Hum Genet (1998) 103:400–404

RAPID COMMUNICATION

Abraham Zlotogorski · Wasim Ahmad
Angela M. Christiano

Congenital atrichia in five Arab Palestinian families resulting from a deletion mutation in the human hairless gene

Received: 31 July 1998 / Accepted: 31 August 1998

Abstract Congenital atrichia is a rare autosomal recessive disorder of hair development, characterized by complete loss of hair shortly after birth. Evidence of linkage to chromosome 8p12 has been established, implicating the human homolog of the mouse hairless (hr) gene as a candidate gene. We have previously identified missense mutations in families with congenital atrichia. Here, we report the first deletion mutation (2147del C) in exon 9 of the human hairless gene leading to a frameshift and downstream premature termination codon in five Palestinian families of Arab origin.

Introduction

The formation of a hair follicle involves a complex series of reciprocal interactions between the dermis and epidermis, resulting in the formation of an epidermal placode and hair plug, a dermal papilla, and finally, the differentiation of epidermal cells to form the inner root sheath and hair shaft of the follicle (Hardy 1992). The initial message is derived from the dermis and instructs the overlying epidermis to thicken, forming a placode and then a downgrowth into the dermis, known as the hair plug. An epidermal message passes from the hair plug to the dermis, resulting in the condensation of a cluster of mesenchymal cells that will eventually 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 either inner root sheath cells or hair shaft cells, depending on their position in relation to the longitudinal axis of the follicle (Hardy 1992). Hair growth proceeds in a cyclical fashion throughout life, with the growth phase being followed by a regression phase (during which the upper portion of the follicle degenerates) and a resting phase. At the end of the resting phase, epidermal stem cells located in the bulge region of the outer root sheath are thought to be recruited by signals from the dermal papilla to form the hair matrix, and a new cycle of growth is initiated (Costarelis et al. 1990; Rochat et al. 1994). Although these events have been described extensively in model systems, the genes governing these processes are largely unknown.

There are many forms of inherited alopecia or hairloss, showing extensive variation in age of onset, severity, and associated ectodermal abnormalities. Congenital alopecia universalis (MIM 203655) or congenital atrichia (MIM 209500) without associated ectodermal defects is very rare and is inherited as an autosomal recessive trait. We and others have recently reported a linkage of this form of atrichia to chromosome 8p12 (Ahmad et al. 1998a; Nothen et al. 1998). Further, we have mapped the human homolog of the hairless gene in the same region of chromosome 8 and have identified pathogenetic mutations in this form of atrichia (Ahmad et al. 1998a, b). The hairless gene product is a putative transcription factor with a single zinc-finger domain and is highly expressed in the brain and the skin. It appears to function in the cellular transition to the first adult hair cycle, and in its absence, hair growth completely ceases, a new hair is never induced, and the result is a complete form of inherited alopecia.

In this study, we have ascertained five Arab Palestinian families, demonstrating autosomal recessive congenital atrichia with papules, from two Palestinian villages east of Jerusalem. We have identified the first deletion mutation in the human hairless gene in affected members from all five families.

A. Zlotogorski
Department of Dermatology, Hadassah University Hospital, Jerusalem, Israel

W. Ahmad
Department of Dermatology, Columbia University, New York, USA

A. M. Christiano (✉)[1]
Departments of Dermatology and Genetics & Development, Columbia University, New York, USA

[1] *Address for correspondence:*
Department of Dermatology, Columbia University, College of Physicians & Surgeons, 630 West 168th Street VC15-1526, New York, NY 10032, USA
e-mail: amc65@columbia.edu, Tel.: +1 212 305 9565, Fax: +1 212 305 7391

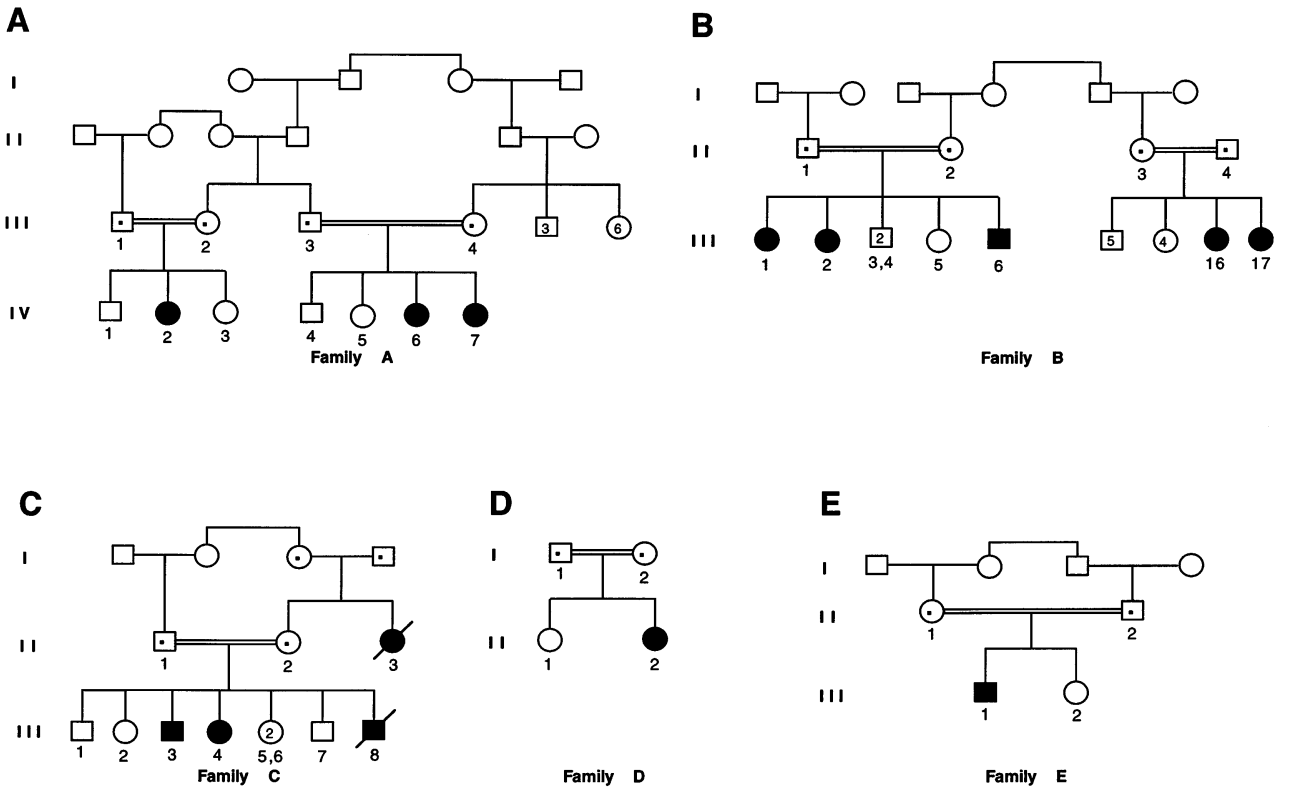

Fig. 1 Pedigrees representing five Arab Palestinian families from the West Bank in Israel. Affected males and females are indicated by *filled squares* and *circles*, respectively, and figures with a *dot in the center* are indicative of obligate heterozygous carriers. *Double lines* between figures are representative of consanguineous unions

Materials and methods

Human subjects

Five Palestinian families of Arab origin from the West Bank in Israel with congenital atrichia were studied (Fig. 1). We obtained DNA from 11 unaffected and 10 affected 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 following informed consent and in accordance with the local Institutional Review Board.

Genotyping

Genomic DNA from 21 members of five families was amplified by the polymerase chain reaction (PCR) with primers for loci D8S258, D8S298, and D8S1786 (Ahmad et al. 1998a). One primer from each pair was labeled with γ^{33}PdATP (NEN, Boston, Mass.). PCR for each marker was performed in a 10 µl volume containing 50 ng DNA, 50 ng each primer, 200 µM dNTP, 1×PCR buffer (Gibco BRL, Gaithersburg, Md.), and 1 U Platinum *Taq* DNA polymerase (Gibco BRL). PCR conditions were an initial denaturation of genomic DNA at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 7 min.

Mutation detection

To screen for a mutation in the hairless gene, a 271 bp PCR fragment containing exon 9 of the human hairless gene was amplified from genomic DNA with the following primers: 5' CTGTTGAATTGTGTCTGCCA 3' (sense strand) and 5' AGTGGAGATTATTGGGGGTG 3' (antisense strand). PCR products were sequenced directly in an ABI Prism 310 Automated Sequencer, with the ABI Prism Rhodamine Terminator Cycle Sequencing Ready Reaction Sequencing Kit (PE Applied Biosystems, Foster City, Calif.), following purification in a Centriflex Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, Md.).

Results

Four of the five families studied are thought to be distantly related, however, we were unable to identify their common ancestor. Collectively, the total number of affected members in these families was more than 50. The large number of affected individuals in the families is attributable to the high rate of consanguinity. The families stated that the lineage originated from three affected brothers. Subsequent marriages within their descendants led to the large number of affected individuals. We studied 21 members of five families, including 11 unaffected and 10 affected individuals, ranging in age from 8 months to 21 years (Fig. 1). Hair was absent from the scalp, axillae, pubis, and other parts of the body, and eyebrows and eyelashes were sparse (Fig. 2a). Natal hairs were present at birth but began to be shed within one month and completely disappeared by 3 months of age. No evidence of hair regrowth was observed.

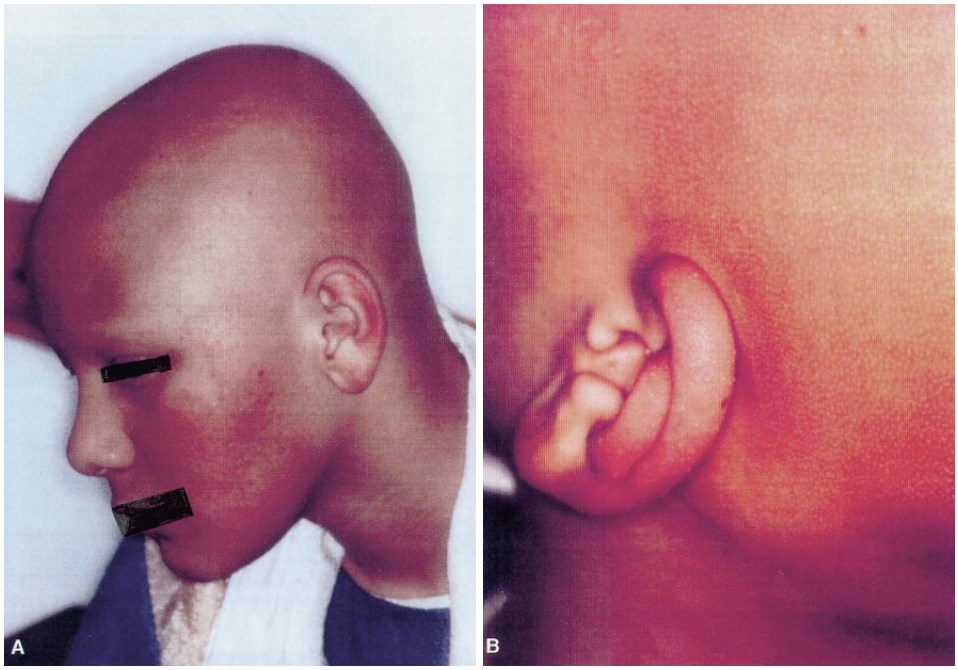

Fig. 2A, B Clinical findings in congenital atrichia. **A** Characteristic appearance of the face of a 12-year-old female from family A (individual IV-2). Note the complete absence of scalp hair, and sparse eyebrows and eyelashes. **B** The skin over the scalp and ear is covered with fine white papules

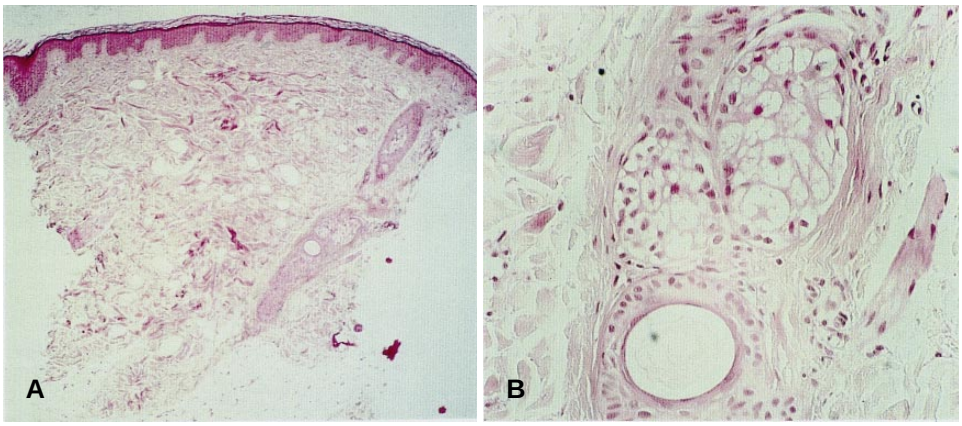

Fig. 3A, B Histopathological findings in congenital atrichia. **A** Scalp biopsy reveals the complete absence of hair follicles and only a single hair follicle remnant. Hematoxylin and eosin staining. ×40. **B** Higher magnification reveals the presence of a characteristic keratin-filled cyst within the hair follicle remnant. Hematoxylin and eosin staining. ×250

Numerous keratin-filled follicular cysts (Fig. 3) developed over extensive areas of the skin of affected individuals, usually between the age of 5–10 years. These papular lesions were most numerous on the cheeks, scalp, arms, forearms, thighs, and shins (Fig. 2b). A wide variability in the number of papules on the body was observed, which was not age-related. One affected member of family A (individual IV-2) had numerous papules on the scalp and face. Papules were not detected in unaffected members of the families. As the pedigree analysis suggests (Fig. 1), the mode of inheritance of atrichia in these families was clearly autosomal recessive.

Genotyping of 21 members of the families, including 10 affected and 11 unaffected individuals, was carried out by using the polymorphic markers D8S258, D8S298, and D8S1786, which are closely linked to the hairless gene on chromosome 8p12. The markers were fully informative, and the ten affected members of the families (individuals IV-2, IV-6, and IV-7 from family A; III-1, III-16, and III-17 from family B; III-3 and III-4 from family C; II-2 from family D; III-1 from family E) were homozygous for the markers D8S298 and D8S1786 with the same linked haplotype, consistent with linkage to the hairless locus. All other members were heterozygous carriers of the linked haplotype (individuals III-2, III-3, and III-4 from family A; II-1 and II-3 from family B; II-1 and II-2 from family C; I-1, I-2, and II-1 from family D; II-1 from family E).

Sequence analysis of exon 9 of the gene from all affected members revealed a recurrent single basepair deletion mutation at position 2147, designated 2197delC (Fig. 4), leading to a frameshift and premature termination codon 544 bp downstream in exon 12. This deletion was present in the heterozygous state in obligate heterozygous carriers within the families.

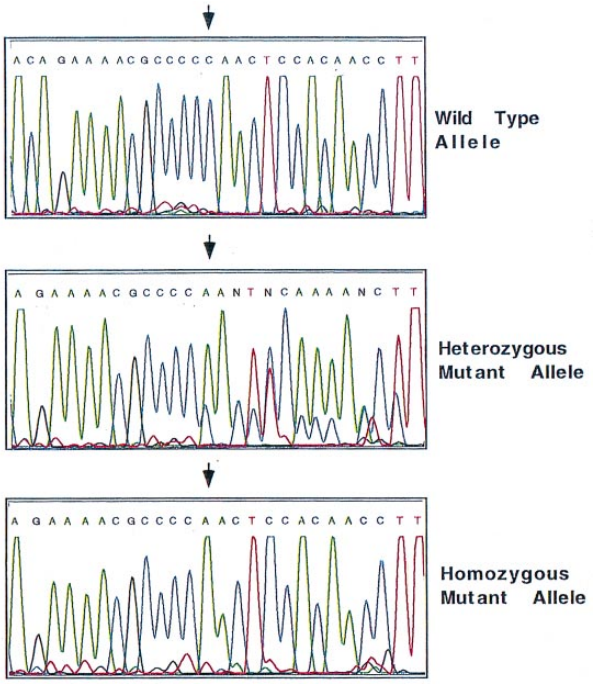

Fig. 4 Sequence analysis of the hairless gene mutation. DNA sequence of exon 9 of the hairless gene from a control individual, a heterozygous carrier, and a homozygous (affected) individual are shown. Note the presence of five consecutive C nucleotides (*arrow*) in the control sequence (upper panel). Note the presence of overlapping sequences downstream of the *arrow*, indicative of a frameshift in the mutant allele in the heterozygous sequence (center panel). The homozygous deletion of a nucleotide C is shown in the mutant sequence, resulting in the occurrence of four instead of five consecutive C nucleotides and a subsequent frameshift and downstream premature termination codon (lower panel)

Discussion

We have recently described the involvement of the human hairless gene in a rare form of hair loss known as congenital atrichia (Ahmad et al. 1998a, b). Here, we report the identification of a single basepair deletion mutation responsible for atrichia in five Palestinian families of Arab origin (Fig. 1). Four of these families live in the same village east of Jerusalem, whereas the fifth resides approximately 30 miles away. The affected individuals were born with normal hair, which was shed during the first three months of life (Fig. 2a). All areas of the body were affected. A scalp skin biopsy of affected persons showed the complete absence of mature hair follicles (Fig. 3). Variations in the structure and shape of hair follicle remnants have been reported in patients with congenital atrichia. These include shortened hair follicles containing horny plugs, and a reduced number of pilosebaceous units containing malformed hairs without cuticles (Birke 1954; Porter 1973). The most characteristic feature of affected individuals with this disorder is the development of numerous keratin-filled follicular cysts over extensive areas of the skin. Other than these hair and skin abnormalities, the remainder of the physical examination revealed no physical or mental developmental abnormalities.

The phenotypic appearance of affected individuals in these Arab Palestinian families is strongly reminiscent of those reported earlier (Damste and Prakken 1954; Landes and Langer 1956; Cantu et al. 1980), including atrichia with cystic papules and an autosomal recessive mode of inheritance. In 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 homolog of the hairless mouse mutation (Sundberg et al. 1989). Cases resembling this disease from Pakistan, with loss of hair over the entire body, have recently been reported under the name alopecia universalis (OMIM 203655; Ahmad et al. 1993; Ahmad et al. 1998a; Nothen et al. 1998); however, congenital atrichia with papules (OMIM 209500) appears be a more precise description of the phenotype resulting from mutations in the human hairless gene.

The molecular basis of the hairless mouse phenotype has previously been shown to be the result of a murine leukemia proviral insertion into intron 6 of the hairless gene, resulting in aberrant splicing and a moderately severe phenotype (Cachon-Gonzalez et al. 1994). A second, phenotypically more severe mouse mutation known as *rhino*, is allelic with hairless and is the result of more deleterious nonsense mutations in the hairless gene (Ahmad et al. 1998c, d; Panteleyev et al. 1998c). The mutation in the Arab Palestinian families results in a frameshift and downstream premature termination codon, thereby predicting an absence of functional mRNA secondary to nonsense-mediated mRNA decay and an absence of the hairless protein (Urlaub et al. 1989; Maquat 1996). However, compared with our previously studied families with two different missense mutations in the hairless gene (Ahmad et al. 1998a, b), the phenotypes in both families are remarkably consistent, despite the deletion mutation in this study being more deleterious at the molecular level. Hairless and rhino mice have a similar pattern of disease progression in that they are indistinguishable from heterozygous (phenotypically normal) littermates at birth. However, at the start of the second hair cycle, which begins at approximately 2 weeks of age, the hair is shed rapidly within a 7-day period in a cephalocaudal pattern and never regrows, because of a series of irreversible cellular events (Panteleyev et al. 1998a, b). Over time, the hair follicles are replaced by cystic structures in the upper and lower portions of the skin in both hairless and more severely in rhino mice. These features are similar to the cystic lesions observed in the Arab Palestinian families.

Expression of the hairless gene in mice has been shown to be restricted to the epidermis and certain hair follicle structures and is absent in the dermis (Cachon-Gonzalez et al. 1994), implying that the molecular defect in hairless

mice is intrinsic to epidermal cells. This is further substantiated by findings that hairless gene expression is restricted to the epithelial cell populations that exhibit a cellular phenotype in hairless mice (Panteleyev et al. 1998b). The precise function of the protein hairless remains elusive; however, recent studies have established that hairless functions as a transcriptional co-repressor in the brain and is regulated directly by thyroid hormone (Thompson 1996; Thompson and Botcher 1997). In hairless mice, the hair matrix cells undergo a premature and massive apoptosis, together with a concomitant decline in Bcl-2 expression, a loss of NCAM positivity, and a disconnection with the overlying epithelial sheath essential for the movement of the dermal papilla (Panteleyev et al. 1998a, b). As a consequence, the hair bulb and dermal papilla remain stranded in the dermis, and indispensable messages between the dermal papilla and stem cells in the bulge are not transmitted, and thus no further hair growth occurs. In hairless mice and in humans with congenital atrichia, we postulate that the absence of the hairless protein initiates a premature and abnormal catagen attributable to abnormal apoptosis, dysregulation of cell adhesion, and defects in dermal papilla-derived signaling that normally control catagen-associated hair follicle remodeling (Panteleyev et al. 1998b). These findings suggest that the hairless gene product plays a crucial role in maintaining the delicate balance between cell proliferation, differentiation, and apoptosis in the hair follicle and in the interfollicular epidermis.

Acknowledgments We are grateful for the participation of the family members in this study. The technical expertise of HaMut Lam is greatly appreciated. We thank Dr. B. Jack Longley for expert dermatopathological assistance, Drs. Andrei Panteleyev, Ralf Paus, and John P. Sundberg for stimulating discussions, and Dr. Spiro Tams for translation and communication with the family members. This work was supported in part by a grant from the National Alopecia Areata Foundation (A.Z. and A.M.C.) and the NIH-NIAMS Skin Disease Research Center (P30-AR44535) at the Department of Dermatology, Columbia University.

References

Ahmad M, Abbas H, Ul Haque S (1993) Alopecia universalis as a single abnormality in an inbred Pakistani kindred. Am J Med Genet 46:369–371

Ahmad W, Ul Haque MF, Brancolini V, Tsou HC, Ul Haque S, Lam H, Aita VM, Owen J, Blaquiere M de, Frank J, Cserhalmi-Friedman PB, Leask A, McGrath JA, Peacocke M, Ahmad M, Ott J, Christiano AM (1998a) Alopecia universalis associated with a mutation in the human hairless gene. Science 279:720–724

Ahmad W, Irvine A, Lam HM, Ahmad M, McGrath JA, Christiano AM (1998b) A missense mutation in the zinc-finger domain of the human hairless gene underlies generalized atrichia in a family of Irish travellers. Am J Hum Genet (in press)

Ahmad W, Panteleyev A, Sundberg JP, Christiano AM (1998c) Molecular basis for the rhino-8 J phenotype: a nonsense mutation in the mouse hairless gene. Genomics (in press)

Ahmad W, Panteleyev AA, Henson-Apollonio V, Sundberg JP, Christiano, AM (1998d) Molecular basis for a novel rhino phenotype: a nonsense mutation in the mouse hairless gene. Exp Dermatol (in press)

Birke G (1954) Über Atrichia congenita und ihren Erbgang. Arch Dermatol Syphil 197:322–343

Cachon-Gonzalez MB, Fenner S, Coffin JM, Moran C, Best S, Stoye JP (1994) Structure and expression of the hairless gene of mice. Proc Natl Acad Sci USA 91:7717–7721

Cantu JM, Sanchez-Corona J, Gonzalez-Mendoza A, Martinez Y, Martinez R, Garcia-Cruz D (1980) Autosomal recessive inheritance of atrichia congenita. Clin Genet 17:209–212

Costarelis G, Sun T-T, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61:1329–1337

Damste J, Prakken JR (1954) Atrichia with papular lesions: a variant of congenital ectodermal dysplasias. Dermatologica 108:114–117

Del Castillo V, Ruiz-Maldonado R, Carnevale A (1974) Atrichia with papular lesions and mental retardation in two sisters. Int J Dermatol 13:261–265

Fredrich HC (1950) Zur Kenntnis der kongenitale Hypotrichosis. Dermatol Wochenschr 121:408–410

Hardy M (1992) The secret life of the hair follicle. Trends Genet 8:55–61

Kanzler MH, Rasmussen JE (1986) Atrichia with papular lesions. Arch Dermatol 122:565–567

Landes E, Langer I (1956) Ein Beitrag zur Hypotrichosis congenita. Hautarzt 7:413–415

Lowenthal LJA, Prakken JR (1961) Atrichia with papular lesions. Dermatologica 122:85–87

Maquat LE (1996) Defects in RNA splicing and the consequence of shortened translational reading frames. Am J Hum Genet 59:279–286

Misciali C, Tosti A, Fanti PA, Borrello P, Piraccini BM (1992) Atrichia and papular lesions: report of a case. Dermatologica 185:284–288

Nothen MM, Cichon S, Vogt IR, Hemmer S, Kruse R, Knapp M, Holler T, Ul Haque MF, Ul Haque S, Propping P, Ahmad M, Rietschel M (1998) A gene for autosomal congenital alopecia maps to chromosome 8p21–22. Am J Hum Genet 62:386–390

Panteleyev AA, Veen C van der, Rosenbach T, Muller-Rover S, Sokolov VE, Paus R (1998a) Towards defining the pathogenesis of the hairless phenotype. J Invest Dermatol 110:903–907

Panteleyev AA, Botchkareva NV, Veen C van der, Christiano AM, Paus R (1998b) Pathobiology of the the hairless phenotype: dysregulation of hair follicle apoptosis and topobiology during the initiation of follicle cycling. J Invest Dermatol 110:577

Panteleyev AA, Ahmad W, Malashenko AM, Ignatieva EL, Paus R, Sundberg JP, Christiano AM (1998c) Molecular basis for the rhino Yurlovo (hr^{rhY}) phenotype: severe skin abnormalities and female reproductive defects associated with an insertion in the hairless gene. Exp Dermatol (in press)

Porter PS (1973) Genetic disorders of hair growth. J Invest Dermatol 60:493–502

Rochat A, Kobayashi K, Barrandon Y (1994) Location of stem cells in human hair follicles by clonal analysis. Cell 76:1063–1073

Rook A, Dawber R (1991) Diseases of the hair and scalp, 2nd edn. Blackwell, Oxford, pp 187–197

Sambrook J, Fritsch EG, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Sundberg JP, Dunstan RW, Compton JG (1989) Hairless mouse, HRS/J hr/hr. In: Jones TC, Mohr U, Hunt RD (eds) Monographs on pathology of laboratory animals. Integument and mammary glands. Springer, Berlin Heidelberg New York, pp 192–197

Thompson CC (1996) Thyroid hormone-responsive genes in developing cerebellum include a novel synaptotagmin and a hairless homolog. J Neurosci 16:7832–7840

Thompson CC, Bottcher MC (1997) The product of a thyroid hormone-responsive gene interacts with thyroid hormone receptors. Proc Natl Acad Sci USA 94:8527–8532

Urlaub G, Mitchell PJ, Ciudad CJ, Chasin LA (1989) Nonsense mutations in the dihydrofolate reductase gene affect RNA processing. Mol Cell Biol 9:2868–2880