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Enlarged vestibular aqueduct: a radiological marker of Pendred syndrome, and mutation of the PDS gene

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Summary

Although the textbook view of Pendred syndrome is that of an autosomal recessive condition characterized by deafness and goitre, it is increasingly clear that not all such patients present this classical clinical picture. Malformations of the inner ear, specifically enlargement of the vestibular aqueduct, are common in Pendred syndrome and mutations in the PDS (Pendred Syndrome) gene have been recorded in patients presenting with deafness and vestibular aqueduct dilatation only, without other features of Pendred syndrome. Since this is the most common radiological malformation of the cochlea in deaf patients, we investigated what proportion of such cases were due to mutation of the PDS gene. We assessed 57 patients referred with radiological evidence of vestibular aqueduct enlargement, by history, clinical examination, perchlorate discharge test and molecular analysis of the PDS locus. Forty-one patients (72%) had unequivocal evidence of Pendred syndrome. The finding of a single heterozygous mutation at the PDS gene in a further eight was strongly suggestive of a critical role for pendrin, the protein product of the PDS gene, in the generation of enlarged vestibular aqueducts in at least 86% (49/57 cases) of patients with this radiological malformation. Securing the diagnosis of Pendred syndrome may be difficult, especially in the single case. Goitre is an inconstant finding, and the perchlorate discharge test, although helpful, is of diagnostic value only if abnormal. Enlargement of the vestibular aqueduct should be considered as the most likely presentation of Pendred syndrome and should prompt specific investigation of that diagnostic possibility. Pendred syndrome might henceforth be recharacterized as deafness with enlargement of the vestibular aqueduct, which is sometimes associated with goitre.

Introduction

The vestibular aqueduct is a narrow, membrane lined channel in the petrous temporal bone through which endolymph drains from the membranous labyrinth of the inner ear to the endolymphatic sac. Due to its narrow diameter, the vestibular aqueduct is not usually identified on radiological examination of the ear. However, improved imaging techniques, initially polytomographic, latterly by CT imaging, have led to a series of reports identifying pathological enlargement of the vestibular aqueduct in some deaf patients.[1–6] These reports initially led to a consensus as to definition of radiological criteria: that a diameter of >1.5 mm in the mid-portion of the descending hind limb is diagnostic of dilatation of the vestibular aqueduct.[7,8] More recently it has been recognized that this abnormality is the single most common imaging abnormality in sensorineural deafness dating from infancy or childhood.[8] While precise prevalence

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figures in respect of this radiological malformation among deaf children are difficult due to the absence of large-scale studies, the current data suggest that up to 12% of deaf children may have vestibular aqueduct enlargement[9] (Figure 1).

Clinical studies on affected patients indicate a range of audiovestibular disturbances incorporating profound congenital deafness, progressive sensorineural deafness and fluctuating threshold levels of hearing.[2–4, 9–11] With the more widespread recognition of the radiological malformation, audiological physicians have recognized a clinical syndrome in affected patients characterized by progressive loss of hearing, often with acute episodes clearly linked to events such as head injury, air travel or diving.[8,12] Despite these clinical and radiological advances, the cause(s) of this emerging condition has remained unidentified.

In the course of a clinical and molecular study of Pendred syndrome, we identified enlargement of the vestibular aqueduct in $>80\%$ of individuals with that condition.[13] Pendred syndrome is an autosomal recessive condition in which deafness is associated with goitre[14] and which has been calculated to cause 7.5% of all congenital deafness.[15] There is rarely much doubt about the diagnosis in the classical presentation of congenital deafness and goitre. However, most children with Pendred syndrome do not develop goitre until the second decade of life and, consequently, the diagnosis is easily missed in childhood.[16] Moreover, up to one-third of affected adults never manifest clinical signs of thyroid enlargement, and the true diagnosis in these individuals is often overlooked.[16,17] Molecular genetic analysis of the PDS (Pendred syndrome) gene on chromosome 7q31 has been proposed as an adjuvant to the investigation of Pendred syndrome.[18–20] However, the effective deployment of this diagnostic approach in clinical practice prerequires full recognition of the range of clinical presentations consequent on PDS mutation. An increasing number of deaf patients have radiological investigation of the inner ear as part of their routine investigation, particularly if being considered for cochlear implant programmes. Consequently the number of cases being recognized with enlargement of the vestibular aqueduct is increasing.

Those series of published cases with enlarged vestibular aqueducts have not drawn attention to associated clinical findings, such as goitre, in the patients reported.[1–4,9] Specifically, the possibility of Pendred syndrome as the underlying diagnosis in the patients reported has not been investigated. Meanwhile, a separate series of reports has suggested that dilatation of the vestibular aqueducts may be observed in deaf siblings, but again, the possibility of Pendred syndrome as the underlying cause has not been considered.[21–23] We investigated a consecutive series of deaf patients in whom enlargement of the vestibular aqueduct was observed radiologically, to specifically establish the proportion of such patients who might have functional defects in pendrin, the protein expressed by the PDS gene, as the underlying diagnosis.

Methods

Eighty-five patients (37 male, 48 female) with enlarged vestibular aqueducts were referred to us for further investigation. All the cochlear CT scans were read by the same consultant radiologist (PDP). Of

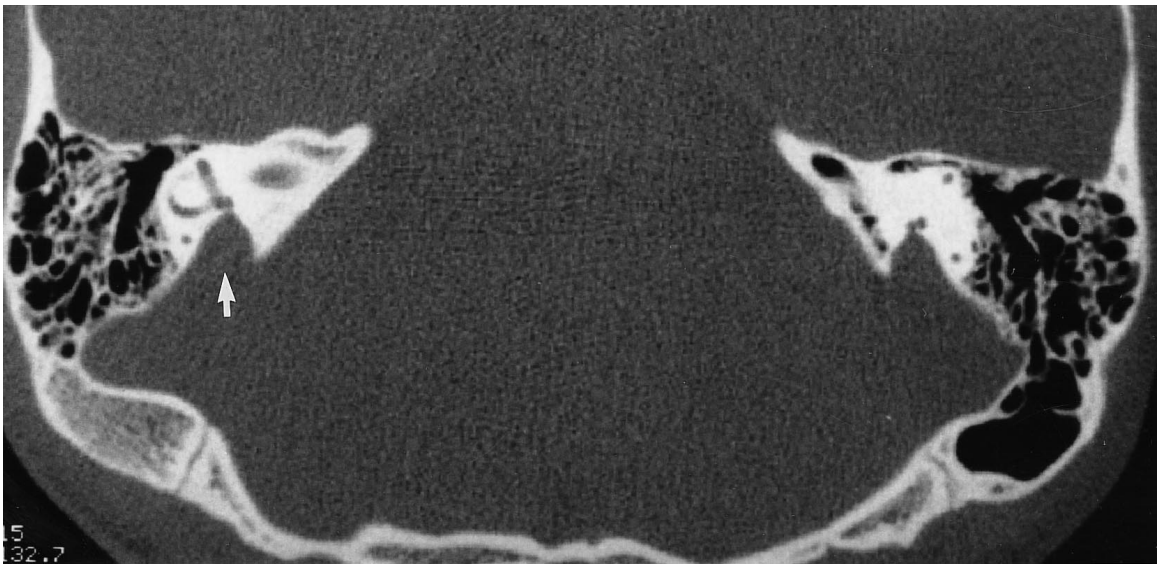

Figure 1. Axial CT section of the petrous temporal bones at the level of the lateral semicircular canal and internal auditory meatus showing enlarged vestibular aqueduct (arrow).

these, 57 patients agreed to attend a genetics consultation at which details of family history, past medical history and clinical examination were recorded. Additional investigations, including perchlorate discharge testing and DNA analysis, were done in 43 patients.

A positive (abnormal) perchlorate discharge test was defined as a discharge exceeding 10% of radioiodide from the thyroid in response to perchlorate challenge.[17,24,25] Mutation analysis of the Pendred syndrome locus was initially performed as published.[20] Briefly, all 21 exons of the PDS gene were PCR amplified to include the intron–exon boundaries. Sequence variants were identified by altered migration patterns on SSCP gel analysis and confirmed through DNA sequencing. To improve the sensitivity of PDS mutation detection, the remaining PDS alleles were analysed by direct PCR sequencing using BIG DYE terminators and ABI 377 automated sequencers. All mutations identified were confirmed by restriction enzyme digestion, and mutant alleles were not observed in a panel of 50 normal individuals.

Results

Fifty-seven patients (age range 6 months to 62 years) were seen further to the identification of vestibular aqueduct enlargement on cochlear CT scan. Two patients (3.5%) did not require investigation because an alternative diagnosis (Branchio-Oto-Renal syndrome in both instances) was apparent when examined clinically. Of the remaining 55 patients, 11 (20%) had evidence of thyroid enlargement on clinical examination.

The family history was suggestive of a diagnosis of Pendred syndrome in 12 (21%), in that there was a deaf sibling with the same radiological abnormality.

The remaining 43 singleton patients underwent perchlorate discharge test, of whom 27 (63%) showed evidence of thyroid abnormality in that the discharge exceeded 10%. The maximum discharge observed was 70%.

Molecular analysis of the PDS locus was done in these 43 patients (Table 1). The 27 patients in whom Pendred syndrome was confirmed by perchlorate discharge comprised 15 in whom both mutant alleles could be identified, eight in whom only one mutant allele could be identified and four in whom no mutation was demonstrable. The 16 patients in whom there was no supportive evidence for Pendred syndrome from the normal perchlorate test comprised two in whom both mutant alleles were identified, eight with a single mutant allele, and six in whom no mutation was found (Tables 1 and 2).

Table 1 Results of mutation analysis at the PDS locus in patients according to perchlorate discharge status

	Perchlorate +	Perchlorate −
Both mutations	15	2
Single mutation	8	8
No mutation recorded	4	6
Total	27	16

Aggregating these different modalities of testing for Pendred syndrome in our study group, we have secured the diagnosis of Pendred syndrome in 41 patients (72%) in the cohort: 12 by family history, 27 by abnormal perchlorate discharge and an additional two in whom perchlorate discharge was normal but molecular analysis identified homozygous mutation at the PDS locus. A further eight patients in whom perchlorate discharge test was normal showed heterozygosity for a single mutant allele at the PDS locus, suggesting that these individuals may also represent examples of PDS mutation. These data suggest that 49/57 (86%) of deaf patients with enlarged vestibular aqueducts have an underlying diagnosis of PDS mutation. Six patients (6/57, 10.5%) remained undiagnosed with neither clinical evidence of Pendred syndrome nor an identifiable PDS mutation. Two patients (2/57, 3.5%) had a diagnosis of Branchio-Oto-Renal syndrome.

Discussion

We found mutation at the PDS gene in 33 of 43 singleton cases with enlargement of the vestibular aqueducts (Table 1). Of these patients, 17 were homozygous/compound heterozygotes, and 16 represented heterozygous mutations in whom a second mutation could not be identified. There were four further patients with positive perchlorate discharge results, strongly suggestive of a diagnosis of Pendred syndrome, in whom we were unable to identify a mutation. Whilst there is no evidence of locus heterogeneity in Pendred syndrome, mutations at the PDS gene have previously been established in only 72% of all familial cases of the condition.[20] Consequently, our inability to establish mutation in all deaf patients with positive perchlorate discharge in the context of enlarged vestibular aqueducts, is not surprising and probably represents the limitations of current mutation detection approaches. However, the alternative possibility of a distinct locus for a form of autosomal recessive deafness with enlarged vestibular aqueduct has to be acknowledged.

Despite the often catastrophic consequences for hearing function which are associated with vestibular

Table 2 PDS sequence variation observed in 10 patients with enlarged vestibular aqueduct and normal perchlorate discharge profile

Sequence	Homo/heterozygous	Result	Known mutation?
A1337G	Homozygous	Q446R	No
C1229T	Homozygous	T410M	Yes (ref 20)
A1246C	Heterozygous	T416P	Yes (refs 19, 20)
T707C	Heterozygous (2 cases)	L236P	Yes (refs 19, 20)
C349T	Heterozygous	L117F	No
T1334G	Heterozygous	L445W	No
C1229T	Heterozygous (2 cases)	T410M	Yes (ref 20)
G626T	Heterozygous	G209V	Yes (ref 29)

aqueduct enlargement, there has been little progress in understanding the causes of this condition since the original description in 1978.[1] Vestibular aqueduct enlargement is an occasional finding in rare dysmorphic conditions associated with deafness, notably Branchio-Oto-Renal syndrome. This is a distinct autosomal dominant condition, in which features of deafness and renal/urinary tract symptoms are observed in patients whose initial presentation is often precipitated by brachial cysts or sinuses, and the true diagnosis is readily identifiable on clinical examination. The vestibular aqueduct abnormality is rarely seen as the sole inner ear malformation in Branchio-Oto-Renal syndrome, usually being described in the context of a range of other, more typical, radiological associations.[26] However, most deaf patients with vestibular aqueduct enlargement have no other clinical stigmata, and several recent observations have firmly implicated Pendred syndrome as one cause of enlarged vestibular aqueduct. This body of evidence ranges from the report that $>80\%$ of patients with documented Pendred syndrome, as evidenced by perchlorate discharge analysis and family linkage studies, were demonstrated to have this radiological phenomenon on cochlear CT scan[13] to subsequent reports of familial cases of deafness with vestibular aqueduct enlargement mapping to the Pendred syndrome region of chromosome 7[27] and reports of mutation at the Pendred locus in patients with enlarged vestibular aqueduct.[20,28,29] These reports represent a compelling case for Pendred syndrome as a cause of vestibular aqueduct enlargement, but do not address the important issue of the overall prevalence of Pendred syndrome among patients with this, the most common inner ear malformation in deaf populations.[8]

The design of this study was such that a consecutive series of patients was ascertained through the radiology department and no details were known of the patients other than that they had hearing impairment associated with enlargement of the vestibular aqueducts on radiology. To address the prevalence of Pendred syndrome in the patient group, we adopted diagnostic criteria depending on whether the cases were familial or sporadic. That 12 (21%) patients were found to have a deaf sibling also shown to have enlarged vestibular aqueducts was taken as diagnostic of Pendred syndrome in those cases. The validity of this diagnostic approach to the recognition of Pendred syndrome, even in the absence of other diagnostic criteria, is now well established.[20,27–30] Moreover, five of these 12 already had positive perchlorate analysis prior to referral to our attention. To investigate the 43 singleton cases for a possible diagnosis of Pendred syndrome, we undertook both perchlorate discharge challenge of the thyroid and molecular analysis of the Pendred syndrome gene.

Of the 43 singleton cases, 27 had a positive perchlorate discharge test ($>10\%$). This has been the gold standard test for Pendred syndrome for over 40 years.[24,25] Occasional cases of false-positive results are known,[31,32] but the general experience has been that the test is reliable,[25] a conclusion mirrored by our own experience in a largescale family study of Pendred syndrome.[17] In the light of this, the finding of a single mutation at the PDS locus in patients with abnormal perchlorate discharge test could be represented as strong evidence for Pendred syndrome. We suggest that a conservative interpretation of our data might be that 12 patients had familial Pendred syndrome, 27 had radioiodide evidence and a further two with normal perchlorate discharge tests had mutational evidence for Pendred syndrome.

Molecular analysis of the PDS locus on chromosome 7 is a much more recent investigation, and several groups have reported mutation at this gene in a variety of patients with classical and nonclassical presentations of Pendred syndrome.[18–20,28,29] The gene comprises 21 exons and to date 36 mutations (mainly point mutations) have been characterized[33] which are predicted to alter the ion transport protein encoded by the PDS gene. Mutations thus far identified have involved 15 exons, although some recurrent mutations have been noted.[19,20,29] The gene is

expressed at high level in the fetal and adult cochlea as well as in the follicular cells of the thyroid gland. Following expression of pendrin in *Xenopus laevis* oocytes by microinjection of PDS cDNA, the rates of transport for iodide and chloride were significantly increased, suggesting that pendrin functions as a transporter for both these ions.[34] The high degree of homology to the DRA gene, mutated in congenital chloride diarrhoea, has led to the suggestion that the vestibular aqueduct enlargement might reflect dysregulation of the PDS ion channel from early cochlear development during embryonic life.

The most surprising finding our study is the observation of sequence variation at the PDS locus in 10 patients with vestibular aqueduct enlargement but normal perchlorate discharge. As can be seen from Table 2, seven nucleotide sequence variations were observed in this group of patients, of which four have already been published as disease-causing mutations in patients with typical and atypical Pendred syndrome.[19,20,29] The other three nucleotide changes have been assumed to be likely mutations because: (i) they have not been observed in the normal control population; (ii) they result in amino-acid substitution within the pendrin protein; and (iii) in the case of A1337G, it is present in the homozygous state in a consanguineous kindred.

Overall, we have identified a total of 41 (72%) cases of Pendred syndrome among the 57 patients with enlarged vestibular aqueducts recruited to the study. The additional finding of a single mutant allele at the PDS gene in a further eight patients with normal perchlorate characteristics strongly suggests that dysfunction of pendrin is critical to vestibular aqueduct enlargement in as many as 86% (49/57) of patients in our study. While some might argue with this interpretation, the data certainly underline the importance of Pendred syndrome as a cause of vestibular aqueduct enlargement in deafness. Moreover, the absence of any clinical thyroid pathology in 80% of the cases in the study confirms the basis of previous claims that the condition is frequently unrecognized,[17] and indeed draws into question the widely cited figure that Pendred syndrome is responsible for 7.5% of all congenital deafness, since this figure was calculated on the basis of clinical findings of thyroid disease in a deaf population.[15] Our data also emphasize the unreliable predictive value of a normal perchlorate test in the diagnosis of Pendred syndrome, in that 10 patients had evidence of mutation despite normal perchlorate analysis. Finally, the study clearly establishes functional defects of the pendrin ion transport protein as the major cause of vestibular aqueduct enlargement. The findings we present also signal the importance of cochlear radiology as a valuable diagnostic tool in the identification of Pendred syndrome. We propose that the identification of vestibular aqueduct enlargement has profound genetic implications for parents of deaf children.

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