Further refinement of Pendred syndrome locus by homozygosity analysis to a 0.8 cM interval flanked by D7S496 and D7S2425

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Abstract

Pendred syndrome is an autosomal recessive disease characterised by congenital sensorineural deafness and goitre. The gene responsible for Pendred syndrome has been mapped to chromosome 7q31 in a 5.5 centimorgan (cM) interval flanked by D7S501 and D7S523. This interval was recently refined to 1.7 cM interval located between D7S501 and D7S692. In the present study, we report linkage analysis data on a large consanguineous family genotyped with eight microsatellite markers located between D7S501 and D7S523. Complete cosegregation with the disease locus was observed with the loci analysed, which further supports locus homogeneity for Pendred syndrome and close linkage to this region. Haplotype analysis placed the Pendred syndrome gene between D7S496 and D7S2425 in a 0.8 cM interval. This additional refinement of the Pendred syndrome region will facilitate the construction of a physical map of the region and will help the identification of candidate genes.

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Pendred syndrome (MIM 274600) is an autosomal recessive disorder characterised by congenital sensorineural hearing loss of variable severity, goitre, variable degrees of hypothyroidism, and a positive perchlorate discharge test.¹ The sensorineural deafness is usually congenital and may be more severe on one side than the other. The cause of the hearing impairment is a congenitally malformed cochlea called Mondini dysplasia (a flattened cochlea with a decreased number of coils and underdevelopment of the vestibular structures).^{1,2} The goitres are generally small to moderate in size and may not appear until late childhood. The patients are either euthyroid or mildly hypothyroid and show a partial discharge of iodide after the administration of perchlorate or thiocyanate. These compounds inhibit active iodide transport and cause the release of the intrathyroidal iodide not bound to thyroid protein.³ In patients with Pendred syndrome, 15 to 85% of iodide is usually discharged from the thyroid after perchlorate administration, indicating abnormal iodine metabolism.⁴ Several biochemical and molecu-

lar studies have excluded the involvement of thyroglobulin and thyroperoxidase in the pathophysiology of Pendred syndrome.^{1,5,6}

The biochemical defect responsible for the iodine metabolism defect and Mondini malformation in Pendred syndrome is as yet unknown. However, it is well known that sensorineural deafness is more frequent among children with endemic hypothyroidism than in the general population.⁴ In addition, in animals, induced hypothyroidism can lead to cochlear malformations.^{1,7}

Pendred syndrome accounts for 5% of the congenitally deaf population.⁴ However, because of the inter- and intrafamilial phenotypic variability, many cases of Pendred syndrome may not be detected and therefore its prevalence may be underestimated. In the British Isles and Scandinavia, Pendred syndrome accounts for 7.5/100 000 and 1/100 000, respectively.⁴

The gene responsible for Pendred syndrome has been mapped by two groups to chromosome 7q31 in an interval of 9.2 and 5.5 cM, respectively.^{5,8} This interval has recently been narrowed down to 2.5 cM and 1.7 cM located between D7S501-D7S525 and D7S501-D7S692, respectively.^{9,10} The present study further reduces the region containing the Pendred syndrome gene to an interval of 0.8 cM.

Methods

CLINICAL EVALUATION

The pedigree of family PenLB1 is shown in fig 1. Diagnosis of Pendred syndrome was based on a detailed family history, audiological investigations, and endocrinological assessment including a perchlorate discharge test. All affected subjects (ages range from 8-30 years) had congenital bilateral sensorineural hearing loss (60 dB or more) with an associated speech defect. Five of them (VI.2, VI.4, VI.6, VI.9, VI.12) developed a multinodular goitre before puberty. The youngest affected member, VII.1 (8 years old), has no evidence of goitre. Three affected members of this family (VI.2, VI.12, VII.1) underwent a perchlorate discharge test and had an organification defect with more than 15% of radioiodine discharged from the thyroid gland.

LINKAGE ANALYSIS

Genomic DNA was purified from peripheral blood samples according to standard techniques. Microsatellite markers were obtained from the Génethon genetic map.¹¹ Microsatel-

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Figure 1 Pedigree and haplotype data for family PenLB1. Haplotypes were constructed assuming a minimal number of recombination events. Markers are listed from the centromere to the telomere. Regions of homozygosity in affected children are boxed.

lite markers were amplified by the polymerase chain reaction and analysed on 6–10% native polyacrylamide gels¹² that were silver stained according to the manufacturer’s instructions (Pharmacia).

Two point linkage analysis was performed using the computer programs MLINK and ILINK of the LINKAGE package version 2.2.¹³ An estimated frequency of 1/25 000 was used for the disease gene. The published allele numbers for the different loci were used¹¹ and their frequencies were set equal.

Table 1 Two point lod scores between Pendred syndrome and 7q markers

Locus	Recombination fraction (θ)					Zmax	θ max
	0.00	0.05	0.1	0.3	0.5		
D7S2459	–∞	2.23	2.16	1.02	0	2.24	0.06
D7S2420	3.49	3.11	2.72	1.13	0	3.49	0
D7S496	3.48	3.10	2.72	1.29	0	3.48	0
D7S692	3.70	3.30	2.89	1.22	0	3.70	0
D7S2425	3.57	3.19	2.81	1.19	0	3.57	0
D7S2456	3.61	3.23	2.85	1.22	0	3.61	0
D7S525	3.73	3.34	2.94	1.26	0	3.73	0
D7S687	3.63	3.27	2.90	1.29	0	3.63	0

Figure 2 Genetic map of the markers used in this analysis. The location of the refined Pendred syndrome region is indicated. The genetic distance between markers is given in cM and is based on previously published data.¹¹

Results

To narrow down the candidate Pendred gene region, 17 members of a large consanguineous pedigree were genotyped with eight microsatellite markers located in the Pendred syndrome candidate region between D7S501 and D7S523.^{5 8 9–11} The results of two point linkage analyses are shown in table 1. A lod score of more than 3 was obtained with seven loci analysed at (θ)=0, supporting the genetic homogeneity of Pendred syndrome and its linkage to this region.

Haplotype analysis was used to establish the smallest region cosegregating with the disease phenotype. The haplotypes of the different members were constructed assuming a minimal number of crossovers (fig 1). Five affected members, VI.2, VI.4, VI.6, VI.9, and VI.12, were homozygous only for two adjacent markers D7S692 and D7S2425. As the parents are consanguineous, the affected members are probably homozygous by descent and have most likely inherited the same mutation from a common ancestor. Since VI.1 shares a common haplotype with V.3, the homozygous region containing D7S692 and D7S2425 is the result of recombination events which had occurred in the relatives of carrier V.4. This hypothesis is supported by the extended homozygosity at all loci between D7S2456 and D7S2420 in patient VII.1, who had received his father’s maternal haplotype and his mother’s paternal haplotype (fig 1).

In the Généthon genetic map, the three loci D7S692, D7S2425, and D7S2456 had been placed at the same position and at a genetic distance of 0.8 cM from markers D7S496, D7S2420, and D7S2459.¹¹ In our consanguineous family, five affected members were homozygous for D7S692 and D7S2425 and heterozygous for D7S2456 (fig 1). This indicates that D7S2456 maps either distal or proximal to the two homozygous loci D7S692/D7S2425. Moreover, D7S2459, D7S2420, and D7S496 were placed at the same position.¹¹ In our family, a recombination event between D7S2459 and the Pendred phenotype was observed in VI.13 (fig 1) giving a maximum lod score of 2.24 (table 1) at a recombination fraction of 0.06. The recombi-

nation occurred during the male (V.4) meiosis. This result allowed us to place D7S2459 proximal to D7S496/D7S2420 (fig 2).

Based on the Généthon genetic map and on the data reported in our study, two possible orders for the Pendred syndrome gene and the loci analysed can be suggested: D7S2459-D7S496/D7S2420-D7S2456-Pendred gene/D7S692/D7S2425-D7S525 or D7S2459-D7S496/D7S2420-Pendred gene/D7S692/D7S2425-D7S2456-D7S525. These results allow the mapping of the Pendred syndrome gene to a 0.8 cM interval located either between D7S2456 and D7S525 or between D7S496/D7S2420 and D7S2456.

Discussion

In this study, we confirm the genetic homogeneity of Pendred syndrome and its linkage to chromosome 7. Haplotype analysis in a large consanguineous family showed a consistent homozygous region in all affected members for two adjacent and very close loci, D7S692 and D7S2425.

In a recently published paper,¹⁰ in family 2 (sibship D), one patient resulting from a first cousin marriage (subject 11) was found to be homozygous at five adjacent loci between D7S2453 and D7S692, and heterozygous at loci D7S2425 and D7S525. By combining these data with our results, we could map D7S2425 distal to D7S692 and exclude the region located between D7S2425 and D7S525 from the Pendred syndrome candidate region. However, the exact position of D7S2456 relative to the Pendred syndrome candidate region could not be drawn from our results and this marker has not been analysed in previously reported Pendred families.⁵⁻⁸⁻¹⁰ Thus, the gene responsible for Pendred syndrome is located in a less than 0.8 cM interval flanked by D7S496/D7S2420 and D7S2425 (fig 2).

Within the Pendred syndrome candidate region, a gene responsible for non-syndromal recessive deafness, DNFB4, has been mapped.¹⁴ Whether the two genes map coincidentally to the same region, or different mutations within the same gene are responsible for both Pendred syndrome and non-syndromic deafness, cannot be answered, since mutations within the same gene, myosin VIIA, were found to be responsible for Usher syndrome and also for non-syndromic deafness.¹⁵ However, it should be noted that since the localisation of DNFB4 to 7q31, no other families with non-syndromic deafness were found to be linked to this region. The re-evaluation of the

diagnosis in DNFB4 families in the light of the clinical variability of Pendred syndrome may help to solve this question.

The underlying basic biochemical defect in Pendred syndrome is not known and, therefore, several genes from the candidate region should be searched for mutations in patients with Pendred syndrome. So far, no obvious candidate genes with known function have been mapped to the refined Pendred syndrome region on human chromosome 7q31 or on its murine homologue on the proximal part of mouse chromosome 6.¹⁶ This emphasises the importance of our study in facilitating the construction of a physical map of the candidate region and in searching for candidate genes.

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- 1 De Groot L. Congenital defects in thyroid hormone formation and action. In: De Groot LJ, ed. *Endocrinology*. Vol I. Philadelphia: Saunders, 1995:871-92.
- 2 Carey JC. Inner ear. In: Stevenson RE, Hall JG, Goodman RM, eds. *Human malformations and related abnormalities*. Vol II. Oxford: Oxford University Press, 1993:231-5.
- 3 Sarne DH, Refetoff S. Thyroid function tests. In: De Groot LJ, ed. *Endocrinology*. Vol I. Philadelphia: Saunders, 1995:617-64.
- 4 Gorlin RJ. Genetic hearing loss associated with endocrine and metabolic disorders. In: Motulski AG, Bobrow M, Harper PS, Scriver C, eds. *Hereditary hearing loss and its syndromes*. New York: Oxford University Press, 1993:337-9.
- 5 Sheffield VC, Kraiem Z, Beck JC, et al. Pendred syndrome maps to chromosome 7q21-34 and is caused by an intrinsic defect in thyroid iodine organification. *Nat Genet* 1996; 12:424-6.
- 6 Reardon W, Trembath RC. Pendred syndrome. *J Med Genet* 1996;33:1037-40.
- 7 Smith SD. Overview of genetic auditory syndromes. *J Am Acad Audiol* 1995;6:1-14.
- 8 Coyle B, Coffey R, Armour JAL, et al. Pendred syndrome (goitre and sensorineural hearing loss) maps to chromosome 7 in the region containing the nonsyndromic deafness gene DNFB4. *Nat Genet* 1996;12:421-3.
- 9 Gausden E, Coyle B, Armour JAL, et al. Pendred syndrome: evidence for genetic homogeneity and further refinement of linkage. *J Med Genet* 1997;34:126-9.
- 10 Coucke P, Van Camp G, Demirhan O, et al. The gene for Pendred syndrome is located between D7S501 and D7S692 in a 1.7-cM region on chromosome 7q. *Genomics* 1997;40:48-54.
- 11 Dib C, Faure S, Fizames C, et al. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nat Genet* 1996;380:152-4.
- 12 Bassem BJ, Caetano-Anolles G, Gresshoff PM. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal Biochem* 1991;196:80-3.
- 13 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-6.
- 14 Baldwin CT, Weiss S, Farrer LA, et al. Linkage of congenital, recessive deafness (DNFB4) to chromosome 7q31 and evidence for genetic heterogeneity in the Middle Eastern Druze population. *Hum Mol Genet* 1995;4:1637-42.
- 15 Weil D, Blanchard S, Kaplan J, et al. Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* 1995;374:60-1.
- 16 Moore DJ, Elliott RW. Encyclopedia of the mouse genome. Mouse chromosome 6. *Mamm Genome* 1996;6:S113-34.