A Missense Mutation in the Zinc-Finger Domain of the Human Hairless Gene Underlies Congenital Atrichia in a Family of Irish Travellers

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Summary

Congenital atrichia is a rare, recessively inherited form of hair loss affecting both males and females and is characterized by a complete absence of hair follicles. Recently, a mutation in the human hairless gene was implicated in the pathogenesis of congenital atrichia. The human hairless gene encodes a putative single zinc-finger transcription-factor protein with restricted expression in brain and skin, which is believed to regulate catagen remodeling in the hair cycle. In this study, we report the identification of a missense mutation in the zinc-finger domain of the hairless gene in a large inbred family of Irish Travellers with congenital atrichia. The mutated arginine residue is conserved among human, mouse, and rat, suggesting that it is of significant importance to the function of the zinc-finger domain.

Introduction

Hair-follicle morphogenesis is a multistep process that requires a series of epithelial-mesenchymal signals to execute the program of developmental events. The initial signal derived from the dermis instructs the overlying epidermis to thicken, form a placode, and then a downgrowth into the dermis, known as the "hair plug." A second signal from the epidermis instructs the dermis to form the dermal papilla. The dermal papilla then stimulates the division of overlying epithelially derived matrix cells in the hair plug. These cells divide rapidly and differentiate into inner root–sheath cells or hair-shaft

cells, depending on their position in relation to the longitudinal axis of the follicle (Hardy 1992). Although these events have been described extensively in model systems, the genes governing these processes are largely unknown.

Hairs grow in a cyclical fashion, with three distinct phases: anagen, catagen, and telogen (Hardy 1992). In anagen, the follicle is regenerated and a new hair grows. At a genetically determined time, the follicles enter the catagen phase, during which elongation ceases and the follicle regresses because the matrix cells stop proliferating. During catagen, the dermal papilla remains intact but undergoes several remodeling events, including degradation of the elaborate extracellular matrix, which is deposited during anagen. At the close of the catagen phase, the hair is loosely anchored in a matrix of keratin, with the dermal papilla just below. Finally, the follicle enters telogen, during which the hair is usually shed. At the end of the resting phase, the dermal papilla migrates toward the epidermal stem cells located in the bulge region of the outer root sheath and recruits them to form the hair matrix, and anagen is reinitiated (Cotsarelis et al. 1990; Rochat et al. 1994). Currently, very little is known about the molecular control of the signals that regulate progression through this morphogenetic cycle, although it is clear that at least some potentially influential regulatory molecules may play a role.

There are many forms of inherited alopecia (i.e., hair loss), which vary in age of onset, severity, and associated ectodermal abnormalities. Congenital alopecia universalis (MIM 203655) or congenital atrichia (MIM 209500) without associated ectodermal defects is a rare autosomal recessive disorder and is the only form of inherited alopecia for which the molecular basis is known. Linkage studies in several large inbred Pakistani kindreds with atrichia (Ahmad et al. 1993) have localized the gene to chromosome 8p12 (Ahmad et al. 1998; Nöthen et al. 1998) and have led to the identification of a human homologue of the mouse hairless gene (Ahmad et al. 1998). In this study, we identified a novel missense mutation in the zinc-finger (ZF) domain of the

human hairless gene in a family of Irish Travellers with congenital atrichia.

Subjects and Methods

Subjects

A large inbred kindred of Irish Travellers was studied, in which five males and four females were affected with congenital atrichia (fig. 1). We obtained DNA from two affected females and seven unaffected individuals. Genomic DNA was isolated from peripheral blood collected in EDTA-containing tubes according to standard techniques (Sambrook et al. 1989). All samples were collected after informed consent had been obtained and in accordance with the local institutional review board.

Genotyping and Mutation Analysis

Genotyping of each member of the family was performed, as described elsewhere (Ahmad et al. 1998), for the markers D8S1786 and D8S298, which are closely linked to the hairless locus. To screen for a mutation in the human hairless gene, exons and splice junctions were PCR amplified from genomic DNA and were sequenced directly in an ABI Prism 310 Automated Sequencer, by means of the ABI Prism Rhodamine Terminator Cycle Sequencing Ready Reaction Sequencing Kit (Perkin-Elmer/Applied Biosystems), after purification in Centri-flex™ gel filtration cartridges (Edge Biosystems). To amplify a 287-bp PCR fragment containing exon 6 of the human hairless gene, the following primers were used: 5′-TTC ACC CTC TGA CCC TGT TC-3′ (intron 5,

sense) and 5′-GAG AGG CAG CCA ACG AAT GA-3′ (intron 6, antisense). The PCR product corresponding to exon 6 was digested with *Pvu*II, according to the manufacturer's recommendations (New England Biolabs), and the products were separated on a 1.5% agarose gel, with a 100-bp molecular-weight ladder (Gibco BRL) used as the size standard.

Results

Clinical Findings

In individuals affected with congenital atrichia, hairs were typically absent from the scalp (individual IV-18; fig. 2A), with shedding of the natal hair shortly after birth, and patients were completely devoid of eyebrows, eyelashes, and axillary and pubic hair. A scalp-skin biopsy from one of the affected individuals revealed the absence of hair follicles, with sparsely distributed sebaceous glands (individual IV-18; fig. 2B). There was no histological evidence of an inflammatory process. All affected individuals had the additional characteristic feature of grouped cystic and papular lesions on the knees and elbows (individuals IV-8 and IV-18; fig. 3A and B), which had the clinical and histopathological appearances of milia (individual IV-8; fig. 3A and C). Affected individuals showed no growth or developmental delay, normal hearing, teeth, and nails, and no abnormalities in sweating. Heterozygous individuals had normal hair and were clinically indistinguishable from genotypically normal individuals. The pedigree is strongly suggestive
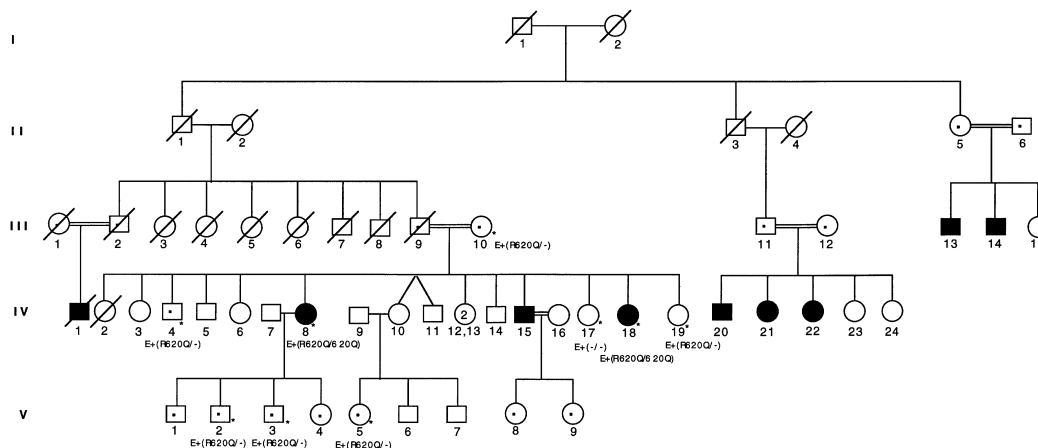
Figure 1 Large inbred pedigree of Irish Travellers displaying autosomal recessive inheritance of congenital atrichia. Affected males and females are represented by blackened filled squares and circles, respectively, and a dot within a symbol denotes that the individual is a carrier. Double lines between figures are representative of consanguineous unions. DNA was obtained from two affected (IV-8 and IV-18) and seven unaffected (III-10, IV-4, IV-17, IV-19, V-2, V-3, and V-5) family members, all of whom were clinically examined (E+) and are marked by an asterisk (*). Genotypes are indicated next to each E+ symbol: (R620Q/R620Q) denotes affected status, (R620Q/−) denotes carrier status, and (−/−) denotes a normal genotype.
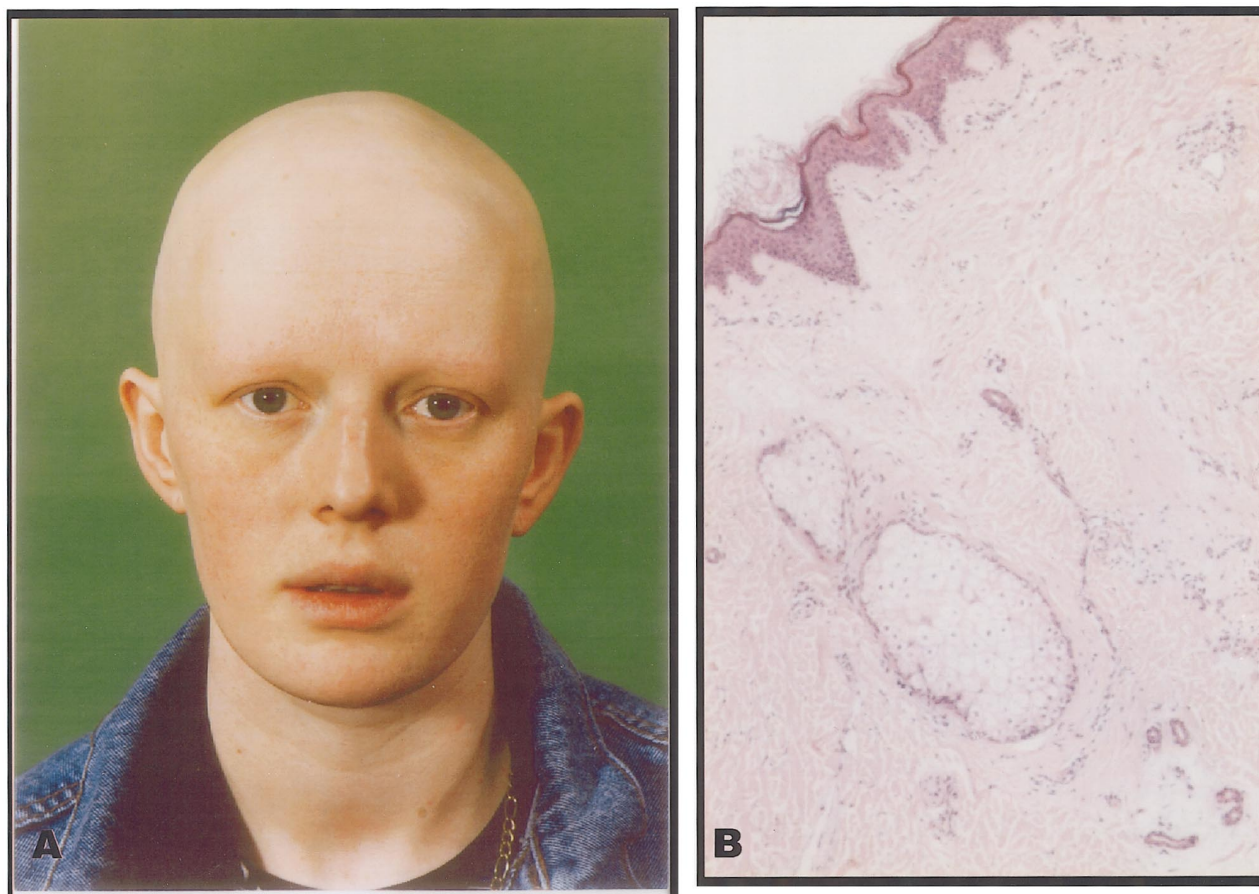
Figure 2 Clinical and histopathological findings in congenital atrichia. *A,* Phenotypic appearance of an affected female (IV-18) at ~25 years of age. Note the complete absence of hair on the scalp, eyebrows, and eyelashes. *B,* Scalp biopsy from the same individual, showing complete absence of hair-follicle structures. A sebaceous gland and some hair-follicle remnants are visible in the dermis (hematoxylin and eosin staining; magnification 128 ×).

of autosomal recessive inheritance with several consanguineous unions (fig. 1).
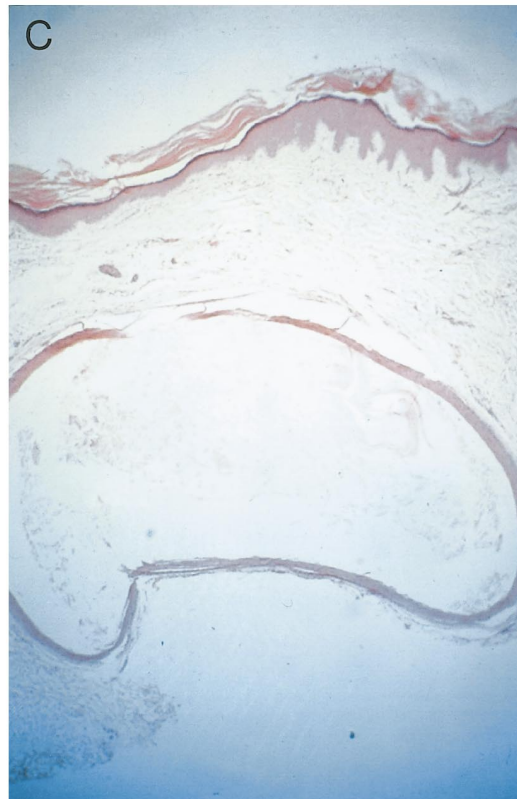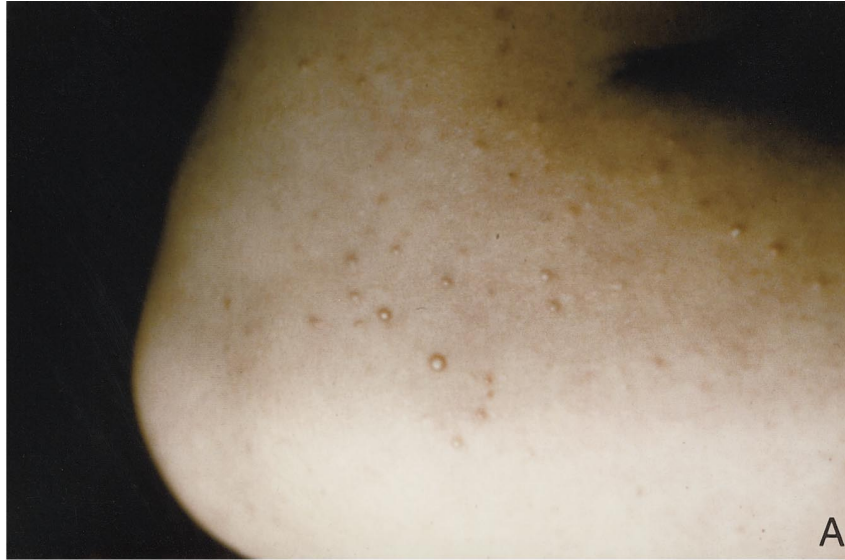
Genotyping

Genotyping of nine members of the family, including two affected and seven unaffected individuals, was performed for the polymorphic markers D8S1786 and D8S298, which are closely linked to the hairless gene on chromosome 8p12. The markers were fully informative, and the two affected female members of the family (individuals IV-8 and IV-18) were homozygous for both the markers, suggesting linkage to the hairless locus. In addition, we found that, of the seven unaffected individuals, six (individuals III-10, IV-4, IV-19, V-2, V-3 and V-5) were heterozygous carriers of the linked haplotype, whereas the seventh (individual IV-17) was both genotypically and phenotypically normal.
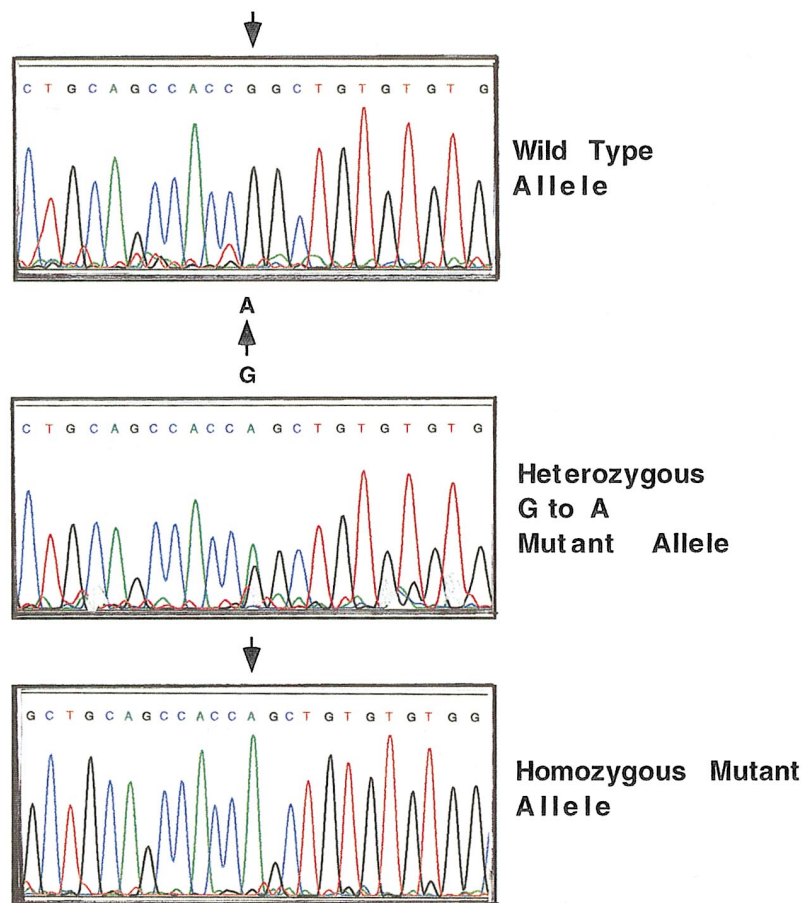
Mutation Analysis

The coding portion and intron-exon borders of the hairless gene were sequenced. Sequence analysis of exon 6 revealed a G→A transition at nucleotide position 1859 (fig. 4), resulting in an arginine-to-glutamine amino acid substitution at codon 620 (R620Q). The mutation created a new restriction site for the endonuclease *Pvu*II (fig. 5). To ensure that the mutation does not represent a neutral polymorphism in this population, a panel of 50 unrelated unaffected individuals (100 chromosomes) of Caucasian and northern European extraction were screened for the mutation using PCR followed by restriction digestion with *Pvu*II, and the mutation was not identified in any individuals outside the family.

Discussion

Elsewhere, we recently have reported a missense mutation in the human hairless gene in a rare form of in-

Figure 3 Clinical and histopathological findings of the cystic lesions associated with congenital atrichia. *A,* Phenotypic appearance of elbow of an affected female (IV-18) at ~6 years of age. Note the numerous and clustered small raised papules on the surface. *B,* Clinical appearance of elbow of an affected female (IV-8) at ~11 years of age. Note the three large raised papules. *C,* Skin biopsy of a papule on elbow of individual IV-8 in panel B, revealing the presence of a large thick-walled dermal cyst filled with keratinaceous material (hematoxylin and eosin staining; magnification 113 ×).

Figure 4   Automated DNA sequence analysis of hairless-gene mutation. Results of DNA sequence analysis of the wild-type allele in an unrelated unaffected control individual are shown in the top panel, for comparison. Results of DNA sequence analysis of a heterozygous carrier are shown in the middle panel. The arrows denote nucleotide position 1859 in the hairless-gene cDNA. Note the overlapping black and green peaks, indicating presence of a heterozygous, G-and-A nucleotide. Results of DNA sequence analysis of the mutant sequence, seen in the lower panel, show the homozygous G-to-A nucleotide substitution, which results in the missense mutation R620Q.

herited hair loss in a large inbred family from Pakistan (Ahmad et al. 1998). In the present article we report the identification of a second, distinct missense mutation responsible for atrichia in a large inbred family of Irish Travellers demonstrating autosomal recessive congenital atrichia, with family members living throughout the Republic of Ireland, Northern Ireland, and England. Irish Travellers have existed as a distinct indigenous ethnic minority within Ireland for centuries (Gmelch and Gmelch 1977). Distinctive cultures and traditions centered on a nomadic lifestyle, as well as resistance to policies of assimilation, have preserved their cultural identity within Ireland. In the Republic of Ireland, ~22,000 Travellers in 4,083 families constitute 0.5% of the total population (Pavee Point home page: Eire). The most recent census in Northern Ireland recorded a total of 1,115 travellers in 239 families (Pavee Point home page: northern Ireland). Consanguineous marriages and

a high fertility rate are common in Irish Traveller families, and previous studies have demonstrated the relative frequency of rare gene defects in this population (Flynn et al. 1989).

The phenotypic appearance of affected individuals in this family is similar to that reported by Landes in 1956 (Landes and Langer 1956) and Cantu in 1980 (Cantu et al. 1980) and include atrichia with cystic papules on the elbows and knees. As early as 1950, this rare human disease was named "atrichia with papular lesions" and was characterized as normal hair formation at birth, followed by hair loss associated with the formation of comedones and follicular cysts (Fredrich 1950; Damste and Prakken 1954; Landes and Langer 1956; Lowenthal and Prakken 1961; Del Castillo et al. 1974; Cantu et al. 1980; Kanzler and Rasmussen 1986; Rook and Dawber 1991; Misciali et al. 1992). In 1989, the human disease was first proposed as a homologue of the *hairless* mouse
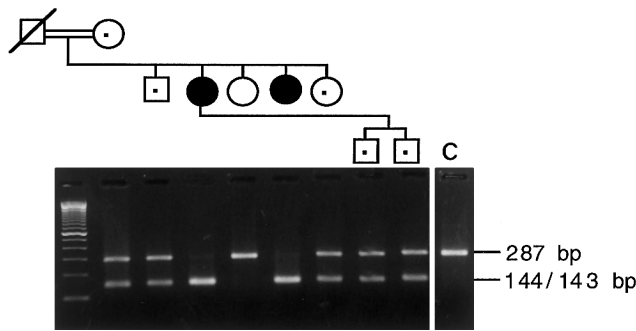
Figure 5 Confirmation and segregation analysis of hairless-gene mutation. Affected female family members IV-8 and IV-18 are represented as blackened circles, heterozygous female (III-10 and IV-19) and male (IV-4, V-2, and V-3) carriers are represented, respectively, as circles with dots and squares with dots, and the single genotypically normal individual, IV-17, is represented as an unblackened circle. The disease status cosegregates with presence of a novel *Pvu*II site generated by the mutation in exon 6. A modified pedigree of the larger kindred in figure 1 is shown (*above*), in which the symbols representing the individuals are aligned with the lanes of the agarose gel (*below*). In the single genotypically unaffected individual (IV-17), only the undigested 287-bp fragment is seen, representative of homozygosity for the normal allele. The carrier individuals (III-10, IV-4, IV-19, V-2, and V-3) display the 287-bp band together with the superimposed 143-bp and 144-bp bands, indicative of heterozygosity for the mutant allele. The two affected individuals (IV-8 and IV-18) display only the superimposed 143-bp and 144-bp bands, representing homozygosity for the mutant allele. A 100-bp ladder, shown in the leftmost lane, was used as the size standard, and the undigested PCR product of an unrelated, unaffected control individual (lane c) is shown in the rightmost lane, for reference.

mutation (Sundberg et al. 1989). Cases resembling this disease, with loss of hair over the entire body, have recently been reported under the name "alopecia universalis" (Ahmad et al. 1993; Ahmad et al. 1998; Nöthen et al. 1998); however, congenital atrichia with papules may be a more precise description of the phenotype. In view of the consistent findings, we propose that the term "congenital atrichia with papules" be used to describe the phenotype caused by mutations in the human hairless gene.

The proteins encoded by the human, mouse, and rat hairless genes contain a single ZF domain with novel spacing of a conserved six-cysteine motif. The mutation in this family, R620Q, resides between the fourth and fifth cysteine residues in the six-cysteine ZF domain (fig. 6). The mutated arginine residue has been conserved during the past 90 million evolutionary years, among human, mouse, and rat, suggesting that it is of significant importance in the function of the ZF domain. The hairless-gene product is a putative transcription factor with a single ZF domain, which is highly expressed in the brain and the skin. It has recently been shown that the suppression of hairless-gene activity in *hairless* mice re-

sults in several basic integument abnormalities at the cellular level, including complete disintegration of the outer root sheath of the hair follicle, failure of upward movement of the dermal papilla and subsequent induction of a new hair, and disruption of the integrity of key functional tissue units in the hair follicle (Panteleyev et al. 1998). In humans, the hairless gene appears to function at the cellular transition from the natal to the first adult hair cycle, and, if compromised, hair growth completely ceases and a new hair is never induced, and the result is a complete form of inherited atrichia.

Available evidence indicates that ZF proteins may act as transcriptional regulators with specific nucleic acid–recognition capabilities. Several lines of evidence suggest that DNA binding is a property specifically conferred by the ZF. It was recently reported that point mutations in the GAL4 ZF domain generated nonfunctional proteins unable to bind DNA by introducing amino acids substitutions clustered in and around the ZF region (Johnston and Dover 1987). Also, mutations within the ZF region of the human glucocorticoid receptor destroy DNA binding in vitro (Hollenberg et al. 1987). The presence of missense mutation in the ZF domain of congenital atrichia patients could diminish the DNA binding activity and could disturb the cascade of hair-cycle events normally triggered by the hairless gene. The possibility also exists that the hairless-gene ZF mutant allele encodes a protein that retains DNA-binding capacity but may have an altered target specificity. Recent studies have established that the hairless gene functions as a transcriptional corepressor in brain and is regulated directly by thyroid hormone (Thompson 1996; Thompson and Bottcher 1997). If the hairless gene also functions as a transcriptional repressor in the skin, then, in this context, dysregulation of repression may be more critical than activation.

Elsewhere, the molecular basis of the *hairless* mouse phenotype had been found to be the result of a murine leukemia proviral insertion, in intron 6 of the hairless gene, that resulted in aberrant splicing (Cachon-Gonzalez et al. 1994). In addition to *hairless*, a second mouse mutation, known as "*rhino*," is known to be allelic at
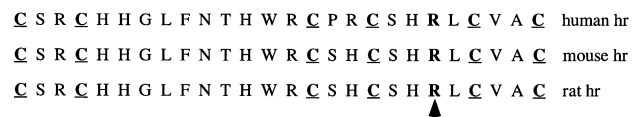
Figure 6 ZF domain harboring six cysteine residues, which is conserved in human, mouse, and rat hairless genes. The six cysteine residues are in boldface and underlined. The arginine residue affected by the mutation in this family is indicated as a boldface "R" (*arrow*) and, also, has been conserved, during the past 90 million evolutionary years, among human, mouse, and rat.

the same locus, and we have recently identified a series of nonsense mutations in the hairless gene in *rhino* mice (Ahmad et al., in press-*a*; in press–*b*; Panteleyev et al., in press–*a*). Both *hairless* mice and *rhino* mice have a similar pattern of disease progression: at birth, they are indistinguishable from heterozygous (normal) littermates, until the second hair cycle, which begins at age ~2 wk. Within a 7-d period, the hair then is shed rapidly in a head-to-tail pattern and, because of a series of irreversible cellular events, never regrows. Over time, the hair follicles are replaced, in the *hairless* mouse, by cystic structures in the upper and lower portions of the skin and, in *rhino* mice, by cystic lesions similar to those observed in the Irish Traveller family (fig. 3) (Panteleyev et al., in press–*b*).

Expression of the hairless gene in mice is restricted to the epidermis and certain hair-follicle structures (Cachon-Gonzalez et al. 1994), implying that the molecular defect in *hairless* mice is intrinsic to that in epidermal cells. This is further substantiated by findings that hairless-gene expression is restricted to the epithelial-cell populations which exhibit a cellular phenotype in *hairless* mice (Panteleyev et al. 1998). In *hairless* mice, the hair matrix cells undergo premature apoptosis and a disconnection with the overlying epithelial sheath essential for the movement of the dermal papilla (Panteleyev et al. 1998; in press–*b*). As a consequence, the hair bulb and dermal papilla remain stranded in the dermis. These findings suggest that a crucial role of the hairless-gene product may be involved in the maintenance of the delicate balance between cell proliferation, differentiation, and apoptosis in the hair follicle as well as in the interfollicular epidermis.

Acknowledgments

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Pavee Point home page, http://homepages.iol.ie/~pavee/fspopul.htm (for Eire, Ireland)
Pavee Point home page, http://homepages.iol.ie/~pavee/fsnorth.htm (for northern Ireland)
Online Mendelian Inheritance in Man (OMIM) http://www.ncbi.nlm.nih.gov.omim (for congenital alopecia universalis [MIM 203655] and congenital atrichia [MIM 209500])

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