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Pendred syndrome: evidence for genetic homogeneity and further refinement of linkage

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Abstract

Pendred syndrome is the association between congenital sensorineural deafness and goitre. The disorder is characterised by the incomplete discharge of radioiodide from a primed thyroid following perchlorate challenge. However, the molecular basis of the association between hearing loss and a defect in organification of iodide remains unclear. Pendred syndrome is inherited as an autosomal recessive trait and has recently been mapped to 7q31 coincident with the non-syndromic deafness locus DFNB4. To define the critical linkage interval for Pendred syndrome we have studied five kindreds, each with members affected by Pendred syndrome. All families support linkage to the chromosome 7 region, defined by the microsatellite markers D7S501-D7S523. Detailed haplotype analysis refines the Pendred syndrome linkage interval to a region flanked by the marker loci D7S501 and D7S525, separated by a genetic distance estimated to be 2.5 cM. As potential candidate genes have as yet not been mapped to this interval, these data will contribute to a positional cloning approach for the identification of the Pendred syndrome gene.
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Pendred syndrome, the association of goitre and congenital hearing loss, was first noted by Vaughan Pendred in 1896 in two sisters,[1] but the disorder was only more widely recognised following the reports of further sibships by Brain in 1927.[2] It is inherited as an autosomal recessive trait and has recently been mapped to 7q31 coincident with the non-syndromic deafness locus DFNB4.[3 4] Fraser,[5] in a study of a larger population based sample, concluded that Pendred syndrome characteristically segregated as an autosomal recessive disorder. The proportion of the severely hearing impaired population affected by Pendred syndrome remains uncertain, but it is likely to be a condition that is significantly underdiagnosed. Phenotypic variability, both between and within sibships, has been noted.[6] Hence, while prelingual hearing loss is a frequent feature of Pendred syndrome, the goitre, typically pubertal in onset, may vary significantly in size, even within families. Although raised levels of thyroid stimulating hormone (TSH) and reduced levels of thyroglobulin have been reported,[7] the majority of patients with Pendred syndrome are clinically and biochemically euthyroid. The biochemical defects leading to Pendred syndrome remain unclear. However, Morgans and Trotter[8] noted an abnormality of iodide handling by the thyroid glands of affected patients. In people affected by Pendred syndrome, between 10 and 80% of accumulated iodide is discharged on the administration of perchlorate. In a normal thyroid, iodide ions are actively transported into the cells and covalently linked to thyroglobulin following oxidation, a process involving thyroid peroxidase (TPO).[9] Homozygous mutations in the TPO gene, located on chromosome 2, lead to the absence of peroxidase activity and the clinical disorder known as total iodide organification defect (TIOD). Linkage of Pendred syndrome to the TPO gene has been previously excluded by analysis of an intragenic minisatellite.[10]

We and others have recently mapped Pendred syndrome to the genetic interval defined by the markers D7S501 and D7S523 at 7q31 in 14 affected sibships.[3 4] As yet, we have not identified candidate genes for the biochemical defects associated with Pendred syndrome that map to this region of chromosome 7. Hence, progress towards defining the molecular basis of this important inherited cause of hearing loss is likely to depend on further genetic refinement of the disease gene location before positional cloning may commence. Before the construction of a physical map of the chromosomal region 7q31, we have refined the linkage interval for Pendred syndrome through the analysis of the microsatellite markers D7S692 and D7S525 and the assessment of five further kindreds. Finally, in a total cohort of 17 Pendred syndrome sibships we found evidence for genetic homogeneity of linkage of Pendred syndrome to 7q31.

Materials and methods

PATIENTS

Four previously unreported kindreds were recruited from the UK, with at least two members with a clinical diagnosis of Pendred syndrome. A further singleton with two unaffected sibs was also identified. Subjects were assigned affected status based on characteristic clinical features of Pendred syndrome, namely the presence of sensorineural hearing loss, typically presenting with audiogram findings of a hearing deficit of 60 dB or more at all standard test frequencies. Additionally, at least one

affected member from each sibship underwent a perchlorate discharge test. A drop in the level of radioiodine of over 10% was considered abnormal with typical observed values in the 20-30% range.[8] A total cohort of 33 patients affected by Pendred syndrome have now been examined, 11 of them male and 22 female. Of this cohort, 24 affected people from 12 independent sibships have previously been linked to 7q31.[3] The mean age of the patients is 27.1 years, ranging from 5.2 years to 53.3 years.

METHODS

PCR products were amplified from 40 ng of genomic DNA using fluorescently labelled primers for markers D7S501, D7S496, and D7S523 and were analysed on a 6% denaturing acrylamide gel on the ABI 373 automated sequencer using the ABI software Genescan™ and Genotyper™. Ten microlitre reactions with 0.5 U *Taq* polymerase (Advanced Biotechnologies), buffered by 50 mmol/l KCl, 10 mmol/l Tris HCl, pH 9, and varying concentrations of $MgCl_2$, were carried out in a 96 well plate on an MJ Research Peltier thermal cycler. The genotypes for D7S692 were generated from 40 ng of genomic DNA using a γ33P radiolabelled primer[11] in 10 μl PCR using the same buffering system as above. D7S692 was taken through 26 cycles of 55°C for 30 seconds, 72°C for 30 seconds, and 94°C for 30 seconds. D7S525 was amplified as for D7S692 but the annealing temperature was raised to 61°C and the number of cycles was lowered to 24. Each reaction was carried out in 0.5 ml microfuge tubes on a Biometra TRIO Thermoblock with a liquid paraffin overlay. The products of these PCR reactions were electrophoresed on a 6% denaturing acrylamide gel, dried on to 3MM Whatman paper, and exposed to *x* ray film overnight.

LINKAGE ANALYSIS

Pairwise lod scores between the markers and the disease were calculated using the MLINK option of FASTLINK version 2.2 of the LINKAGE computer package run on a SUN O S 5.4 microsystem. Homogeneity tests were carried out using HOMOG[12] and tests for allelic association using the HRRLAMB.[13] The linkage calculations assume Pendred syndrome to be a fully penetrant autosomal recessive disorder with a disease allele frequency of 0.1%. Allelic frequencies were estimated from the distribution in families in the present data set. No new mutations were allowed and equal male and female recombination rates were used.

Table 1 Individual family and cumulative pairwise linkage analysis between Pendred syndrome and microsatallite marker D7S496

	Recombination fractions (q)				
Family No	*0.00*	*0.02*	*0.10*	*0.20*	*0.30*
23	0.60	0.57	0.43	0.27	0.13
24	1.46	1.38	1.06	0.67	0.34
25	0.98	0.92	0.71	0.44	0.21
27	0.20	0.18	0.11	0.04	0.01
28	0.08	0.08	0.06	0.03	0.01
Cumulative lod score	3.32	3.13	2.37	1.45	0.70

Results

The microsatellite marker D7S496 generated significant evidence of linkage when genotyped in the five families previously untyped (Zmax=3.3, θ=0) (table 1). Genotypes at four additional microsatellite loci (fig 1) were analysed in a total cohort of 17 sibships with Pendred syndrome. The cumulative maximum pairwise lod scores for markers D7S496 and D7S692 at θ=0 was 10.6 and 8.08 respectively, with no recombinants observed at either of these loci (table 2).

Novel recombinants on disease bearing haplotypes were identified in three of the families, 23, 27, and 28. Family 23 (fig 2A) shows a recombination event in II.1 between the markers D7S501 and D7S496. Affected offspring in families 27 (fig 2B) and 28 (fig 2C) both have recombinant alleles at D7S523, but II.2 in family 28 also shows a maternally derived recombinant genotype at locus D7S525. This now allows us to define the critical linkage interval for the Pendred syndrome gene to the region

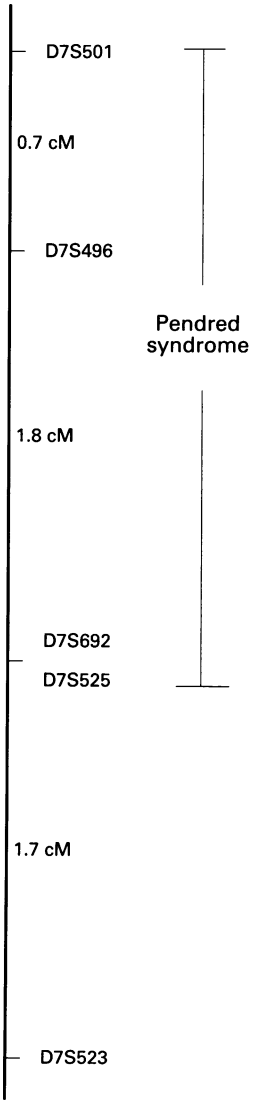

Figure 1 Linkage map of the microsatellite markers from chromosome 7q31. Genetic distances are indicated in centimorgans (cM). Loci were placed using the FLIPS option of CRIMAP on the published order.[14] The location of the refined genetic linkage interval for Pendred syndrome is indicated.

between microsatellite markers D7S501 and D7S525, an estimated genetic distance of 2.5 cM.

Family 27 shows a further interesting observation. III.2 and III.4 are first cousins, the progeny of non-consanguineous parents, and are both affected by Pendred syndrome. Only one (maternally transmitted) haplotype is shared between the affected people. A further recombinant chromosome, carrying maternal haplotypes in III.3, is likely to aid the definition of the minimal interval for the location of the Pendred syndrome disease gene. However, genotyping at additional loci within the newly defined critical interval, D7S501 to D7S525, will be required as the maternal genotypes for D7S496 and D7S692 are uninformative.

In a test for genetic heterogeneity given linkage (HOMOG), α (the proportion of families linked to that marker) equalled 1.0 at $\theta = 0.0$ for D7S496 and D7S692, and thus we found no evidence for genetic heterogeneity within our cohort of families. Allelic association was not detected at any of the five loci within the linkage interval. For example, at D7S496 in a chi-square test of transmitted against non-transmitted alleles $\chi^2=4.8$, $p=0.44$.

Table 2 Maximum lod scores and recombination fractions for chromosome 7 markers and Pendred syndrome for a combined cohort of 17 independent sibships

	0.00	0.1	0.2	0.3	Zmax	θmax
D7S501	−∞	3.14	2.36	1.26	3.15	0.092
D7S496	10.56	7.52	4.68	2.29	10.56	0.000
D7S692	8.08	5.79	3.63	1.80	8.08	0.000
D7S525	−∞	6.15	4.16	2.19	7.09	0.025
D7S523	−∞	6.20	4.01	2.04	7.56	0.017

Discussion

Genetic linkage of Pendred syndrome to 7q31 has now been reported in a total of 19 independent families, including two originally from Asia and three residing in Israel.[3 4] In addition to the geographical separation of these families, detailed haplotype analysis suggests that the Pendred syndrome gene mutations have arisen independently. The lack of absolute allelic association for any marker from the linked region further supports the absence of a major founder effect to explain both the prevalence and homogeneity in Pendred syndrome between populations.

The presence of recombinations at both D7S501 and D7S523 within the five new families reported here supports the evidence previously published that Pendred syndrome maps to the interval D7S501-D7S523. Family 28 shows a recombination event at D7S525 and this allows us to refine the linkage region for Pendred syndrome to a 2.5 cM interval flanked by markers D7S501 and D7S525 (fig 1). The appearance of five recombinations at D7S501 within the cohort of families suggests that with the development of new markers between D7S501 and D7S496 the linkage interval for Pendred syndrome could be further refined.

The appearance of affected cousins, III.2 and III.4 in family 27 (fig 2B), the offspring of non-consanguineous parents, suggests that mutant alleles for Pendred syndrome may be more prevalent than previously considered as three independent disease bearing haplotypes are identified within this family.

Marked genetic heterogeneity is found in both syndromic and non-syndromic hearing loss. Although at least nine genetically distinct forms of autosomal recessive hearing impairment have been mapped,[15-23] typically by a search for autozygosity using isolated consanguineous families, no genes have yet been identified. One of the loci, DFNB4, maps to an interval that includes the region containing the Pendred syndrome gene. A similar observation has recently been reported in the heterogeneous disorder Usher syndrome; three clinically distinct forms of Usher syndrome are recognised, but all are characterised by the association of sensorineural hearing loss and retinitis pigmentosa. Six different genetic loci have been linked to Usher syndrome, one coding for Usher syndrome type III,[24] two coding for Usher syndrome type II,[25 26] and three coding for Usher syndrome type I.[27 28] Mutations in an unconventional myosin gene,[29] which are proposed to affect the connecting cilium of photoreceptor cells and the hair stereocilia of the inner ear, were recently identified in Usher syndrome type IB (USH1B) patients linked to 11q13.5. Non-syndromic hearing loss, DFNB2, maps to the same genetic interval, and is likely to be allelic to USH1B.

The locus for DFNB4 has been defined by autozygosity mapping to a region of homozygosity extending over a 5.5 cM interval at 7q31. Co-localisation of both Pendred syndrome and non-syndromic hearing loss, DFNB4, to the same interval could be explained by different mutations in the same gene. However, it is not clear what common molecular lesion could underlie both the organification of iodide defect and the abnormal development of the inner ear. Alternatively, Pendred syndrome may represent a contiguous gene deletion of the DFNB4 gene and another gene critical in thyroid peroxidase function. Although no markers are deleted in our study, we cannot exclude microdeletions within the linkage interval, as the maximum intermarker distance extends to 1.8 cM. Pendred syndrome and DFNB4 may therefore be caused by separate, but closely linked, genes coincidentally lying within the same 5.5 cM region. Finally, the single large Druze family used to define DFNB4 may represent a phenotypic variant of Pendred syndrome. While no affected members have been investigated for abnormalities of perchlorate discharge, they do not have clinically detectable goitres (B Bonné-Tamir, personal communication, 1996).

The linkage of all the Pendred syndrome families so far examined to 7q31 and this further genetic refinement of the linked region significantly increases the prospect of identifying the gene responsible for this important disorder using positional cloning techniques. The identification of the Pendred syndrome gene may also be a route to the cloning of the gene for DFNB4, as the genetic heterogeneity of non-syndromic hearing loss limits the progress

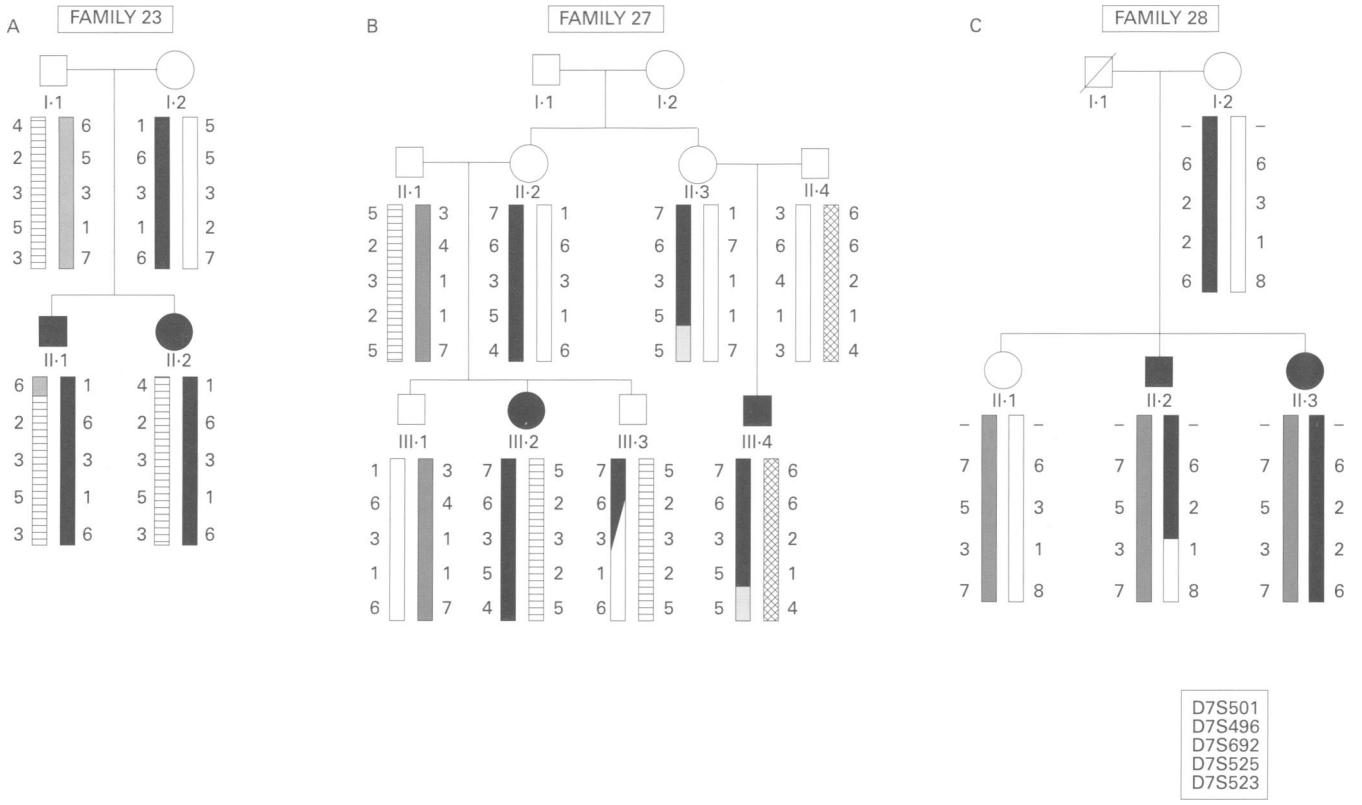

Figure 2 Pedigree structure and haplotype data for three of the families analysed, 23 (A), 27 (B), and 28 (C). Subjects affected by Pendred syndrome are shaded. Genotypes at all five microsatellite loci are shown for families 23 and 27; however, in family 28 genotypes for marker D7S501 are unresolvable for parental origin and are not shown.

being made towards identifying the genes involved.

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