Compound Heterozygous Mutations in the Hairless Gene in Atrichia with Papular Lesions

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Abbreviations: APL, atrichia with papular lesions; AU, alopecia universalis; *HR*, hairless gene

To the Editor:

Inherited hair loss can follow both Mendelian and complex patterns of inheritance. Alopecia areata is a nonscarring form of hair loss with a prevalence of 0.1%-0.2% in the USA (Safavi, 1992). Its most severe form, alopecia universalis (AU) leads to total hair loss from the whole body. Alopecia areata is inherited as a complex genetic trait in which coinheritance of more than one gene is believed to confer susceptibility to the phenotype. In contrast, atrichia with papular lesions (APL) (OMIM#209500) is an autosomal recessive Mendelian disorder characterized by the onset of complete alopecia during infancy (Zlotogorski *et al*, 2002b), leading to its misdiagnosis as AU. Although the genetic basis of alopecia areata remains unknown, APL results from mutations in a single gene, hairless (*HR*).

The differentiation between AU and APL is of extreme importance. Although many patients with AU respond to available therapies, APL patients fail to respond to any treatment modality over several years, which eventually leads to the diagnosis of APL. The final proof, however, is based upon identification of mutations in the *HR* gene. To date, these mutations have all (with one exception) been homozygous mutations in APL patients from consanguineous families (Ahmad*et al*, 1998; Cichon *et al*, 1998; Zlotogorski *et al*, 1998; Henn *et al*, 2002).

The increasing number of reports of patients with APL (Ahmad *et al*, 1999a; 1999b; Kruse *et al*, 1999; Sprecher *et al*, 1999; Aita *et al*, 2000; Zlotogorski *et al*, 2002a) led us to hypothesize that APL is much more common than previously considered and in some cases is misdiagnosed as AU.

This misdiagnosis is most probably due to lack of awareness, lack of fixed diagnostic criteria until recently (Zlotogorski *et al*, 2002b), and the belief that patients with APL are found only rarely and in consanguineous families.

In this study, we identified three small nonconsanguineous families with only one offspring affected by early onset hair loss that never regrew. Importantly, these patients either had an associated papular eruption and/or failed to respond to treatment for AU. Upon examination, the clinical findings in all three families were consistent with APL.

The proband in family A is a 22-y-old woman (Figure 1a). Her father and mother originate from Iraq and Morocco, respectively. The proband denied any history of consanguinity. The patient was born with few hairs that were shed during the first months of life. Topical treatments failed to induce hair regrowth. Papules appeared at 3 y of age on the scalp, cheeks, arms, and shins. The number of nonfacial papules has steadily declined with time. On examination, we observed complete scalp atrichia, absence of body hair, and sparse eyebrows and eyelashes. She had numerous papules on the arms, thighs, elbows (Figure 2a, b), knees, lower neck, and back. A few papules were present on the scalp, as well as hypopigmented marks.

The proband in family B is a 4-y-old boy who was adopted by his family at the age of 19 mo from Russia, and had complete alopecia at that time. The patient was presumed to have AU, and was treated with class II topical corticosteroids, topical 2% minoxidil, short contact anthralin, and squaric acid dibutylester immunotherapies, but no hair was generated. Examination showed atrichia of the entire scalp, sparse thin eyelashes and eyebrows, a 2.5 mm papule on the elbow, and no hypopigmented patches. Nails and dentition were normal.

Finally, the proband in family C is a 13-mo-old boy (II-3). Both parents are Australian of English descent and denied any history of consanguinity. The proband was born with only four strands of hair on his head that fell out within 10 d and failed to regrow. He had no eyebrows at birth and had only a few eyelashes. A papular eruption appeared on the arms and legs at 3mo of age and eruptions gradually increased in number until 12 mo of age. Examination showed scalp atrichia (Figure 2c) with only a single strand of blonde hair on the vertex. He had scattered eyebrows and sparse eyelashes (Figure 2d). Papules were noted on the arms, thighs, and lower legs. Teeth, nails, and sweating were normal.

To screen for mutations in the *HR* gene, all exons and splice junctions were PCR amplified (Ahmad *et al*, 1999b) in the patients and their relatives. The proband in family A was found to carry a maternally derived C-to-T transition in exon 4 resulting in the nonsense mutation Q478X, and a

paternally derived G-to-T transversion at the acceptor splice site of intron 4, 1557–1G → T (Figure 1a).

The proband in family B carried a previously described T-to-G transversion at nucleotide position 1864 within exon 6, resulting in the missense mutation C622G, and a C-to-G transversion at position –3 at the acceptor splice site of intron 13, 2847–3C → G (Figure 1b).

Finally, the proband from family C was a compound heterozygote for the maternally derived nonsense mutation Q478X, identical to that in family A, and a paternally derived T insertion at position +2 at the splice donor site of intron 12, 2776 + 2insT (Figure 1c).

We tested 40–83 control individuals (80–166 alleles) for the presence of these mutations, to rule out the unlikely possibility that they were polymorphisms. The absence of these mutations in the control chromosomes, the nature and severity of the mutations, and the recurrence of some of them in unrelated probands provide evidence that they represent the pathogenetic mutations in these families.

In summary, we have identified compound heterozygous mutations in the *HR* gene in three individuals. The type of hair and papular involvement were not different from those seen in patients carrying homozygous mutations.

The nonsense mutation Q478X was first described in a consanguineous Pakistani family (Sprecher *et al*, 1999). Here, we find the same mutation recurring in two unrelated individuals of Moroccan and Australian origin. The occurrence of this mutation on such diverse backgrounds suggests that Q478X may represent a mutational hotspot.

A second recurrent mutation was the missense C622G, previously identified in a consanguineous family of Polish descent (Aita *et al*, 2000). The mutation alters the third of four invariant cysteine residues in the zinc-finger domain, with high homology to the C-X-X-C-(X)17-C-X-X-C structure of the zinc fingers of the GATA family of transcription factors.

Finally, we identified splice site mutations in each proband, 1557–1G → T (family A), 2847–3C → G (family B), and 2776 +2insT (family C). As mRNA samples from the patients were not available, we calculated the splicing efficiency score for all of them (Shapiro and Senapathy, 1987). The mutation in family A is predicted to abolish normal splicing of exon 5 on this allele, as the invariant AG immediately preceding the exon is changed to AT. The scores for the wild-type and mutant sequences are 89.13 and 73.11, respectively. In family B, the mutation 2847–3C → G results in the splice site change from ccctaccctgaCag/exon to ccctaccctgaGag/exon and a reduction in the

score from 90.00 to 78.30. Finally, the mutation 2776 + 2insT in family C changes the consensus sequence from exon/gtaagt to exon/gtTaag, with scores of 78.65 and 49.64, respectively.

Furthermore, identification of *HR* mutations in the proband in family B excludes the possibility of an alternative autosomal recessive form of hypotrichosis, specific to the Mari population in the Volga-Ural region of Russia. Affected individuals with Mari hypotrichosis have sparse scalp hairs that are wiry and twisted. The eyelashes and eyebrows are shed after the first year of life and remain absent. Body hair is generally sparse and thin, and axillary and pubic hair is essentially absent. Mutations in the *HR* gene have recently been excluded in 21 families (Rogaev *et al*, 1999).

Prior to this study, only one APL patient with compound heterozygous mutations had been described in a small nonconsanguineous family (Henn *et al*, 2002). Three additional compound heterozygous patients from families without consanguinity further support the hypothesis that isolated cases of APL may be more common than previously thought.

These data extend our knowledge of mutations in the *HR* gene, and may suggest that infants with presumed AU in small nonconsanguineous families may warrent testing for *HR* gene mutations, particularly before embarking on therapeutic modalities that will fail in APL.

References

Ahmad, W, ul Haque, MF, Brancolini, V, *et al*: Alopecia universalis associated with a mutation in the human hairless gene. *Science* 1998 279: 720–724,

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* 1999a 113: 281–283,

Ahmad, W, Zlotogorski, A, Panteleyev, A, *et al*: Genomic organization of the human hairless gene and identification of a mutation underlying congenital atrichia in an Arab Palestinian family. *Genomics* 1999b 56: 141–148,

Aita, VM, Ahmad, W, Panteleyev, AA, *et al*: A novel missense mutation (C622G) in the zinc-finger domain of the human hairless gene associated with congenital atrichia with papular lesions. *Exp Dermatol* 2000 9: 157–162,

Cichon, S, Anker, M, Vogt, IR, *et al*: Cloning, genomic organization, alternative transcripts and mutational analysis of the gene responsible for autosomal recessive universal congenital alopecia. *Hum Mol Genet* 1998 7: 1671–1679,

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 different diagnosis of alopecia universalis. *J Am Acad Dermatol* 2002 47: 519–523,

Kruse, R, Cichon, S, Anker, M, *et al*: Novel hairless mutations in two kindreds with autosomal recessive papular atrichia. *J Invest Dermatol* 1999113: 954–959,

Rogaev, EI, Zinchenko, RA, Dvoryachikov, G, Sherbatich, T, Ginter, EK: Total hypotrichosis: Genetic form of alopecia not linked to hairless gene.*Lancet* 1999 354: 1097–1098,

Safavi, K: Prevalence of alopecia areata in the First National Health and Nutrition Examination Survey (letter). *Arch Dermatol* 1992 128: 702,

Shapiro, MB, Senapathy, P: RNA splice junctions of different classes of eukaryotes: Sequence statistics and functional implications in gene expression. *Nucl Acids Res* 1987 15: 7155–7174,

Sprecher, E, Lestringant, GG, Szargel, R, *et al*: Atrichia with papular lesions resulting from a nonsense mutation within the human hairless gene. *J Invest Dermatol* 1999 113: 687–690,

Zlotogorski, A, Ahmad, W, Christiano, AM: Congenital atrichia in five Arab Palestinian families resulting from a deletion mutation in the human hairless gene. *Hum Genet* 1998 103: 400–404,

Zlotogorski, A, Martinez-Mir, A, Green, J, Lam, H, Panteleyev, AA, Sinclair, R, Christiano, AM: Evidence for pseudodominant inheritance of atrichia with papular lesions. *J Invest Dermatol* 2002a 118: 881–886,

Zlotogorski, A, Panteleyev, AA, Christiano, AM: Clinical and molecular diagnostic criteria of papular atrichia. *J Invest Dermatol* 2002b 118: 887–890,

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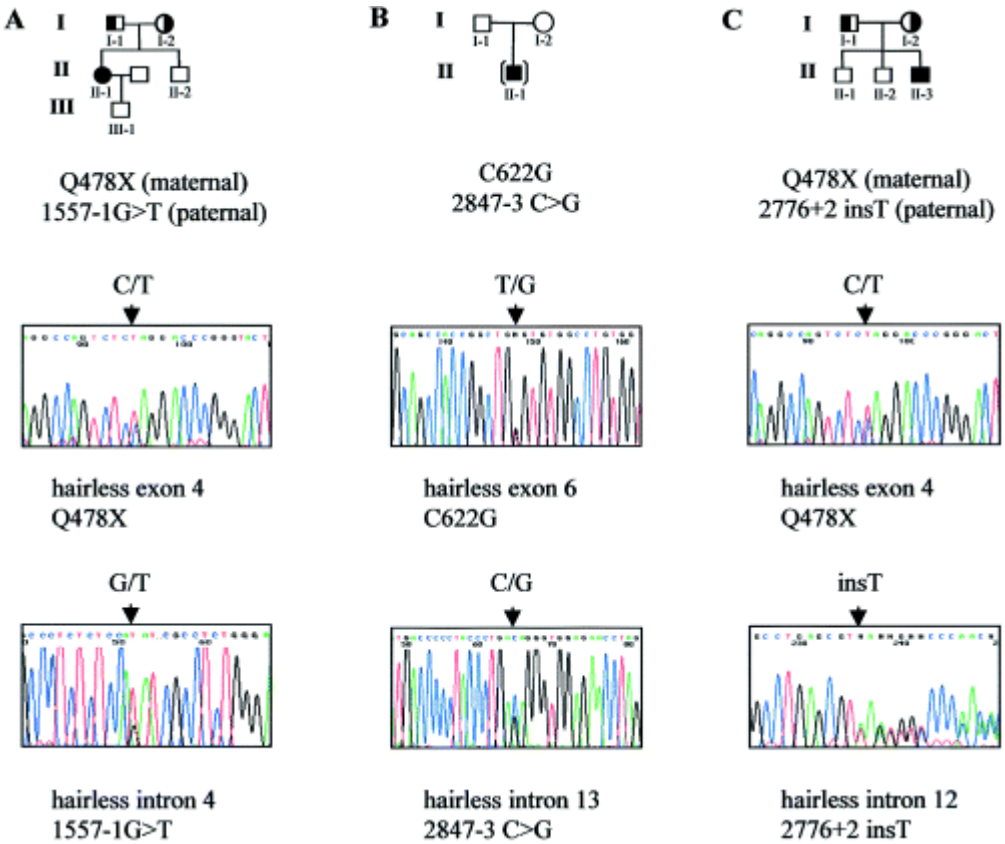
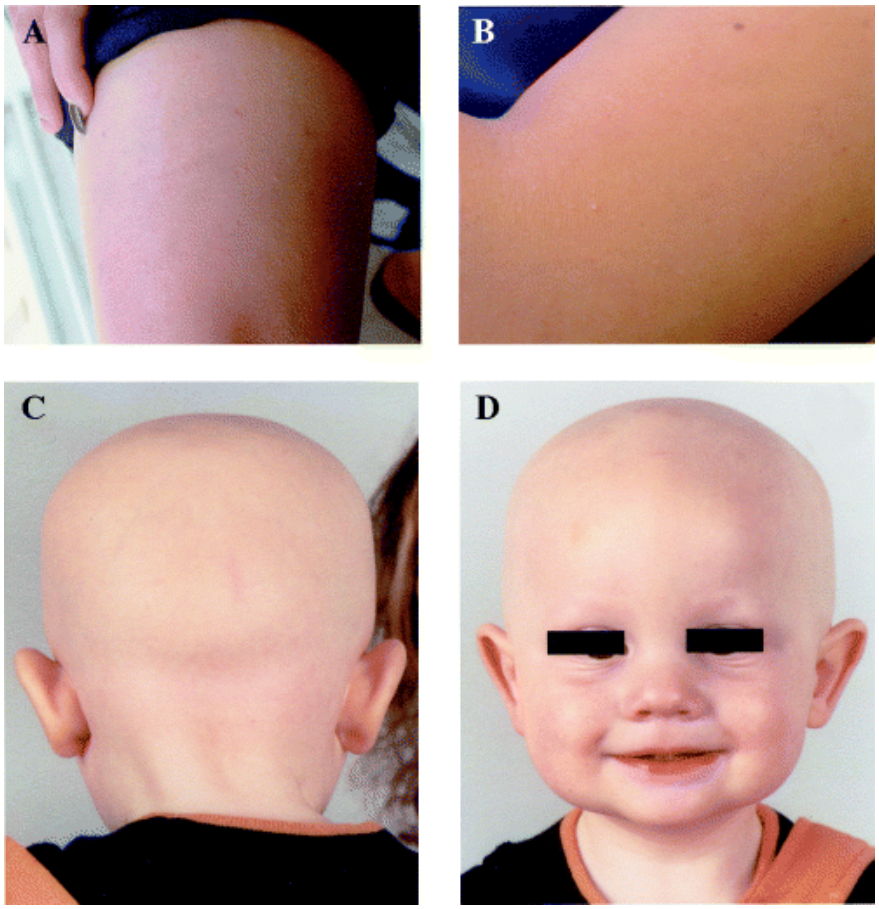
Figures

Figure I



Mutation analysis of the three families with APL. Pedigrees representing families A, B, and C with the proband affected with APL are shown. Note the absence of parental consanguinity. Circles and squares represent female and male patients, respectively. The black half-filled figures represent mutation carriers. Automated DNA sequence analysis of the HR gene is shown for each mutation. (A) DNA sequence of exon 4 and intron 4 from compound heterozygous individual II-1 revealed the nonsense mutation Q478X on the maternal allele and the splice site mutation 1557–1G → T on the paternal allele (arrows). (B) DNA sequence of exon 6 and intron 13 from the compound heterozygous individual II-1 revealed the missense mutation C622G and the splice site mutation 2847–3C → G (arrows). (C) Sequence analysis of exon 4 and intron 12 from the compound heterozygous individual II-3 demonstrated the presence of the nonsense mutation Q478X on the maternal allele and the splice site mutation 2776 + 2insT on the paternal allele (arrows).

Figure II



Clinical presentation of atrichia with papular lesions (families A and C). In patient II-1 from family A, there is complete atrichia, with several papules on the thighs (A) and around the elbow (B). In patient II-3 from family C, complete atrichia (C) with absent eyebrows and sparse eyelashes (D) are noted.