

ARTICLE

SLC26A4 gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations

Sébastien Albert¹, Hélène Blons², Laurence Jonard², Delphine Feldmann^{2,3}, Pierre Chauvin⁴, Nathalie Loundon^{1,3}, Annie Sergent-Allaoui⁵, Muriel Houang⁶, Alain Joannard⁷, Sébastien Schmerber⁸, Bruno Delobel⁹, Jacques Leman¹⁰, Hubert Journal¹¹, Hélène Catros¹², Hélène Dollfus¹³, Marie-Madeleine Eliot¹⁴, Albert David¹⁵, Catherine Calais¹⁶, Valérie Drouin-Garraud¹⁷, Marie-Françoise Obstoy¹⁸, Patrice Tran Ba Huy¹⁹, Didier Lacombe²⁰, Françoise Duriez²¹, Christine Francannet²², Pierre Bitoun²³, Christine Petit^{3,24}, Eréa-Noël Garabédian^{1,3}, Rémy Couderc^{2,3}, Sandrine Marlin^{3,25} and Françoise Denoyelle^{*,1,3}

¹Service d'ORL et de Chirurgie Cervico-faciale, Hôpital d'Enfants Armand-Trousseau, AP-HP, Paris, France; ²Service de Biochimie et de Biologie Moléculaire, Hôpital d'Enfants Armand-Trousseau, AP-HP, Paris, France; ³INSERM U587, Paris, France; ⁴Unité d'Epidémiologie et de Sciences de l'Information – INSERM U707, Faculté de Médecine Pierre et Marie Curie, Université Paris 6, Paris, France; ⁵Service de médecine nucléaire, Hôpital d'Enfants Armand-Trousseau, AP-HP, Paris, France; ⁶Service d'endocrinologie, Hôpital d'Enfants Armand-Trousseau, AP-HP, Paris, France; ⁷Service de Pédiatrie, CHU, Grenoble, France; ⁸Service d'ORL, CHU, Grenoble, France; ⁹Centre de Génétique, Hôpital St Antoine, Lille, France; ¹⁰Centre Rochin, Lille, France; ¹¹Unité de Génétique Médicale, CHR, Vannes, France; ¹²Centre G Deshayes, Auray, France; ¹³Service de Génétique médicale, Hôpital de Haute-pierre, Strasbourg, France; ¹⁴Service d'ORL, Hôpital de Haute-pierre, Strasbourg, France; ¹⁵Service de Génétique, Hôtel Dieu, Nantes, France; ¹⁶Service d'ORL, Hôtel Dieu, Nantes, France; ¹⁷Service de Génétique, Hôpital Charles Nicolle, Rouen, France; ¹⁸Service d'ORL, Hôpital Charles Nicolle, Rouen, France; ¹⁹Service d'ORL, Hôpital Lariboisière, AP-HP, Paris, France; ²⁰Unité de Génétique Médicale, Hôpital Pellegrin, Bordeaux, France; ²¹Service d'ORL, Hôpital Pellegrin, Bordeaux, France; ²²Unité de Génétique Médicale, Hôtel Dieu, Clermont Ferrand, France; ²³Service de Pédiatrie, Hôpital Jean Verdier, AP-HP, Bondy, France; ²⁴Unité de Génétique des Déficits Sensoriels, Institut Pasteur, Paris, France; ²⁵Unité de Génétique Médicale, Hôpital d'Enfants Armand-Trousseau, AP-HP, Paris, France

Sensorineural hearing loss is the most frequent sensory deficit of childhood and is of genetic origin in up to 75% of cases. It has been shown that mutations of the *SLC26A4* (*PDS*) gene were involved in syndromic deafness characterized by congenital sensorineural hearing impairment and goitre (Pendred's syndrome), as well as in congenital isolated deafness (DFNB4). While the prevalence of *SLC26A4* mutations in Pendred's syndrome is clearly established, it remains to be studied in large cohorts of patients with nonsyndromic deafness and detailed clinical informations. In this report, 109 patients from 100 unrelated families, aged from 1 to 32 years (median age: 10 years), with nonsyndromic deafness and enlarged vestibular aqueduct, were genotyped for *SLC26A4* using DHPLC molecular screening and sequencing. In all, 91 allelic variants were observed in 100 unrelated families, of which 19 have never been reported. The prevalence of *SLC26A4* mutations was 40% (40/100), with biallelic mutation in 24% (24/100), while six families were homozygous. All patients included in this series had documented deafness, associated with EVA and without any evidence of syndromic disease. Among patients with *SLC26A4* biallelic mutations,

*Correspondence: Professor F Denoyelle, Department of Pediatric Otolaryngology, INSERM U587, Armand Trousseau children's hospital, 26 avenue Arnold Netter, 75571 Paris cedex 12, France. Tel: +33 1 44 73 69 25; Fax: +33 1 44 73 61 08; E-mail: f.denoyelle@trs.ap-hop-paris.fr
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deafness was more severe, fluctuated more than in patients with no mutation. In conclusion, the incidence of *SLC26A4* mutations is high in patients with isolated deafness and enlarged vestibular aqueduct and could represent up to 4% of nonsyndromic hearing impairment. *SLC26A4* could be the second most frequent gene implicated in nonsyndromic deafness after *GJB2*, in this Caucasian population.

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Introduction

Sensorineural hearing impairment is the most frequent sensory defect of childhood: one child in 1000 is born deaf in developed countries¹ and deafness is mainly prelingual. Recent studies suggested that more than 75% of childhood deafness is of genetic origin. About one quarter of genetic forms are syndromic (deafness is a part of genetic syndrome). The remaining forms are classified as non-syndromic, but in some cases classification is not obvious due to late onset of some symptoms. To date, over 80 loci for nonsyndromic hearing impairment (NSHI) have been identified, but despite this wide heterogeneity, *GJB2* genetic alterations are causative in up to 50% of prelingual NSHI.² Over 400 syndromes associated with deafness have been reported. Several genes are implicated in syndromic or nonsyndromic forms. For example, *GJB2*, the most common NSHI gene, has also rarely been found in several syndromic forms of deafness.²

Similar findings apply to the *SLC26A4* gene (*PDS* gene), encoding pendrin. Indeed, *SLC26A4* mutations are causative in two autosomal recessive disorders, Pendred's syndrome and a NSHI with inner ear malformation.^{3,4} Pendred's syndrome is characterized by congenital sensorineural hearing loss and goitre that usually develops in the second decade of life (the median age of goitre appearance was 14.9 years in a study performed in a cohort of French patients with Pendred's syndrome⁵) and is associated with hypothyroidism in 50% of cases.^{5,6} Pendred's syndrome and DFNB4 patients have in common similar deafness features: bilateral, typically prelingual or early postlingual, frequently severe or profound with variable evolution, and associated with inner ear anomalies, such as enlarged vestibular aqueduct (EVA) or Mondini's dysplasia.^{7,8} Many studies have focused on Pendred's syndrome, in which the role of *SLC26A4* is well defined, but the prevalence of *SLC26A4* gene alterations in NSHI remains unclear and is certainly underestimated.

The *SLC26A4* gene, located on the long arm of chromosome 7,⁹ encodes pendrin, a protein expressed in various tissues, including principally the inner ear, thyroid and kidney. Pendrin is a transmembrane anion exchanger, with 12 predicted transmembrane domains, that belongs to the solute carrier 26 family. It was shown to exchange chloride, iodide,¹⁰ bicarbonate¹¹ and formate.¹² Recently, Wangemann *et al*, showed that the absence of pendrin led

to altered inner ear tissue pH through impaired HCO₃-transport, enhanced oxidative stress, absence of KCNJ10 expression and absence of endolymphatic potential.¹³

In Pendred's syndrome, the *SLC26A4* gene has been extensively studied and over 100 different mutations, spanning the entire coding region, have been described. The prevalence of *SLC26A4* mutation in Pendred's syndrome is very high in different studies, up to 90%.¹⁴ Screening of this gene in large cohorts of hearing impaired patients has been previously performed on the basis of the presence or not of an enlarged vestibular aqueduct (EVA) detected on CT-scan, but without precise informations concerning the thyroid involvement and the ages of the patients.^{15–17} Indeed, in the Pendred syndrome, the goitre usually appears in the second decade of life and the probability to develop or not goitre depends of the age of patients.

The prevalence of *SLC26A4* mutations in patients with NSHI and inner ear anomalies has never been studied in large and standardized series of Caucasian patients.

From 1995 to 2002, a national research program on deafness allowed a prospective collection of clinical data and samples of 109 children (100 unrelated families) with NSHI and enlarged vestibular aqueduct. Molecular *SLC26A4* screening was undertaken in this series of patients in order to determine the prevalence and the spectrum of *SLC26A4* gene mutations in this pathology and to identify genotype/phenotype correlations.

Methods

We enrolled, in a French national collaborative study, 109 deaf children from 100 unrelated families, who presented with a bilateral prelingual (less than 3 years of age) or an early postlingual (during the first decade) nonsyndromic sensorineural hearing impairment associated with an EVA on CT-scan (some patients had other inner ear anomalies like Mondini dysplasia). In all patients, the mode of inheritance was compatible with an autosomal recessive inheritance. None of patients had goitre or other thyroid anomaly. For each patient, a complete medical history was obtained to determine the age of onset of the deafness and to exclude the possibility of environmental causes. The deaf subjects underwent an otoscopic examination of the

ear, nose and throat and a general examination, with systematic assessment for signs suggestive of a syndromic form of deafness (in particular, goitre and signs of hypothyroidism) by a clinical geneticist specializing in the management of deaf children. They also had an ophthalmological evaluation, investigation for haematuria and proteinuria and an electrocardiogram. None of patients had *GJB2* mutation. Deaf children underwent pure-tone audiometry with a diagnostic audiometer in a sound-proof room, with recording of pure-tone air- and bone-conduction thresholds. Air-conduction pure-tone average (ACPTA) thresholds in the conversational frequencies (0.5, 1, 2 and 4 kHz) were calculated for each deaf ear and were used to define the severity of deafness: mild ($20\text{ dB} < \text{ACPTA} \leq 39\text{ dB}$), moderate ($40\text{ dB} < \text{ACPTA} \leq 69\text{ dB}$), severe ($70\text{ dB} < \text{ACPTA} \leq 89\text{ dB}$) and profound ($\geq 90\text{ dB}$). The severity of deafness in each child was defined by the degree of hearing loss of the best ear. In accordance with the European Working Group on Genetics of Hearing Impairment criteria, hearing loss was considered as progressive when the patient lost more than 15 dB in the ACPTA thresholds in the conversational frequencies by comparison of two reliable audiometric tests carried out at least 10 years apart, to which we added the further criterion of more than 8 dB loss in tests at least 5 years apart. We considered deafness as fluctuant when the mean hearing level in conversational frequencies had risen by more than 10 dB between two successive audiograms.

Genomic DNA was isolated from whole blood. The protocol was accepted by the Committee for the Protection of Individuals in Biochemical Research as required by French legislation and informed consent was obtained from all patients or their parents.

SLC26A4 mutation screening was performed by Denaturing High-Performance Liquid Chromatography (DHPLC), on a Wave™ DNA Fragment Analysis System (Transgenomic™, USA). All 20 protein-encoding exons (2–21) were previously amplified by Polymerase Chain Reaction (PCR).

Variant profiles were sequenced on a new amplification, using an ABI 310 Genetic Sequencer (Applied Biosystems). Sequences were analysed by Sequence Analysis 3.0 (Applied Biosystems). Exon 20 was directly sequenced for all patients. Wherever possible, mutation segregation was studied by direct sequencing of the variant exon in parents. New variants were tested in 50 unrelated healthy controls.

To analyse correlations between genotype and phenotype, patients were classified according to the presence of biallelic *SLC26A4* mutations or the absence of mutations. Groups were compared using χ^2 and Student's tests.

Results

The 109 patients from 100 unrelated families were aged 1–32 years (mean age: 10.2 years and median age: 10 years) at the time of the genetic assessment.

Allowing one allele in each consanguineous family, we detected 91/198 (46%) allelic variants, 53 of which were different. For the study of clinical phenotype and the prevalence of *SLC26A4* mutations, we considered as possibly deleterious frameshift mutations, missense mutations of the coding sequence and splice site mutations. Intronic variants with unknown pathogenicity were not taken in account in the prevalence determination. The L597S missense mutation was found in four alleles from 50 healthy controls and was then considered as non-pathogenic.

Prevalence of *SLC26A4* mutation in this series was 40% (40/100 families), detailed in Table 1. Prevalence of biallelic mutation was 24% (24/100 families, including six families with homozygous mutations, two of them with consanguinity), prevalence of monoallelic mutation was 16% (16/100 families). Among 33 patients tested with caloric vestibular tests because of clinical suspicion of vestibular disorders, 10 had alterations and among them three patients were in the group with monoallelic or biallelic mutation.

A total of 19 variants have never been described (Tables 2 and 3), consisting of nine missenses (T99R; M147T; I199T; M283I; T307M; Q421L; F355S; C565R; V690A: these mutations were not detected in 50 unrelated healthy controls), two insertions (129InsC; IVS12–3InsCAGT), one deletion (IVS7–12DelTTATT), one splice site (IVS11 + 1 G > C) and six intronic variants (IVS3 + 14 A > C; IVS4–7 A > G; IVS12–14 T > C; IVS13 + 9 C > T; IVS13–5 T > G; IVS20–22 T > C). Some of them were not conserved when compared to proteins with significant sequence homology to human pendrin (Table 3).

Three mutations were frameshift: one deletion (2127delT), and two insertions (IVS12–3InsCAGT, 129InsC). These mutations led to a stop codon at X719 for 2127delT, at X467 for IVS12–3InsCAGT and at X85 for 129InsC. One particular mutation was IVS7–12DelTTATT: using two splice site prediction software,^{18,19} the acceptor splice site prediction of exon 7 decreased from 0.95 in wild type to 0.19 in mutated type. The mutation was then considered as pathogenic.

The clinical features of patients with biallelic mutations compared to patients without mutation are detailed Table 4. Difference between groups was statistically significant for the following items: the age of deafness discovery, the walking age, the fluctuating evolution and the hearing loss severity.

Discussion

In 198 alleles tested from unrelated families, 91 allelic variants were found and 53 were different. The analysis of the segregation of mutations between families always gave coherent results. The nine new missense mutations

Table 1 Details of Phenotype and genotype of the 45 patients with *SLC26A4* mutation

Patient number	Age ^a	Deafness		IEM	Perchlorate test ^b	Spo or MAF	SLC26A4 mutations	
		Degree	Evolution				Allele 1	Allele 2
F1P1	10			EVA		MAF	IVS8+1 G>A	—
F2P1	6	Prof	Prog	EVA+vestibular dilatation	Normal	Spo	I199T	—
F3P1	25	Prof	Prog	EVA+Mondini		Spo	G209V	—
F4P1	8	Sev		EVA	Normal	Spo	Y530H	—
F5P1	30	Prof	Prog	EVA		MAF	IVS8+1 G>A	—
F6P1	12	Sev	Prog	EVA	Normal	MAF	F355S	—
F7P1	14	Coph	Fluct	EVA		Spo	M147T	M147T
F8P1	5	Prof		EVA+Mondini		Spo	V609G	—
F9P1	7	Prof	Prog	EVA+Mondini	Normal	Spo	T99R	—
F10P1	8	Sev	Fluct	EVA	Normal	Spo	IVS12–3 Ins CAGT	—
F11P1	8			EVA+Mondini	Normal	MAF	Q421L	—
F12P1	12	Sev	Fluct	EVA+Mondini	Normal	Spo	T307M; G740V	Y530H
F13P1	24	Prof	Prog	EVA	Normal	MAF	G209V	G209V
F13P2	24	Prof	Stable	EVA	Normal	MAF	G209V	G209V
F14P1	4			EVA		Spo	L117F	—
F15P1	12	Prof	Prog	EVA		MAF	M147T	M147T
F15P2	12	Prof	Prog	EVA		MAF	M147T	M147T
F16P1	23	Prof	Fluct	EVA+Mondini	Normal	Spo	M1T	L445W
F17P1	11	Coph	Fluct	EVA		MAF	G740V	Del5nt
F17P2	16	Mild	Fluct	EVA		MAF	G740V	Del5nt
F18P1	10	Prof	Prog	EVA	Normal	MAF	T193I	G209V
F18P2	12			EVA		MAF	T193I	G209V
F19P1	12	Prof	Prog	EVA		Spo	S28R	S391R
F20P1	12	Mild		EVA		Spo	F335L	—
F21P1	28	Coph	Stable	EVA		Spo	L627R	—
F22P1	4			EVA		Spo	G209V	Y530H
F23P1	7	Sev	Fluct	EVA+Mondini	25%	MAF	T410M	—
F24P1	2		Fluct	EVA+Mondini	Normal	MAF	V138F	V138F
F25P1	9	Prof	Fluct	EVA+Mondini	Normal	MAF	T416P	2127delT
F25P2	8	Prof	Fluct	EVA	Normal	MAF	T416P	2127delT
F26P1	6	Prof	Fluct	EVA	Normal	Spo	S391N	—
F27P1	7	Prof	Fluct	EVA		Spo	E29Q	L445W
F28P1	3			EVA	Normal	Spo	G209V	L236P
F29P1	13	Prof	Fluct	EVA+Mondini	20%	Spo	Y78C	Y530H
F30P1	1			EVA		Spo	R409H	L445W
F31P1	2			EVA+Mondini		Spo	C565R	T721M
F32P1	16	Prof	Prog	EVA+Mondini		MAF	IVS1–3–2 A>G	L445W
F33P1	4	Sev	Fluct	EVA+Mondini	29%	Spo	IVS 11+1 G>C	V690A
F34P1	7	Sev		EVA	32%	Spo	IVS 14+1 G>A	IVS 18+1 G>A
F35P1	9	Sev	Prog	EVA	Normal	Spo	129insC	T721M
F36P1	3		Fluct	EVA	33%	Spo	S694P	D724N
F37P1	15	Prof	Prog	EVA+Mondini		Spo	G209V	IVS8+1 G>A
F38P1	12	Prof	Stable	EVA		Spo	G209V	G209V
F39P1	6	Mild		EVA	Normal	MAF	M283I	—
F40P1	32	Prof		EVA		Spo	S133T	S133T

F: family number; P: patient number; IEM: inner ear malformation; Coph: cochotic; Prof: profound; Sev: severe; Prog: progressive; Fluct: fluctuating deafness; EVA: enlarged vestibular aqueduct; Spo: sporadic case; MAF: Multi Affected Family.

^aAge (in years) at genetic consultation for study inclusion.

^bThe percentage indicates the decrease in radioactivity in case of pathologic perchlorate discharge test.

identified in this study showed a variable degree of conservation in their amino acid sequence when compared to proteins with high-sequence homology, and therefore may or not be disease causing. However, because of the low incidence of *SLC26A4* mutations in DFNB4 hearing loss, only a very large study in people with normal hearing, combined with a study in patients DFNB4 deafness, could establish the pathogenicity of *SLC26A4* mutations.

Mutations found in our series were compared to a study of patients with Pendred's phenotype⁵ realized in our

centre, in patients with a similar origins. Many of the mutations found were common: E29Q, V138F, G209V, L236P, IVS8 + 1 G>A, R409H, T410M, T416P, Y78C, T193I, F355S, L445W, Y530H, S694P, D724N, 2127delT. Moreover, among the four frequent mutations (G209V, L445W, Y530H, IVS8 + 1) in the present study, all were also found in Pendred families.⁵ Molecular studies of Pendred syndrome were performed in other populations. The frequent mutations were: T416P, L236P, E384G.^{16,20,21} These mutations have been investigated by *in vitro* functional studies

Table 2 Details of the 19 new allelic variants

Type of new mutation	Details of new mutation
Missense	T99R; M147T; I199T; M283I; T307M; Q421L; F355S; C565R; V690A
Frameshift	129InsC; IVS12–3InsCAGT
Splice site	IVS11+1 G>C
Intronic mutation	IVS3+14 A>C; IVS4–7 A>G; IVS7–12DelTTATT; IVS12–14 T>C; IVS13+9 C>T; IVS13–5 T>G; IVS20–22 T>C

Table 3 Characteristics of mutated amino acid of the nine new missense mutations compared to proteins with significant sequence homology to human pendrin

Missense	Localization	Characteristics of mutated amino acid			
		Proteins with significant sequence homology			Rat-SAT
		Hum-DRA	Hum-DTD	Mse-ST-OB	
T99R	TMD	0	0	0	0
M147T	TMD	+	+	+	+
I199T	TMD	+	0	0	0
M283I	TMD	+	+	+	+
T307M	TMD	+	0	0	0
F355S	TMD	+	+	+	+
Q421L	ECL	+	+	+	+
C565R	C-term	0	0	0	0
V690A	C-term	+	+	+	0

TMD = transmembrane domain, C-term = C terminal region, ECL = extra cellular loop; +: same amino acid; 0: different amino acid.

Table 4 Clinical phenotypes of the two distinct groups with biallelic *SLC26A4* mutations and without *SLC26A4* mutation

	Biallelic mutation of <i>SLC26A4</i> gene 29 patients 24 families		No mutation of <i>SLC26A4</i> gene 64 patients 60 families		P-value
Median walking age (months)	12		14.5		<0.001 ^a
Deafness					
Median age at discovery ^b (months)	12		24		<0.001 ^a
	No.	%	No.	%	
Mode					
Fluctuations	12/22	54.5	8/36	22.2	0.03 ^c
Progressive	8/22	36.4	14/36	38.9	0.85 ^c
Stable	2/22	9.1	14/36	38.9	0.03 ^c
Degree					
≥ Severe	21/22	95.5	27/52	51.9	<0.001 ^c
Steeply sloping audiometric profile	16/20	80.0	31/52	59.6	0.10 ^c
Associated vestibular disorders	2/24	8.3	7/64	10.1	1.0 ^c
Anomalies of thyroid function tests	0/21	0.0	0/28	0.0	—
Anomalies of PDT	4/14	28.6	1/24	4.2	0.03 ^c
TB CT-scan ^d					
EVA	29/29	100.0	57/57	100.0	—
Cochlear anomalies	10/29	34.5	11/57	19.3	0.12 ^c
Vestibular anomalies	1/23	4.3	4/57	7.0	1.0 ^e
Sporadic cases	17/29	58.6	41/64	64.1	0.61 ^c
Autosomal recessive transmission	12/29	41.4	23/64	35.9	0.61 ^c
Consanguinity	4/29	13.8	0/64	0.0	<0.01 ^e

For the clinical phenotype study, all children from families with biallelic mutations or no mutations were taken into account.

PDT: Perchlorate Discharge Test; TB: Temporal bone; EVA: enlarged vestibular aqueduct; NS: non significant.

^aStudent's *t*-test.

^bDocumented among 29 and 64 subjects respectively.

^c χ^2 test.

^dAll the 109 CT-scans showed inner ear malformation but only 105 underwent central review by our expert radiologist.

^eFisher exact test.

and compared to frequent mutations implicated in DFNB4 patients (V480D, V653A and I490D/G497S):^{3,11,22} Significant differences were shown in terms of *in vitro* activity: the V480D, V653A and I490D/G497S mutations allowed continued pendrin activity, whereas the L236P, T416P and E384G mutations did not. These *in vitro* results are in contradiction with the *SLC26A4* molecular results in DFNB4 and Pendred's syndrome populations (similar spectrum of mutations). In the functional studies, the results reflect the consequences of a single mutation. Thus, the type of mutation in trans or the intervention of a regulatory element, either genetic or external, could be key in determining the degree of activity of pendrin and hence the clinical phenotype (for both DFNB4 deafness, as well as Pendred's syndrome).

The prevalence of *SLC26A4* biallelic mutations in our series of 100 families was 24% (24/100 families) and prevalence of monoallelic mutation was 16% (16/100). Comparison with other series is difficult, the criteria of inclusion of the patients for *SLC26A4* screening being usually the presence of an EVA, without detailed data about the thyroid status and the age of the patients. Tsukamoto reported a prevalence of 78.5% in a Japanese study of 32 deaf patients with EVA,²³ however, it should be noted that the age of subjects is unclear and that the H723R mutation represents 53% of the mutated alleles in this population.²³ In a Caucasian population, Scott¹¹ found that 15% of the 20 patients with a DFNB4 type hearing impairment had a *SLC26A4* mutation. Finally, the prevalence of *SLC26A4* mutations in our series is lower compared to that seen in Pendred's syndrome, estimated to be 90% (40 patients with Pendred's syndrome from 30 unrelated families recruited in the same population).⁵

Comparing the prevalence of bi and monoallelic mutations to the data reported in subjects with Pendred's syndrome recruited in the same population⁵ (biallelic mutations: 24% in *SLC26A4* versus 77% in Pendred's syndrome; monoallelic: 16 versus 13%), it raises the question of the pathogenicity of *SLC26A4* in the cases of simple heterozygosity. Tsukamoto in a Japanese series showed similar data: the prevalence of heterozygotes was 0% in Pendred's syndrome patients, 31% in presumed DFNB4 patients.²³ Pryor¹⁷ studied 18 subjects with non-syndromic EVA, and only detected monoallelic mutations in these patients.

Nevertheless, some arguments support the role of this gene in the clinical symptomatology of patients with monoallelic mutations: the incidence is higher than that estimated for heterozygotes in the general population, indicating that the second mutation may not have been found. Indeed, the existence of abnormalities in the regions regulating transcription, the promotor site and in exon 1 (which is not translated), as well as deletions involving one or several exons which cannot be amplified by standard PCR, have not been studied. A frequent

mutation or deletion in one of those regions could explain the high rates of heterozygotes. In addition, another explanation could be the presence of molecular defects within the regulator genes for *SLC26A4*: indeed, a murine study of *Foxi1* (*Fkh10*), a positive gene regulator of pendrin, found that in *Foxi1*(-/-) mutants, there was no expression of pendrin in the inner ear.²⁴ However, there is no evidence in the literature to suggest a digenic aetiology.

With respect to the individuals without mutations, there could be another gene for NSHI, although no other gene responsible for deafness with EVA has yet been identified. In addition, EVA and Mondini malformations of the cochlea are not specific for Pendred's syndrome or DFNB4 type deafness, but can also be found in BOR (branchio-otorenal) and Waardenburg's syndromes. Thus, it can be postulated that some patients who do not have *SLC26A4* mutations may represent minor sporadic, or incomplete forms of BOR or Waardenburg's syndromes. Finally, several studies have implicated a number of ionic channels in the homeostasis of the endolymph. Alterations in these proteins could be responsible for some cases of deafness with dilatation of the inner ear.¹³

The detailed phenotypic study revealed significant differences between the group with biallelic mutation and the group without mutation. Biallelic mutations of the *SLC26A4* gene were associated with a more severe hearing loss at the time of the first consultation, an earlier age of discovery of the hearing impairment (that may be due to a more severe deafness) and a fluctuating course for the disease. This leads to the definition of a phenotype that is largely associated with DFNB4 type hearing loss and thereby directs the choice of molecular tests.

It is of interest that the median age for learning to walk was lower in the biallelic group than the group without mutation (12.7 versus 15.4 months, respectively), although both are normal. As vestibular tests were not systematically performed, it is difficult to establish if any real vestibular defects occurred more frequently in one or the other group.

Differentiating an isolated deafness with EVA from Pendred's syndrome in children, and in particular in very young children, can be difficult. This is especially the case if the thyroid signs (goitre and hypothyroidism) of Pendred's syndrome, which usually appear in the second decade,⁶ only manifest themselves late and hence the diagnosis can only be definitively established as the disease progresses. The median age of our cohort being 10 years, we can expect that some of the younger patients may develop goitre.

The perchlorate discharge test has been used to try to differentiate between the two entities. However, there are both false negatives and false positives. In our study, five out of 23 perchlorate tests were positive, four of which in group with biallelic mutations. Both the clinical examination of the neck and the thyroid function tests were normal in the patients with positive perchlorate discharge test,

whose mean age was younger compared to the mean age of goitre onset in Pendred's syndrome (6.5 years).^{5,6} Thyroid signs can present at a later date and it is essential that children with a mutation of the *SLC26A4* gene are followed up with thyroid function tests (LT4 and TSH levels have to be normal) and clinical search for a goitre.

An ongoing study in our department has shown that out of 888 cases with a nonsyndromic prelingual or early postlingual deafness, 91 had an associated EVA that is, 10.2%. In the literature, 6–15% of the nonsyndromic deafness carry EVA.^{7,25} From our series, in the same population, it can be estimated, considering biallelic or bi and monoallelic mutations, that up to 4% of nonsyndromic deafness in children is attributable to mutations of *SLC26A4*. Thus, the *SLC26A4* gene is the second most frequent cause of nonsyndromic deafness, the commonest being *GJB2*.²⁶

The identification of this form of deafness can now allow reliable genetic counseling, advice on prognosis and implementation of measures to prevent fluctuations in the deafness (avoidance of trauma). Management requires regular monitoring of the deafness and thyroid function.

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References

- Morton NE: Genetic epidemiology of hearing impairment. *Ann NY Acad Sci* 1991; **630**: 16–31.
- Connexin-Deafness Homepage, <http://davinci.crg.es/deafness/>.
- Li XC, Everett LA, Lalwani AK *et al*: A mutation in PDS causes non-syndromic recessive deafness. *Nat Genet* 1998; **18**: 215–217.
- Usami S, Abe S, Weston MD, Shinkawa H, Van Camp G, Kimberling WJ: Non-syndromic hearing loss associated with enlarged vestibular aqueduct is caused by PDS mutations. *Hum Genet* 1999; **104**: 188–192.
- Blons H, Feldmann D, Duval V *et al*: Screening of *SLC26A4* (PDS) gene in Pendred's syndrome: a large spectrum of mutations in France and phenotypic heterogeneity. *Clin Genet* 2004; **66**: 333–340.
- Reardon W, Coffey R, Chowdhury T *et al*: Prevalence, age of onset, and natural history of thyroid disease in Pendred syndrome. *J Med Genet* 1999; **36**: 595–598.
- Cross NC, Stephens SD, Francis M, Hourihan MD, Reardon W: Computed tomography evaluation of the inner ear as a diagnostic, counselling and management strategy in patients with congenital sensorineural hearing impairment. *Clin Otolaryngol* 1999; **24**: 235–238.
- Phelps PD, Coffey RA, Trembath RC *et al*: Radiological malformations of the ear in Pendred syndrome. *Clin Radiol* 1998; **53**: 268–273.
- Everett LA, Glaser B, Beck JC *et al*: Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat Genet* 1997; **17**: 411–422.
- Scott DA, Wang R, Kremann TM, Sheffield VC, Karniski LP: The Pendred syndrome gene encodes a chloride-iodide transport protein. *Nat Genet* 1999; **21**: 440–443.
- Scott DA, Wang R, Kremann TM *et al*: Functional differences of the PDS gene product are associated with phenotypic variation in patients with Pendred syndrome and non-syndromic hearing loss (DFNB4). *Hum Mol Genet* 2000; **9**: 1709–1715.
- Park HJ, Karniski LP: Human pendrin expressed in *Xenopus laevis* oocytes mediates chloride/formate exchange. *Am J Physiol Cell Physiol* 2000; **278**: C207–C211.
- Wangemann P, Itza EM, Albrecht B *et al*: Loss of KCNJ10 protein expression abolishes endocochlear potential and causes deafness in Pendred syndrome mouse model. *BMC Med* 2004; **2**: 30.
- The *SLC26A4* homepage, http://www.medicine.uiowa.edu/pendredandbor/slc26a4_mutations.htm.
- Park HJ, Shaikat S, Liu XZ *et al*: Origins and frequencies of *SLC26A4* (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness. *J Med Genet* 2003; **40**: 242–248.
- Campbell C, Cucci RA, Prasad S *et al*: Pendred syndrome, DFNB4, and PDS/*SLC26A4* identification of eight novel mutations and possible genotype-phenotype correlations. *Hum Mutat* 2001; **17**: 403–411.
- Pryor SP, Madeo AC, Reynolds JC *et al*: *SLC26A4*/PDS genotype-phenotype correlation in hearing loss with enlargement of the vestibular aqueduct (EVA): evidence that Pendred syndrome and non-syndromic EVA are distinct clinical and genetic entities. *J Med Genet* 2005; **42**: 159–165.
- Center for biological sequence analysis, NetGene2 Server: <http://www.cbs.dtu.dk/services/NetGene2/>.
- GeneSplicer Web Interface, http://www.tigr.org/tdb/GeneSplicer/gene_spl.html.
- Coyle B, Reardon W, Herbrick JA *et al*: Molecular analysis of the PDS gene in Pendred syndrome. *Hum Mol Genet* 1998; **7**: 1105–1112.
- Van Hauwe P, Everett LA, Coucke P *et al*: Two frequent missense mutations in Pendred syndrome. *Hum Mol Genet* 1998; **7**: 1099–1104.
- Rotman-Pikielny P, Hirschberg K, Maruvada P *et al*: Retention of pendrin in the endoplasmic reticulum is a major mechanism for Pendred syndrome. *Hum Mol Genet* 2002; **11**: 2625–2633.
- Tsukamoto K, Suzuki H, Harada D, Namba A, Abe S, Usami S: Distribution and frequencies of PDS (*SLC26A4*) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese. *Eur J Hum Genet* 2003; **11**: 916–922.
- Hulander M, Kiernan AE, Blomqvist SR *et al*: Lack of pendrin expression leads to deafness and expansion of the endolymphatic compartment in inner ears of *Foxi1* null mutant mice. *Development* 2003; **130**: 2013–2025.
- Antonelli PJ, Varela AE, Mancuso AA: Diagnostic yield of high-resolution computed tomography for pediatric sensorineural hearing loss. *Laryngoscope* 1999; **109**: 1642–1647.
- Denoyelle F, Weil D, Maw MA *et al*: Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. *Hum Mol Genet* 1997; **6**: 2173–2177.