Is the Loose Anagen Hair Syndrome a Keratin Disorder?

A Clinical and Molecular Study

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Objectives: To report the clinical features of the loose anagen hair syndrome and to test the hypothesis that the typical gap between the hair and the inner root sheath may result from hereditary defects in the inner root sheath or the apposed companion layer.

Design: Case series.

Setting: A pediatric dermatology unit (referral center).

Patients: A consecutive sample of 17 children (13 girls). For 9 of them and their first-degree relatives, molecular analyses were performed in the K6HF gene with 50 appropriate controls.

Intervention: Minoxidil therapy (5% lotion) in 11 patients for 1 to 12 months.

Main Outcome Measures: Clinical and follow-up features and determination of mutations in the K6HF gene.

Results: Most patients had easily pluckable hair with no sign of scalp inflammation or scarring. Ten patients seldom cut their hair, and 4 had unmanageable hair. One patient had hypodontia. Two patients had an additional clinical phenotype of diffuse partial woolly hair. The family history was positive for loose anagen hair syndrome in 5 patients. Marked improvement was noted after treatment with 5% minoxidil lotion in 7 of the 11 patients treated. Polymerase chain reaction analysis of the gene segments encoding the α-helical 1A and 2B subdomains of K6hf, the type II cytokeratin exclusively expressed in the companion layer, was performed in 9 families. In 3 of these 9 families, a heterozygous glutamic acid and lysine mutation, E337K, was identified in the L2 linker region of K6HF.

Conclusions: Diffuse partial woolly hair can be associated with loose anagen hair syndrome. A keratin mutation, E337K in K6HF, was possibly causative in 3 of the 9 families studied. Another keratin, and possibly the type I partner of K6hf, could be responsible for loose anagen hair syndrome in other patients, or the gene involved may be a minor gene.

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Since 1990, it has been known that mutations in keratin genes can cause various hereditary epithelial disorders. The deleterious mutations are not randomly distributed along a keratin molecule, but occur preferentially in those parts of the molecule that are essential for ordered filament formation. The main areas involved in this process are the helix initiation motif or the helix termination motif. The hereditary hair disease monilethrix can be caused by a mutation in hair keratins.1

Loose anagen hair syndrome (LAHS) is a recently described hair disease, characterized by easily pluckable hair. It was first reported by Zaun in 1984,2 and then by Nödl et al in 1986,3 and was then published in the US literature by Hamm and Traupe4 and Price and Gummer5 in 1989. The essential finding is the ability, by gentle pulling, to painlessly extract anagen hairs that lack the inner root sheath (IRS) and the outer root sheath.

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Previous analyses of hair morphologic features in patients with LAHS have shown that the main defect is a gap between the cuticle of the IRS and the cuticle of the hair, without abnormalities of the hair follicle structure. We hypothesized that the typical gap between the hair and the IRS may result from defects in the IRS or in the apposed companion layer (CL). Both compartments are tightly connected and are thought to stabilize the growing hair. Thus, mutations in keratins expressed in these follicular compartments may compromise the stable anchor-

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PATIENTS AND METHODS

PATIENTS

From January 1988 to December 1998, 17 cases of LAHS originating from 17 different families were diagnosed in our unit because of easily pluckable hair, alopecia, and/or slow-growing hairs. The diagnosis was established on clinical grounds associated with a trichogram in 16 patients. More than 50% of dystrophic anagen hairs in the trichogram was considered as diagnostic of LAHS in 14 of the 16 patients investigated.

DNA

After ethic committee approval, blood was drawn from consenting affected and unaffected members of 9 nuclear families (the propositus and parents and sometimes brothers and sisters) of this series and from 30 unrelated healthy individuals, without a history of hair disorder, to whom the aim and nature of the study had been explained. Genomic DNA was isolated (Blood and Tissue Culture DNA Extraction System; QIAGEN GmbH, Hilden, Germany).

MUTATION ANALYSIS

The gene segments encoding the α-helical 1A and 2B subdomains of K6HF were amplified by the polymerase chain reaction using the primer pairs 5’-TCAGTGCCGACCTCCCTGTGTT-3’ (forward primer) and 5’-TGGTTTCTCATAGCTTCAAGCTGC-3’ (reverse primer) for amplification of the IA region and the primer pairs 5’-GGCAAGGCTTGAAGCTGACTGAGGA-3’ (forward primer) and 5’-GGAGAACAACTCAGCTAGGAGACTGACAA3’ (reverse primer) for amplification of the 2B region. Polymerase chain reaction analysis was performed (Expand Long Template PCR System; Roche, Mannheim, Germany). The polymerase chain reaction conditions consisted of incubation for 2 minutes at 94°C, followed by 30 cycles for 10 seconds at 94°C, 30 seconds at 60°C, and 2 minutes at 68°C. Polymerase chain reaction products were separated by agarose gel electrophoresis, purified using silica gel beads (Roche), and sequenced directly according to a radiolabeled chain terminator cycle-sequencing protocol (Thermo Sequenase; Amersham Biosciences, Braunschweig, Germany) by using the gene-specific primers as sequencing primers.

RESULTS

CLINICAL FINDINGS

The main complaint for 13 patients was easily pluckable hair. The number of haircuts ranged from none from birth to 1 every 2 months. Nine patients had thin, lusterless, and/or dry hair. Unmanageable hair was noted in 4 patients; hair was described as being rough, messed up, or hard to comb. It was more evident in the occipital region in 2 patients. Last, when noted, hair length was reported as short or variable. It did not exceed 20 cm. All children were in good health. Eyebrows and eyelashes were normal. Nails were normal, except for one patient who had fragile nails. One patient had hypodontia and lacked incisors and premolars (Table). A trichogram was obtained in 16 patients according to a routine technique of sampling hairs in 3 sites (frontal, temporal, and occipital). Examination by light microscopy revealed a prevalence of dystrophic anagen hairs, and a low count of telogen hairs for 14 of the 16 patients (Table). These dystrophic anagen hairs were devoid of root sheaths, had a distorted bulb, and had a tapered end (Figure 1). For the 2 other patients, results showed mostly normal anagen hairs. Nevertheless, dystrophic anagen hairs were still numerous (37.3% and 19.3%, respectively) and clinical examination and pull test results were positive for LAHS.

The family history was positive for LAHS in 5 patients. Three of them had 1 adult relative (1 mother, 1 father, or 1 paternal aunt) who displayed clinical signs.
of LAHS. The 2 other patients with a positive family history of LAHS (1 brother for one; a father, a brother, and a sister for the other one, corresponding to family 7 detailed in Figure 2) displayed, among normal or loose anagen hairs, a special type of curly hairs. These were shorter, hypopigmented, and easy to pluck, corresponding to the clinical phenotype of diffuse partial woolly hair (DPWH) (Figure 3).

INDIRECT IMMUNOFLUORESCENCE MICROSCOPY STUDIES

Sections of a scalp biopsy specimen of a patient with LAHS (patient 2) were reacted with specific K6 and K6hf antibodies. Figure 4 shows a near-longitudinal section in which the K6hf antibody decorated the entire CL. K6 was coexpressed with K6hf in the CL; however, this occurred only above the widening of the outer root sheath. At this level, the outer root sheath also started to be positive for K6. The appearance of the CL is not visibly different from that published previously for hair follicles of healthy individuals.

IDENTIFICATION OF A GLUTAMIC ACID AND LYSINE MUTATION, E337K, IN THE L2 LINKER REGION OF K6HF

The pedigrees of the 3 families positive for LAHS (families 2, 3, and 7) are shown in Figure 2. Analysis of the region encoding the helix initiation motif of K6HF did not reveal alterations from the reported sequence in affected and unaffected members of the 9 families studied. The primer pair used amplifies not only the helix initiation motif coding region but also considerable parts coding for the head domain and the α-helical 1A subdomain of K6HF. Except for some polymorphisms, these sequences were identical in all families. The same holds true for the K6HF helix termination motif coding region contained in the approximately 9000-base pair polymerase chain reaction product obtained with primer pair 2 (results not shown). However, successive sequence analysis of the 9000-base pair segment, which spans from exon 2 to exon 9 of the K6HF gene, revealed a heterozygous G→A point mutation and an adjacent T→C mutation (sequence ladder b in Figure 5) in affected individual II:1 of family 3. While the T→C mutation was silent, the G→A mutation led to an E337K substitution in the K6HF molecule (Figure 5). Whereas both point mutations were clearly absent in clinically unaffected control individual I:1 of the family (sequence ladder a in Figure 5), the same mutation pattern, including the silent T→C mutation, could be detected in clinically unaffected individuals I:2 and II:2 of family 3. The G→A point mutation affects the fourth amino acid position (glutamic acid) of the L2 linker region, which spans from S334 to W341 of keratin K6hf (Figure 5). Investigations in 50 healthy individuals did not reveal the presence of the T→C or the G→A mutation in the K6HF gene (results not shown).

The results of subsequent analysis for the K6HF E337K mutation in the other families were completely negative for members of families 1, 4 to 6, 8, and 9. In contrast, in family 7, the G→A mutation and the resulting E337K substitution could be detected in clinically affected individual I:2 and children II:1, II:2, and II:3. None of these individuals exhibited the silent T→C point mutation observed in some members of family 3.

In family 2, the K6HF E337K mutation was clearly present in affected individual II:2 and in the clinically

Figure 1. A, A typical trichogram showing almost only dystrophic anagen hairs. B, Detail showing ruffled cuticles and distorted bulbs.

Figure 2. Pedigrees of families 2, 3, and 7 who are affected by loose anagen hair syndrome. DNA samples were obtained from all individuals. Black symbols indicate clinically affected members of the families; gray symbols, individuals who are clinically not affected but carry the K6HF E337K mutation; and open symbols, individuals who are not clinically affected and who do not carry the mutation. In family 7, diffuse partial woolly hair was diagnosed in I:1, II:1, II:2, and II:3.
Loose anagen hair syndrome may not be as rare as has been thought. Sinclair et al., in a large series of 43 patients, estimated the incidence to be 2 to 2.25 cases per million per year. The clinical presentation of children in our series is similar to that of other cases reported in the literature.5,8-20 The children have slow-growing hair that seldom requires cutting.5,8-19,21,22 Parents may also be worried by the patients’ diffuse hair loss or localized patches of alopecia.4,5,8-16,19,22,23 They may have easily pluckable hair, although this sign is not always obvious.9,10,12-15,19,21,23 However, as in our series, the pull test result is usually positive, with more than 50% of dystrophic anagen hairs per pull in pediatric cases of LAHS. The presence of loose anagen hairs in children on a gentle hair pull is not diagnostic of LAHS when 1 to 2 dystrophic anagen hairs per hair pull are found.22 Additional findings include thin, dry, lusterless, or unmanageable hair.9,12,13,17-19 with the occipital area being the most frequently affected region. Sometimes, hair is also reported as sticky5,11 or tacky.8

Loose hair anagen syndrome may be an inherited condition. The pattern of affected family members seems to be that of an autosomal dominant inheritance.5,8-12,14-16,18,19,21-23. For 3 of them, relatives displayed clinical characteristics of LAHS. For the 2 others, family members had hairs of the DPWH type.25-27 The anomaly comprises apparently normal straight hairs and abnormal curly hairs diffusely intermingled. Some characteristics of LAHS and of DPWH are quite similar (age at onset, female predominance, hair loss, thin hair, curly or unmanageable hair, and longitudinal grooves on microscopic examination). These 2 diseases may, therefore, be somehow related. Furthermore, Sinclair et al.8 recently noted a woolly hair appearance for 21 of their 24 patients studied.

Based on the pathological features of LAHS, we anticipated that mutations in hair keratins should not be the cause for the syndrome. Indeed, mutation analyses of all known hair keratins of both types in the available families with LAHS did not reveal alterations of the gene structure in affected individuals (results not shown). In view of the cleavage zone between the IRS and the hair shaft in the hair follicles of persons with LAHS, we speculated that mutations in keratins expressed in the IRS might be responsible for the observed defect. Unfortunately, keratins expressed in the IRS are not yet known. In contrast, in the apposed CL, connected via desmosomal junctions with the Henle layer of the IRS, a specific keratin, K6hf, has recently been described.6 Indeed, mutation analysis of the K6HF gene revealed a G→A substitution in the region coding for the L2 linker of the rod domain of K6HF, leading to a replacement of glutamic acid residue 337 by lysine in 3 of the 9 families analyzed. Up to now, keratin mutations in linker regions, causative for epidermal genodermatoses, ie, the Weber-Cockayne syndrome type epidermolysis bullosa, have only been reported for linker L1/2.28 However, because recent investigations emphasized the importance of the highly conserved linker L2 for the correct axial alignment of keratin molecules,29 mutations in this non-α-helical part of the K6HF rod domain may also affect intermediate filament assembly. Because our immunofluorescence studies did not reveal discernible alterations of the CL in the follicles of those with LAHS, it might be that, unlike mutations in the helix initiation or termination motif of the rod domain of keratins, the observed L2 mutation does not lead to a visible breakdown of the intermediate filament network, but rather to a general destabilization of the latter.

Based on molecular studies, a dominant inheritance of LAHS was confirmed in the 3 families in whom

References 4, 9, 10, 12-14, 16, 18, 19, 21-23.
the mutation was found, because both children and fathers bore the mutation. However, father I:2 and child II:2 in family 3 seemed clinically unaffected. In family 7, in which the association of LAHS and DPWH was found in propositus II:1, the father (I:2) had DPWH and the other children (II:2 and II:3) had mild LAHS and associated DPWH. This could be due to a variable penetrance of the condition. Another explanation could be provided, for the father of family 3 only, by the natural improvement of LAHS.

When comparing phenotypically tested patients according to the presence or absence of the E337K mutation of K6hf, characteristics were not different (age at onset, hair growth, positive family history of LAHS, association with the DPWH phenotype, and trichogram results). Another gene may, therefore, be implicated in the patients who tested negative for the mutation. Because the type I partner of K6hf is still unknown, we restricted our mutation analysis to the K6HF gene. Another explanation could be provided by the coexistence of major and minor genes. The K6HF gene involved may be one of the minor ones, the major one yet to be discovered.

Loose anagen hair syndrome is a recently described hair disease, well defined by its clinical findings and trichogram characteristics. The results in our series of 17 pediatric patients are similar to other findings in the literature. Other hair disorders, such as DPWH, may be associated with LAHS, or even linked in a yet unknown manner. Future case reports may confirm our findings. Our experience with minoxidil therapy is encouraging, but needs to be confirmed in a controlled trial. We have, for

Figure 4. Expression studies of K6hf and K6 in a follicle with loose anagen hair syndrome (LAHS). A near-longitudinal section of a follicle with LAHS was reacted with an antibody against K6hf (red) and K6 (green), as described by Winter et al. ORSsb indicates outer root sheath, suprabasal; ORS, outer root sheath, basal; cl, companion layer; co, cortex; and dp, dermal papilla.

Figure 5. Mutation analysis of the type II keratin K6HF gene in family 3, affected by loose anagen hair syndrome. Excerpts from DNA sequencing gels show K6HF sequences from either unaffected individual I:1 (a) or affected individual II:1 (b). The affected individual is heterozygous for a silent C→T transition (solid arrow) and an adjacent G→A transition (open arrow), which leads to a E337K substitution in the L2 linker region of K6HF (amino acid residues S334 through W341).
the first time to our knowledge, authenticated a keratin mutation, E337K in K6HF, in patients with LAHS, which is possibly causative for this syndrome in 3 of the 9 families studied. It remains to be seen if another keratin, and possibly the type I partner of K6h, is responsible for LAHS in other patients or if the gene involved is a minor gene, the major one yet to be discovered.

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