Atrichia with papular lesions (APL) (OMIM#209500) is a rare form of irreversible alopecia that is inherited in an autosomal recessive pattern (Fredrich, 1950; Damste and Prakken, 1954; Loewenthal and Prakken, 1961). In individuals affected with this form of hair loss, hairs are typically absent from the scalp, axilla, and body. Patients are almost completely devoid of eyebrows and eyelashes. Histologic examination of affected scalp skin shows the absence of mature hair follicle structures. Although alopecia may accompany several different forms of congenital ectodermal dysplasias, APL patients are unique in that, along with total atrichia, papules and follicular cysts filled with cornified material represent a unique cutaneous abnormality among inherited alopecias. Apparently normal hairs are present at birth in most APL patients, but these neonatal hairs are usually shed within the first months of life and are never replaced. In individual cases, the shedding of the hair occurs during the first 2–3 y of life. At approximately 2 y of age, affected patients begin to develop multiple follicular papules, and variations in the structure and morphology of the hair follicle remnants have been reported.

Recently, we and others reported linkage of this form of atrichia to chromosome 8p12 (Ahmad et al, 1998a; Cichon et al, 1998; Sprecher et al, 1998), and subsequently, a large number of pathogenetic mutations in the hairless (hir) gene have been implicated in this disorder. Published estimates of the prevalence of this disorder remain surprisingly low considering pathogenetic mutations in hir have been found in distinct ethnicities around the world. Therefore, it is likely that congenital atrichia with papular lesions is far more common than previously thought and is often mistaken for its phenocopy, the putative autoimmune form of alopecia universalis. To clarify this discrepancy, we propose criteria for the clinical diagnosis of congenital atrichia with papular lesions.

Among these is the novel report of the consistent observation of hypopigmented whitish streaks on the scalp surface of affected individuals. Additionally, we report the identification of a novel missense mutation in hir from a family of Arab Palestinian origin that exhibits the pathognomonic features of atrichia with papular lesions. Collectively, we anticipate that an increased recognition of this disorder will result in more accurate diagnosis and the sparing of unnecessarily treatment to patients. Key words: alopecia/atrichia with papular lesions/diagnosis/hairless/mutation.

**MATERIALS AND METHODS**

**Family history** We studied an Arab Palestinian family originating from a village near Jerusalem with a single female affected with congenital atrichia (Fig 1A). The family history was significant for parental consanguinity and consistent with autosomal recessive inheritance. The proband was a 6-y-old-girl who was born with normal scalp hair, which began to shed during the second to third month (Fig 2). It progressed to complete and permanent hairlessness at the age of 1 y. The patient did not respond to standard topical steroid therapy used for treatment of alopecia universalis.

**Clinical material** Blood samples were collected from the family and a scalp biopsy was performed in the proband to confirm the diagnosis. Specifically, a punch biopsy was taken from the left parietal region of the scalp under local anesthesia. The sample was fixed in 4% paraformaldehyde and embedded in paraffin. Four micrometer sections were processed and stained with hematoxylin and eosin according to standard protocols. All procedures were performed in accordance with the guidelines of the local Institutional Review Board.

**Communications**

**Clinical and Molecular Diagnostic Criteria of Congenital Atrichia with Papular Lesions**

Abraham Zlotogorski, Andrei A. Panteleyev,* Vincent M. Aita,* and Angela M. Christiano*

Department of Dermatology, Hadassah Medical Center, Jerusalem, Israel; Departments of Dermatology and Genetics & Development, Columbia University, New York, New York, U.S.A.

Congenital atrichia with papular lesions is a rare, autosomal recessive form of total alopecia and mutations in the hairless (hir) gene have been implicated in this disorder. Published estimates of the prevalence of this disorder remain surprisingly low considering pathogenetic mutations in hir have been found in distinct ethnicities around the world. Therefore, it is likely that congenital atrichia with papular lesions is far more common than previously thought and is often mistaken for its phenocopy, the putative autoimmune form of alopecia universalis. To clarify this discrepancy, we propose criteria for the clinical diagnosis of congenital atrichia with papular lesions.
293 bp fragment. Wild-type alleles are cleaved into products of 122 bp and 171 bp.

Figure 1. Genetic analysis of an Arab family with APL. (A) A pedigree representing a family with a single member affected with APL. Circles and squares represent females and males, respectively. (B) Automated DNA sequence analysis of the hairless gene. DNA sequence of exon 16 from control, heterozygous carrier, and homozygous affected individuals. Note the arrow denoting the mutation at nucleotide position 3166 of the hairless cDNA. (C) Restriction endonuclease analysis of the hairless gene with the enzyme Pml I. Note the mutation prevents cleavage by Pml I, resulting in a 293 bp fragment. Wild-type alleles are cleaved into products of 122 bp and 171 bp.

RESULTS

Clinical findings consistent with papular atrichia At the time of examination, very few hairs remained on the scalp of the proband (Fig 2A). Careful examination of this patient led to a novel clinical observation that has not been described earlier in congenital atrichia, namely, hypopigmented whitish streaks on the scalp surface (Fig 2A). Hair was absent from the axillae and other parts of the body, and the eyebrows and eyelashes were sparse (Fig 2B). She had the additional characteristic feature of grouped diffuse papular eruption of approximately 100 papules, which began to appear at age 2, on the scalp, cheeks, arm, elbows, thighs, and knees (Fig 2C). The patient showed no growth or developmental delay, normal teeth and nails, and no abnormalities in sweating.

Histopathology of the scalp Histologic studies revealed a normal but slightly hyperkeratotic epidermis, normal sweat (swg) and sebaceous (sg) glands, and the absence of any signs of inflammation. Only small fragments of hair follicle epithelia (Fig 2D, arrowheads), however, were found on serial sections of skin sample. Two remnants of the upper hair follicle portion (Figs 2D, 1 and 2) reminiscent of utriculi in hairless mouse skin were found in the upper dermis. Both these remnants did not contain hair shafts and instead were filled with amorphous cornified material.

Mutation detection To screen for a mutation in the hairless gene, PCR amplification of all exons of the human hairless gene were performed as described previously (Ahmad et al, 1999). PCR products were sequenced directly in an ABI Prism 310 Automated Sequencer, using the ABI Prism Rhodamine Terminator Cycle Sequencing Ready Reaction Sequencing Kit (Applied Biosystems, Foster City, CA), following purification in a Centriflex Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, MD). The mutation was verified in the patient, the parents, and unaffected, unrelated control individuals using restriction enzyme digestion with Pml I (New England Biolabs, Beverly, MA).

Identification of a mutation in the hairless gene We identified a homozygous G-to-A transition at nucleotide 3166 in the hairless gene leading to the conversion of a valine residue (GTG) to a methionine residue (ATG) (Fig 1B). This mutation, designated V1056M, abolishes a restriction endonuclease site for the enzyme Pml I, which was used as a screening assay in 40 Caucasian control individuals, and 98 Arab Palestinian control individuals, to rule out the possibility that this mutation is a polymorphism (Fig 1C). Furthermore, the mutation V1056M resides in a region of the hairless protein (amino acids 946–1157) recently shown to have homology to the cupin family of metalloenzymes, which are candidates for enzymes that regulate chromatin remodeling and the integrity of chromatin structure (Clissold and Ponting, 2001). The absence of this mutation in 276 alleles, the conservation of this amino acid across all species where hairless has been cloned, and its location within a domain of potential important function, supports that it is the pathogenetic mutation in this family.

DISCUSSION

Establishing the diagnosis of a disease requires rigid criteria, and based on the large number of families we have personally examined and on the reported literature, we propose an algorithm of diagnostic criteria for congenital APL (Table I). The first of these is the importance of family history, including the pattern of inheritance established as autosomal recessive, with careful questioning for consanguinity. Another important hallmark of the clinical history is that patients were born either without hair or with normal hair that is shed during the first several months and never regrew, and that papules started to appear during the first years of life. The examination should reveal the complete lack or near complete lack of scalp hair, sparse eyebrows and eyelashes, and lack of secondary axillary, pubic, or body hair. In addition, small to large numbers of papules should be found distributed over some or all of the following areas: scalp, cheeks, arm, elbows, buttocks, thighs, and knees, and of particular note, under the midline of the eye. Whitish hypopigmentary fine marks or streaks may be present on
the scalp, and patients should have normal nails, teeth, and sweating, and no major growth or developmental deficiencies. Finally, two definitive laboratory tests include (i) a scalp biopsy to reveal the absence of hair follicles and the presence of cysts filled with cornified material upon histologic examination, and (ii) the molecular diagnosis of mutations in the \textit{h} gene.

We utilized these criteria in the identification of a novel missense mutation in an Arab Palestinian patient now definitively diagnosed with congenital APL. The patient presented here exhibits the pathognomonic features of congenital APL. These include the absence of scalp hair, following its shedding within the first months of life, the persistence of papular lesions, and the histology demonstrating the absence of mature hair follicles (Fig 2). In addition to the atrichia, the most characteristic feature of affected individuals with APL is the development of the cornified cysts, usually appearing by age 5 (Fig 2D). In our patient, the popular eruption started by age 2, and following repeated questioning of other patients with APL, we discovered that papules may appear even earlier, with a range between 1 and 10 y of age. In some cases, the lesions are few in number, and therefore are initially ignored by

Table I. Diagnostic criteria for APL

I. FAMILY HISTORY
*Family history, pattern of inheritance established as autosomal recessive, possible history of consanguinity.
*Patients are sometimes born without hair and none ever grows. More typically, patients are born with normal hair that shed after several months and never regrow.
*Papules that start to appear during the first year of life, particularly under the midline of the eye, on the face, extremities.

II. EXAMINATION
*Complete lack or almost complete lack of scalp hair.
*Sparse eyebrows and eyelashes.
*Lack of secondary axillary, pubic, or body hair.
*Papules – few to many distributed over some or all of the following areas: scalp, cheeks, arms, elbows, thighs, and knees.
*Normal nails and teeth, normal sweating, and no growth or developmental problems.
*Whitish hypopigmented streaks on the scalp.

III. LABORATORY TESTS
*Lack of response to any treatment modality.
*Biopsy – absence of mature hair follicle structures, cysts filled with cornified material.
*Mutation in the \textit{h} gene.

Figure 2. Clinical and histopathologic presentation of APL. (A) The scalp is almost completely devoid of hair, with few papules and hypopigmented whitish streaks. (B) Papules on the cheek. Note the common infra orbital location of papules. Sparse eyebrows and eyelashes are also visible. (C) Diffuse papular eruption on the upper arm. (D) Histologic examination reveals the complete absence of normal hair follicle structures. Note the sebaceous glands (\textit{sg}) and sweat glands (\textit{swg}) appear normal.
the patients, the parents, and the clinician. In general, there is an increase in the number of papules in the individual patient with age; however, there is a wide inter- and infrafamilial variability in the number and distribution of papules on the body, which is not age related. Papules are observed mainly on the scalp, arms, elbows, thighs, knees, cheeks, and under the midline of the eye (Fig 2A).

Following examination of this patient, we noticed a clinical sign that was not described in earlier reports of APL, specifically, hypopigmented whitish streaks on the scalp surface (Fig 2A). These marks are also found in the other affected members of APL families of Arab origin, as well as in the patients from previous reports of APL (Table I) and therefore we believe it is not an incidental finding. Hypopigmentation could develop as a secondary lesion (e.g., a post-inflammatory response), but none of our patients admitted scratching or inflammation. It is also noteworthy that these streaks are persistent and become a permanent feature of the scalp. In the future, it will be interesting to note whether atrichia patients of non-Arab origin share these whitish streaks.

The mutation identified in this study extends our knowledge of mutations in the hairless gene which define the pathogenetic basis of this disease. It is noteworthy that the clinical picture is identical in patients carrying all types and combinations of mutations, including deletion, missense, splice site, and nonsense mutations (Ahmad et al., 1998a, b, 1999a, b; Cichon et al., 1998; Zlotogorski et al., 1998; Kruse et al., 1999; Sprecher et al., 1999a, b; Aita et al. 2000). Specifically, based on examination of 10 Arab families, including the family in this report, we find that the clinical phenotype of complete scalp atrichia manifesting during the first months, sparse eyebrows and eyelashes and papular eruption, is remarkably consistent irrespective of the type of mutation, and in contrast to previous reports we observed no clinical evidence that would warrant splitting the disease into subphenotypes on the basis of the type of mutation and the number and distribution of papules (Zlotogorski et al., 1998; Ahmad et al., 1999a; Sprecher et al., 1999a, b). Unlike the paradigm in the mouse, where a splicing defect leading to reduction of hairless mRNA levels results in the hairless mouse phenotype (Cachon-Gonzalez et al., 1994, 1999) and complete null mutations result in the more severe rhino phenotype (Ahmad et al., 1998c, d; Panteleyev et al., 1998c), such genotype-phenotype correlations have not yet emerged in human APL.

We have noticed two clinical features that display minor interfamilial variation. These include (i) the amount of hair on the scalp, ranging from complete absence to a few grouped hairs remaining on the vertex; and (ii) variability in the number and distribution of the papules. Importantly, these variations do not differ from the infrafamilial variation, e.g., two affected members in the same family may have 50 or 500 papules. Understanding this variation and taking into consideration the sometimes incomplete clinical phenotype description is crucial.

Furthermore, we have encountered several patients (A.Z., A.M.C.) who have been mistakenly diagnosed as alopecia universalis, and treated accordingly. In fact, APL could easily be dismissed by the clinician as a case of AT/AU and the associated papular rash could be attributed to a form of keratosis pilaris as an explanation for the papular lesions. We strongly urge those examining these patients to use the guidelines we propose for making the important distinction between APL and AT/AU. We believe that the phenotype of atrichia with papular lesion may be more common than was earlier believed, and that in the near future, clinicians will discover some APL patients that were labeled and treated accordingly as AU. Therefore, we propose the criteria outlined above so that patients with this disorder may be diagnosed accurately and spared the battery of treatments that are destined to fail.

We thank HaMut Lab for expert technical assistance. We very much appreciate the participation of the patient and family members in this study. We are grateful to Dr. Benjamin Glaser, Jerusalem, Israel, for the generous gift of unrelated, unaffected Arab control DNA. This work was supported in part by the National Alopecia Areata Foundation (A.Z., A.M.C.), the Dermatology Foundation, and the NIH Skin Disease Research Center Grant P30-44535 at Columbia University.

REFERENCES


