A Gene for Pili Annulati Maps to the Telomeric Region of Chromosome 12q

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Pili annulati (PA) is a rare hair shaft disorder characterized by discrete banding of hairs. We studied two families with PA in which the disorder segregated in an autosomal dominant fashion. All family members were clinically examined and hair samples were examined under the light microscope. In family G, of 19 individuals examined, ten were affected, over three generations. In family B, there were three affected individuals of seven examined over three generations. A genome-wide scan of family G revealed a maximum logarithm of odds (LOD) of linkage score of 3.89 at marker D12S1723 at the telomeric region of chromosome 12q. From one critical recombinant in family G, the locus was narrowed down to a 9.2 cM region between D12S367 and the end of chromosome 12q. In family B linkage at the telomeric region of chromosome 12q also revealed a maximum LOD score of 0.89 at marker D12S1723. A combined LOD score, assuming no locus heterogeneity between the families was 4.78. Frizzled 10, which is located within the region, was sequenced but we were unable to detect a mutation causing PA. This study, for the first time, identifies a genetic locus for PA.

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Pili annulati (PA) (MIM %180600) is a rare autosomal dominant hair disorder characterized by hairs displaying a banded appearance (Fig 1). This is caused by cavities located in the cortex of the hair shaft both within and in between cortical cells (Price *et al*, 1968). It is often an incidental finding with alternating light and dark bands causing a slightly spangled appearance of the hair. There is no association with hair fragility. Most reported familial cases have been inherited in an autosomal dominant fashion (Cady and Trotter, 1922; Snell and Foley, 1922; Reyn, 1934; Ashley and Jacques, 1950; McCleary and Montgomery, 1955; Dawber, 1972; Lama *et al*, 1988; Misciali *et al*, 1993). There have been no previously published genetic linkage studies of PA to date.

Here, we report the results of a linkage study of two families with PA segregating in an autosomal dominant fashion using a 5 cM spaced genome-wide scan using 763 microsatellite markers.

Nineteen members of family G and seven members of family B were clinically examined for banded hairs typical of PA. Given that PA is not usually associated with hair fragility it is a subtle phenotype and can often be missed. We found the banding most easily assessed by light microscopy of fixed hair specimens. At least 20 hairs were cut at the scalp surface of each family member. These were fixed in Ultramount (Fronine, Riverstone, Australia) and examined under light microscopy. Individuals with hair containing bands typical of PA were assigned with a positive phenotype. If the sample did not display any banded hairs a negative phenotype was only assigned after hair microscopic examination of a further three specimens (of at least 20 hairs taken from different scalp sites) also proved negative. Clinical and microscopic hair examination revealed that in family G, of 19 individuals examined, 10 displayed banded hairs and were classed as affected (Fig 2). Initial hair samples taken

Figure 1
Clinically detectable banding in pili annulati.

Abbreviations: FZD10, Frizzled 10; LOD, logarithm of odds; PA, pili annulati

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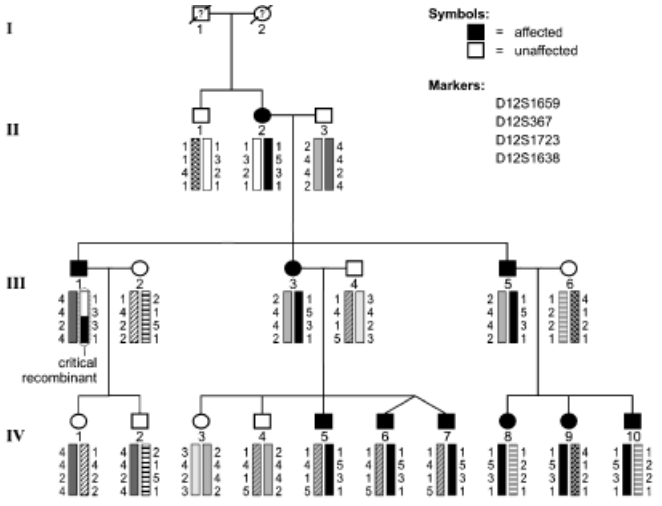

Figure 2
Pedigree of family G with haplotype at the teloremic region of chromosome 12q.

from IV-10 in the first 2 months of life did not reveal any banding; however, re-examination at age 2 years subsequently displayed the PA phenotype. In family B, there were three affected individuals of seven examined over three generations. Informed consent was obtained from all research participants and ethics committee approval was provided by the St Vincent's Hospital Ethics Committee, Protocol Number: 70/99.

Genotyping for the genome-wide scan and subsequent fine mapping was performed at the Australian Genome Research Facility. For genotyping, genomic DNA was isolated from peripheral blood using the Nucleon BACC2 DNA extraction kit (Amersham Life Sciences, Buckinghamshire, UK). Reactions were amplified using a PTC-225 DNA Engine Tetrad (MJ Research, Waltham, Massachusetts) and PCR products pooled to run more than one marker per lane. PCR products were electrophoresed on an ABI PRISM 377 DNA Sequencer equipped with Genescan software version 3.1.2 (Applied Biosystems, Foster City, California). Files were then imported into Genotyper Version 2.1 (AB, Foster City, California) software that interpreted the electropherogram and assigned genotypes.

A two-point analysis was done using the MLINK program (version 5.1) of the LINKAGE package (Lathrop and Lalouel, 1984). An autosomal dominant model with a disease allele frequency of 1 in 50,000, a disease penetrance of 99% and equal marker allele frequencies was used. A maximum logarithm of odds (LOD) of linkage score of 3.89 was obtained at marker D12S1723 at the telomeric region of chromosome 12q in family G (Table I). In family B linkage at this same locus also revealed a peak value at D12S1723 with a LOD score of 0.89 (Table II) producing a combined LOD score of 4.78 (Table III). A critical recombination seen in individual III-1 between markers D12S367 and D12S1723 narrowed the locus harboring a causative gene to a 9.2 cM region between D12S367 and the end of chromosome 12q.

There are over 40 genes found in the region. Several of these could be considered good candidate genes as they are involved with epidermal cell proliferation or differentiation. Frizzled 10 (FZD10) was selected as a primary candidate gene in the region for sequencing analysis. In *Drosophila*, *frizzled* functions to transmit polarity signals in epidermis cells during hair and bristle development. Members of the *FZD* family have an extracellular Wnt binding domain. The *WNT* gene family encodes a set of signaling molecules thought to play an important role in embryonic development as well as hair follicle morphogenesis (Terasaki *et al*, 2002).

The exons and 5'UTR of the *FZD10* gene were investigated for the presence of polymorphisms via direct sequencing. PCR primers were designed based on sequence information found in NCBI. Individuals IV-3, IV-5, and IV-9 from family G (Fig 2) were used for mutation detection and one control sample. The PCR product was then sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). The sequenced products were run on 6.5%

Table I. Two-point LOD of linkage scores for markers at the telomeric region of chromosome 12q in family G

	Recombination fraction (θ)						
Marker	**0**	**0.01**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
D12S1259	1.99	2.01	2.04	1.97	1.68	1.25	0.69
D12S367	−3.87	1.54	2.01	2.02	1.72	1.26	0.69
D12S1723	3.59	3.53	3.28	2.96	2.27	1.53	0.77
D12S1638	1.99	2.01	2.04	1.97	1.68	1.25	0.69

LOD, logarithm of odds.

Table II. Two-point LOD of linkage scores for markers at the telomeric region of chromosome 12q in family B

	Recombination fraction (θ)						
Marker	**0**	**0.01**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
D12S1259	0.60	0.59	0.55	0.51	0.41	0.29	0.16
D12S367	0.89	0.88	0.81	0.72	0.54	0.35	0.17
D12S1723	0.89	0.88	0.81	0.72	0.54	0.35	0.17
D12S1638	0.00	0.00	0.00	0.00	0.00	0.00	0.00

LOD, logarithm of odds.

Table III. Two-point LOD of linkage score for markers at the telomeric region of chromosome 12q in families B and G combined

	Recombination fraction (θ)						
Marker	**0**	**0.01**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
D12S1259	3.13	3.13	2.99	2.81	2.32	1.69	0.93
D12S367	0.25	2.71	3.10	2.99	2.47	1.76	0.94
D12S1723	4.78	4.71	4.73	3.01	3.01	2.02	1.01
D12S1638	2.53	2.52	2.44	1.91	1.91	1.40	0.77

LOD, logarithm of odds.

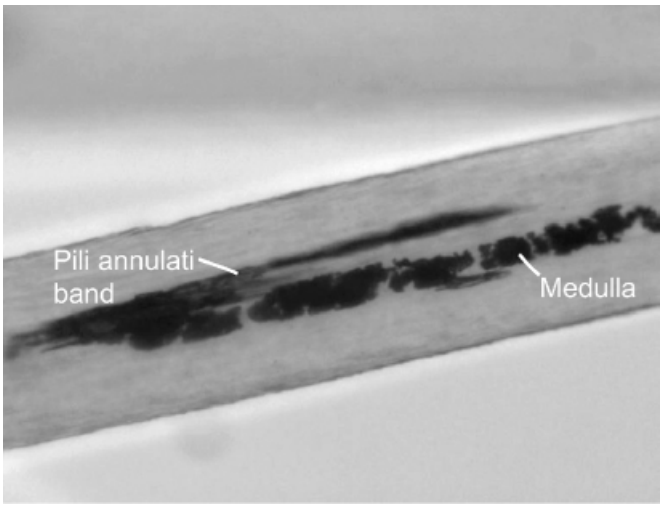

Figure 3
Pili annulati banding compared with the medulla seen on light microscopy of fixed hair specimens.

polyacrylamide gels, dried and autoradiographed. No mutations were found in any exons or splice sites.

A successful linkage study relies on the phenotype being clearly defined with affected individuals distinguished reliably from those not affected. In PA the phenotype can be so subtle that the banding may only be detected on microscopic examination of hairs. We found that microscopy of fixed hair specimens in a clear medium (and therefore viewed with transmitted light) was a more reliable method of examining the hairs than of unfixed hair in reflected light. This was because the pathological bands, due to internal light reflection, were darker than the surrounding normal hair shaft and more easily detected than in unfixed hairs. We needed, however, to be careful to distinguish the bands from pigmented medulla, particularly when this was present intermittently (Fig 3). We have also observed that age has some bearing on the expression of the PA bands. Banding does not appear to be expressed until approximately two years of age (as was seen in individual IV-10). Thereafter we have previously noted that younger affected individuals tend to display a more robust phenotype and so are either detectable clinically or have obvious banding on microscopy (unpublished observation). So inspections of families B and G would find only individual II-1 (family G) as being at risk of phenotype error.

Cady and Trotter (1922) were the first to suggest an autosomal dominant inheritance describing three families with PA. Since then there have been multiple reports of familial PA, segregating in an autosomal dominant fashion. We have now, for the first time, found significant evidence of a locus for PA at the telomeric region of chromosome 12q. This is the beginning of the search for mutations causing this disease. Future research at this locus may include studying the regulatory and non-coding regions of the *FZD 10* gene for mutations that have not been screened and prioritizing other candidate genes within this interval for sequencing. To date the only isolated hair shaft disorder for which a gene has been found is monilethrix, which causes beading and fragility. This disorder is caused by mutations of hair keratin genes (Winter *et al*, 1997). PA is only the second isolated hair shaft disorder for which linkage has been found and it is the first such disorder in the non-fragile class for which linkage has been found. The chromosome 12q locus does not contain structural genes akin to the keratin genes. Given the intermittent nature of the pathological banding observed in PA, it is more likely that this abnormality would be caused by a mutation in a regulatory gene affecting hair follicle cell proliferation or differentiation.

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