NIH Public Access
Author Manuscript
J Dermatol Sci. Author manuscript; available in PMC 2010 August 3.

Published in final edited form as:
J Dermatol Sci. 2008 April ; 50(1): 25–30. doi:10.1016/j.jdermsci.2007.10.012.

Mutations in the hairless gene underlie APL in three families of Pakistani origin

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

[a] Department of Dermatology, Columbia University, New York, NY, United States

[b] Department of Genetics and Development, Columbia University, New York, NY, United States

Summary

Background—Atrichia with papular lesions (APL) (OMIM#209500) is a rare autosomal recessively inherited form of irreversible alopecia characterized by papular lesions of keratin-filled cysts on various regions of the body. Males and females are equally affected and present with a distinct pattern of total hair loss on scalp, axilla and body. It begins shortly after birth with the development of hair loss, and patients are normally devoid of eyelashes and eyebrows. Mutations in the *hairless* (*HR*) gene have been previously shown to be responsible for APL.

Objective—In this study, we studied the molecular basis of APL in three unrelated families of Pakistani origin.

Method—Molecular analysis of the *HR* genes was performed on genomic DNA from probands and family members.

Results—DNA sequencing of the *HR* gene in family A revealed a novel homozygous 2 bp deletion in exon 6 leading to a frameshift and a downstream premature termination codon in exon 8 (1782-83delAG). In family B, we identified a novel homozygous deletion of a G nucleotide at the exon 15–intron 15 boundary, termed 3097delG. Family C carries a previously reported missense mutation consisting of an A-to-G transition at nucleotide 276 resulting in the mutation N970S in exon 14.

Conclusion—Two mutations identified in this study are novel mutations in the *HR* gene and extend the body of evidence implicating the hairless gene family in the pathogenesis of human skin disorders. The one previously reported mutation suggests it may represent a recurrent mutation, or alternatively, an allele that is widely dispersed around the world.

Keywords

Missense mutation; Deletion; Hairless gene; Atrichia with papular lesions; Autosomal recessive; Alopecia; Hairless

1. Introduction

To date, the genetic basis of several forms of congenital hair loss has been identified, many of which are inherited in an autosomal recessive manner [1]. One example is atrichia with papular lesions (APL) (OMIM#209500), characterized by complete hair loss which begins shortly after birth. The scalp, axillae and the entire body become completely devoid of hairs,

*Corresponding author at: Department of Dermatology and Genetics & Development, Columbia University, College of Physicians & Surgeons, 630 West 168th Street VC15 204A, New York, NY 10032, United States. Tel.: +1 212 305 9565; fax: +1 212 305 7391. E-mail address: amc65@columbia.edu (A.M. Christiano).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

whereas the eyelashes and eyebrows may be just partially affected [2]. This form of hair loss is irreversible and histology confirms an absence of mature hair follicles. After approximately 2 years of age, affected individuals begin to develop papular lesions of keratin-filled cysts on various regions of the body, which represents a unique cutaneaous finding in APL among inherited alopecias. Other ectodermal structures such as nails, sweat glands or teeth are not affected [3,4].

The human homolog of the *hairless* gene has been identified and mapped to human chromosome 8p12, a region syntenic to mouse chromosome 14 [5–7]. The *hairless* gene product is a transcriptional co-regulator with a single zinc-finger domain, which is highly expressed in the skin and the brain [7–9]. Furthermore, mutations in the *hairless* (*HR*) gene were identified in many APL families and to date, more than 30 *hairless* mutations have been reported [10–18]. Although APL could potentially be diagnosed by its clinical features alone, or by skin biopsy, APL remains frequently misdiagnosed as the more common disorder, alopecia universalis (AU). This is maybe due to a lack of awareness, and/or the notion that APL patients are rare and found exclusively in consanguineous families. Therefore, many more mutations are likely to be discovered, since APL appears to be more common than previously considered [15]. To date, *HR* mutations have been found in APL patients from various ethnic backgrounds, including Pakistani, Mediterranean, Arab Palestinian, Caucasians, Japanese and Polish [10–18]. In this study, we identified three Pakistani families originating from different regions of Pakistan with clinical manifestations of APL and high degree of consanguinity. All affected individuals were identified by generalized scalp and body alopecia, sparse eyebrows and lashes as well as papules (Fig. 1A). Direct DNA sequencing of the *HR* gene in APL patients identified unique *HR* mutations in three families of Pakistani origin.

2. Materials and methods

2.1. Preparation of nucleic acids and 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 the exons of *HR* as previously described [10]. Briefly, PCR products were purified using Rapid PCR Purification Systems (Marligen Biosciences) and eluted in H_2O. Sequencing PCR was performed using purified fragments and either forward or reverse primers (10 pmol) with BigDye® Terminator v3.1 Cycle Sequencing Kits (ABI). Samples were purified using Centriflex Gel Filtration Cartridges (Edge Biosystems), resuspended in Hi Dye.

In order to analyze the homozygous deletion G at the intron/exon boundary 15, designated 3097delG in family B, mismatch allele-specific PCR was performed using a forward primer 5′-GACACACCACTGCCTGCCTGGCACCGG**C**C**T**CAGAAA-3′, and a reverse primer 5′-CTGAGGAGGAAAGAGCGCTC-3′. Note that the G > C and A >T substitutions were introduced into the forward primer to generate the *Eco*NI restriction enzyme site only in the PCR product from the wild-type allele (shown in bold and underlined). The amplification conditions were 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 7 min. The PCR products were digested by the *Eco*NI at 37 °C for overnight, which were analyzed on 1.5% agarose gels.

To confirm the missense mutation, direct PCR was used. The DNA mutation N970S was confirmed as a mutation by digestion of the corresponding PCR-amplified product with *DdeI* (New England Biolabs, Beverly, MA). Fifty unrelated, unaffected control DNAs from Pakistani individuals were checked for the mutation N970S by PCR, digestion and run on a 3% agarose gel and visualized by ethidium bromide staining.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

3. Results

In this study, we identified mutations in affected individuals in three families of Pakistani origin. To screen for mutations in the *HR* gene, all exons and splice junctions were PCR amplified and sequenced directly. Sequence analysis of each exon of the hairless gene in the proband of family A revealed a homozygous 2 bp deletion in exon 6 (Fig. 1). The father was a heterozygous carrier. The mutation was designated 1782delAG, and results in a frameshift and a premature termination codon 230 bp downstream in exon 8. All affected members from family B show a homozygous deletion G at the intron/exon boundary 15, termed 3097delG (Fig. 2). This mutation most likely abolished normal splicing of exon 15, and leads to a frameshift and premature stop codon 214 bp downstream in exon 17. The mismatch allele specific PCR showed that 50 unrelated healthy control individuals (100 chromosomes) from Pakistan do not carry the mutation (Fig. 2).

The two affected individuals of family C (Fig. 3) carried a missense mutation consisting of an A-to-G transition (AAC to AGC) resulting in the substitution N (asparagine) to S (serine) at amino acid 970 in exon 14, and is designated N970S. The mutation creates a restriction endonuclease site for the enzyme *DdeI* in exon 14, resulting in a cleavage into products of 145 and 151 bp. The wild-type PCR product contains no restriction site for the enzyme, resulting in a band of 296 bp. The carrier individuals display the 296 bp band together with the superimposed 145 and 151 bp bands, indicative of heterozygosity for the mutant allele.

4. Discussion

Since so many disorders of congenital hair loss exist, it is important to establish the correct diagnosis in order to avoid unnecessary and ineffective treatment options. Since APL is resistant to any treatment modalities due to destruction of the hair follicle, a careful family history, clinical exams and genetic analysis in the hairless gene (*HR*) on chromosome 8p21 would help to establish a definitive diagnosis.

Although cases from small non-consanguineous families have been reported [17], consanguinity and autosomal recessive inheritance still remains a distinguishing feature of APL. Interestingly, there is no genotype–phenotype connections in APL patients. The various *HR* mutations that have been discovered in APL patients, in this report as well as previous reports, do not indicate a correlation between the type of mutation, its location and the severity of the phenotype. All patients with APL are affected to the same degree of clinical serverity, irrespective of the nature of the mutation.

One possible consequence of the mutation 3097delG (which likely abolished normal splicing of exon 15) in the patient of family B is an out-of-frame skipping of exon 15. This mutation results in a deletion of G of the receptor splice side of exon 15 (3097delG). The wild-type sequence of this splice site is: (exon 15) … GCACAGAAAG/**g**taggtcctcggcca … (intron 15). The mutation results in creation of the mutant splice junction: (exon 15) GCACAGAAAG/taggtcctcggcc (intron 15), or alternatively (exon 15) GCACAGAAA/gtaggtcctcggcc (intron 15), in which the consensus G is deleted either within the exon, or within the gt of the donor splice site. The consequence of this mutation could result in the production of several mutant mRNA species. Exon skipping is a well-known potential consequence of mutations which disrupt consensus splicing sequences [18–20]. The presence of exon skipping in this case could not be assessed as tissue samples for analysis of mRNA species generated by this mutation were not available for study, however, exon 15 is 119 bp in size, predicting its skipping would be out-of-frame. The other possibility could be splicing after the *AAA* at the end of the exon, creating a 1 bp deletion, and therefore generating a frameshift mutation.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

N970 is a conserved amino acid in the HR sequence compared among mouse, rat, human and monkey. Interestingly, this mutation has previously been reported in a South Tyrolian APL family [16,21], suggesting it may represent a recurrent mutation, or alternatively represents an allele that is widely dispersed around the world. The recurrent missense mutation reported in exon 14 of the family C and the previously reported South Tyrolian family suggests the C-terminus, including the second TR-ID and the jumonji C domain, to be critical regions for HR function. The C-terminus of HR is a highly conserved region among mouse, rat, monkey and human protein sequences. It is possible that alterations in or loss of this highly conserved region of the *HR* protein results in *HR* inactivation, which suggests a critical domain to *HR* function, resides in this region. The C-terminus of hairless contains a highly conserved region related to the JmjC domain. Five mutations with impaired activity have been reported to be located in this JmjC domain (N970S, D1012N, V1056M and V1136D) showing that these mutations disrupt binding to the vitatmin D receptor [21].

Furthermore, the fact that this mutation was present in this previously reported patient of South Tyrolian origin and in our patient, suggest that they may share a common founder allele.

By expanding the database of allelic series of *HR* mutations (Fig. 4), diagnosis of APL becomes increasingly accurate, and the invariably ineffective treatment of APL patients can be minimized. A refined clinical history, including autosomal recessive inheritance and the findings of small to large numbers of papules distributed over some or all of the following areas: scalp, cheeks, arms, elbows, buttocks, thighs and knees, and under the midline of the eye, as well as a scalp biopsy with the presence of dermal cysts, and in particular the molecular diagnosis of mutations in the HR gene, will help to make a definitive diagnosis of APL. Patients will benefit from genetic analysis as in many cases the diagnosis is done in retrospect, following the lack of response to any treatment modality over several years.

Acknowledgments

We are especially grateful to the patients and their families for their interest in this study. We highly appreciate the skillful help and excellent technical assistance of Ha Mut Lam. This study was supported in part by USPHS NIH grant from NIH/NIAMS R01 AR47338 (to AMC).

References

1. Irvine AD, Christiano AM. Hair on a gene string: recent advances in understanding the molecular genetics of hair loss. Clin Exp Dermatol 2001;26:59–71. [PubMed: 11260183]
2. Zlotogorski A, Panteleyev AA, Aita VM, Christiano AM. Clinical and molecular diagnostic criteria of congenital atrichia with papular lesions. J Invest Dermatol 2001;117:1662–5. [PubMed: 11886538]
3. Paradisi M, Masse M, Martinez-Mir A, Lam H, Pedicelli C, Christiano AM. Identification of a novel splice site mutation in the human hairless gene underlying atrichia with papular lesions. Eur J Dermatol 2005;15:332–8. [PubMed: 16172039]
4. Damste J, Prakken JR. Atrichia with papular lesions: a variant of congenital ectodermal dysplasia. Dermatologica 1954;108:114–7. [PubMed: 13150805]
5. Ahmad W, Faiyaz ul Haque M, Brancolini V, Tsou HC, ul Haque S, Lam H, et al. Alopecia universalis associated with a mutation in the human hairless gene. Science 1998;279:720–4. [PubMed: 9445480]
6. Nothen MM, Cichon S, Vogt IR, Hemmer S, Kruse R, Knapp M, et al. A gene for universal congenital alopecia maps to chromosome 8p21-22. Am J Hum Genet 1998;62:386–90. [PubMed: 9463324]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

7. Cachon-Gonzalez MB, Fenner S, Coffin JM, Moran C, Best S, Stoye JP. Structure and expression of the hairless gene of mice. Proc Natl Acad Sci USA 1994;91:7717–21. [PubMed: 8052649]
8. Stoye JP, Fenner S, Greenoak GE, Moran C, Coffin JM. Role of endogenous retroviruses as mutagens: the hairless mutation of mice. Cell 1988;54:383–91. [PubMed: 2840205]
9. Potter GB, Beaudoin GM 3rd, DeRenzo CL, Zarach JM, Chen SH, Thompson CC. The hairless gene mutated in congenital hair loss disorders encodes a novel nuclear receptor corepressor. Genes Dev 2001;15:2687–701. [PubMed: 11641275]
10. Ahmad W, Zlotogorski A, Panteleyev AA, Lam H, Ahmad M, ul Haque MF, et al. Genomic organization of the human hairless gene (HR) and identification of a mutation underlying congenital atrichia in an Arab Palestinian family. Genomics 1999;1(56):141–8. [PubMed: 10051399]
11. Sprecher E, Bergman R, Szargel R, Friedman-Birnbaum R, Cohen N. Identification of a genetic defect in the hairless gene in atrichia with papular lesions: evidence for phenotypic heterogeneity among inherited atrichias. Am J Hum Genet 1999;64:1323–9. [PubMed: 10205263]
12. Ahmad W, Nomura K, McGrath JA, Hashimoto I, Christiano AM. A homozygous nonsense mutation in the zinc-finger domain of the human hairless gene underlies congenital atrichia. J Invest Dermatol 1999;113:281–3. [PubMed: 10469319]
13. Masse M, Martinez-Mir A, Lam H, Geraghty MT, Christiano AM. Identification of a recurrent mutation in the human hairless gene underlying atrichia with papular lesions. Clin Exp Dermatol 2005;30:363–5. [PubMed: 15953070]
14. Betz RC, Indelman M, Pforr J, Schreiner F, Bauer R, Bergman R, et al. Identification of mutations in the human hairless gene in two new families with congenital atrichia. Arch Dermatol Res 2007;299:157–61. [PubMed: 17372750]
15. Zlotogorski A, Panteleyev AA, Aita VM, Christiano AM. Clinical and molecular diagnostic criteria of congenital atrichia with papular lesions. J Invest Dermatol 2002;118:887–90. [PubMed: 11982770]
16. Kruse R, Cichon S, Anker M, Hillmer AM, Barros-Nunez P, Cantu JM, et al. Novel hairless mutations in two kindreds with autosomal recessive papular atrichia. J Invest Dermatol 1999;113:954–9. [PubMed: 10594736]
17. Henn W, Zlotogorski A, Lam H, Martinez-Mir A, Zaun H, Christiano AM. Atrichia with papular lesions resulting from compound heterozygous mutations in the hairless gene: a lesson for differential diagnosis of alopecia universalis. J Am Acad Dermatol 2002;47:519–23. [PubMed: 12271294]
18. Terracina M, Posteraro P, Schubert M, Sonego G, Atzori F, Zambruno G, et al. Compound heterozygosity for a recessive glycine substitution and a splice site mutation in the COL7A1 gene causes an unusually mild form of localized recessive dystrophic epidermolysis bullosa. J Invest Dermatol 1998;111:744–50. [PubMed: 9804332]
19. Kon A, McGrath JA, Pulkkinen L, Nomura K, Nakamura T, Maekawa Y, et al. Glycine substitution mutations in the type VII collagen gene (COL7A1) in dystrophic epidermolysis bullosa: implications for genetic counseling. J Invest Dermatol 1997;108:224–8. [PubMed: 9008239]
20. Paller AS, Varigos G, Metzker A, Bauer RC, Opie J, Martinez-Mir A, et al. Compound heterozygous mutations in the hairless gene in atrichia with papular lesions. J Invest Dermatol 2003;121:430–2. [PubMed: 12880440]
21. Wang J, Malloy PJ, Feldman D. Interactions of the vitamin D receptor with the corepressor hairless: analysis of hairless mutants in atrichia with papular lesions. J Biol Chem 2007;282:25231–9. [PubMed: 17609203]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

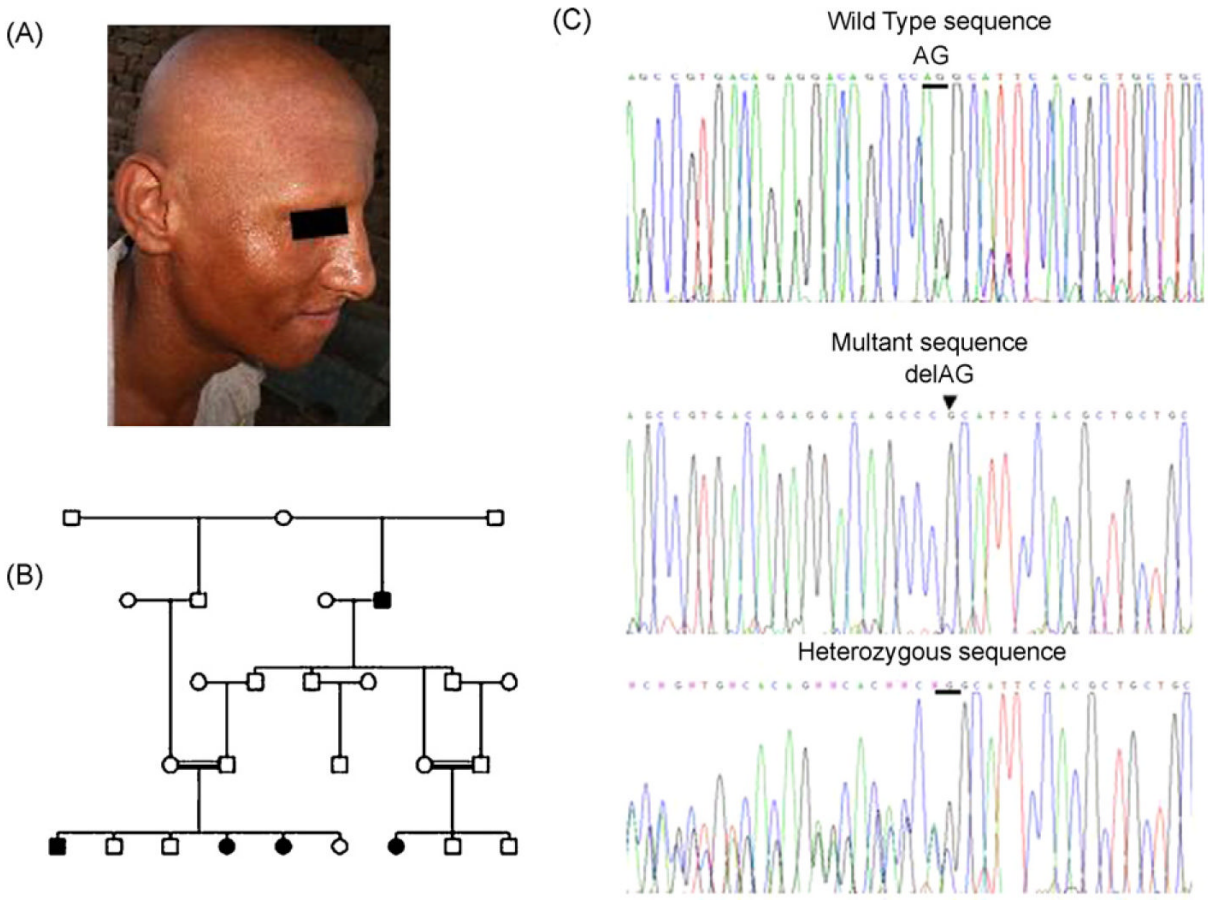

Fig. 1.

(A) Clinical appearance of atrichia with papular lesions in an individual of Pakistani origin (family A). The patient is nearly devoid of scalp hair, eyebrows and eyelashes. Note the papules and dermal cysts on face and scalp. (B) Pedigree of family A of Pakistani origin. (C) The mutation was identified as a deletion AG resulting in a frameshift and a premature termination codon.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

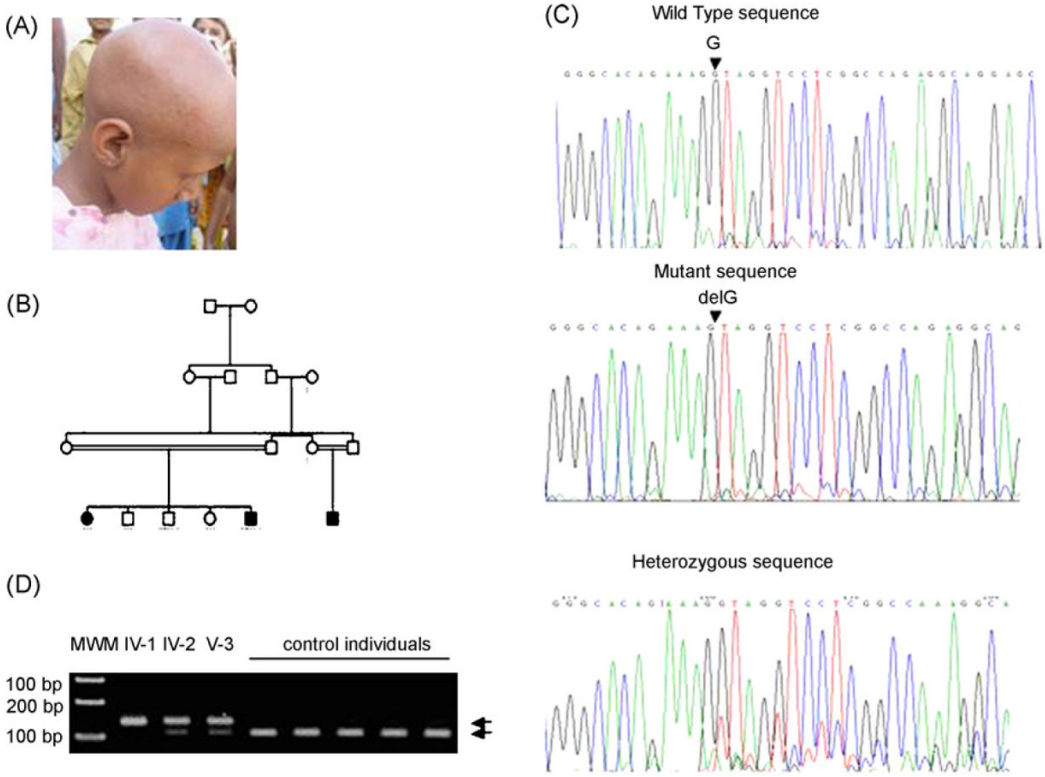

Fig. 2.

(A) Clinical appearance of atrichia with papular lesions in an individual of Pakistani origin (family B). Note the papules and dermal cysts on face and scalp. (B) Pedigree of family B is of Pakistani origin. (C) The mutation identified was a homozygous deletion G at the intron/exon boundary 15, designated 3097delG. (D) For screening of the 3097delG mutation, mismatch allele-specific PCR was performed. The PCR product, 135 bp in size, was subsequently digested with the *Eco*NI restriction enzyme. Note that only the PCR product from the wild-type allele was digested into two fragments, 103 and 32 bp in size. The 32 bp fragment was not shown. MWM, molecular weight marker.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

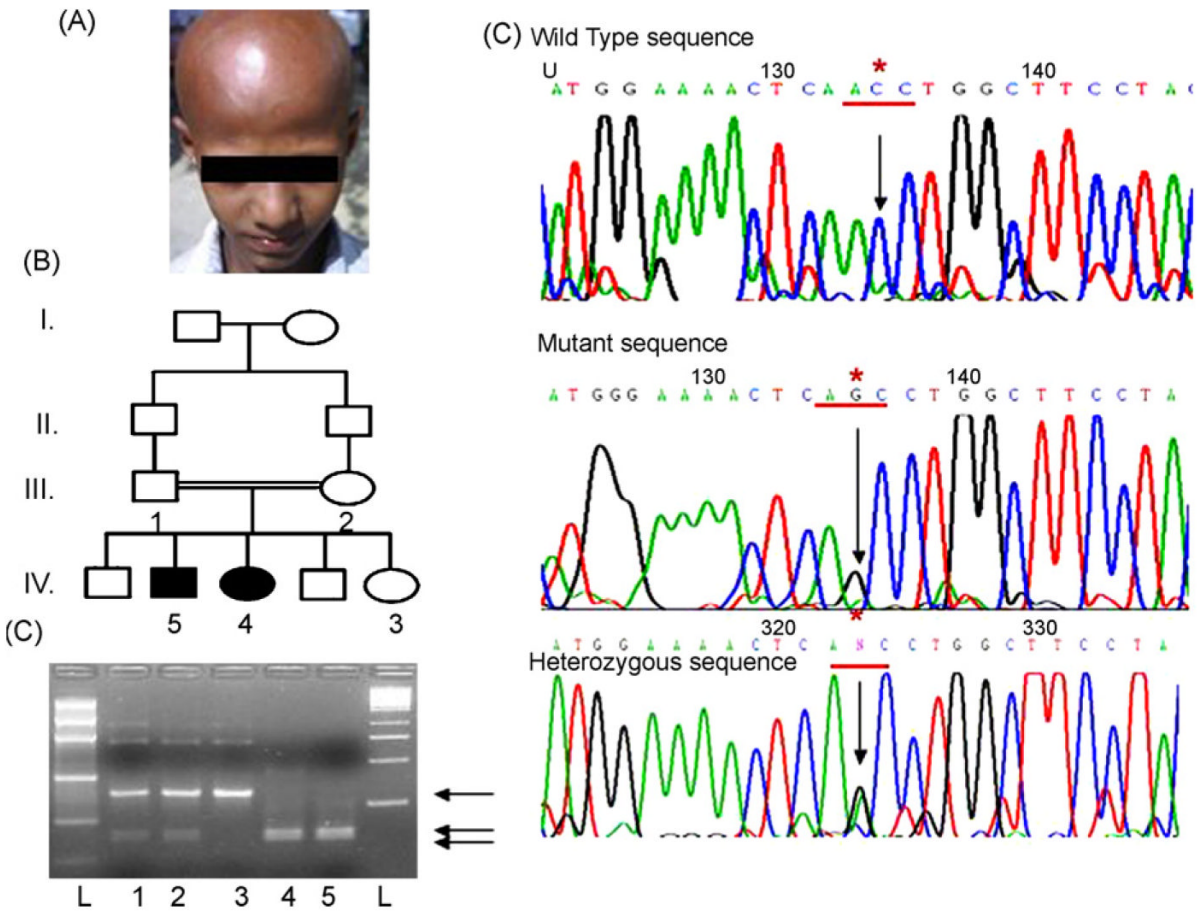

Fig. 3.

Family C is from the Punjab region of Pakistan. (A) Clinical appearance of atrichia with papular lesions in an individual of Pakistani origin (family C). (B) Pedigree of family C. (C) Restriction endonuclease analysis of the hairless gene with the enzyme *DdeI*. Note the mutation creates a restriction endonuclease site for the enzyme *DdeI* in exon 14, resulting in a cleavage into products of 145 and 151 bp. The wild-type allele abolishes a restriction site for that enzyme, resulting in a product of 296 bp. The carrier individuals display the 296 bp band together with the superimposed 145 and 151 bp bands, indicative of the heterozygosity for the mutant allele. (D) The mutation was determined to be a A-to-G transition resulting in the conversion of N970 (AAC) to a S970 codon (AGC) in exon 14, designated N970S.

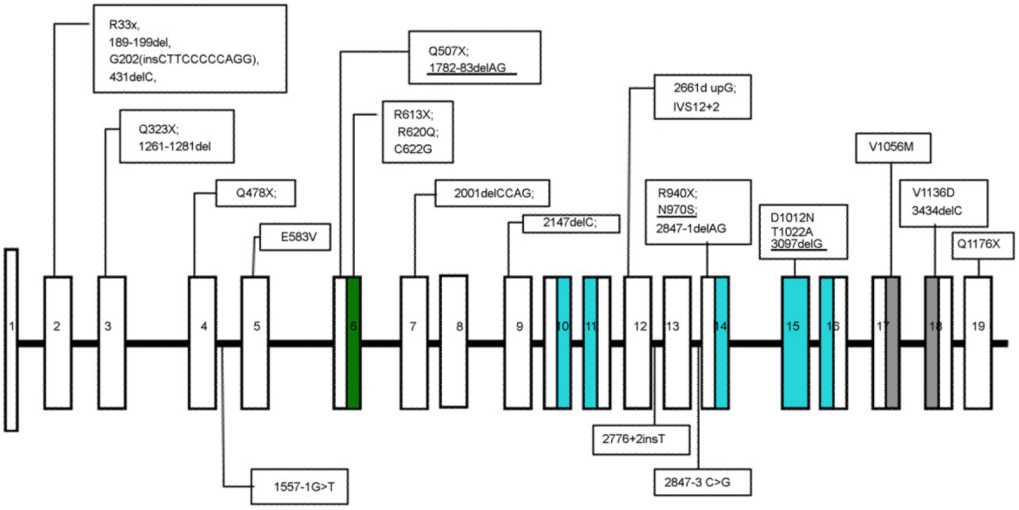

Fig. 4.

The spectrum of mutations in the human hairless gene causing atrichia with popular lesions are superimposed on the genomic organization of the human hairless gene. Exons are represented by bars, introns by the horizontal line. Exon 1 contains the 5′ UTR, exon 2 contains the initiation methionine, and exon 19 the 3′ UTR termination codon. The zinc-finger domain is in exon 6 and highlighted in green, the TR-ID domain is in exon 10, 11, 14, 15 and 16 and is highlighted in blue. The Jumonji C domain is in exon 17 and 18 and highlighted in grey. In this setting the mutations in the hairless gene are listed, including the findings in this paper (underlined).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript