduction

Atrichia with papular lesions (APL) (OMIM#209500) is a rare autosomal recessive disorder characterized by complete hair loss including scalp, axilla and body shortly after birth in combination with the development of papular lesions of keratin-filled cysts on various regions of the body [1]. This form of hair loss is irreversible and histology is consistent with an absence of mature hair follicles. APL was mapped to chromosome 8p12 and mutations in the *Hairless (HR)* gene have been found in a growing number of APL patients [2-18]. The diagnosis of APL requires detailed family history, especially of consanguinity, a clinical history, DNA sampling, and identification of a *HR* mutation. Using these methods, we and others have identified several types of pathogenic *HR* mutations in multiple families of various ethnic backgrounds including nonsense, missense, insertion and deletion mutations [2-18].

Recently, we identified five Pakistani families with clinical manifestations of APL and high degree of consanguinity. Four of the families described herein originated from different geographic cities in the Punjab region of Pakistan. The family APL9 derived from the Kashmir region, approximately 500km away from Punjab. All affected individuals were identified by generalized scalp and body alopecia, sparse eyebrows and lashes in combination with papules. Direct DNA sequencing of the *HR* gene in APL patients revealed *HR* mutations in each family. Here, we report three novel nonsense mutations among these five unrelated families, and haplotype analysis for the shared alleles.

Materials and Methods

Preparation of Nucleic Acids and Direct Sequencing

Genomic DNA was isolated from blood following informed consent using the Pure-Gene DNA Isolation Kit (Gentra Systems) and PCR was performed using *HR* specific primers to amplify all exons and splice junctions of *HR* as previously described (6). Briefly, PCR products were purified using the Rapid PCR Purification Systems (Marligen Biosciences) and eluted in H_20. Sequencing PCR was performed using purified amplicon and either forward or reverse primer (10pmol) with BigDye® Terminator v3.1 Cycle Sequencing Kits (ABI). Samples were purified using Centriflex Gel Filtration Cartridges (Edge Biosystems), resuspended in Hi Dye (ABI), and sequenced using the 310 Genetic Analyzer (ABI Prism).

Haplotype Analysis

In order to analyze whether the mutations Q323X and Q502X were found on the same haplotype, genomic DNA from members of APL1, APL2, APL16 and APL19 was amplified by PCR using primers for three polymorphic markers close to the *HR* gene, D8S282, D8S560 and D8S1786. The amplification conditions for each PCR 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. PCR products were run on 8% polyacrylamide gels and genotypes were assigned by visual inspection.

Results

We identified three novel nonsense mutations in affected individuals in five families. All affected individuals were homozygous for nonsense mutations due to C-to-T transitions at different positions in the amino acid sequence. All affected individuals presented with the same clinical manifestations of generalized scalp and body hair as well as the presence of papules (Fig.1). The mutations in the APL1 and the APL16 families (Fig.2) are located in exon 3 and are the result of a C-to-T transition (CAG-TAG) in the glutamine residue at position 323, changing to a stop codon, designated Q323X. Further, we identified a nonsense mutation in exon 6 in the affected members of the APL2 and APL19 families (Fig. 2). All of them showed a C-to-T transition (CAG-TAG) in the glutamine residue at position 502, changing to a stop codon, designated Q502X. The affected members of the APL9 family (Fig.3) were found to have a nonsense mutation (CGA-TGA) in exon 14 with a C-to-T transition in an arginine residue at position 940, changing to a stop codon, and designated R940X. Further, genotyping with microsatellite markers (Fig.2) revealed a homozygous haplotype for the Q323X mutation in the APL1 and APL16 families, and for the Q502X mutation in the APL2 and APL19 families.

Discussion

We have identified three novel nonsense mutations in the *HR* gene resulting in APL in five large families from Pakistan. The location and the severity of the phenotype do not indicate a difference between nonsense mutations or other types of mutations, similar to previous reports [2-18]. Because all of the nonsense mutations reported here are upstream of the last and penultimate exons of the *HR* gene, it is likely the mRNAs bearing the nonsense mutations will be degraded via nonsense mediated mRNA decay [19]. One of the three mutations resides at a CpG dinucleotide (R940X), suggesting it may result from the spontaneous deamination of cytosine to thymine [20,21].

The continued identification of novel mutations is important to the understanding and diagnosis of APL. There are several other genetic forms of alopecia, which may be misdiagnosed as APL [1] and the discovery of new mutations in *HR* allows for a streamlined and thorough screening of the *HR* gene to confirm a diagnosis of APL. The recurrence of particular *HR* mutations in certain populations, as exemplified herein, will not only create a database of *HR* mutations, resulting in a faster and correct diagnosis of APL in future patients from special populations, but also contributes to our understanding of allelic variations in *HR* as well as the identification of founder mutations, which will continue to be informative in understanding the pathogenesis of APL.

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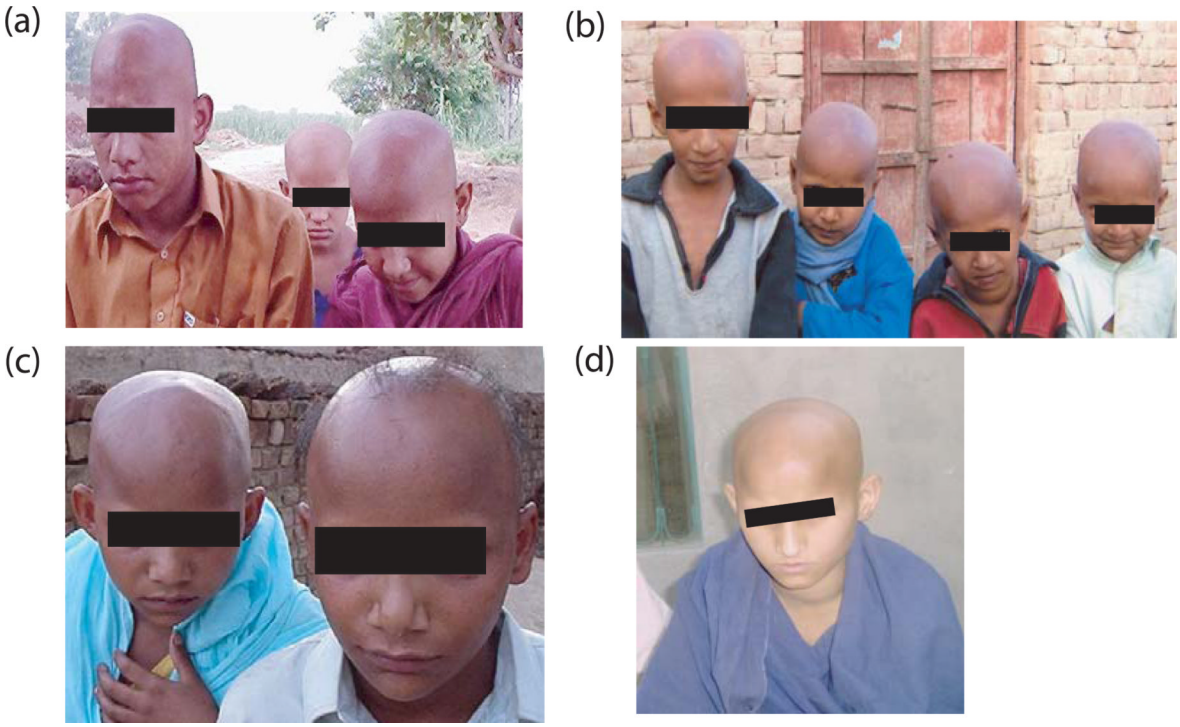

Fig. 1. Clinical presentation of APL

Family APL1 and APL2 are from the Punjab region of Pakistan. The clinical photograph of members of the (a) APL1, (b) APL16, (c) APL2 and (d) APL19 show features of APL, including the complete alopecia seen here.

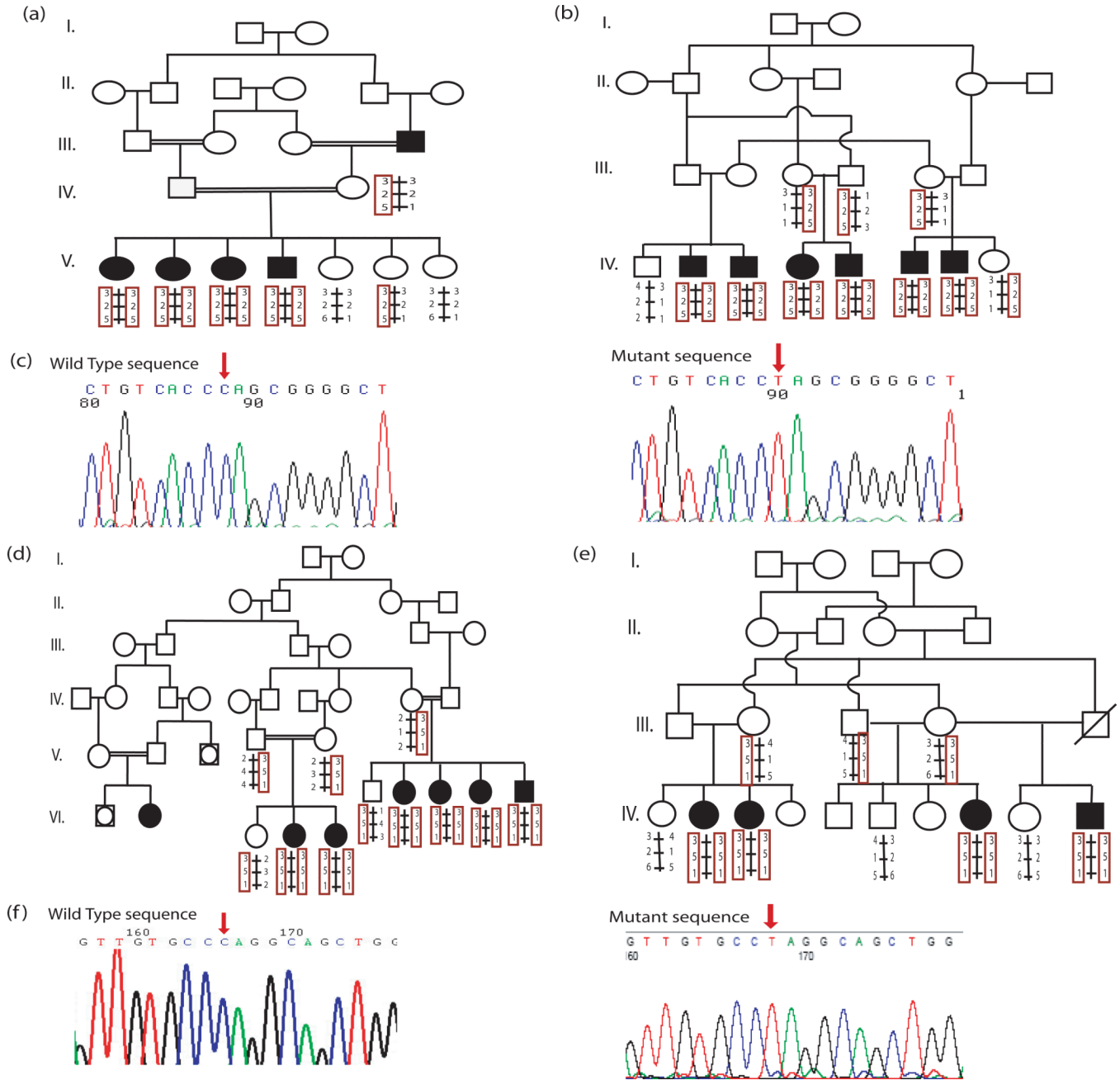

Fig. 2. Pedigrees, mutation and haplotype analysis

Pedigree and haplotype analysis of (a) APL1 and (b) APL16 families. Analysis of individuals genotyped in the pedigrees shows that APL1 and APL16 families have a shared haplotype for the mutant allele. (c) The mutation was identified as a C- to- T transition resulting in the conversion of Q323 (CAG) to a stop codon (TAG) in exon 3, designated Q323X. Pedigree and haplotype analysis of (d) APL2 and (e) APL19 families. Analysis of individuals genotyped in the pedigrees shows that APL2 and APL19 families have a shared haplotype for the mutant allele. (f) The mutation was identified as a C- to-T transition resulting in the conversion of Q502 (CAG) to a stop codon(TAG) in exon 4, designated Q502X.

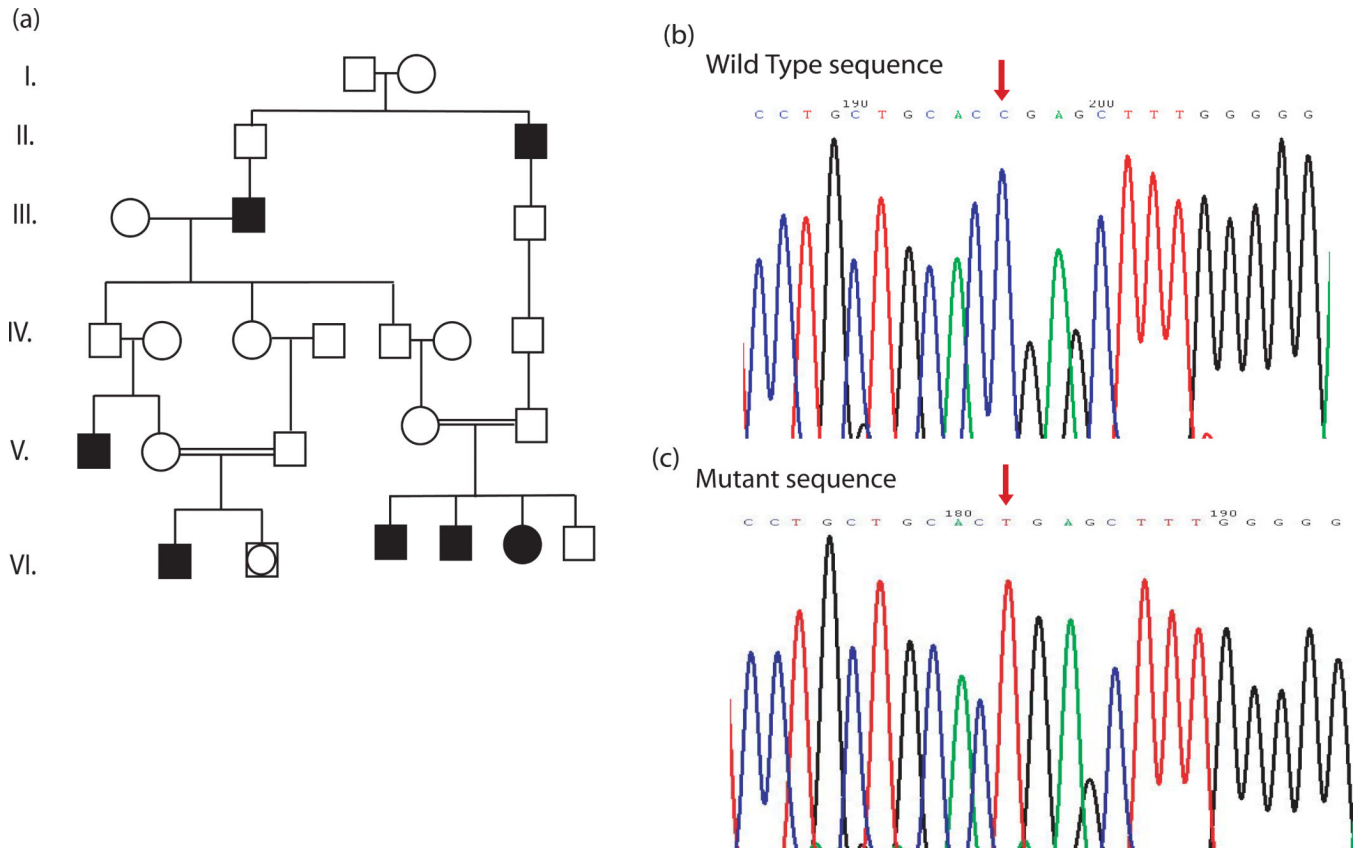

Fig. 3. Family APL9 is from the Kashmir region of Pakistan

(a) Pedigree of APL9. (b,c) The mutation was determined to be C- to- T transition resulting in the conversion of R940 (CGA) to a STOP codon (TGA) in exon 14, designated R940X.