# Pendred Syndrome: study of three families

## *Sindrome di Pendred: studio di tre famiglie*

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Key words

Inner ear • Malformations • Pendred syndrome • Deafness • Goitre

Parole chiave

*Orecchio interno • Malformazioni • Sindrome di Pendred • Sordità • Gozzo*

Summary

Although the textbook view of the Pendred syndrome is that of an autosomal recessive condition characterised by deafness and goitre, it is increasingly clear that not all patients present this classical clinical description. Malform-ations of the inner ear, specifically, enlargement of the vestibular aqueduct, are common in the Pendred syndrome. Mutations in the Pendred syndrome gene have been observed in patients with deafness and vestibular aqueduct dilatation, in the absence of other Pendred syndrome features. In our study, all patients with congenital profound or severe sensory-neural deafness were evaluated using computed tomography and magnetic resonance imaging, followed by genetic examinations and blood tests. The procedure followed was the sensory-neural child deafness protocol elaborated by the Joint Committee for Infant Hearing based on skull and petrous bone. In 3 families, the computed tomography scans (performed on 7 out of 8 of these deaf subjects) showed enlarged vestibular aqueducts. The present study evaluates whether or not enlargement of the vestibular aqueduct should be considered as the most likely presentation of the Pendred syndrome.

*Riassunto*

*Nonostante la maggior parte dei testi descriva la Sindrome di Pendred come una patologia autosomica recessiva caratterizzata da sordità e gozzo, è sempre più evidente che non tutti i pazienti presentano questo caratteristico quadro clinico. Malformazioni dell'orecchio interno, soprattutto la dilatazione dell'acquedotto vestibolare, sono frequenti nella Sindrome di Pendred e mutazioni del gene della Pendrina sono state rinvenute in pazienti che presentano soltanto sordità e dilatazione dell'acquedotto vestibolare, senza la presenza delle altre caratteristiche classiche della sindrome. Tutti i pazienti portatori di sordità neurosensoriale congenita profonda o grave sono stati esaminati basandosi sul protocollo di studio delle sordità infantili elaborato dalla Joint Committee for Infant Hearing, basato sull'esecuzione di TC e RMN di cranio rocche petrose, esami ematici e ricerca genetica. Grazie a questo protocollo abbiamo identificato tre famiglie nelle quali la Tomografia Assiale Computerizzata (eseguita su sette degli otto individui sordi analizzati) evidenzia una dilatazione dell'acquedotto vestibolare. In definitiva vogliamo sostenere che la dilatazione dell'acquedotto vestibolare può essere considerata la manifestazione più frequente della Sindrome di Pendred.*

## Introduction

In 1896, while examining some patients, Vaughan Pendred [1] observed a significant correlation between goitre and congenital hearing loss. It is only in the last century, thanks to developments in the medical diagnostic field that it has been possible to identify two other important pathological components i.e., the impaired thyroid iodine uptake and the presence of vestibular aqueduct expansion. In the present study, attention was focused on the presence of this inner ear malformation since most Authors [2 3] hold that this is the most frequent and most sensitive diagnostic finding related to this particular disease.

When considering the congenital hearing impairment [4], it is important to stress that most cases involve profound and bilateral hearing loss which appears in the child prior to language acquisition. Only on rarer occasions does the deafness appear in an advanced age and, therefore, it can have a progressive course

Goitre [5 6] is a very frequent clinical finding (75% of subjects). However, it generally appears only in adults and almost never before the age of puberty. This thyroid disorder can cause various degrees of damage from an undetectable form of goitre to a severe multinodular form that frequently requires surgical treatment at a young age due to the compression effect on the main neck structures (larynx, vascular-nervous bundle). Even if the thyroid mass is very clearly evident, only in 10% of cases is glandular functionality impaired, whereas in the remaining cases, thyroid function tests reveal euthyroidism.

It was only in 1956 that the presence of an impaired thyroid iodine uptake was highlighted when, for the

first time, a perchlorate discharge test was performed on Pendred syndrome $(PDS)^{-/-}$ subjects. Perchlorate can induce the release of the iodine accumulated in the thyroid in a ratio proportional to the efficiency of the accumulation mechanism. Subjects with normal thyroid function at most discharge 10% of the iodine stored in the follicles whereas, subjects with accumulation problems discharge more than 60% of the content. Until a few years ago, it was believed that this was the most sensitive non-genetic test for diagnosis of the syndrome; however, recent studies have shown that the sensitivity of this perchlorate discharge test does not exceed 60%.

The discovery of a significant relationship between PDS and vestibular aqueduct expansion is even more recent. The vestibular aqueduct is defined as "wide" when the diameter exceeds 1.5 mm at the mid-point (i.e., between the common crus and the intracranial duct aperture). In 1999, Reardon et al. [2] showed, at computed tomography (CT) and magnetic resonance imaging (MRI) that, in a group of 85 Pendred carrier-subjects, 86% showed a visible aqueductal expansion. Hence, it can be seen that, prior to childhood, the aqueductal malformation is certainly more frequent then the thyroid disorder. Furthermore, it should be pointed out that a study on 85 subjects constitutes an extremely large and representative cohort for this rare disease.

According to Fraser's studies [7 8], PDS accounts for 7.5% of congenital deafness conditions. However, recent studies tend to suggest a higher percentage. Indeed, one of the salient traits of the disease is the extreme variability of the phenotype which makes it difficult to establish its real incidence without performing genetic examinations.

## Patients and methods

### Patients

Studies were carried out on 8 patients with sensory-neural deafness (Table I) and 6 unaffected family members from 3 non-related Italian families. All patients were evaluated according to the sensory-neural child deafness protocol elaborated by the Joint Committee for Infant Hearing. Since March 2001, this protocol has been in use in the Audiology Unit of the Florence University Oto-Neuro-Ophthalmology Surgical Sciences Department. The experimental protocol comprises audiology and clinical examinations, radiological evaluation and molecular analysis of the Pendrin gene.

### Audiology and clinical examination

All patients were evaluated to establish the onset of hearing loss (or the age at which hearing-loss was first noted). If patients were under 6 years of age, they were studied using children's tests: the choice of the test was made based on the mental age and behaviour of the child or individual as well as on the basis of whether or not neuropsychological problems were present. Generally, if the child is over 30 months old, the "peep show" technique is used. Complete medical histories were taken, and physical, ENT examinations were also carried out. To study thyroid function, serum free $T_4$ and thyroid stimulating hormone (TSH) were measured using the Axsym System.

### Radiological evaluation

To study the presence of Mondini malformations or EVA, all subjects apart from A.C. underwent high-resolution CT of the temporal bone together with MRI of

**Table I.** Outline of patients with sensorineural deafness and results of audiology and clinical examinations, radiological evaluation and molecular analysis of the Pendrin gene.

| | Mutation | Deafness | Thyroid function | Vestibular aqueduct |
|---|---|---|---|---|
| Family L. | | | | |
| M.L. | T132I/IVS2-2A > G | Severe | Normal | Enlarged |
| A.L. | T132I/IVS2-2A > G | Profound | Normal | Enlarged |
| Family C. | | | | |
| D.C. | T721M/T721M | Profound | Hypothyroid goitre | Enlarged |
| F.C. | T721M/T721M | Severe | Hypothyroid goitre | Enlarged |
| A.C. | T721M/T721M | Severe | Euthyroid goitre | Not performed |
| Family P. | | | | |
| S.P. | T721M/X781W | Profound | Hypothyroid goitre | Enlarged |
| M.P. | T721M/X781W | Severe | Hypothyroid goitre | Enlarged |
| L.P. | T721M/X781W | Profound | Hypothyroid goitre | Enlarged |

the brain. Subjects were considered to have enlarged vestibular aqueducts if the diameter measured midway between the operculum and the common crus was > 1.5 mm. Radiological evaluation was performed at the Radiology Department of the Meyer Paediatric Hospital of Florence. Radiological evaluation of all subjects with sensory-neural deafness revealed bilateral vestibular aqueduct enlargement.

### DNA ANALYSIS

The 3 families received genetic counselling in order to evaluate the risk of hereditary hearing loss. Initially, the Connexin 26 gene was analysed for mutations as this is the gene which most frequently presents mutation, and it is responsible for hereditary deafness, together with the A1555G and A7445G mitochondrial mutations. Later, since radiological evaluations showed bilateral enlargement of the vestibular aqueduct in all deaf subjects, this finding is considered a marker of PDS, a further DNA analysis was carried out in order to find a mutation of the PDS gene SLC26A4.
Blood samples were obtained from deaf and hearing relatives. Written informed consent to the study was obtained from the patients. DNA was extracted using standard techniques.

1. PCR connexin 26

Amplification reactions were performed in a final volume of 50 µl, containing 150 ng of genomic DNA, 200 µmol/l dNTPs, 20 pmol each primer, 1 mmol/l $MgCl_2$, 2% DMSO, and 2 U Taq polymerase. Thermal cycle conditions included 5 minutes of denaturation at 95°C, followed by 30 polymerase chain reaction (PCR) cycles at 94°C for 45 seconds, 57°C for 45 seconds, and a final extension at 72°C for 7 minutes.
The four PCR primers were:
F1: TTCTGTCTTACCTGTTTTG (169 to 149), and
R2: AGCCTTCGATGCCGGACCT (374 to 392) 564 bp PCR product;
F3: ACCGGAGACATGAGAAGAAG (290 to 309);
R4: TCTAACAACTGGGCAATGC (684 to 702) 412 bp PCR product.

*Connexin 26 Sequence*
The PCR products obtained were directly sequenced on both strands, after purification on an automated DNA sequencer (ABI Prism 377, Perkin Elmer).

2. Polymerase A1555G and A7445G chain reactions

DNA was amplified using 100 ng DNA, 10 pmol of each primer, 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 $MgCl_2$, 200 µM dNTPs, and 2 U Taq DNA polymerase (Perkin Elmer, Foster City, CA, USA) in a volume of 50 µl with an initial 5-minute denaturation at 95°C, followed by 40 cycles at 94°C for 30 seconds, at 54°C for 1 minute, and at 65°C for 2 minutes in a thermal cycler.
The primers used for the two amplification products were 1039-1058 and 3099-3080, in order to obtain the DNA responsible for the synthesis of 12 S rRNA subunity; 6900-6919 whereas 8721-8702, was used to obtain the DNA for $tRNA^{SER}$. Numbers reported are based on those published in the Cambridge mitochondrial sequence.

*Restriction Enzyme Analysis*
A total of 5 µl of PCR product obtained with the primer pairs 1039-1058 and 3099-3080 (fragment 12S mt DNA) was digested with 10 U BsmA1 and was incubated at 56°C overnight.
In addition, 5 µl of PCR product obtained with primer 6900-6919 and 8721-8702 was digested with 50 U Xba 1, and was incubated at 37°C for 3 hours.
The products underwent electrophoresis with 1.5% agarose and were visualized with ethidium bromide.

3. PDS Analysis

Intronic primers were used to amplify the 21 exons and flanking regions as described by Coyle et al. 1998 [10]. PCR was performed in 25 µl reaction volumes containing 10 pmol per primer, 100 µM dNTP, 2.5 µl reaction buffer (100 mM Tris pH 8.3, 500 mM KCl, 15 mM MgCl2, 0.01% gelatin), 2 U Ampli Taq Gold Polymerase (Perkin Elmer, Foster City, CA, USA) and 50 ng DNA. The reaction was performed in an automated Thermal Cycler 9700 (Pelkin Elmer, Foster City, CA, USA) with the following cycling profile: an initial denaturation step at 94°C for 12 min followed by 35 cycles each of which was characterised by a denaturation at 94°C for 30 seconds, annealing at a specific temperature for 30 seconds, extension at 72°C for 30 seconds, A final extension step at 72°C for 10 min was performed. PCR products were sequenced directly on an automated sequencer (ABI 3100; Perkin Elmer, Foster City, CA, USA) using the ABI-PRISM big-dye terminator cycle sequencing ready reaction kit (Perkin Elmer, Foster City, CA, USA).

## Results

### FAMILY L.

Family L. is composed of 2 children (A.L. and M.L.) with sensory-neural deafness and the two parents without hearing-loss, goitre or any thyroid metabolism disorder. The children were submitted to audiological evaluation.
M.L. was evaluated at 2 years of age using the "peep show" technique which revealed profound bilateral deafness (Fig. 1). Clinical examination showed no evidence of goitre, and serum free $T_4$ and TSH were normal. Radiological imaging revealed, bilaterally, an

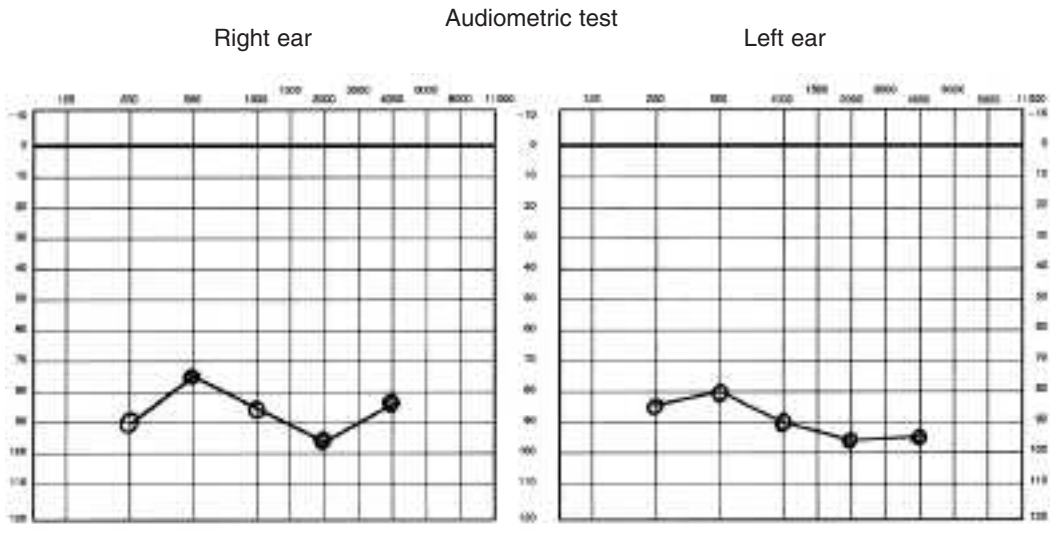


**Fig. 1.** L.M. Audiometric test.

aqueduct, the dimensions of which exceeded normal values, clearly visible at CT and MRI. The CT (Fig. 2) result received showed: "No mastoid cell alterations. Regular middle-ear conformation with normal auditory ossicle structure. Vestibular aqueduct expansion bilaterally. Modest bilateral cochlear dysplasia". A genetic investigation showed no mutation in the connexin 26 gene but displayed a compound heterozygous mutation T132I/IVS2-2A > G on the PDS gene.

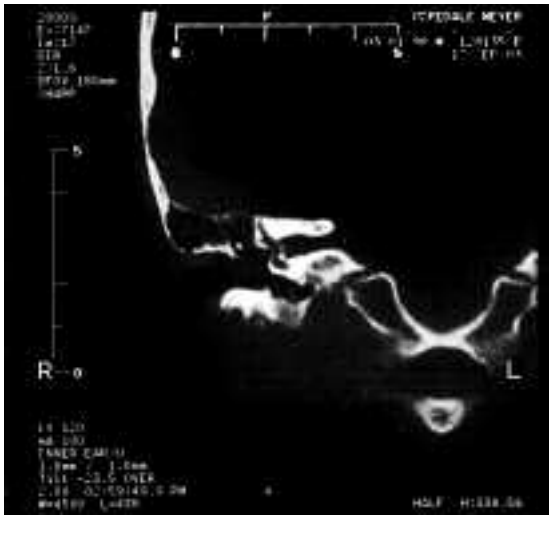


**Fig. 2.** Petrosal bone CT image of L.M.

A.L. was evaluated at 7 years of age with a free-field survey and showed a 90-dB hearing level loss in the right ear, a 100-dB loss in the left ear, and no response at higher frequencies in either ear (Fig. 3). Haematic examination performed to detect possible thyroid dysfunction was found to be negative and, as in the objective examination, it was not possible to determine any goitre. MRI and CT (Fig. 4) highlighted "recognizable vestibular aqueduct expansion" and "modest cochlear dysplasia with a number of turns less than normal". Furthermore, as far as concerns A.L., a genetic investigation showed no mutation in the connexin 26 gene but revealed a compound heterozygous mutation T132I/IVS2-2A > G on the PDS gene.
Parents are not blood-related but the genetic examination showed that they are both heterozygous for T132I (395 C > Ts) and IVS2-2A > G mutation, respectively.

### Family C.

This family is composed of 3 children with sensory-neural hearing loss (F.C., A.C. and D.C.), one normal child (M.C.) and two unaffected parents.
F.C. was submitted to audiological examination at 6 years of age using the "peep show" technique and displayed a bilateral 70-dB hearing-loss. Haematic thyroid function parameters highlighted hypothyroidism, and the objective examination showed a goitre of appreciable dimensions. A bilateral aqueduct expansion was discovered using CT and MRI imaging. DNA

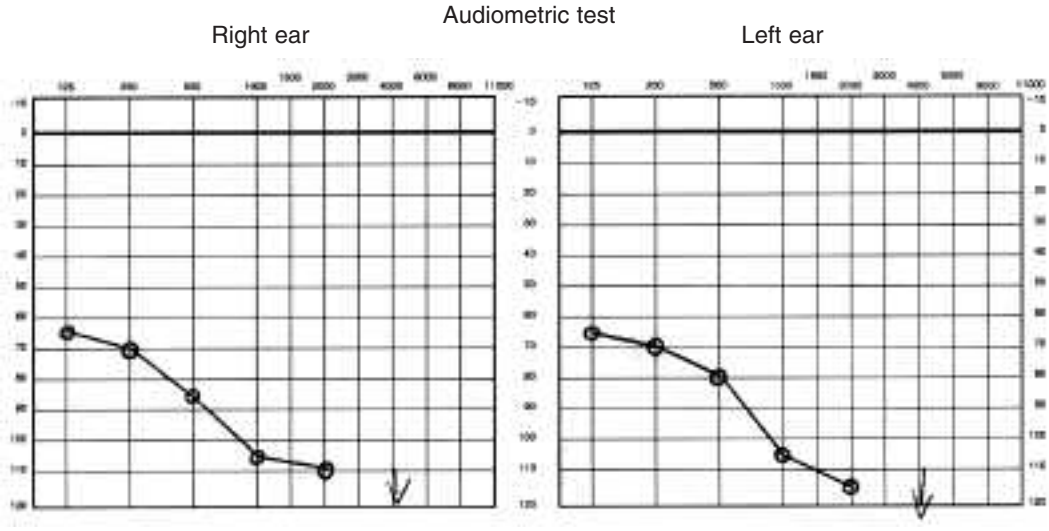


Fig. 3. L.A. Audiometric test.

analysis highlighted the presence of homozygous parental mutation T721M.

A.C. and D.C. were submitted to audiological and clinical examinations, respectively, at 7 and 8 years of age. At the "peep show", A.C. displayed a bilateral 90-dB hearing-loss, and D.C. a bilateral 60-dB hearing loss. Haematic thyroid function parameters highlighted hypothyroidism in D.C. whereas, in A.C., serum free $T_4$ and TSH values were normal. In both subjects, the objective examination showed a goitre of appreciable dimensions, which is more evident in D.C. than in A.C. Furthermore, CT and MRI of the petrous bone were recommended for both children but to-date A.C. has refused to undergo the examinations; in D.C., a bilateral aqueduct expansion was found.

The parents are second-degree cousins. On examining the genealogical tree (Fig. 5), we discovered the presence of 2 uncles (brothers of both the paternal and maternal grandmothers) who were both deaf from birth. Following the genetic examination, it was possible to highlight the heterozygosis of both parents for the mutation T721M which determines the substitution of Threonin with Methionin in Protein Position 721.

The presence of goitre must be carefully considered since the family resides in an endemic goitre area and has a family history with a non-deafness-correlated goitre.

### Family P.

Family P. is composed of 3 deaf subjects (S.P., M.P. and L.P.) and the 2 parents who do not show either hearing-loss or thyroid dysfunction. The first audiological examination, which, in all cases, was conducted prior to 2 years of age using the "peep show" technique, highlighted 2 severe cases (71-90 dB) of bilateral deafness (M.P. and S.P.) together with one case of profound bilateral deafness (L.P). At follow-up, at three years of age, S.P. showed a slight bilateral deterioration, fulfilling bilateral profound deafness criteria (> 90-dB). As far as concerns the state of thyroid in each subject, all 3 daughters are hypothyroid goitre carriers and all use Eutirox to correct the hormonal dysfunction. It should be pointed out that, contrary to observations in the majority of our clinical cases, and to reports in the literature, the glandular involvement is manifested at a young age and is not manifested in the second part of puberty. In one of the cases we observed, the onset of goitre occurred at approximately 6 years of age (L.P.), while, in another case, it occurred around the age of 13 (M.P.) whereas, in the third case, it occurred immediately after birth (S.P.). CT and MRI demonstrated that there was a bilateral vestibular aqueduct expansion in the 3 children.

Both parents are heterozygous for different SLC26A4 mutations: the father shows a T721M mutation, while the mother presents an X781W mutation.

## Discussion

The gene responsible for the disease is known as SLC26A4 and was only identified [9] in 1999 on chromosome 7, in position 31 of the long arm. It is an extremely large gene (21 exons). The mutation is inher-

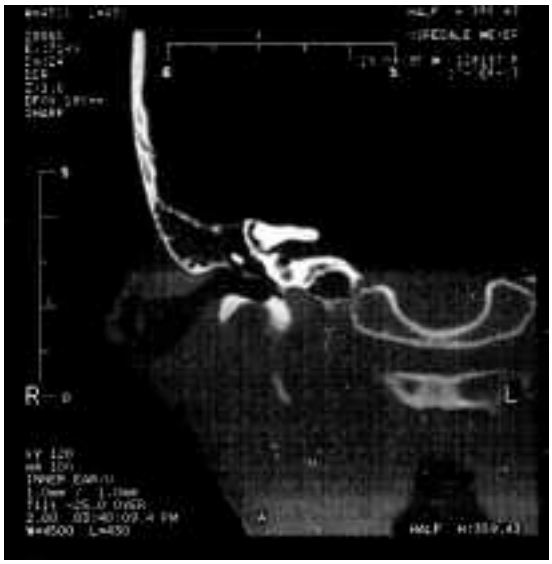


**Fig. 4.** Petrosal bone CT image of L.A.

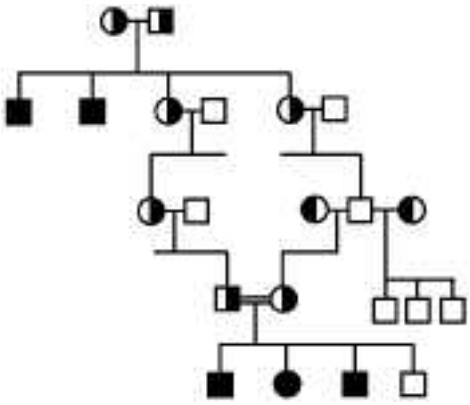

**Fig. 5.** Genealogical tree.

ited in an autosomal recessive manner as with the majority of the hereditary-syndrome deafness conditions. To-date, at least 70 different mutations capable of causing the syndrome have been identified. The protein [11-14] produced by this gene is large and has been called Pendrin (Pds): it is made up of 780 aminoacids and weighs 86 kDa. According to computer studies, it has been identified as a surface 780-amino acid (86 kDa) protein composed of 12 transmembrane domains. Even if the protein structure is very similar to the sulphur transporter proteins group, it is possible to exclude any Pendrin involvement in movements of this iodine element. The protein is involved in iodine and chloride flow in some important districts. Thanks to the numerous studies aimed at showing the protein expression points, it was possible to highlight a concentration of the same in the thyroid, kidney and the inner ear. In the thyroid, the protein develops a crucial role in iodine follicle accumulation; in the kidney, it acts as a formic acid-chloride exchanger in the proximal tubule; finally, in the inner-ear, endolymphatic duct and sac, the protein is essential for the maintenance of endolymph homeostasis serving as a chloride and water carrier.

In 1999, to better understand the role of the protein in the inner ear, Everett et al. [13] generated *knockout mice* for the Pendrin gene (Pds -/-) to study their embryonic and postnatal development. Until the 15$^{th}$ day of pregnancy, it was not possible to determine any alteration therein, but the Pds -/- mice inner-ear contrast coloration performed on the 15$^{th}$ day showed a significant expansion of the endo-lymphatic duct and sac. At the time of birth, the mice showed severe balancing problems: they were observed running around in circles, in a constant unidirectional sense and displayed severe persistent head tilting. After 30 days of life, electronic microscope studies showed a severe cochlear sensory-neural cell degeneration and severe malformation of the otoliths that, in the majority of cases, appeared abnormally enlarged. To-date, it is not clearly understood just how the Pendrin mutation is responsible for cellular degeneration. However, the most convincing hypothesis is that the Cl and $H_2O$ reabsorption-arrest, mediated by the protein in the endolymphatic duct and sac, produces an excess of hyperosmotic endolymph that damages the cochlear sensorial structures following its passage into the auditory duct. With regard to the genesis of megaotolitis, unfortunately, very little is known. According to Reardon et al. [2], in 2000, the enlargement of the vestibular aqueduct should be considered as the most likely presentation of the PD. They found that 49 out of 57 cases of deafness, with enlarged vestibular aqueducts, had unequivocal evidence of PDS.

The presence of EVA, at CT/MRI, has been demonstrated to be a reliable radiological marker, and has become one of the most reliable tools in the diagnostic workup of PDS. The Mondini deformity, which had been thought to be a characteristic radiological feature of this syndrome, is not frequent. Moreover, it was never found in our study. Vestibular aqueduct enlargement is an occasional finding in rare dysmorphic conditions associated with deafness, notably the Branchio-Oto-Renal syndrome. This syndrome can be easily distinguished from the PDS on account of the characteristic features of deafness and renal/urinary tract symptoms.

## Conclusion

In conclusion, in our study, on 8 cases of PDS mutation, 7 (87.5%) later showed also vestibular bilateral aqueduct expansion, with a very similar percentage to that discovered by Reardon in 1999. This percentage could increase radiological evaluation if A.C. is performed. Thus, we agree with the majority of Authors who hold that vestibular aqueduct enlargement is the most frequent finding in the PDS. In our experience, with the aid of this new radiological marker, CT and MRI allow us to identify a probable Pendred syndrome in good time, while genetic tests allow us to confirm the diagnosis. However, a genetic check can be performed at a later stage but not as a diagnostic point of departure. In conclusion, to-date, the mutation search on a gene of 21 exons such as that of PDS is a long time-consuming procedure. Carrying out a large number of these tests requires a considerable time-use of laboratories whereas this time might be spent more wisely in the search for more frequent mutations, such as those of connexin 26.
Hopefully, the future holds the possibility of greater research in the area of genetic studies which will lead to faster and less expensive procedures able to promote screening for the entire population It is essential to find a disease-marker for quick diagnosis (as in the case of vestibular aqueduct enlargement) for the audiological treatment of PDS since it is mandatory that an early prosthesis together with a rapid rehabilitation programme be carried out in order to ensure a normal social life and a normal linguistic development for these children.

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■ Received: July 5, 2004
Accepted: May 31, 2005

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