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Main Article

Clinical findings and *PDS* mutations in 15 patients with hearing loss and dilatation of the vestibular aqueduct

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Abstract

Following systematic skull imaging of hundred and sixty seven individuals attending a medical referral centre for the deaf in Brussels, Belgium, fifteen patients (9 per cent) aged between two and 25 years were diagnosed with dilatation of the vestibular aqueduct. Careful audiological study, with a baseline assessment then longitudinal follow up, indicated mild to profound deafness with a progressive course (i.e. an average loss of 3.3 dB per year) and frequent dizziness. Sequencing of *PDS* was performed in all individuals. Alterations of this gene (either homozygous, heterozygous or compound heterozygous base changes) were found in 53 per cent of patients with a large vestibular aqueduct. Four new mutations (two missense, a splice site and a four base pair insertion) were described. We were unable to confirm a correlation between homozygosity, heterozygosity and a Pendred or deafness-only phenotype.

Key words: Vestibular Aqueduct; Sensorineural Hearing Loss; PDS Protein, Human genetics

Introduction

The large vestibular aqueduct syndrome (online mendelian inheritance in man accession number 603545) was defined by Valvassori and Clemis in 1978.[1] Although it appears to be an anatomical description, it actually represents the association of hearing loss and an enlargement of the vestibular aqueduct.[2,3]

The vestibular aqueduct is an 8 mm long bone channel in the petrous temporal bone, which originates from the medial wall of the vestibule and extends towards the cerebellar aspect of the petrous pyramid. It contains a vein, an artery and an endolymphatic duct ended by a sac.[2]

At the fourth week of gestation, the vestibular aqueduct appears as a diverticulum from the primordial otocyst. It enlarges and reaches its largest size at approximately the fifth week of gestation. Thereafter, it gradually decreases in volume. It has been proposed that arrested development around the fifth week results in a large vestibular aqueduct.[4]

The diagnosis of large vestibular aqueduct is usually made by radiological examination. On computed tomography (CT) scanning, a vestibular aqueduct with a diameter larger than 1.5 mm at a point midway between the endolymphatic sac and the vestibule is considered abnormal.[4] On magnetic resonance imaging (MRI), a large vestibular aqueduct corresponds to an aqueduct diameter exceeding that of the posterior semicircular duct.[2] A large vestibular aqueduct is generally observed on both sides and is often associated with other malformations of the inner ear, such as an enlarged vestibule or semicircular canal or an underdeveloped cochlea.[5,6]

Clinically, hearing loss is variable, ranging from mild to profound, and is generally bilateral. In rare instances, the large vestibular aqueduct syndrome is found in the absence of hearing loss.[7] The sensorineural hearing loss often shows a sloping audiometric profile (with marked impairment for high frequencies). Occasionally, a mixed hearing loss occurs.[2] The auditory course usually waxes and wanes but eventually results in severe hearing impairment. In the process, sudden hearing loss is common and transient recovery frequent. Hearing fluctuations may also occur as a consequence of minor head trauma.[3]

A large vestibular aqueduct may be an isolated occurrence but is also found as a component of syndromes such as Pendred syndrome[8] (OMIM 274600) or branchio-oto-renal dysplasia[9] (OMIM 113650). Pendred syndrome was first described in 1896. It is an autosomal recessive disorder resulting in sensorineural deafness and goitre, caused by mutations in *PDS* (OMIM 274600).[10] Mutations in *PDS* can also cause DFNB4 (DFN is an abbreviation for deafness, B means autosomal recessive and 4 means that it is the fourth such entity described) (OMIM 600791), a type of autosomal recessive, non-syndromic hearing loss.[11,12] It has been hypothesised that residual pendrin activity

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(dependent on the type of *PDS* gene mutation) might induce a deafness-only clinical picture, whereas a complete loss of activity would be associated with the full blown Pendred syndrome.[11–13]

In large vestibular aqueduct syndrome, the precise mechanism by which progressive sensorineural hearing loss occurs is unknown. Various aetiological theories have been proposed, namely: increased intraluminal pressure, endolymphatic sac reflux and perilymphatic fistula.[3,7,14]

Increased intraluminal pressure has been advocated based on MRI findings that show dilatation of the endolymphatic sac and duct, with the occasional bony, 'moth-eaten' appearance of their surroundings.[3] However, endolymphatic hydrops is usually observed in the apical region of the cochlea. Consequently, one should expect an involvement of the low frequencies, exactly the opposite of what is observed *in vivo*.[7]

Some authors have suggested that the congenitally weakened inner-ear membranes rupture, resulting in mixed perilymph and endolymph. They speculate that a large vestibular aqueduct may be a coincidental radiological finding, when actually a membranous inner-ear malformation is the underlying cause of the hearing loss.[14]

Hyperosmolar endolymphatic sac content reflux into the cochlea is also thought to result in damage to the cochlear neuroepithelium.[3] Likewise, perilymphatic fistula has been considered as a possible cause of sudden hearing loss in affected patients. Belenky *et al*. provided imaging support for this mechanism, with round window anomalies being observed during surgery in some patients with large vestibular aqueduct.[15]

Surgical attempts at shunting endolymph through a subarachnoid bypass or performing endolymphatic sac occlusion have both proved unsuccessful.[7] In cases of profound deafness, cochlear implantation remains an option.[2,16,17]

This paper reports 15 cases of large vestibular aqueduct, with emphasis on the clinical, audiological and genetic findings. In particular, four new mutations are described.

Materials and methods

Between 1983 and 2004, 167 radiological investigations were carried out for hearing loss at the Centre Comprendre et Parler national hearing rehabilitation centre, Brussels. Fifteen cases of large vestibular aqueduct were found (9 per cent). Fourteen of these have been followed longitudinally by us from infancy. The fifteenth patient began her follow up at the age of 23 years. The fifteen patients belong to 13 unrelated families (two monozygotic twins, two remote cousins). At the time of study, the average age of the fourteen children was eight years and two months (range, three years and four months to 13 years). At the time of writing, the adult patient was 43 years old. The sex ratio was seven males to eight females. The mean follow up of the fifteen patients was five years five months (range, nine months to 15 years).

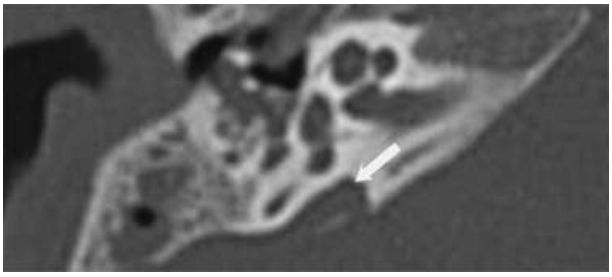

FIG. 1

Computed tomograph scan illustrating enlargement of the vestibular aqueduct (arrow).

All patients underwent CT of the petrous temporal bone (Figure 1); seven also underwent a MRI of the inner ear. Audiological tests were performed with a Madsen OB 822 audiometer (GN Otometrics, Taastrup, Denmark) in a soundproof room. In the fourteen children, the first audiometry was performed at an average age of three years four months (range, 24 to 81 months). The adult patient received her first audiometry at the age of 23 years. Serial audiograms were performed yearly, and the average hearing level was expressed as the average score of three frequencies (500, 1000, 2000 Hz). Some patients also underwent electrocardiogram, click-evoked oto-acoustic emissions recordings, auditory brainstem response testing, ophthalmological examination and blood chemistry (data not shown). Two patients underwent a perchlorate discharge test.

Two hundred micrograms of deoxyribonucleic acid, extracted from peripheral leukocytes using the salting-out method, were analysed. Sequencing of the 21 exons of *PDS* was performed after polymerase chain reaction amplification, according to standard methods and using the primers reported in literature.[18] Amplification products were visually confirmed by agarose gel electrophoresis. Bidirectional sequencing reactions were analysed on an ABI 377 sequencer (Applied Biosystems, Foster City, California, USA).

Results

Table I shows the clinical data for the fifteen patients. Hearing loss was congenital in twelve patients, postlingual in one patient (after four years) and unspecified in two patients (diagnosed at five and six years). A conductive component ($\geq$20 dB) with normal tympanometry was present for at least two frequencies (of 250, 500, 1000 and 2000 Hz) in eleven patients. In one patient, there was no conductive component present. Hearing loss was bilateral in all fifteen patients, and only one patient (a child) had a unilateral large vestibular aqueduct. At the time of diagnosis, thirteen children with bilateral large vestibular aqueduct had an average hearing threshold (500, 1000 and 2000 Hz) in the better ear of 63 dB (range 28–87) and in the poorer ear of 79 dB (range 38–108). A hearing asymmetry of 16 dB was therefore observed. For the adult patient, the average hearing threshold was 73 dB in

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TABLE I

CLINICAL CHARACTERISTICS OF PATIENTS WITH LARGE VESTIBULAR AQUEDUCT

Patient	Gender	Hearing loss onset	Bilateral LVA?	Inner-ear associated malformation?	Thyroid hormones*	Perchlorate discharge test	Vertigo?	Cochlear implantation?	*PDS* mutation
1	F	Congenital	Yes	No	ND	ND	Yes	Yes	nhl
2	M	Congenital	Yes	No	Normal	ND	No	No	E29Q/V138F
3	M	Congenital	Yes	SCD-VD	Normal	ND	No	Yes	V138F/A411T†
4	F	Congenital	Yes	No	Normal	ND	No	No	E29Q/2177–2178 ins CTAT†
5	M	Congenital	Yes	CM-VD	Normal	ND	No	No	nhl
6	F	Postlingual	Yes	CM	ND	ND	Yes	No	nhl
7	F	Congenital	Yes	CM	Normal	ND	Yes	No	nhl
8	M	Congenital	Yes	No	ND	ND	No	No	nhl
9	M	Unspecified	Yes	SCD-VD	ND	ND	No	No	ND
10	F	Congenital	Yes	CM-VD	Normal	Abnormal	No	Yes	IVS 8 + 1 G > A/L 445 W†
11	M	Congenital	No	VD	Normal	ND	No	Yes	nhl
12	M	Congenital	Yes	VD	Normal	ND	No	Yes	H723R/H723R
13	F	Congenital	Yes	VD-SCD	ND	ND	No	No	G209V/nhl
14	F	Congenital	Yes	CM	Low	ND	No	No	IVS 6 + 1 G >A†/ IVS 8 + 1 G >A
15	F	Unspecified	Yes	No	Normal	Normal	No	No	H723R/nhl

*Serum concentration. †New mutation. LVA = large vestibular aqueduct; F = female; ND = not done; nhl = nihil; M = male; SCD = semicircular canal dilatation; VD = vestibular dilatation; CM = cochlear malformation

the right ear and 105 dB in the left ear. For four children and the single adult, the difference was ≥20 dB.

The average hearing loss (same frequencies) was calculated for eleven patients with a follow-up period of over two years. In an early case of cochlear implantation, the auditive follow up was performed in the unimplanted ear. For the patient presenting with a unilateral large vestibular aqueduct, the hearing loss was calculated only for the malformed ear.

The average loss of hearing calculated among the nine patients with follow up of more than two years was 2.5 dB/year for the better ear (Figure 2) and 3.7 dB/year for the poorer ear (Figure 3). In eleven patients, the progressive level of hearing loss (from 250 to 4000 Hz) at the time of diagnosis and then three years later was determined (Figure 4). A hearing loss was observed at all frequencies: 7 dB at 250 Hz, 14 dB at 500 Hz, 11 dB at 1000 Hz, 12 dB at 2000 Hz and 9 dB at 4000 Hz.

Free thyroxine and thyroid-stimulating hormone (TSH) serum concentrations were normal in ten patients. One patient had congenital hypothyroidism. A perchlorate discharge test carried out in a patient without thyroid enlargement and normal thyroid hormones was normal. One patient with a euthyroid goitre had an abnormal perchlorate discharge test, with a 50 per cent reduction of thyroid radioactivity, compatible with Pendred syndrome (Table I).

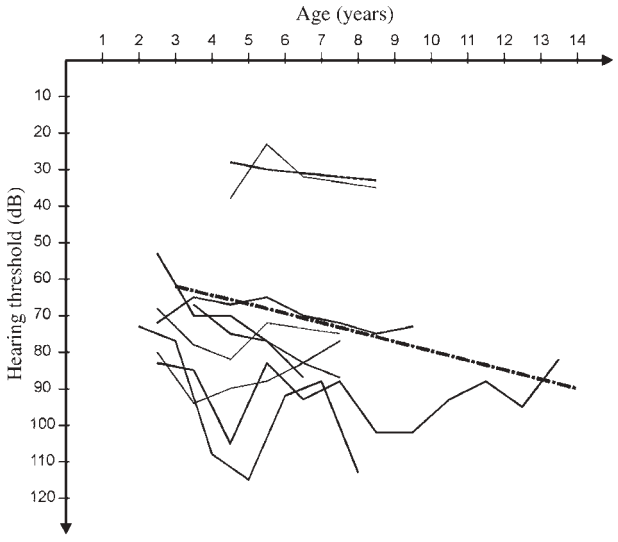

FIG. 2

Hearing in the best ear for nine children with over two years' follow up. The lines represent the best ear's hearing thresholds (average 500, 1000, 2000 Hz) for each child over time. The broken line is the average auditory loss in the best ear for these nine children.

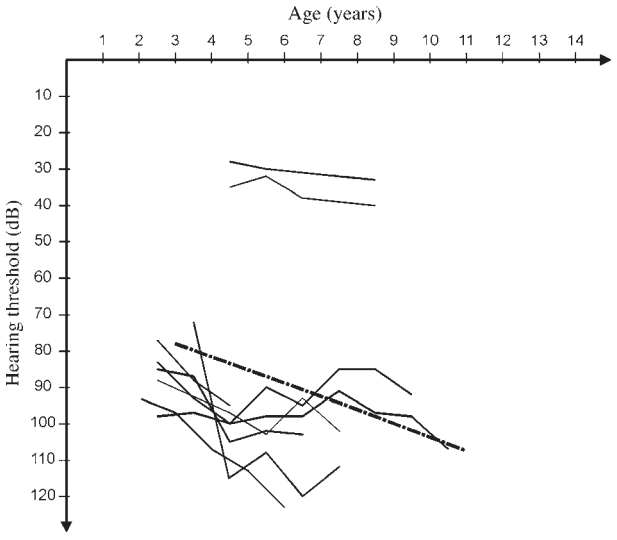

FIG. 3

Same parameters for the worse ear.

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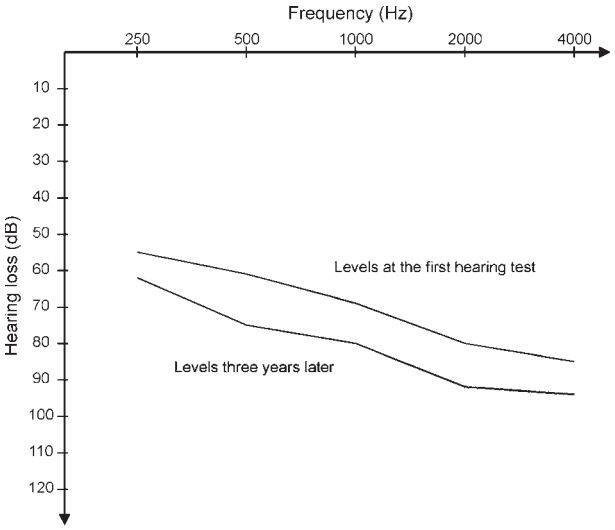

FIG. 4
Mean level of hearing loss at the first hearing test and then three years later, for the frequencies 250, 500, 1000, 2000 and 4000 Hz (11 children).

Three of the fifteen patients complained of vertigo during follow up (Table I). Of these patients, the monozygotic twins had episodes associated with left-sided neck stiffness and vomiting. These bouts of dizziness relapsed every three to four months. For one twin, vestibular tests, including the Hallpike test and electronystagmography, were normal. For her sister, electronystagmography indicated right canal paresis (46 per cent) without directional preponderance. Another child had similar crises, with prostration of seven to nine hours' duration and vomiting, every two to three months.

Radiological imaging showed bilateral malformations of the aqueduct in 14 patients.

Large vestibular aqueduct syndrome was isolated in five of the fifteen patients (Table I). Inner-ear associated malformations comprised an enlarged vestibule in 13/29 inner ears (45 per cent), dysplastic cochlea in 9/29 (31 per cent) and an enlarged semicircular canal in 6/29 (21 per cent).

The patient with Pendred syndrome was diagnosed by a perchlorate discharge test and had bilateral enlarged aqueduct and vestibule with dysplastic cochlea. In the patient with congenital hypothyroidism, bilateral large vestibular aqueduct and dilatation of the left cochlea were observed (data not shown).

Complete sequencing of *PDS* was performed in fourteen patients. Eight of them had nucleotide changes in the *PDS* gene. Six patients had two mutated alleles and two were found to be heterozygous. Ten different sequence changes were observed: seven missense mutations (E29Q; V138F; G209V; A411T; L445T; L597S and H723R), an insertion (CTAT in position 2177–2178) and two splice site mutations (G/A +1 IVS6 and G/A +IVS8)(Table I).

Discussion

In the present series, fifteen patients (9 per cent) were found to have a dilatation of the vestibular aqueduct, out of 167 investigated for presumed congenital deafness. This proportion is a higher figure than those published in two previous reports.[4,6] In a study of one eighty one patients with hearing loss, Okumura found 7 per cent with large vestibular aqueduct syndrome.[6] In Fahy and colleagues' study of 170 children, assessed by MRI within a paediatric cochlear implant programme, only 4 per cent were identified with large vestibular aqueduct syndrome.[4] However, this is six times higher than the 1.5 per cent of patients observed to have large vestibular aqueduct syndrome in a retrospective study of 3700 temporal bone X-ray polytomographs carried out by Valvassori and Clemis,[1] and nine times higher than the 1 per cent of patients with large vestibular aqueduct syndrome observed in a study of two thousand six hundered and eighty three patients with cochleovestibular complaints.[5] The discrepancies between the findings of the three latter studies are readily explained by the fact that patients were recruited from a normal hearing population.

The congenital nature of deafness was suggested in the majority of our patients (12/15), with an onset of symptoms prior to three years of age, similar to Tong and colleagues' findings.[19]

Equally, our audiological findings, with relatively less affected low frequencies and an interaural average difference of 16 dB, are consistent with those of previous reports.[2] Dizziness is also a well known component of large vestibular aqueduct syndrome. Jackler and de la Cruz reported a rate of 29 per cent in the seventeen patients they studied,[7] whilst dizziness was a complaint in 20 per cent of our patients.

If we now consider the type of hearing loss observed in our series, we find a mixed loss in eleven/twelve (92 per cent) patients, similar to the findings of Govaerts *et al.*[2] and Nakashima *et al.*[20] In our series, there was bilateral involvement in 93 per cent (14/15) of patients, which is in accordance with figures of 72–91 per cent found in the literature.[5,19] We observed a higher percentage of associated inner-ear malformations (65.5 per cent) than the 33 per cent reported by Tong *et al.*[19] Enlargement of the vestibule was prominent in our series (45 per cent)(Table I), compared with the 30.4 per cent reported by Okumura *et al.*,[6] who found abnormalities of the lateral semicircular canal (47.8 per cent) and cochlea (26.1 per cent) to be the most frequent.

DFNB4 is a progressive disorder, and we thus found an average loss of 3.3 dB per year, which is in the same range (4–5.8 dB) as the findings of Govaerts *et al.*,[2] Jackler and de la Cruz[7] and Au and Gibson.[18]

- **This study investigated fifteen patients with deafness in association with dilated vestibular aqueduct**
- **Hearing loss was usually mild to moderate and sensorineural in character, with a progressive course and frequent vertigo**
- **Mutations of *PDS* were found in 53 per cent of patients**

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TABLE II

PERCENTAGE OF PDS MUTATION-POSITIVE PATIENTS BETWEEN PENDRED SYNDROME AND DILATED VESTIBULAR AQUEDUCT POPULATIONS FROM THE LITERATURE

Study	Population	*n*	Homozygosity or heterozygosity for *PDS* mutations	%
Everett *et al.*[10] (1997)	PDS	3	3	100
Van Hauwe *et al.*[22] (1998)	PDS	14	14	100
Kopp *et al.*[23] (1999)	PDS	1	1	100
Lopez-Bigas *et al.*[24] (1999)	PDS	4	4	100
Masmoudi *et al.*[25] (2000)	PDS	2	2	100
Blons *et al.*[27] (2004)	PDS	32	28	87.5
Li *et al.*[11] (1998)	LVA	1	1	100
Usami *et al.*[12] (1999)	LVA	6	4	66.6
Scott *et al.*[13] (2000)	LVA	3	3	100
Fugazzola *et al.*[26] (2000)	LVA	1	1	100
Campbell *et al.*[18] (2001)	LVA	23	9	39
Bogazzi *et al.*[28] (2004)	LVA	15	5	33
Pryor *et al.*[29] (2005)	LVA	39	11	28

PDS = patients with pendred syndrome; LVA = patients with large vestibular aqueduct

This regular trend may be interrupted by episodes of sudden hearing loss followed by a partial or total recovery.[2] A history of cranial trauma was not detected in our population, similar to previous reports.[4,7,21] Consideration of the mechanism by which cranial trauma might cause auditory deterioration has raised speculation concerning the possible role of cerebrospinal fluid pressure fluctuations, transmitted directly to the inner ear via the widely patent vestibular aqueduct and resulting in cochlea membrane rupture.[7]

Fifty three per cent of our patients with dilatation of the aqueduct were found to harbour one or two mutations in the *PDS* gene. This figure is comparable with, although higher than that of previous reports. Previous studies have given a variable yield of molecular analyses in patients presenting with either deafness and an enlarged vestibular aqueduct, or Pendred syndrome (Table II).[10–13,18,22–29] Of the 10 nucleotide changes observed in our series, six have been already reported: the E29Q,[22] V138F,[23] G209V,[24] L597S[22] and H723R[23,25] missense mutations, and the IVS8 +1G > A splice site mutation affecting an acceptor site. Interestingly, the two prevalent mutations described in 17 and 12 families from the literature (L236P and T416P) were not observed in our series.[15,17] The four new mutations observed in our series were: a splice site mutation in intron 6 (IVS6 + 1G > A); a four base pair insertion in position 2177; and two missense mutations resulting in an essential amino-acid transition at the level of exons 10 and 11 (A411T and L445T). For the latter mutation, a different nucleotide change resulting in the same substitution has already been reported.[18]

Conclusion

Our results suggest that *PDS* mutations account for a large proportion of the large vestibular aqueduct syndrome cases detected by systematic investigation of deaf patients, using inner-ear imaging. We provide additional evidence supporting consideration of this syndrome as a progressive disorder and highlighting the need for careful assessment of inner-ear morphology at diagnosis. Four novel mutations (two missense, an insertion and a splice site mutation) provide further evidence for heterogeneity. We were unable to confirm L236P and T416P as major recurrent mutations. Their high prevalence may be limited to the North American population by a founder effect.

Electronic resource for Online Mendelian Inheritance in Man: http://www.ncbi.nlm.nih.gov

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