bridization from a cDNA library of 21-day-old Co plants using a PCR fragment spanning the 5′ part of *RSW1* transcript. Ath-A is on YAC CIC9H6, chromosome 4. The EST T20782 clone was from *Arabidopsis* Biological Resource Center (Columbus, OH); rice EST D39394, from the MAFF DNA Bank (Tsukuba, Japan). The sequences were analyzed at the Australian National Genome Information Service with the use of Wisconsin GCG software [J. Devereux, P. Haeberley, O. Smithies, *Nucleic Acids Res.* **12**, 387 (1984)]. Sequences for *RSW1*, Ath-A, Ath-B, and rice D39394 have been deposited at GenBank (AF027172, AF027173, AF027174, and AF030052, respectively); comparisons are provided as supplementary material (*17*).
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22. We thank R. Schmidt and C. Lister for sharing mapping information and clones. Supported by the Australian National University (A.C., J.P., L.P., J.R., and R.E.W.), Australian Research Council (J.P.), Cooperative Research Centre for Plant Science (T.A., R.B., A.C., J.B., and L.P.), Cotton Seed Distributors (T.A. and J.G.), Deutsche Forschungsgemeinschaft (A.S.B., W.H., and W.W.), Groupe Limagrain Pacific (A.S.B.), Groupement de Recherches et d'Etudes sur les Génomes and Actions Coordonnées Concertées dans le Domaine des Sciences du Vivant (C.C. and H.H.), and North Eucalypt Technologies (J.B. and J.G.).

20 August 1997; accepted 18 December 1997

Alopecia Universalis Associated with a Mutation in the Human *hairless* Gene

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There are several forms of hereditary human hair loss, known collectively as alopecias, the molecular bases of which are entirely unknown. A kindred with a rare, recessively inherited type of alopecia universalis was used to search for a locus by homozygosity mapping, and linkage was established in a 6-centimorgan interval on chromosome 8p12 (the logarithm of the odds favoring linkage score was 6.19). The human homolog of a murine gene, *hairless*, was localized in this interval by radiation hybrid mapping, and a missense mutation was found in affected individuals. Human *hairless* encodes a putative single zinc finger transcription factor protein with restricted expression in the brain and skin.

The human hair follicle is a dynamic structure that generates hair through a complex and exquisitely regulated cycle of growth and remodeling (*1*). Despite the extensive descriptive understanding of the hair cycle, currently, very little is known about the molecular control of the signals that regulate progression through the hair cycle, although it is clear that at least some potentially influential regulatory molecules may play a role (*1*). For example, a knock-out mouse with targeted ablation of the gene encoding the fibroblast growth factor 5 (FGF5) provided evidence that FGF5 is an inhibitor of hair elongation, and the mouse had an increase in hair length due to an increase in the time that follicles remain in anagen. The FGF5 gene was also deleted in the naturally occurring mouse model, *angora* (*2*). Another member of the FGF family, FGF7 or keratinocyte growth factor, was disrupted by gene targeting, and the resultant mouse had hair with a greasy, matted appearance, similar in phenotype to the *rough* mouse (*3*). A transgenic mouse was engineered that disrupted the spatial and temporal expression of the gene encoding the lymphoid enhancer factor 1, a transcription factor that binds to the promoter region of several published hair keratin promoters. Disruption of this potential master regulator of hair keratin transcription resulted in defects in the positioning and angling of the hair follicles (*4*). More recently, a mutation in a structural protein, mouse desmoglein 3 (encoded by the gene *dsg3*), was found to be the underlying mutation in the naturally occurring mouse phenotype, *balding* (*5*). Finally, the *nude* mouse phenotype, characterized by hairlessness and athymia, was found to be the result of mutations in the winged-helix *nude* (*whn*) gene, a member of the winged-helix class of transcription factors (*6*). In addition to the complexity of the signaling pathways, in sheep, there are over 100 distinct structural proteins synthesized by the hair cortex and cuticle cells that produce the keratinized structure of a wool fiber (*1*). Despite these examples of recent progress in animal models, we have only begun to understand the control and molecular complexity of the hair follicle and its cyclic progression in humans.

There are several forms of hereditary human hair loss, known collectively as alopecias, which may represent a dysregulation of the cycle of hair growth and remodeling (*1*), yet the molecular basis of the alopecias has remained largely unexplored (*7*). The most common form of hair loss, known as androgenetic alopecia (male pattern baldness), is believed by some to affect ~80% of the population (*7*). Alopecia areata is a common dermatologic disease affecting about 2.5 million individuals in the United States alone, which causes round, patchy hair loss on the scalp (*7*). Alopecia areata can progress to involve hair loss from the entire scalp; this condition is referred to as alopecia totalis. Alopecia universalis (AU) is the term for the most extreme example of disease progression, which results in the complete absence of scalp and body hair (*7*). Although an autoimmune pathomechanism for alopecia areata has been

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suggested, the precise etiology is unknown, and no autoantigen or causative gene has been identified (8). The inheritance patterns of these forms of alopecia are also unclear, although a polygenic model with variability in penetrance and expressivity would appear most plausible, perhaps modulated by superimposed hormonal or immune factors.

In an effort to understand the molecular basis of a simple, recessively inherited form of AU (Online Mendelian Inheritance in Man accession number 203655) with no evidence of a confounding autoimmune component, we studied a large Pakistani kindred with AU segregating as a single Mendelian abnormality without associated ectodermal defects and containing four affected males and seven affected females (Fig. 1). The affected individuals were in good general health, with no evidence for immune system dysfunction or unusual susceptibility to skin tumors. At birth, the hair usually appears normal on the scalp but never regrows after a ritual shaving, usually performed a week after birth (Fig. 2, A and B). A skin biopsy from the scalp of an affected person revealed very few hair follicles, which were dilated and without hairs (Fig. 2C), and the absence of an inflammatory infiltrate. Affected individuals are born completely devoid of eyebrows and eyelashes (Fig. 2B) and never develop axillary and pubic hair. The pedigree is strongly suggestive of autosomal recessive inheritance, and the large number of consanguineous loops account for all affected persons being homozygous for the abnormal allele (Fig. 1).

To identify the alopecia locus segregating in this family, we initiated a genome-wide search for linkage by homozygosity mapping (9). During the initial screening, DNA samples from four affected individuals (IV-20, V-2, V-11, and VI-2 in Fig. 1) were genotyped with 386 highly polymorphic microsatellite markers spaced at 10-centimorgan (cM) intervals (*10*). In the course of this screen, 13 genomic regions were found to be homozygous for three to four affected individuals; each of these genomic regions were tested further in 32 additional family members, and 12 of the regions were excluded. One marker, D8S136 on chromosome 8p12, was found to be homozygous in all seven living affected individuals. Further analysis with markers from this region resulted in the identification of homozygosity in all affected individuals for the markers D8S1786 and D8S298 (*11*). Allele patterns obtained with the markers D8S136 and D8S1786 indicated that these two markers are placed very close to each other on chromosome 8p12. A maximum two-point logarithm of the odds ratio for linkage (lod) score of 6.19 at zero recombination was achieved with the marker

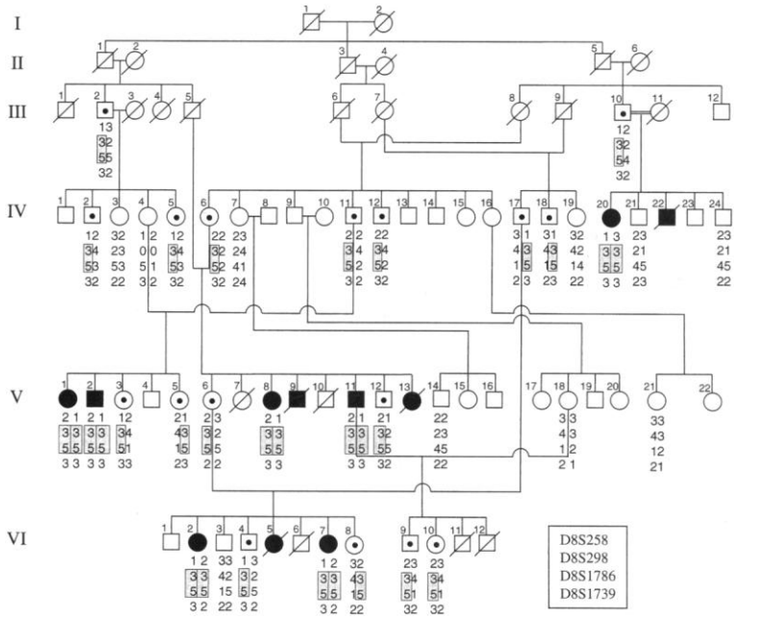


Fig. 1. Pedigree of the AU family over six generations. Black circles and squares represent affected females and males, respectively, figures with a black dot at the center represent heterozygous carriers, and figures with diagonal lines represent deceased individuals. The gray shaded box beneath the pedigree characters indicates the haplotype on chromosome 8p that cosegregates with the disease. The order of the markers is indicated in the lower right corner.

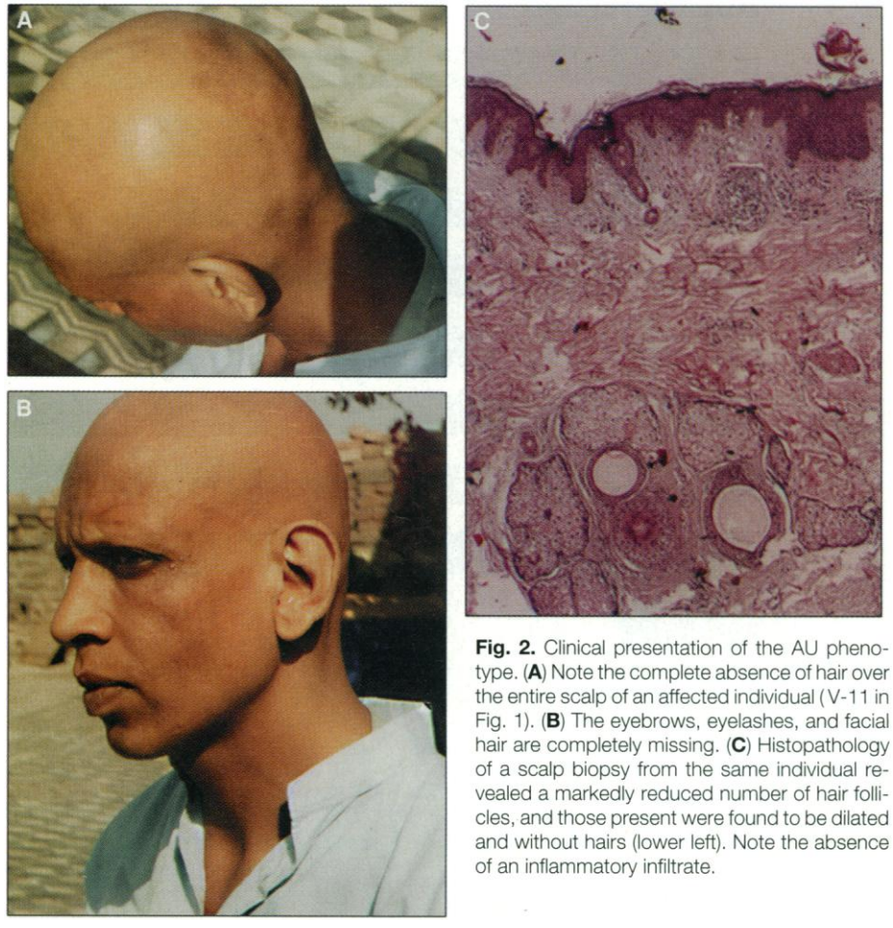


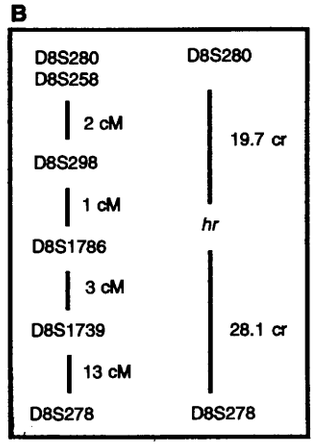
Fig. 2. Clinical presentation of the AU phenotype. (**A**) Note the complete absence of hair over the entire scalp of an affected individual (V-11 in Fig. 1). (**B**) The eyebrows, eyelashes, and facial hair are completely missing. (**C**) Histopathology of a scalp biopsy from the same individual revealed a markedly reduced number of hair follicles, and those present were found to be dilated and without hairs (lower left). Note the absence of an inflammatory infiltrate.

Fig. 3. (**A**) The lod score calculations for the linkage of AU to chromosome 8p12 markers for the AU family. The α signifies infinity. (**B**) Comparison of the linkage interval defined in the AU family (left) with the location of the human *hairless* (*hr*) gene (right) established by radiation hybrid mapping. By linkage analysis, the locus of the gene in the AU family was predicted to lie within the 6-cM interval defined by the markers D8S258 and D8S1739 (left). By radiation hybrid mapping, the *hairless* gene was predicted to lie within the 19-cM interval between the markers D8S280 and D8S278 (right), thus making it a strong candidate gene in the AU family. Physical distance is reported in centirads (cr).

A

Locus	Recombination fraction θ						
	0	0.01	0.05	0.1	0.2	0.3	0.4
D8S258	α	2.57	2.85	2.63	1.87	1.01	0.32
D8S298	6.19	6.04	5.45	4.7	3.16	1.65	0.47
D8S1786	4.92	4.83	4.43	3.92	2.87	1.79	0.76
D8S1739	α	1.74	2.64	2.61	1.92	1	0.22

B



D8S298 (Fig. 3A) by means of the FASTLINK 3.0 package (*12*), indicating that the alopecia gene in this family maps to chromosome 8p12. Recombinant haplotypes observed in individuals VI-2 and VI-7 placed the alopecia locus within a 6-cM interval between the distal and proximal markers, D8S258 and D8S1739, respectively (Fig. 3, A and B), with no obvious candidate genes in this interval.

In an independent line of investigation, we had developed an interest in the *hairless* mouse (*13*) as a potential model for inherited human alopecias and had begun to clone the human homolog of *hairless* with polymerase chain reaction (PCR) primers based on the available murine cDNA sequence (GenBank accession number Z32675) (*13*). We reverse transcriptase (RT)–PCR amplified a segment corresponding to exons 13 to 18 in the murine sequence using human skin fibroblast mRNA as template (*14*) and subsequently delineated the entire coding sequence of human *hairless*, which consists of

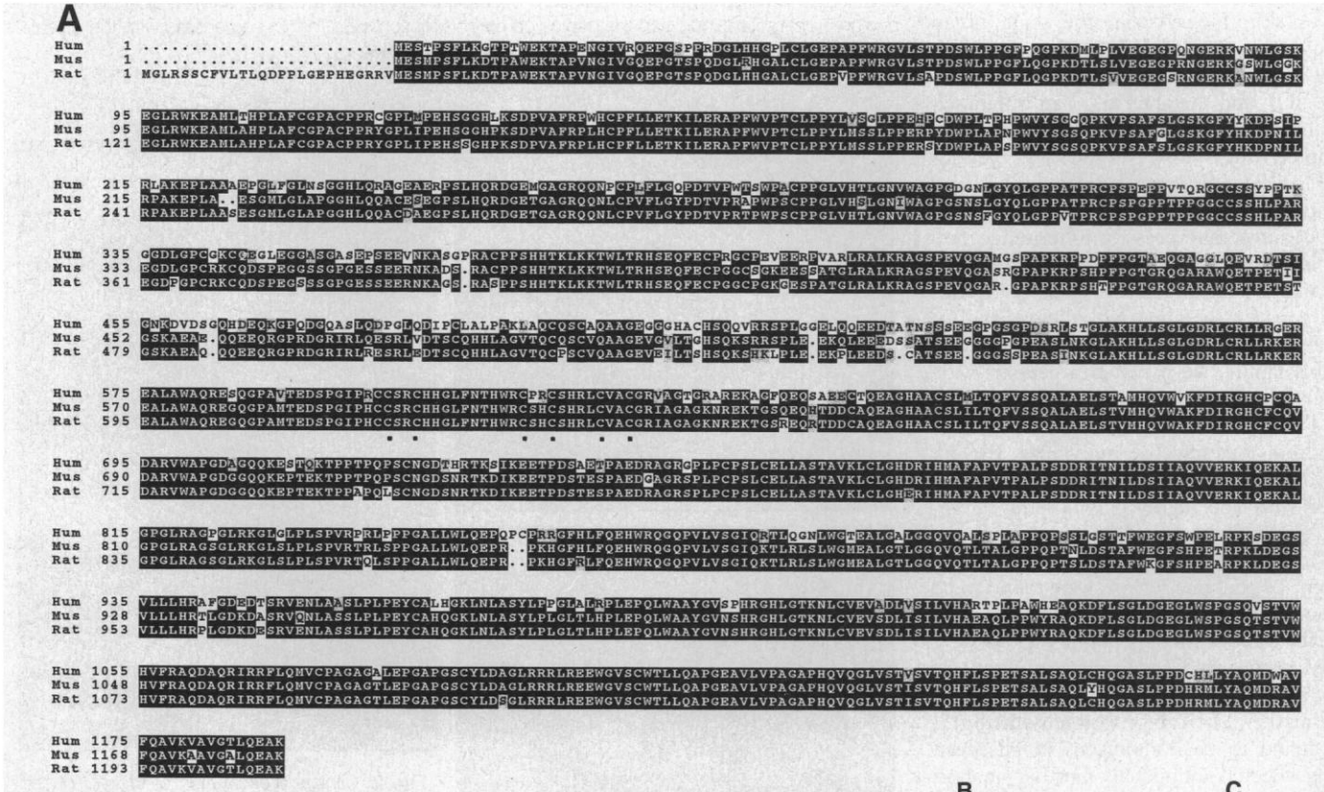


Fig. 4. (**A**) Sequence comparison of human (H), mouse (M), and rat (R) *hairless*. Areas shaded in black represent regions of complete homology, those shaded in grey represent conservative amino acid substitutions, and areas in white represent nonconservative substitutions. The homology of human *hairless* compared with mouse and rat was 84% and 83%, respectively. The conserved six-cysteine motif is indicated by asterisks beneath the sequence. (**B**) Northern blot analysis of human *hairless* (*hr*) in poly(A)$^+$ mRNA from eight different tissues, revealing a ~5-kb message (arrow). Lanes 1 to 8 show heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas, respectively. Substantial expression is noted only in the brain (lane 2), with trace expression elsewhere (lanes 1 and 3 to 8). (**C**) Northern blot analysis of human *hairless* in poly(A)$^+$ mRNA from cultured fibroblasts derived from hair-bearing skin reveals the same size *hairless* message (arrow).

1189 amino acids (Fig. 4A). The expression pattern of human *hairless* is consistent with that observed in mouse (*13*) and rat (*15*), with substantial expression in the brain and skin (Fig. 4, B and C) and trace expression elsewhere (*16*). The human and mouse amino acid sequences are 84% homologous and 80% identical, and the human and rat sequences are 83% homologous and 78% identical (Fig. 4A). The murine *hairless* gene resides on mouse chromosome 14 (*13*), which shares synteny with human chromosomes 8p and 14q, among others (*17*). To determine the precise chromosomal localization of the human homolog of *hairless*, we used radiation hybrid mapping (*18*) with the GeneBridge 4 panel consisting of 93 radiation-induced human-hamster cell hybrids (Research Genetics), which placed the human homolog of the mouse *hairless* gene on chromosome 8p, between the two polymorphic markers D8S280 and D8S278, spanning a 19-cM region (Fig. 3B). The 6-cM candidate region obtained for the AU gene by linkage analysis with flanking markers D8S258 and D8S1739 lies between markers D8S280 and D8S278 on the basis of the Genome Data Base (*17*), the Center for Medical Genetics database (*19*), and the radiation hybrid map constructed by the Human Genome Mapping Center at Stanford University (*20*). On the basis of this genomic colocalization, the human *hairless* gene became a major candidate gene responsible for AU in this family, and the search for a mutation was initiated.

Direct sequence analysis (*21*) of exon 15 (amino acids 993 to 1032, Fig. 4A) revealed a homozygous A-to-G transition in all affected individuals, which was present in the heterozygous state in obligate carriers within the family and not found in unaffected family members (Fig. 5). The A-to-G transition occurred at the first base of a threonine (T) residue at position 1022 (ACA), leading to a missense mutation and converting the threonine residue to an alanine (A) residue (GCA), and was designated T1022A. The mutation created a new cleavage site for the restriction endonuclease Hga I (GACGC), which was used to confirm the presence of the mutation in genomic DNA, in addition to direct sequencing (*21*). To verify that the missense mutation was not a normal polymorphic variant, we screened for the mutation by a combination of heteroduplex analysis (*22*), direct sequencing, and restriction digestion in a control population consisting of 142 unrelated, unaffected individuals, 87 of whom were of Pakistani origin. No evidence for the mutant allele was found in these individuals.

The *hairless* mouse, *hr/hr*, arose from spontaneous integration of an endogenous murine leukemia provirus into intron 6 of the *hairless* gene (*23*), resulting in aberrant splicing and only about 5% normal mRNA transcripts present in *hr/hr* mice (*13*). The protein encoded by the human, mouse, and rat *hairless* genes contains a single zinc finger domain with a novel and conserved six-cysteine motif and is therefore thought to function as a transcription factor (*13*, *15*), with structural homology to the GATA family (*24*) and to TSGA, a protein encoded by a gene expressed in rat testis (*25*). In addition to the total body hair loss that bears striking resemblance to AU, the *hr/hr* mouse exhibits a number of phenotypic effects not observed in the AU family, including defective differentiation of thymocytes (*26*), as well as a unique sensitivity to ultraviolet radiation and chemically induced skin tumors (*27*). Similar to previous studies in mouse and rat, human *hairless* was substantially expressed in fibroblasts from hair-bearing skin and most highly expressed in brain, where its importance remains unknown (*15*). Evidence in support of a role for multifunctional transcription factors involved in hair loss can be drawn from genetic studies of the *whn* gene in *nude* mice, in which mutations in a winged-helix transcription factor result in the absence of hair and athymia (*6*). Molecular evolutionary studies of *whn* have shown that a homolog is present in the puffer fish, *Fugu rubripes*, which has a thymus, but not hair, therefore suggesting that its role in hair keratinization may represent a new function for *whn* in mammals (*6*). A COOH-terminal activation domain was identified in *whn* by comparative genome analysis. The essential function of this domain could be obliterated by site-directed mutagenesis of acidic residues to alanine, analogous to the missense mutation we describe in the AU family (Fig. 5). It is possible that in humans, the AU mutation disrupts a similar potential activation domain within *hairless* with restricted specificity in the skin, whereas the *hr/hr* mouse displays a more pleiotropic defect because of the near absence of *hairless* mRNA and protein (*6*, *28*). We anticipate that further studies into the biology of human *hairless* and its transactivation targets may illuminate potential therapeutic opportunities.

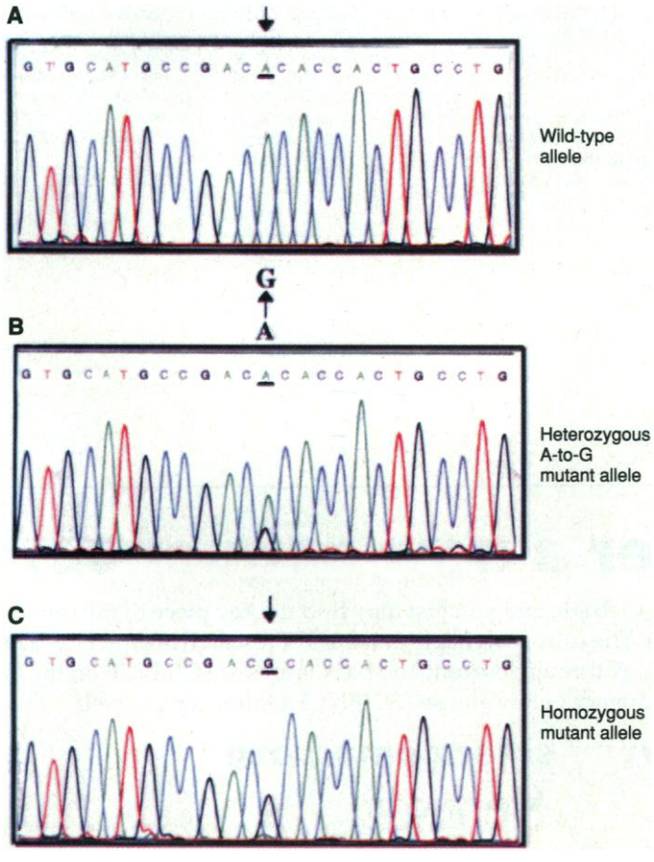


Fig. 5. Mutation analysis of exon 15 of the human *hairless* gene in the AU family. (**A**) The wild-type sequence contains a homozygous A (arrow), at the first base of a threonine codon (ACA). (**B**) Sequence analysis of heterozygous carriers in the AU family reveals the presence of a G as well as the wild-type A at this position (arrow). (**C**) Sequencing of all affected individuals in the AU family reveals a homozygous mutant G at this position (arrow), resulting in the substitution of threonine by alanine (GCA).

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10. Blood samples were collected from 36 members of the AU family, according to local informed consent procedures. DNA was isolated according to standard techniques (*29*). Fluorescent automated genotyping for the genome-wide linkage search was carried out at as a service by Research Genetics, with 386 markers covering the genome at about 10-cM intervals.
11. Refined and more extensive screening of all regions showing homozygosity in affected and unaffected family members was carried out with primers obtained from Research Genetics or in the Genome Data Base (*17*). Analysis of microsatellite markers consisted of end-labeling one primer with [γ-^{33}P]deoxyadenosine triphosphate; a PCR reaction consisting of 7 min at 95°C, followed by 27 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C; and electrophoresis in a 6% polyacrylamide gel (Sequa-gel; Action Scientific, Atlanta, GA). Microsatellite markers were visualized by exposure of the gel to autoradiography, and genotypes were assigned by visual inspection.
12. Statistical calculations for linkage analysis were carried out with the computer program FASTLINK version 3.0P [A. A. Schaffer, *Hum. Hered.* **46**, 226 (1996)], which enables all inbreeding loops in the family to be retained and has the capability for two-point analysis. Autosomal recessive with complete penetrance was assumed with a disease allele frequency of 0.0001. The lod scores were calculated with equal allele frequencies; however, results did not change when the frequency of the marker allele in association with the disease allele was set as high as 0.9. Multipoint analysis was not possible because of the large number of inbreeding loops and the complexity of the pedigree.
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16. The human multiple tissue Northern blot containing 2 μg of polyadenylated [poly(A)$^+$] mRNA from eight tissues was obtained from Clontech (Palo Alto, CA) and hybridized according to the manufacturers' recommendations with a random primed radiolabeled probe containing exons 13 to 18 of human *hairless*, generated as described in (*14*) and hybridized with ExpressHyb Solution (Clontech). Poly(A)$^+$ mRNA was extracted from cultured skin fibroblasts from hair-bearing skin from a control individual according to standard methods (*29*), and a Northern blot containing 2 μg of poly(A)$^+$ mRNA was hybridized with the same probe under identical conditions.
17. See the Genome Data Base at www.bis.med.jhmi.edu
18. A segment of human *hairless* intron 13 was PCR-amplified and used for radiation hybrid mapping with the G3 panel by Research Genetics. Primers were as follows: 5′-TATGTCACCAAGGGCCAGCC-3′ (sense) and 5′-TCAGGGTAGGGGGTCATGCC-3′ (antisense). PCR conditions were 5 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, with AmpliTaq Gold DNA polymerase (Perkin-Elmer). PCR primers specifically amplified human *hairless* and did not cross-hybridize with the hamster DNA used in the radiation hybrid panel.
19. See www.marshmed.org/genetics/maps/ss-maps/8ss.txt
20. See www.shgc.stanford.edu
21. Primers for specific amplification of exon 15 were placed in the flanking introns: 5′-AGTGCCAGGATTACAGGCGT-3′ (sense, intron 15) and 5′-CTGAGGAGGAAAGAGCGCTC-3′ (sense, intron 16). PCR fragments were purified on AGTC Centriflex columns (Edge BioSystems, Gaithersburg, MD) and sequenced directly with POP-6 polymer on an ABI Prism 310 Automated Sequencer (Perkin-Elmer). The mutation was verified by restriction endonuclease digestion with Hga I, according to the manufacturer's specifications (New England Biolabs).
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30. We sincerely thank the family members for their participation in this work, S. Malik and S. M. Zaidi for their assistance during our stay in Chakwal, B. J. Longley for expert dermatopathological advice, and M. Grossman and P. Schneiderman for their infinite patience. Informed consent for the publication of the photographs as well as the pedigree was obtained through personal visits to the family members. Supported in part by grants from Quaid-i-Azam University, Islamabad, Pakistan (M.A.), the National Alopecia Areata Foundation (A.M.C.), NIH–National Human Genome Research Institute HG-00008 (J.O.), and NIH–National Institute of Arthritis and Musculoskeletal and Skin Diseases Research Center P30AR44535 (M.P., J.O., and A.M.C.).

22 October 1997; accepted 5 January 1998