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

Hyunmi Kim [a], Muhammad Wajid [a], Liv Kraemer [a], Yutaka Shimomura [a], Angela M. Christiano [a,b,*]

[a] *Department of Dermatology, Columbia University, College of Physicians & Surgeons, 630 West 168th Street VC15 204A, New York, NY 10032, United States*
[b] *Department of Genetics and Development, Columbia University, College of Physicians & Surgeons, 630 West 168th Street VC15 204A, New York, NY 10032, United States*

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Alopecia;
Hairloss

Summary

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.
Objective: Here, we studied five unrelated large Pakistani families with clinical manifestations of APL.
Methods: Based on previous reports of *HR* mutations in APL, we performed direct DNA sequencing analysis.
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 same haplotype.
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

* Corresponding author. Tel.: +1 212 305 9565; fax: +1 212 305 7391.
E-mail address: amc65@columbia.edu (A.M. Christiano).

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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.

1. 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 500 km 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.

2. Materials and methods

2.1. 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_2O. Sequencing PCR was performed using purified amplicon and either forward or reverse primer (10 pmol) with BigDye® Terminator v3.1 Cycle Sequencing Kits (ABI). Samples were purified

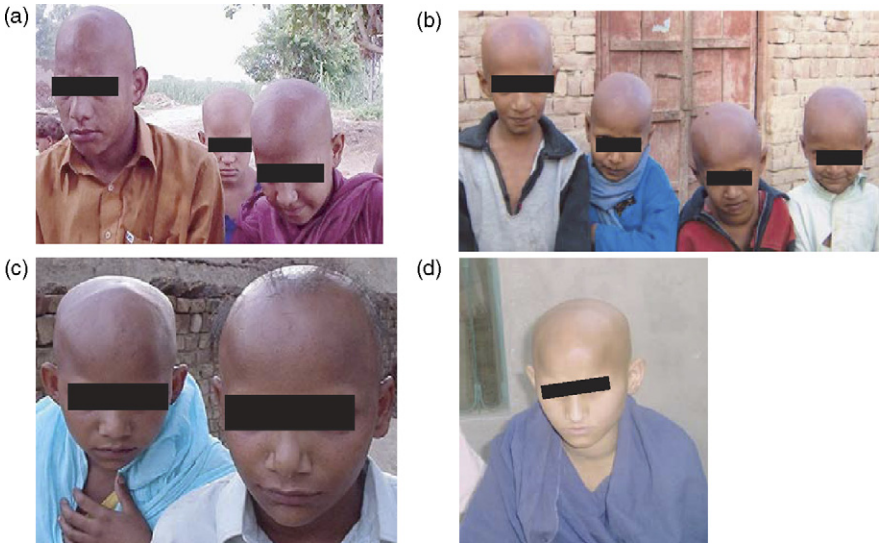

Fig. 1 Clinical presentation of APL. Family APL1 and APL2 are from the Punjab region of Pakistan. The clinical photograph of members of the (a) APL1, (b) APL16, (c) APL2 and (d) APL19 show features of APL, including the complete alopecia seen here.