The Bjornstad Syndrome (Sensorineural Hearing Loss and Pili Torti) Disease Gene Maps to Chromosome 2q34-36

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Summary

We report that the Bjornstad syndrome gene maps to chromosome 2q34-36. The clinical association of sensorineural hearing loss with pili torti (broken, twisted hairs) was described >30 years ago by Bjornstad; subsequently, several small families have been studied. We evaluated a large kindred with Bjornstad syndrome in which eight members inherited pili torti and prelingual sensorineural hearing loss as autosomal recessive traits. A genomewide search using polymorphic loci demonstrated linkage between the disease gene segregating in this kindred and *D2S434* (maximum two-point LOD score = 4.98 at $\theta = 0$). Haplotype analysis of recombination events located the disease gene in a 3-cM region between loci *D2S1371* and *D2S163*. We speculate that intermediate filament and intermediate filament-associated proteins are good candidate genes for causing Bjornstad syndrome.

Introduction

The incidence of severe prelingual hearing impairment is ~1/1,000 births (Morton 1991). In developed countries, ~60% of these cases are thought to have a genetic origin (Marazita et al. 1993), with ~30% occurring as part of a syndrome (Reardon 1992). The genetics of hearing loss is complex. Mutations at >40 loci can cause nonsyndromic deafness (autosomal and sex linked). In addition, there are >300 syndromes associated with hearing loss, and the disease genes responsible for a few of them have been identified (Gorlin, Toriello, and Cohen 1995; Van Camp and Smith 1998). Mutations in myosin 7A, Pendrin, PAX3, and COL 4A have been shown to cause Usher 1B, Pendred, Waardenburg, and Alport syndromes, respectively (Petit 1996). However, the mutated genes responsible for many syndromes associated with deafness have yet to be identified.

In 1965, Bjornstad described a new syndrome characterized by the presence of pili torti (brittle, broken hair) and congenital sensorineural hearing loss (designated "Bjornstad syndrome"; OMIM 262000). In the initial description of several families, five patients demonstrated both pili torti and sensorineural hearing loss (Bjornstad 1965). The terms "pili torti" and "twisted hair" had already been applied 33 years earlier (Ronchese 1932) to describe a rare hair abnormality in which the hair shafts are flattened at irregular intervals and twisted through 180° about the axes, causing them to break spontaneously (Petit et al. 1993). Pili torti may be congenital or acquired (the latter form secondary to patchy alopecia from a variety of causes). The congenital form may be isolated and determined by an autosomal dominant gene or associated with various rare syndromes, including Menkes syndrome, ectodermal dysplasia, neurological defects, and metabolic disturbances (Kurwa and Abdel-Aziz 1973; Birnbaum and Baden 1987). Pili torti, which may involve all or part of the scalp, is usually recognized during the 2d year of life (Robinson and Johnston 1967). Typically, hair trimming is not required, because it fractures at a short length (Petit et al. 1993).

Twenty-one cases of Bjornstad syndrome have been described (Bjornstad 1965; Reed et al. 1967; Robinson and Johnston 1967; Munro 1971; Cremers and Geerts 1979; Voigtlander 1979; Scott et al. 1983; Petit et al. 1993). Ten of these cases were familial (Reed et al. 1967; Cremers and Geerts 1979; Voigtlander 1979; Petit et al. 1993), with the largest families composed of three af-

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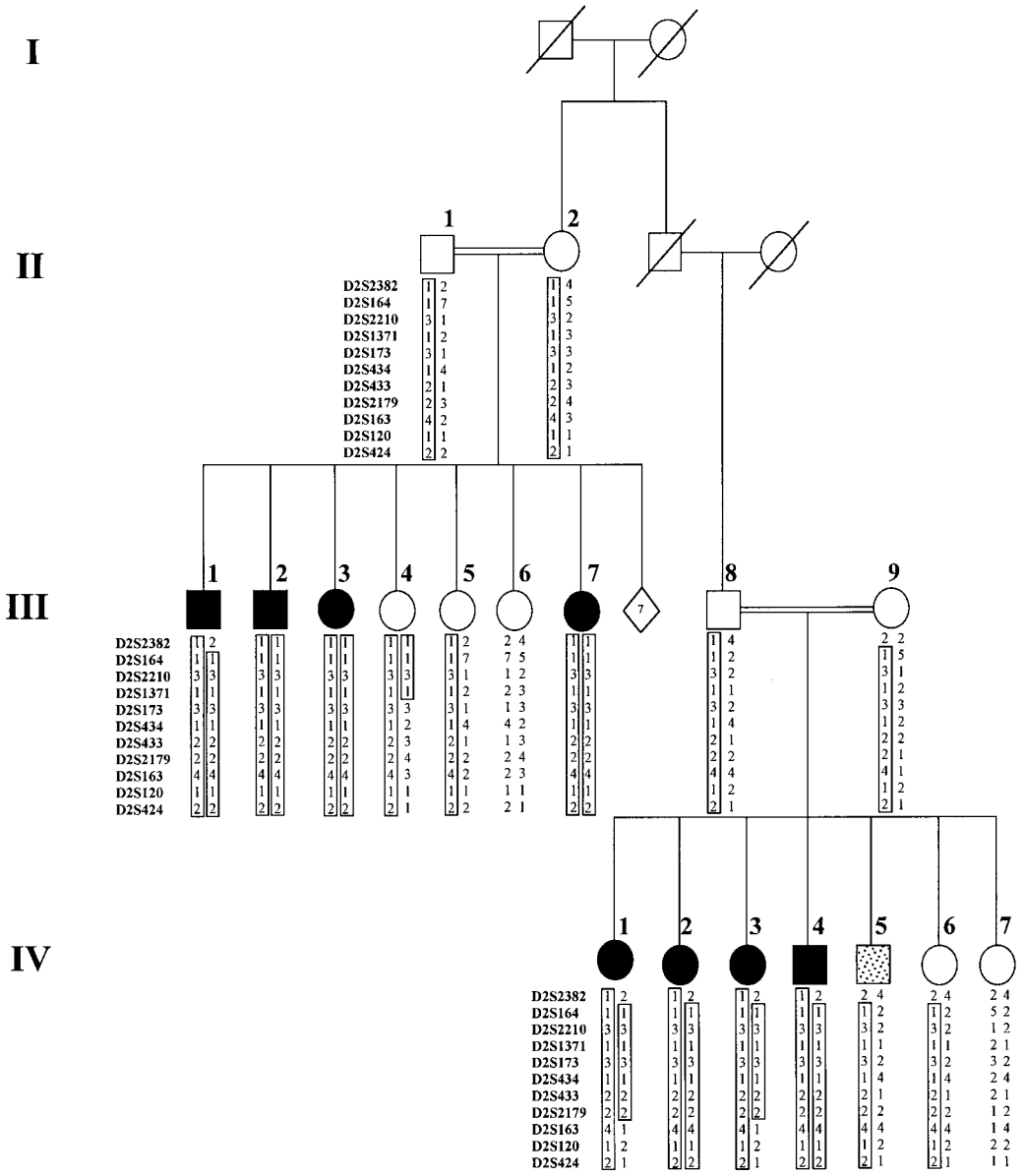

Figure 1 Pedigree of the kindred with Bjornstad syndrome. Open symbols = unaffected; solid symbols = affected; stippled = unknown clinical status; squares = men; circles = women; diamond = siblings of generation III not studied; double lines indicate consanguinity (in both cases, the marriages occurred between second cousins); symbols representing deceased individuals are slashed.

fected members (Reed et al. 1967; Petit et al. 1993). Although there are two reports of pili torti and deafness being inherited in a pattern consistent with an autosomal dominant mode of transmission (Cremers and Geerts 1979; Petit et al. 1993), the pattern seen in most families suggests an autosomal recessive mode of inheritance (Bjornstad 1965; Reed et al. 1967; Voigtlander 1979).

Despite detailed clinical descriptions of this disorder, the molecular cause of Bjornstad syndrome remains unknown. To elucidate the underlying defect and inheritance of this syndrome, we performed a genetic linkage study in a large family with eight affected members. Our analysis demonstrated that the locus responsible for Bjornstad syndrome maps to chromosome 2q34-36.

Subjects and Methods

Patients

This study was reviewed by and conducted in accordance with the policy of the Institutional Review Board of the Massachusetts Eye and Ear Infirmary. All family

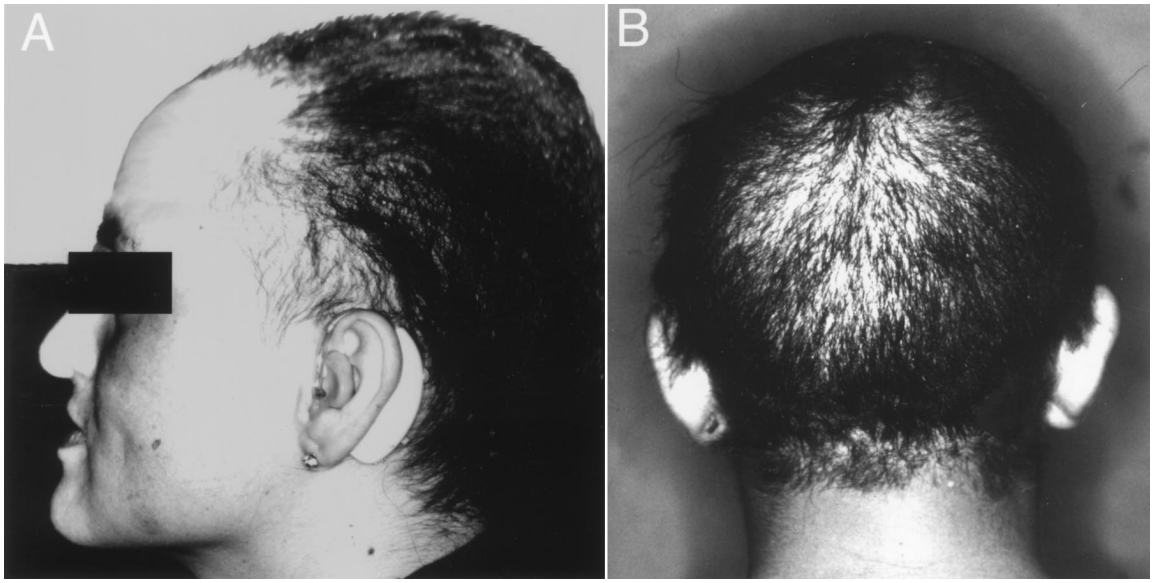

Figure 2 Photograph of an affected member. *A*, Characteristic short, frizzled hairs and the use of a hearing aid. *B*, Posterior view of the patient, showing sparse and short hair.

members of legal age signed informed consent forms to participate in this study. Eighteen family members from three generations of a Mexican family (mixed Spanish and native Indian heritage) were evaluated (by J.F.L.N. and M.A.M.F.) with a questionnaire (to assess other known causes and risk factors for hearing loss), a limited physical examination including auscultation and fundoscopy, and a comprehensive otolaryngologic evaluation (exam, audiogram, and vestibular testing). Puretone audiometry was performed (by R.M.C.) with air conduction at 250, 500, 1,000, 2,000, 4,000, 6,000, and 8,000 Hz and with bone conduction at 500, 1,000, 2,000, and 4,000 Hz. Hair and blood samples were obtained for microscopy and genetic analyses, respectively, and were transported into the United States with knowledge of the Mexican Health Ministry. Family members with prelingual hearing loss and pili torti were diagnosed with Bjornstad syndrome.

Genetic Analyses

Karyotype analyses were performed on samples from two affected individuals (III-1 and IV-1, fig. 1) as described by Hook (1977). Genomic DNA was extracted from whole blood samples by standard techniques (Tanigawa et al. 1990), and diluted to 50 ng/liter for amplification by PCR with fluorescein-labeled primers from the Cooperative Human Linkage Center Human Screening Set/Weber Version 8 (Research Genetics). The volumes of final reactions were 5 μl and contained 10 ng of template genomic DNA, 0.25 U of *Taq* polymerase, 2 pmol of forward and reverse primers, 0.2 mM deoxynucleotides, and 2.5 mM $MgCl_2$; 1 × Cetus PCR buffer was used. Samples were denatured at 95°C for 10 min, followed by PCR reactions (10 cycles: 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; 20 cycles: 89°C for 30 s, 55°C for 30 s, and 72°C for 1 min). The pooled products were supplemented with Tamra red–labeled internal-size standards and were electrophoresed on an Applied Biosystems model 377 Sequencer and analyzed by Genescan version 2.1 peak calling software and by the Applied Biosystems Genotyper version 2.0 program.

Additional polymorphic markers from Research Genetics were tested to confirm the linkage, narrow the disease interval, and construct a haplotype. The PCRs were performed with 3 μl (150 ng) of DNA in a 7-μl reaction mixture containing 1 μl 10 × Cetus buffer (100 nM Tris-HCL, pH 8.3, 500 mM KCl, and 25 nM $MgCl_2$), 1 μl of nucleotide mix (1.25 mM each of dATP, dCTP, dGTP, and dTTP); 2 pmol of forward (γ^{32}P–labeled) and 2 pmol of reverse primers; and 1 U (0.2 l) *Taq* polymerase. Thirty-two cycles of amplification were completed at 94°C for 20 s, 55°–58°C for 30 s, and 72°C for 30–45 s. Reaction products were separated on 6% denaturing polyacrylamide gels (7.7 M urea) and analyzed by autoradiography.

Linkage Analyses

Two-point LOD scores were performed between the disease gene and each marker, using the MLINK (version 5.1) program, assuming allele frequencies derived from 25 unrelated individuals. The Bjornstad syndrome gene mutation (both pili torti and sensorineural hearing loss)

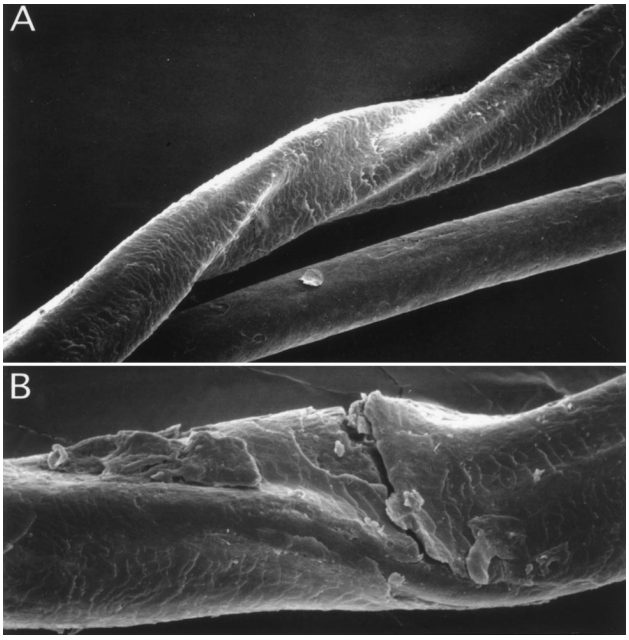

Figure 3 Scanning electron micrograph of hairs. *A*, Hair twisted 180° on its own axis (X200). *B*, Broken hair at a twist (X500).

was assumed to be a fully penetrant under a recessive model. The Bjornstad syndrome gene frequency was estimated at .001 for LOD score calculations. Equal recombination frequencies between males and females were assumed.

Scanning Electron Microscopy

Hair samples were mounted on conductive carbon tape, sputter coated with gold/paladium, and viewed with an Amray 1000A scanning electron microscope.

Results

Clinical Evaluations

Eight individuals in one large kindred (designated "family BS-1") were diagnosed as affected with Bjornstad syndrome on the basis of hair and hearing analysis. All affected individuals had nonprogressive prelingual hearing loss and used hearing aids. Two used sign language for communication (IV-1 and IV-4) and had audiometric profiles of profound sensorineural deafness. The audiological examination in the remaining six demonstrated a pattern consistent with mild to moderate hearing impairment in lower to middle frequencies and moderate to severe impairment in higher frequencies. These six individuals had relatively normal language and speech.

All eight affected individuals had hair abnormalities involving the entire scalp that were usually recognized by the parents during the 2d year of life. Hair was sparse, coarse, dry, lusterless, and fragile (fig. 2). Affected individuals had never required haircuts. A scanning electron micrograph of a scalp hair sample from one affected individual is shown in figure 3. Eyebrows and eyelashes and axillary, pubic, and body hair were normal.

The hormonal status of affected individuals appeared normal. All affected women denied menstrual abnormalities; two girls (ages<14 years) had secondary sexual characteristics that were normal for age. One affected male (individual III-1) is married and has two unaffected children (data not shown).

There was no history of pili torti and congenital sensorineural hearing loss in other surviving or deceased family members. Remarkable findings in other family members included one child with isolated and profound sensorineural hearing loss (individual IV-5, fig. 1) necessitating sign language; one adult (individual II-1) had vitiligo of both hands. Histories and physical examinations were otherwise unremarkable in both affected and unaffected family members. Neither tinnitus nor dizziness was reported by any family member. There were no nail or teeth malformations and no individual had a white forelock. No nystagmus, pinna malformations, or seventh cranial nerve dysfunction was observed.

Genetic Studies

Pedigree analyses were consistent with autosomal recessive transmission of Bjornstad syndrome in family BS-1 (fig. 1). Karyotype analyses were performed on samples from individuals III-1 and IV-1 and revealed no abnormalities. Genetic studies were performed with DNA samples from 18 individuals. Because Bjornstad syndrome

Table 1

Linkage between the Disease Gene and Chromosome 2 Loci

	LOD SCORE AT θ =					
LOCUS	0	.01	.05	.10	.20	.30
D2S2382	$-\infty$	−.79	.33	.58	.43	.04
D2S164	$-\infty$	2.88	3.23	3.07	2.38	1.49
D2S2210	$-\infty$	2.85	3.18	2.99	2.26	1.32
D2S1371	$-\infty$	1.71	2.13	2.07	1.61	.96
D2S173	2.49	2.44	2.25	2.01	1.50	.95
D2S434	4.98	4.88	4.49	3.99	2.92	1.77
D2S433	4.04	3.97	3.65	3.25	2.39	1.48
D2S2179	$-\infty$	1.44	1.88	1.84	1.41	.84
D2S163	$-\infty$	−.38	.78	1.07	1.01	.66
D2S120	$-\infty$	−1.44	-.20	.20	.40	.33
D2S424	$-\infty$	−.38	.78	1.07	1.01	.67
D2S2372	$-\infty$	1.92	2.34	2.27	1.80	1.16
D2S313	$-\infty$	−1.3	.48	.97	1.02	.64
D2S360	$-\infty$	−.46	.72	1.04	1.05	.76
D2S351	$-\infty$	−1.12	.67	1.18	1.24	.87
D2S1363	$-\infty$	−1.29	.48	.96	.99	.59

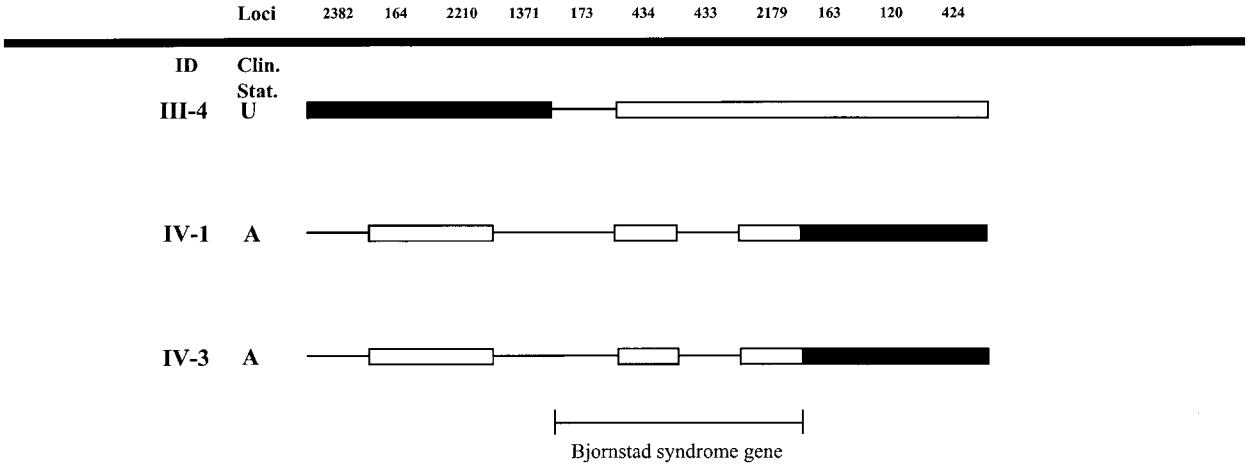

Figure 4 Schematic representation of the genotypes of three individuals who were discordant with clinical status in the 5-cM interval between *D2S2382* and *D2S424*. Pedigree identification numbers (ID) and clinical status (clin. stat.: U = unaffected; A = affected) are shown. Black filled bars represent nonconcordance between a DNA locus and disease status; white bars represent concordance; lines represent uninformative genotype. The 3-cM interval containing the Bjornstad syndrome gene is shown. The locations of loci *D2S2382, D2S164, D2S2210, D2S1371, D2S173, D2S434, D2S2179*, *D2S163*, *D2S120*, and *D2S424* are taken from the chromosome 2 gene map. The distances between these loci are not drawn to scale.

involves both sensorineural hearing loss and pili torti, individual IV-5, who had hearing loss but normal hair, was considered as having an unknown clinical status for the purposes of LOD score calculations.

A genomewide search was performed using polymorphic loci. Evidence of linkage was initially detected between the Bjornstad syndrome gene and *D2S434* (LOD score = 4.98; $\theta = 0$). Linkage between the disease gene and other chromosome 2q loci was then analyzed. Two-point LOD scores obtained with 16 polymorphic loci are shown in table 1. On the basis of linkage analyses with the chromosome 2 anchor locus, *D2S434*, the Bjornstad disease gene was localized to chromosome 2q34-36.

To refine further the location of the disease locus, haplotypes of three individuals who exhibited recombination across this region were studied (fig. 4). The genotypes of individuals III-4, IV-1, and IV-3 defined recombination events and indicated that the disease locus resided in the 3-cM interval between *D2S1371* and *D2S163*.

Discussion

We report that the recessive gene mutation responsible for Bjornstad syndrome in one large kindred maps to chromosome 2q34-36. All affected individuals with both pili torti and sensorineural hearing loss were homozygous for alleles between *D2S1371* and *D2S163*. This interval excludes the nearby PAX3 gene, which is mutated in disorders (Waardenburg syndrome and craniofacial-deafness-hand syndrome) that share some clinical features with Bjornstad syndrome (Tassabehji et al. 1992; Asher et al. 1996).

Clinical analyses of seven heterozygous disease gene carriers exhibited neither hearing nor hair abnormalities, but one individual carried the disease haplotype and had profound prelingual hearing loss. However, his hair texture, strength, and morphology were normal. Although his hearing loss might be accounted for by the disease allele, we suspect that another etiology caused his deafness. Evaluations of other Bjornstad syndrome families should help to determine whether Bjornstad syndrome gene mutations cause other hearing defects.

Hair consists of 50–100 proteins (Rogers and Powell 1993), of which keratins and intermediate filament–associated proteins (IFAP) predominate (Emonet et al. 1997). Transgenic mice overexpressing IFAP gene products have brittle, easily fractured hair (Rogers and Powell 1993), a finding that further supports the model that abnormalities in these proteins can alter hair structure. Defects in keratins, IFAP, or the enzymes involved in posttranslational modification of these molecules might be expected to cause the ultrastructural defects of hair in Bjornstad syndrome.

Sensorineural deafness and pili torti in Bjornstad syndrome could be independent phenotypes that result from the coinheritance of closely linked gene defects. Although our studies do not exclude the possibility of a contiguous gene syndrome, the recognized immunoreactivity of cytokeratins and other IFAPs in the cochlea (Anniko et al. 1990; Bauwens et al. 1991) makes it ap-

pealing to consider a single-gene defect that accounts for both phenotypes. Genes encoding the major structural components of hair (cytokeratins and IFAPs) therefore appear to be particularly good candidates for mutations that account for the distinct manifestations of this disorder.

At present, only villin encoded on chromosome 2q35 (Rousseau-Merck et al. 1988), colocalizes to the disease gene interval defined in this study (Schuler et al. 1996). Although this actin-binding protein is widely expressed in microvilli (Hofer and Drenckhahn 1996), villin has not been found in the specialized microvilli, known as "stereocilia," of the inner ear (Flock et al. 1982; Alberts et al. 1994). We therefore anticipate that ongoing analyses of the *D2S1371* and *D2S163* interval will lead to the identification of a gene that has a critical role in the structure of scalp hair and inner-ear cells.

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References

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994) Molecular biology of the cell, 3d ed. Garland, New York

Anniko M, Arnold W, Thornell LE, Virtanen I, Ramaekers FC, Pfaltz CR (1990) Regional variations in the expression of cytokeratin proteins in the adult human cochlea. Eur Arch Otorhinolaryngol 247:182–188

Asher JH Jr, Sommer A, Morell R, Friedman TB (1996) Missense mutation in the paired domain of PAX3 causes craniofacial-deafness-hand syndrome. Hum Mutat 7:30–35

Bauwens LJ, Veldman JE, Bouman H, Ramaekers FC, Huizing EH (1991) Expression of intermediate filament proteins in the adult human cochlea. Ann Otol Rhino Laryngol 100: 211–218

Birnbaum PS, Baden HP (1987) Heritable disorders of the hair. Dermatol Clin 5:137–153

Bjornstad RT (1965) Pili torti and sensory-neural loss of hearing. Paper presented at the 17th Combined Scandavian Dermatological Association Meeting, Copenhagen, Denmark, May 27–29

Cremers CW, Geerts SJ (1979) Sensorineural hearing loss and pili torti. Ann Otol 88:100–104

Emonet N, Michaille JJ, Dhouailly D (1997) Isolation and characterization of genomic clones of human sequences presumably coding for hair cysteine-rich proteins. J Dermatol Sci 14:1–11

Flock A, Bretscher A, Weber K (1982) Immunohistochemical localization of several cytoskeletal proteins in inner ear sensory and supporting cells. Hear Res 7:75–89

Gorlin RJ, Toriello HV, Cohen MM (eds) (1995) Hereditary hearing loss and its syndromes, 2d ed. Oxford University Press, Oxford

Hofer D, Drenckhahn D (1996) Cytoskeletal differences between stereocilia of the human sperm passageway and microvilli/stereocilia in other locations. Anat Rec 245:57–64

Hook EB (1977) Exclusion of chromosomal mosaicism: tables of 90%, 95%, and 99% confidence limits and comments on use. Am J Hum Genet 29:94–97

Kurwa AR, Abdel-Aziz AH (1973) Pili torti—congenital and acquired. Acta Derm Venereol (Stockh) 53:385–392

Marazita ML, Ploughman LM, Rawlings B, Remington E, Arnos KS, Nance WE (1993) Genetic epidemiological studies of early-onset deafness in the US school-age population. Am J Med Genet 46:486–491

Morton NE (1991) Genetic epidemiology of hearing impairment. Ann NY Acad Sci 630:16–31

Munro DD (1971) Disorders of the hair. In: Fitzpatrick TB, Arndt KA, Clark WH, Eisen AZ, VanScott EJ, Vaughan JH (eds) Dermatology in general medicine. McGraw-Hill, New York, pp 395–418

Petit A, Dontenwille MM, Bardon CB, Civatte J (1993) Pili torti with congenital deafness (Bjornstad's syndrome): report of three cases in one family, suggesting autosomal dominant transmission. Clin Exp Dermatol 18:94–95

Petit C (1996) Genes responsible for human hereditary deafness: symphony of a thousand. Nat Genet 14:385–391

Reardon W (1992) Genetic deafness. J Med Genet 29:521–526

Reed WB, Stone VM, Boder E, Ziprkowski L (1967) Hereditary syndromes with auditory and dermatological manifestations. Arch Dermatol 95:456–461

Robinson GC, Johnston MM (1967) Pili torti and sensory neural hearing loss. J Pediatr 70:621–623

Rogers GE, Powell BC (1993) Organization and expression of hair follicle genes. J Invest Dermatol 101 Suppl:50S–55S

Ronchese F (1932) Twisted hairs (pili torti). Archives of Dermatology and Syphilology 26:98–109

Rousseau-Merck MF, Simon-Chazottes D, Arpin M, Pringault E, Louvard D, Guenet JL, Berger R (1988) Localization of the villin gene on human chromosome 2q35-q36 and on mouse chromosome 1. Hum Genet 78:130–133

Schuler GD, Boguski MS, Stewart EA, Stein LD, Gyapay G, Rice K, White RE, et al (1996) A gene map of the human genome. Science 274:540–546

Scott MJ, Bronson DM, Esterly NB (1983) Bjornstad syndrome and pili torti. Pediatr Dermatol 1:45–50

Tanigawa G, Jarcho JA, Kass S, Solomon SD, Vosberg HP, Seidman JG, Seidman CE (1990) A molecular basis for familial hypertrophic cardiomyopathy: an α/ß cardiac myosin heavy chain hybrid gene. Cell 62:991–998

Tassabehji M, Read AP, Newton VE, Harris R, Balling R, Guss P, Strachan T (1992) Waardenburg's syndrome patients have mutations in the human homologue of the PAX3 paired box gene. Nature 355:635–636

Van Camp G, Smith RJH (1998) Hereditary hearing loss homepage. http://dnalab-www.uia.ac.be/dnalab/hhh

Voigtlander V (1979) Pili torti with deafness (Bjornstad syndrome): report of a family. Dermatologica 159:50–54