Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1279–1284
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Analysis of the *SLC26A4* gene in patients with Pendred syndrome in Taiwan

Chien-Chung Lai[a,b,1], Chih-Yang Chiu[c,d,1], An-Suey Shiao[b,e], Yi-Chu Tso[c,d], Yi-Chi Wu[c,d], Tzong-Yang Tu[b,e], Tjin-Shing Jap[b,c,d,f,*]

[a]*Division of Otorhinolaryngology, Department of Surgery, Taipei City Hospital, Taipei 111, Taiwan, ROC*
[b]*Faculty of Medicine, National Yang-Ming University, Taipe 112, Taiwan, ROC*
[c]*Section of Biochemistry, Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei 112, Taiwan, ROC*
[d]*Faculty of Medical Technology, National Yang-Ming University, Taipe 112, Taiwan, ROC*
[e]*Division of Otology, Department of Otorhinolaryngology, Taipei Veterans General Hospital, Taipei 112, Taiwan, ROC*
[f]*Division of Metabolism and Endocrinology, Department of Medicine, Taipei Veterans General Hospital, Taipei 112, Taiwan, ROC*

Received 9 January 2007; accepted 22 May 2007

Abstract

Pendred syndrome (PS) is an autosomal recessive disease that is characterized by congenital sensorineural hearing loss, goiter, and a partial iodine organification defect. In this study, we characterized the thyroid status and identified mutations in the *SLC26A4* gene in Chinese subjects with PS. We evaluated 7 unrelated Chinese subjects who had PS. Biochemical analysis, formal audiogram, ultrasonography of the thyroid gland, perchlorate discharge test, computerized tomography scan of the vestibular aqueducts, and DNA sequence analysis of *SLC26A4* were performed. Levels of thyroid hormones were essentially normal in all patients: 2 patients had goiters and/or elevated serum thyroglobulin levels, whereas 2 other patients had positive thyroid antibodies and a positive perchlorate discharge test. We identified *SLC26A4* gene mutations in 6 of 7 probands and their affected relatives. The affected subjects in family I was compound heterozygous for 2 missense mutations: a mutation in exon 9 (1079C>T) that resulted in the replacement of alanine by valine at codon 360 (A360V) and a mutation in exon 19 (2168A>G) that resulted in the replacement of histidine by arginine at codon 723 (H723R). The affected subjects in families II and III all were homozygous for a mutation in intron 7. The probands IV and V were compound heterozygotes for the mutation in intron 7 and in exon 19, and the proband VI was compound heterozygous for the intron 7 mutation and a missense mutation in exon 12 (1343C>T) that resulted in the replacement of serine by leucine at codon 448 (S448L). One novel mutation was identified (A360V). We identified biallelic mutations in the *SLC26A4* gene in 6 of 7 probands with PS in Taiwan, including a novel missense mutation. The mild thyroid dysfunction in these patients suggests that PS should be considered in all patients with congenital or early-onset hearing impairment.

1. Introduction

Pendred syndrome (PS) is an autosomal recessive disease that is characterized by congenital sensorineural hearing loss, goiter, and a partial iodine organification defect [1]. Inner ear malformations can be identified in most cases using high-resolution computed tomography (CT) of the temporal bones [2], and hearing loss may range from mild to profound. An enlarged vestibular aqueduct (EVA) and widened endolymphatic sac and duct are the most common structural abnormalities [2,3]. In about 80% of the patients, the iodine organification defect causes thyroid problems, most commonly in late childhood to early adulthood, ranging from euthyroid goiter to overt hypothyroidism [4,5]. The diagnosis of PS is supported by the perchlorate discharge test (PDT) in which administration of oral perchlorate 2 hours after a dose of iodine 131 leads to a partial discharge of 15% to 80% of the radioactivity from the thyroid gland in patients with PS compared with less than 10% in normal subjects [4]. The identification of defective iodine organification in thyroid tissue from a PS patient [6] suggested that the underlying defect involved impaired organification of iodine in thyroglobulin (Tg).

* Corresponding author. Section of Biochemistry, Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei 112, Taiwan, ROC. Tel.: +886 2 28757158; fax: +886 2 28717680.
E-mail address: tsjap@vghtpe.gov.tw (T.-S. Jap).
[1] These authors contributed equally to this work.

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.05.013

Molecular genetic studies have resulted in the localization of the PS locus on chromosome 7 [6,7]; and in 1997, Everett et al [8] identified mutations in the *SLC26A4* gene at 7q22-31.1 to be the molecular cause of PS. The PDS/*SLC26A4* gene encompasses 21 exons and encodes pendrin, a 780–amino acid transmembrane protein that is expressed in the inner ear, the thyroid gland, the kidneys, and the endometrium [8,9]. Most importantly, pendrin functions as an iodide-chloride transporter in thyroid and other polarized mammalian cells [10,11].

The incidence of PS is estimated to be 7.5 to 10 in 100 000 individuals [12]. A previous study has demonstrated a high prevalence of specific *SLC26A4* mutations in East Asians, and some distinct *SLC26A4* gene mutations have also been found in subjects of different ethnic backgrounds; for example, T416P and IVS8+1G>A accounted for the 2 most frequent mutations in Iowa, USA [13], and IVS7-2A>G accounted for 84% of mutations in Taiwan, suggesting the possibility of a founder effect [14,15]. About 150 different *SLC26A4* gene mutations have been found in patients with PDS and nonsyndromic deafness. The H723R is among the independently recurring mutations and has been identified in numerous probands from Asia [16-18] and several individuals from the Netherlands and the United States [19,20].

Although the genetic and otologic features of PS have been extensively studied in Taiwan [14,15], the thyroid function status of these patients had yet to be clarified. We therefore sought to identify *SLC26A4* gene mutations and characterize the thyroid status in Chinese patients with PS in Taiwan.

2. Patients and methods

2.1. Patients

Seven unrelated affected Chinese subjects (5 male and 2 female; age range, 12-27 years) were identified by the authors (CCL, ASS, TYT,) during routine evaluation in otorhinolaryngology clinics. These patients were suspected to have PS on the basis of the classic findings of sensorineural deafness and EVAs. Most of these patients have been previously described as having otologic features of PS [21].

An additional 100 unrelated normal Chinese subjects were recruited as control subjects to determine whether any novel sequence changes might be common polymorphisms. Demographic data and a detailed family history were obtained from each individual. This study was approved by the institutional review board of the hospital, and informed consent was obtained from each individual.

2.2. Clinical investigations

Thyroid size was measured by ultrasonography using a Toshiba Power-vision 7000 Instrument with an 8-MHz probe (Toshiba instruments, Tokyo, Japan). The total volume of the thyroid gland was calculated using the following equation: volume of each lobe (in milliliters) = anteroposterior diameter (in centimeters) × mediolateral diameter (in centimeters) × craniocaudal diameter (in centimeters) × 0.5233, and the lobe volumes were summed.

All probands with PS underwent additional studies that included a sensory hearing test, high-resolution CT of the temporal bones, and thyroid function tests, including thyroid antibodies (Abs), serum free thyroxine (FT4), thyrotropin (TSH), and Tg. An oral PDT was performed on all individuals except case VI.

2.3. Radiological examination

The vestibular aqueducts were examined by high-resolution CT (GE Medical System, Chicago, IL; HiSpeed Adv CT scanner, canthomeatal axis, 1-mm thickness in each slide). The midway diameter of the vestibular aqueduct (MDVA) was measured at the midway of the vestibular duct from the common crus to the petrosal orifice by a magnifier (Pea Scale Lupe ×10, Fremont, CA; the unit of measurement is 0.1 mm). The petrosal orifice diameter of the vestibular aqueduct was also measured using the same method. Enlarged vestibular aqueduct was diagnosed when the MDVA was more than 1.4 mm [21].

2.4. Endocrine studies

Serum TSH, FT4, anti–thyroid peroxidase (anti-TPO), and anti-Tg Abs were measured by chemiluminescent immunoassays (ADVIA Centaur; Diagnostic Division, Bayer Healthcare, East Walpole, MA), whereas serum Tg was measured by an immunoradiometric assay (CIS Bio International, Gif-sur-Yvette Cedex, France). Thyrotropin receptor autoantibodies were measured using commercially available kits (RSR, Cardiff, UK). To perform the PDT, basal radioactive iodine uptake was measured 2 hours after the administration of 0.925 mBq radioactive iodine (iodine 131) and again 2 hours after oral administration of 1.5 g $KCIO_4$. A *positive test* was defined as a discharge of greater than 15% of radioactivity at 2 hours.

2.5. Analysis of the SLC26A4 gene

Genomic DNA was isolated from EDTA-containing whole blood using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Piscataway, NJ). The 20 coding exons (numbered from 2 through 21) of the *SLC26A4* gene were amplified by polymerase chain reaction (PCR) from peripheral leucocytes using primers and conditions as previously described. For all reactions, the 25-μL reaction mixture contained 200 ng of genomic DNA, 2.0 mmol/L $MgCl_2$, 0.2 mmol/L of each deoxynucleoside triphosphate (dNTP), 0.15 μmol/L of each primer, 1× reaction buffer, and 1 U of FastStart Taq DNA polymerase (Roche, Indianapolis, IN). The PCR products were purified by spin column using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) and sequenced by automated DNA sequencing analysis with fluorescence-labeled

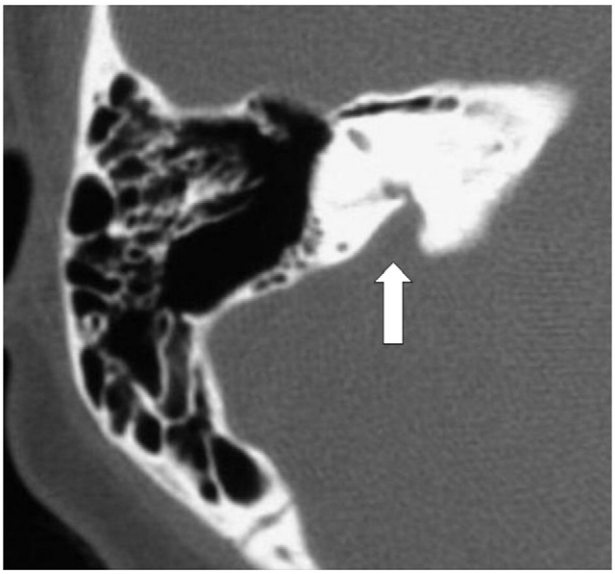

Fig. 1. An EVA (arrow) is shown in the axial high-resolution CT of the right-side temporal bone.

dideoxyterminators (BigDye Terminator V3.1 Cycle Sequencing Kits, Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations (ABI 377-36 Autosequencer, Applied Biosystems).

To screen the genomic DNA from index patient 7 for potential large mutations, we performed long-range PCR using the Expand Long Template PCR System (Roche, Mannheim, Germany). A 25-μL reaction contained 250 ng DNA, 0.3μmol/L of each primer, 0.5 mmol/L of each dNTP, 2.75 mmol/L $MgCl_2$,, and 4 U of enzyme mix. Amplification was set as denaturation at 94°C for 30 seconds, primer annealing at 56°C for 30 seconds, and extension at 68°C for 15 minutes.

2.6. Mutation confirmation

To confirm the presence of the novel exon 9 mutation in the *SLC26A4* gene in our proband and the absence of this mutation in 100 normal subjects, we performed PCR using a modified primer that introduces a base substitution adjacent to codon 360 to create an artificial *Fnu*4HI restriction site only if codon 360 contained the wild-type sequence. After digestion with *Fnu*4HI, the products were analyzed by electrophoresis through a 12.5% polyacrylamide gel with fragments visualized by silver staining kit (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

2.7. Case report

A 17-year-old adolescent boy (I-3) came to the hospital for evaluation of a hearing impairment that developed during early childhood. He was found to have abnormal right and left vestibular aqueducts with midway diameters of 4.38 and 3.67 mm (normal, <1.4), respectively (Fig. 1). Physical examination showed normal development without goiter; and a hearing test showed a pure tone average of 95 and 92 dB in the right and left ear, respectively (normal, <14). Thyroid function tests showed an FT4 level of 18.0 pmol/L (normal, 10.3-21.9), TSH of 0.5 mU/mL (normal, 0.4-4), Tg of 14.5 g/L (normal, <40), anti-TPO Abs of 33.30 U/mL (normal, <60), anti-Tg Abs of 17.01 U/mL (normal, <60), TSH receptor autoantibodies of 8% (normal, <10%), and a

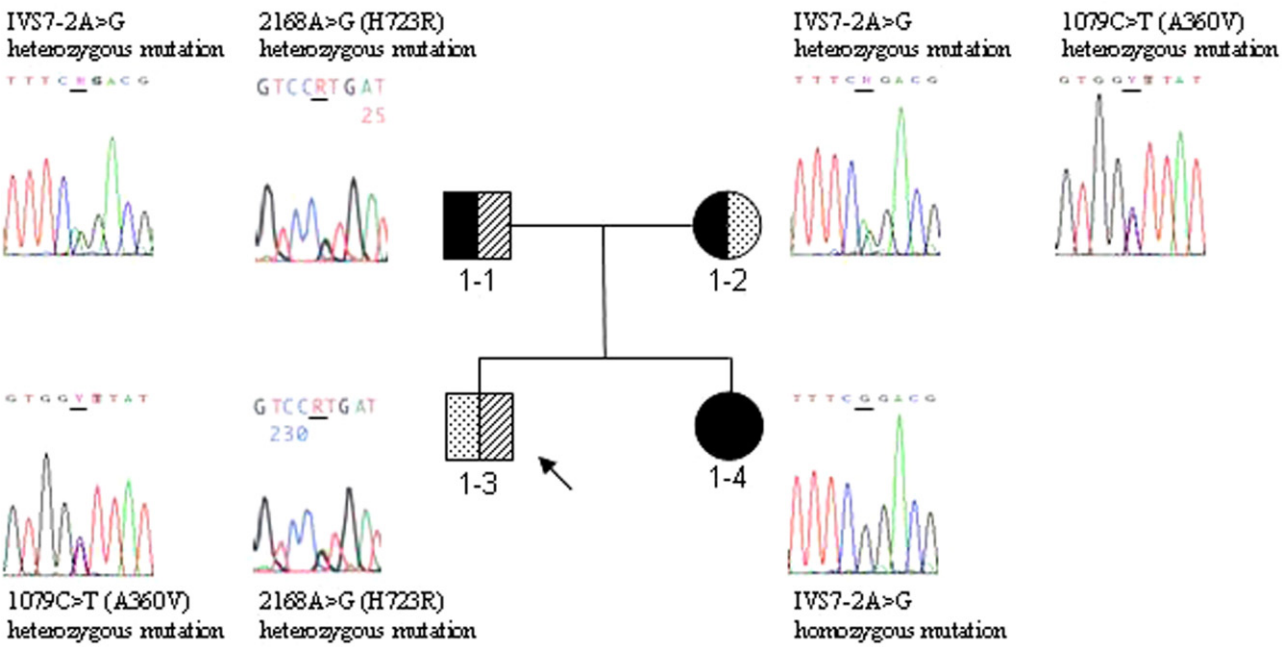

Fig. 2. Pedigree of family with *SLC26A4* gene mutations. All individuals are affected. The mutant alleles are indicated by shaded symbols ▨◍ (A360V), hatched symbols ▨◍(H723R), and solid symbols ■ ● (IVS7-2A>G). The sequence of homozygous and compared heterozygous *SLC26A4* gene mutations.

negative PDT. His sister also had hearing impairment and EVA on CT scan. His affected mother (I-2) and sister (I-4) had PDT results of 19.6% and 20.4%, respectively. The echographic pattern of the thyroid of his mother and sister, who both had positive anti-thyroid Abs, showed a normal structure. The pedigree is shown in Fig. 2.

3. Results

The clinical features, thyroid studies, and mutational analysis of *SLC26A4* gene are shown in Table 1. All probands studied had hearing impairment and increased MDVAs, findings that are characteristic of PS. Thyroid abnormalities were common but varied: patient I-2 had an elevated serum anti-TPO titer of 275.8 U/mL (normal, <60), and patient I-4 had an elevated titer of 363.20 U/mL (normal, <60). These 2 individuals—mother (I-2) and daughter (I-4)—also had a slightly positive PDT. The serum Tg level was increased in 2 individuals (III and V), whereas goiter was identified in another 2 individuals (I-2 and V). Only one subject (V) had both a goiter and elevated serum Tg levels. We identified compound heterozygous or homozygous mutations of the *SLC26A4* gene in 6 probands but no such mutations in any of the 50 normal subjects. The mutations included a compound heterozygous missense mutation in exon 9 (1079C>T) that resulted in the replacement of alanine by valine at codon 360 (A360V) and a mutation in exon 19 (2168A>G) that resulted in the replacement of histidine by arginine at codon 723 (H723R) (family I). The affected subjects in families II and III shared a homozygous mutation in intron 7 (IVS7-2A>G). Probands IV and V had a compound heterozygous mutation in intron 7 (IVS7-2A>G) and a mutation in exon 19 (2168A>G) that resulted in the replacement of histidine by arginine at codon 723 (H723R). Proband VI had a compound heterozygous mutation in intron 7 (IVS7-2A>G) and a missense mutation in exon 12 (1343C>T) that resulted in the replacement of serine by leucine at codon 448 (S448L). One of the mutations identified (A360V) was novel.

4. Discussion

In contrast to the typical thyroid disturbances that develop in late childhood to early adulthood in 80% of patients with PS ranging from subclinical hypothyroid to euthyroid goiters [4], we found more modest thyroid problems in our patients. Of the 5 patients who were in late childhood (I-3, V) or adulthood (I-1, I-2, and II), only 2 (I-2 and V) had goiters (40%) and one (I-2) also had positive TPO Abs. Elevation of serum Tg, which can often be found in patients with PS [22], was also identified in 2 individuals. The perchlorate test was slightly positive in the 2 patients of family I, indicating a partial iodide organification defect.

There are several possible explanations for the low incidence of thyroid impairment in our study: First, thyroid disturbance may have been mild because the daily dietary intake of iodine in Taiwan is much higher than the recommended intake [23]. This hypothesis is consistent with reports of a lower incidence of thyroid dysfunction in patients with *SLC26A4* mutations in Japan, where the iodine

Table 1
Summary of clinical features and mutational analysis of all individuals

Family	Mutation	Age (y)	Sex	PDT (%)	TFT (FT4, TSH)	Tg (μg/L)	Thyroid Size (mL)	Hearing (dB) (right, left)	MDVA (mm) (right, left)
I-1	Intron 7, IVS7-2A>G Exon 19, H723R	42	M	0	(12.5, 2.2)	26.0	22.9	ND	ND
I-2	Intron 7, IVS7-2A>G Exon 9, A360V	40	F	24.3	(16.9, 1.3)	21.7	48.2	ND	ND
I-3	Exon 9, A360V Exon 19, H723R	17	M	5.8	(18.0, 0.5)	14.5	18.8	(95, 92)	(4.38, 3.67)
I-4	Intron 7, IVS7-2A>G Intron 7, IVS7-2A>G	12	F	20.4	(18.0, 3.6)	19.1	14.5	(105, 95)	(4.11, 4.01)
II	Intron 7, IVS7-2A>G Intron 7, IVS7-2A>G	27	M	13.6	(18.0, 0.9)	30.2	15.7	(98, 102)	(2.08, 2.50)
III	Intron 7, IVS7-2A>G Intron 7, IVS7-2A>G	12	M	6.3	(19.3, 1.8)	81.7	23.3	(98, 100)	(2.31, 2.31)
IV	Intron 7, IVS7-2A>G Exon 19, H723R	14	M	11.8	(15.4, 0.9)	32.5	27.7	(75, 88)	(2.45, 2.65)
V	Intron 7, IVS7-2A>G Exon 19, H723R	17	F	9.5	(15.4, 0.7)	88.2	42.1	(97, 110)	(2.08, 1.88)
VI	Intron 7, IVS7-2A>G Exon 12, S448L	12	M	ND	(16.7, 1.3)	8.4	8.6	(82, 57)	(2.34, 2.34)
VII	Normal Normal	15	F	0	(18.0, 2.7)	32.0	6.0	(85, 87)	(4.22, 3.44)

A *positive PDT* was defined as a discharge of greater than 15% at 2 hours. Values for thyroid-stimulating hormone are expressed in milli–international units per liter (0.4-4.0); FT4, picomoles per liter (10.3-21.9); Tg, micrograms per liter (<35); hearing: pure tone average, decibels (<20); and MDVA, millimeters (<1.4). TFT indicates thyroid function test; TSH, thyroid-stimulating hormone; ND, not detected.

intake is also high [17]. These authors have speculated that *SLC26A4* mutations in countries with a high iodide intake are more likely to be associated with familial EVA rather than PS [17]. Second, the young age of the patients in our study may also contribute to the low incidence of goiter.

Of the 7 families we studied, only the proband of family VII did not show any genetic abnormality including a large deletion, insertion, or rearrangement in the *SLC26A4* gene (data not shown). All affected individuals in families I through VI carried mutation in both *SLC26A4* alleles. All mutations had been previously identified except for A360V.

Proband 1 had compound heterozygous A360V and H723R mutations. Evidence that the compound heterozygous A360V and H723R mutations were causally related to PS in this family is based on the following: (1) the A360V residue is highly conserved in multiple species, suggesting that any substitution at this codon would have a biological consequence; (2) the A360V was not found in 100 unrelated subjects, suggesting that it is not a common polymorphism (data not shown); and (3) there is an absence of any other mutations in the rest of coding sequence. The A360V mutation occurs in the putative eighth transmembrane domain [8].Both parents (I-1 and II-2) and one sibling also had hearing impairment; the mother and the daughter were positive for both PDT and anti-TPO Abs. Therefore, both PS and positive anti-TPO Abs can coexist in members of a family [24]. The phenotypic overlap between PS and autoimmune thyroiditis is well known to cause diagnostic difficulties [24].

The consequence of the IVS7-2A>G mutation, resulting in the loss of the entire exon 8 and in the splicing of exons 7 and 9, had been proven by using the reverse transcriptase PCR with RNA from the peripheral blood [15]. Remarkably, thyroid function in our 3 homozygous patients (patients I-4, II, and III) remained normal, except for patient I-4 who had abnormal thyroid Abs, a positive PDT, and wider MDVA. Accordingly, this mutation seems to have very modest effects on the protein in the thyroid gland. The low incidence of thyroid impairment in these 3 patients may also be attributed to the higher iodine intake in Taiwan.

Probands IV and V had identical compound heterozygous IVS7-2A>G and H723R genotypes, but different phenotypes. Patient V, who was 16 years old, had an elevated Tg level and a goiter, but normal results in the thyroid function tests and PDT. Both he and proband IV had average hearing impairment in the right and left pure tone audiometry and increased midway diameter of the right and left vestibular aqueducts. Consistent with our findings, phenotypic variability with respect to thyroid findings has previously been reported in 2 families carrying the same PDS mutation [25] as well as intrafamilial variability in carriers of the same mutation [26]. The interaction between different environmental and genetic factors is another potential explanation for such variability [13,22].

We did not identify any mutations in the *SLC26A4* gene in individual VII. However, because only exons 2 to 21 of the *SLC26A4* gene were sequenced, the possibility for the presence of mutation(s) in the other regions of the gene cannot be excluded. It is also possible that other genetic factors may account for the deafness in this individual.

In conclusion, we have identified mutations in the *SLC26A4* gene in 6 of 7 probands with PS in Taiwan, including a novel mutation (A360V). Because goiter and thyroid dysfunction are not always present in PS patients, it is likely that *SLC26A4* gene defects are responsible for a significant proportion of nonsyndromic deafness with EVA. This is supported by the observation that the DFNB4, an autosomal recessive form of nonsyndromic deafness, is allelic to PS [27]. The clinical diagnosis of PS and DFNB4 can be challenging, making mutation screening of *SLC26A4* a valuable test.

Acknowledgments

This study was supported by grants from Medical Research-VGH-94-287 and National Science Council-NSC-95-2314-B-075-070 Taiwan, ROC.

We gratefully acknowledge the assistance of Prof Michael A Levine for critical reading of the manuscript.

References

[1] Reardon W, Trembath RC. Pendred syndrome. J Med Genet 1996;33:1037-40.
[2] Phelps PD, Coffey RA, Trembath RC, et al. Radiological malformations of the ear in Pendred's syndrome. Clin Radiol 1998;53:268-73.
[3] Fugazzola L, Mannavola D, Cerutti N, et al. Molecular analysis of the Pendred's syndrome gene and magnetic resonance imaging studies of the inner ear are essential for the diagnosis of true Pendred's syndrome. J Clin Endocrinol Metab 2000;85:2469-75.
[4] Reardon W, Coffey R, Chowdhury T, et al. Prevalence, age of onset, and natural history of thyroid disease in Pendred's syndrome. J Med Genet 1999;36:595-8.
[5] Morgans ME, Trotter WR. Association of congenital deafness with goiter: the nature of the thyroid defect. Lancet 1958;1:607-9.
[6] Sheffield VC, Kraiem Z, Beck JC, et al. Pendred syndrome maps to chromosome 7q21-34 and is caused by an intrinsic defect in thyroid iodine organification. Nat Genet 1996;12:424-6.
[7] Coyle B, Coffey R, Armour JAL, et al. Pendred syndrome (goitre and sensorineural hearing loss) maps to chromosome 7 in the region containing the nonsyndromic deafness gene DFNB4. Nat Genet 1996;12:421-3.
[8] Everett LA, Glaser B, Beck JC, et al. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet 1997;17:411-22.
[9] Li XC, Everett LA, Lalwani AK, et al. A mutation in PDS causes nonsyndromic recessive deafness. Nat Genet 1998;18:215-7.
[10] Scott DA, Wang R, Kreman TM, Sheffield VC, Karniski LP. The Pendred syndrome gene encodes a chloride-iodide transport protein. Nat Genet 1999;21:440-3.
[11] Gillam MP, Sidhaye AR, Lee EJ, et al. Functional characterization of pendrin in a polarized cell system. J Biol Chem 2004;279:13004-10.
[12] Fraser GR. Association of congenital deafness with goiter (Pendred's syndrome): a study of 207 families. Ann Hum Genet 1965;28:201-49.
[13] 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;17:403-11.

[14] Wu CC, Yeh TH, Chen PJ, Hsu CJ. Prevalent SLC26A4 mutations in patients with enlarged vestibular aqueduct and/or Mondini dysplasia: a unique spectrum of mutations in Taiwan, including a frequent founder mutation. Laryngoscope 2005;115:1060-4.

[15] Yang JJ, Tsai CC, Hsu HM, et al. Hearing loss associated with enlarged vestibular aqueduct and Mondini dysplasia is caused by splice-site mutation in the PDS gene. Hear Res 2005;199:220-30.

[16] Yong AML, Goh SS, Zhao Y, et al. Two Chinese families with Pendred's syndrome—radiological imaging of the ear and molecular analysis of the pendrin gene. J Clin Endocrinol Metab 2001;86: 3907-11.

[17] Sato E, Nakashima T, Miura Y, et al. Phenotypes associated with replacement of His by Arg in the Pendred syndrome gene. Eur J Endocrinol 2001;145:697-703.

[18] Park HJ, Shaukat 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: 242-8.

[19] Hauwe PV, Everett LA, Coucke P, et al. Two frequent mutations in Pendred Syndrome. Hum Mol Genet 1998;7:1099-104.

[20] Prasad S, Kolln KA, Cucci RA, et al. Pendred syndrome and DFNB4-mutation screening of SLC26A4 by denaturing high-performance liquid chromatography and the identification of eleven novel mutations. Am J Med Genet 2004;124:1-9.

[21] Lai CC, Shiao AS. Chronological changes of hearing in pediatric patients with large vestibular aqueduct syndrome. Laryngoscope 2004;114:832-8.

[22] Kopp P, Karamanoglu AO, Sabacan L. Phenocopies for deafness and goiter development in a large inbred Brazilian kindred with Pendred's syndrome, associated with a novel mutation in the PDS gene. J Clin Endocrinol Metab 1999;84:336-41.

[23] Lin HD, Lo JG, Ching KN. Amount of urinary iodine excretion in residents of Taipei city: a hospital-based study. J Chin Med Assoc 1991;48:20-4.

[24] Vaidya B, Coffey R, Coyle B, et al. Concurrence of Pendred syndrome, autoimmune thyroiditis, and simple goiter in one family. J Clin Endocrinol Metab 1999;84:2736-8.

[25] Masmoudi S, Charfedine I, Hmani M, et al. Pendred syndrome: phenotypic variability in two families carrying the same PDS missense mutation. Am J Med Genet 2000;90:38-44.

[26] Napiontek U, Borck G, Müller-Forell W, et al. Intrafamilial variability of the deafness and goiter phenotype in Pendred syndrome caused by a T416P mutation in the SLC26A4 gene. J Clin Endocrinol Metab 2004;89:5347-51.

[27] Scott DA, Wang R, Kreman 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;9:1709-15.