

## ORIGINAL ARTICLE

# Mutation spectrum and genotype–phenotype correlation of hearing loss patients caused by *SLC26A4* mutations in the Japanese: a large cohort study

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Mutations in *SLC26A4* cause a broad phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss associated with enlarged vestibular aqueduct. Identification of these mutations is important for accurate diagnosis, proper medical management and appropriate genetic counseling and requires updated information regarding spectrum, clinical characteristics and genotype–phenotype correlations, based on a large cohort. In 100 patients with bilateral enlarged vestibular aqueduct among 1511 Japanese hearing loss probands registered in our gene bank, goiter data were available for 79, of whom 15 had Pendred syndrome and 64 had nonsyndromic hearing loss. We clarified the mutation spectrum for the *SLC26A4* mutations and also summarized hearing levels, progression, fluctuation and existence of genotype–phenotype correlation. *SLC26A4* mutations were identified in 82 of the 100 patients (82.0%). Of the Pendred syndrome patients, 93% (14/15) were carriers, as were 77% (49/64) of the nonsyndromic hearing loss patients. Clinical characteristics of patients with *SLC26A4* mutations were congenital, fluctuating and progressive hearing loss usually associated with vertigo and/or goiter. We found no genotype–phenotype correlations, indicating that, unlike in the case of *GJB2* mutations, the phenotype cannot be predicted from the genotype. Our mutation analysis confirmed the importance of mutations in the *SLC26A4* gene among hearing loss patients with enlarged vestibular aqueduct and revealed the mutation spectrum, essential information when performing genetic testing.

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## INTRODUCTION

Based on our genetic screening, *SLC26A4* is the second most common responsible gene in Japanese deafness patients.<sup>1</sup> Mutations in the *SLC26A4* gene are known to be responsible for a broad phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss with enlarged vestibular aqueduct (EVA). The prevalent association of *SLC26A4* mutations in these patients (90% in Pendred syndrome and 78.1% in nonsyndromic hearing loss associated with EVA) indicates the importance of this gene in the pathophysiology of this category of hearing impairment.<sup>2</sup> More than 160 mutations have been found in *SLC26A4* (Pendred/BOR Homepage, <http://www.healthcare.uiowa.edu/labs/pendredandbor/>), and different mutational spectrums among different ethnic groups have been reported.<sup>2</sup> The identification of *SLC26A4* mutations enables more appropriate genetic counseling and proper medical management for these patients. For such clinical application, updated information regarding mutation spectrum, clinical characteristics and

genotype–phenotype correlations based on a large cohort is needed. In addition to our previous reports,<sup>1–7</sup> the present study was performed using a large cohort of patients to collect updated data and summarize these data to enable more precise decision making by ear, nose and throat clinicians.

## MATERIALS AND METHODS

### Subjects

Data on 1511 independent probands and 1545 family members were collected from 33 ear, nose and throat departments nationwide in Japan and registered in our gene bank. All subjects or next of kin, caretakers or guardians on behalf of the minors/children gave prior written informed consent for participation in the project, and the Shinshu University Ethical Committee as well as the respective Ethical Committees of the other participating institutions of the Deafness Gene Study Consortium (Hokkaido University, Hirosaki University, Iwate Medical University, Tohoku University, Yamagata University, Fukushima Medical University, Jichi Medical University, Gunma University, Nihon University, Nippon Medical School, Nippon Medical School Tama Nagayama

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Computerized tomography scan was used to diagnose EVA (according to the criteria of EVA: a diameter of > 1.5 mm at the midpoint between the common crus and the external aperture), and they were clinically well characterized by repeated auditory examinations.

The 100 subjects (51 males and 49 females) from among the 1511 probands who met the criteria of bilateral EVA and who ranged in age from 0 to 59 years with a mean age of 13.9 years at the time of examination were enrolled in the current study. Fifteen subjects had Pendred syndrome and 64 had nonsyndromic hearing loss.

The controls were 192 unrelated Japanese healthy individuals with normal hearing evaluated by auditory testing.

### Mutation analysis

To identify *SLC26A4* mutations, a DNA fragment containing all the exons of *SLC26A4*, including flanking intronic sequences, was sequenced as described elsewhere.<sup>4</sup> New variants were tested in 192 unrelated normal hearing controls.

Possible pathologic mutations were defined as (1) mutations found to be homozygotes or compound heterozygotes (and determined by segregation study); (2) variants that were not found, or were very few, in the 192 control subjects; and (3) amino acids that were well conserved among various species.

### Clinical evaluations

Hearing levels were determined by pure-tone audiometry in adults. For the young patients, conditioned orientation response audiometry or auditory steady-state response was used. Clinical data, including hearing loss progression, fluctuation, episodes of tinnitus and vestibular dysfunction (vertigo, dizziness) and goiter, were collected by anamnestic evaluation. For genotype–phenotype correlation analysis, one-way analysis of variance (Tukey's honest significant difference (HSD) test), Kruskal–Wallis test and multivariate statistics (multiple regression analysis and logistic regression analysis) were used.

## RESULTS

### *SLC26A4* mutation spectrum

There were a total of 39 *SLC26A4* mutations found in the probands with bilateral EVA (Table 1). These mutations were either homozygous, compound heterozygous or heterozygous with no other mutations being detectable. There were two nonsense mutations (p.S610X, p.L727X), three deletion frameshift mutations (c.322delC, c.917delT, c.1219delCT) and three insertion frameshift mutations (c.139insC, c.1652insT, c.2111ins GCTGG). Seven splice site mutations were found (c.416-1G>A, c.600+1G>T, c.601-1G>A, c.919-2A>G, c.1001+1G>A, c.1002-9A>G and c.1707+5G>A).

There were 24 missense mutations (p.P76S, p.T94I, p.P123S, p.M147V, p.P297Q, p.K369E, p.A372V, p.N392Y, p.G396E, p.T410M, p.A434T, p.G439R, p.S448L, p.T527P, p.I529S, p.S532I, p.C565Y, p.R581S, p.S657N, p.V659L, p.S666F, p.T721M, p.H723R and p.H723Y). To evaluate the evolutionary conservation of the amino acids affected by these missense mutations, we made an alignment of the *SLC26A4* amino acid sequence of four mammalian species: human, rat, cow and mouse. On the basis of this alignment, all missense mutations had changed evolutionary conserved amino acids. Of these mutations, nine variants had not been reported. We checked the 192 control subjects with normal hearing, but with the exception of p.H723R in 1 case, no mutations were detected.

Sequencing identified mutations in 82 of the 100 patients (82.0%). Mutations were detected in 93% of those with Pendred syndrome (14/15) and 77% (49/64) of those with nonsyndromic hearing loss. Of these, 15/100 (15.0%) were homozygous, 51/100 (51.0%) were compound heterozygous and 16/100 (16.0%) were heterozygous (Table 2).

The most frequent mutation was p.H723R that accounted for 36.0%, and the second was c.919-2A>G found in 7.0%, followed by c.1707+5G>A (4.0%). Frequency of the other 36 mutations was very low (0.5–2.0%).

### Clinical findings

Table 2 shows the clinical details for the 100 subjects.

The subjects had an average hearing level of 80.9 dB (7.5–112.5 dB), with hearing loss that was mild in 5, moderate in 22, severe in 37, profound in 19 and unknown in 12. Regarding onset age of hearing loss, 45 patients were congenital, 18 were prelingual (1–3 years old), 20 were from 4 to 14 years and 17 were unknown. These results clearly indicated that early onset is dominant in patients with EVA. Also, 70 patients (70%) showed progressive hearing loss and 56 patients (56.0%) felt fluctuation of hearing. With regard to the 79 patients for whom data on vertigo were available, 41 patients complained of vertigo and 38 did not. Of the 79 patients for whom data on goiter were available, 15 had goiter and 64 did not, with an onset age from 12 to 33 years. As to family history, all families were recessive inheritance or sporadic cases.

Genotype–phenotype correlations for diagnostic age, fluctuation, vertigo, tinnitus and goiter are summarized in Figure 1.

We defined nonsense or frameshift mutations as truncating (T) and missense mutations as nontruncating (NT) and classified the genotypes as truncating/truncating (T/T), truncating/nontruncating (T/NT) or nontruncating/nontruncating (NT/NT). Significant differences were not found between the groups in any of the clinical features (Tukey's HSD test was used for diagnostic age and Kruskal–Wallis test was used for fluctuation, vertigo, tinnitus and goiter, all tests indicated  $P > 0.05$ ; Figure 1). Figure 2 shows the relationship between hearing loss severity and the mutation (T or NT) that also showed no significant differences (Tukey's HSD test,  $P > 0.05$ ). We also performed multivariate statistics (multiple regression analysis and logistic regression analysis) and we found that only the age of the patients correlated with the hearing loss severity while the genotype of *SLC26A4* mutations did not significantly affect the hearing loss severity ( $P > 0.05$ ).

### DISCUSSION

The present large cohort study revealed a high prevalence (82%; 82/100) of *SLC26A4* mutations in sensorineural hearing loss patients with EVA in Japanese. The frequency (8.7%) is the second most common next to *GJB2* that is found in 16.2% of overall and 25.6% of congenital hearing loss patients.<sup>1</sup>

Our mutation analysis results confirmed the previous reports that indicated the importance of this gene among hearing loss patients with EVA. This study also added novel mutations and summarized updated data for the precise molecular diagnosis.

First, the high prevalence (82%) of *SLC26A4* mutations in EVA patients is compatible with the high prevalence of *SLC26A4* mutations reported in eastern Asians; that is, 97.9% in Chinese,<sup>8</sup> and 92% in Koreans.<sup>9</sup> These frequencies are higher than those reported in Caucasoid populations (20% in Americans,<sup>10</sup> 40.0% in French<sup>11</sup> and 28.4% in Spanish<sup>12</sup>). It is still an open question whether other genes are involved in the EVA patients without *SLC26A4* mutations.

**Table 1 Possible pathogenic variants found in enlarged vestibular aqueduct (EVA) subjects (n = 100)**

Nucleotide change	Amino acid change	Exon	Frequency (n = 100)			Allele frequency (in 200 alleles)	References
			Homozygote	Compound heterozygote	Heterozygote		
c. 139insC		1		1		0.50	This study
c. 266C>T	p. P76S	2		1		0.50	Suzuki <i>et al.</i> <sup>5,6</sup>
c. 281C>T	p. T94I	3		1		0.50	Wang <i>et al.</i> <sup>7,8</sup>
c. 322delC		4		1		0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 367C>T	p. P123S	4		1		0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 416-1G>A		Intron 4		2		1.00	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 439A>G	p. M147V	5		2		1.00	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 600 + 1G>T		Intron 5		1		0.50	This study
c. 601-1G>A		Intron 5		1		0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 890C>A	p. P297Q	7		1		0.50	This study
c. 917delT		7			1	0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 919-2A>G		Intron 7	1	11	1	7.00	Coucke <i>et al.</i> <sup>21</sup>
c. 1001 + 1G>A		Intron 8		2		1.00	Coyle <i>et al.</i> <sup>22</sup>
c. 1002-9A>G <sup>a</sup>		Intron 8		1		0.50	This study
c. 1105A>G	p. K369E	9		1		0.50	Usami <i>et al.</i> <sup>2,3</sup>
c. 1115C>T	p. A372V	9		1		0.50	Usami <i>et al.</i> <sup>2,3</sup>
c. 1174A>T	p. N392Y	10		3		1.50	Park <i>et al.</i> <sup>14,16</sup>
c. 1187G>A	p. G396E	10		1		0.50	This study
c. 1219delCT		10		1		0.50	This study
c. 1229C>T	p. T410M	10	1	1		1.50	Coyle <i>et al.</i> <sup>22</sup>
c. 1300G>A	p. A434T	11			1	0.50	This study
c. 1315G>A	p. G439R	11		1		0.50	Suzuki <i>et al.</i> <sup>5,6</sup>
c. 1343C>T	p. S448L	11		1		0.50	Wang <i>et al.</i> <sup>7,8</sup>
c. 1579A>G	p. T527P	14		2		1.00	Suzuki <i>et al.</i> <sup>5,6</sup>
c. 1586T>G	p. I529S	14		1		0.50	Wang <i>et al.</i> <sup>7,8</sup>
c. 1595G>T	p. S532I	14		2		1.00	Usami <i>et al.</i> <sup>3,17</sup>
c. 1652insT		15		3	1	2.00	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 1694G>A	p. C565Y	15		1		0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 1707 + 5G>A		Intron 15	1	6		4.00	Park <i>et al.</i> <sup>8,9</sup>
c. 1743G>C	p. R581S	16		2		1.00	Iwasaki <i>et al.</i> <sup>5,18</sup>
c. 1829C>A	p. S610X	17		1		0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 1970G>A	p. S657N	17		1		0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 1975G>C	p. V659L	17		3		1.50	Wang <i>et al.</i> <sup>7,8</sup>
c. 1997C>T	p. S666F	17		1		0.50	Tsukamoto <i>et al.</i> <sup>2,4</sup>
c. 2111ins GCTGG		19		1	1	1.00	Usami <i>et al.</i> <sup>2,3</sup>
c. 2162C>T	p. T721M	19		1	1	1.00	Usami <i>et al.</i> <sup>2,3</sup>
c. 2168A>G	p. H723R	19	11	40	10	36.00	Usami <i>et al.</i> <sup>2,3</sup>
c. 2168C>T	p. H723Y	19	1			1.00	This study
c. 2180T>A	p. L727X	19		1		0.50	This study

<sup>a</sup>c. 1002-9A>G, uncertain pathogenicity.

Mutations in *FOXII*,<sup>13</sup> a modulatory gene of *SLC26A4*, were not found in our series of patients (data not shown). As seen in previous mutation screening reports, we encountered a significant number of heterozygous cases without a second mutation even after direct sequencing of the coding region of the gene. It is highly likely that there is one more occult mutation somewhere because patients with heterozygous mutation are associated with EVA.

Second, it is evident that the mutation spectrum found in the Japanese population is quite different from that in Caucasoid populations, but similar to the mutation spectrum reported in the Asian populations, especially Koreans.<sup>8-12,14</sup> There are two frequent mutations in east Asians, namely p.H723R and c.919-2A>G. p.H723R is most prevalent in the Japanese and Korean populations,<sup>8</sup> whereas c.919-2A>G is most common in the Chinese.<sup>7</sup>

The existence of a genotype-phenotype correlation is still controversial.<sup>6,12,15</sup> Mutations in *SLC26A4* can cause a broad phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss associated with EVA. In the present study, various features of the phenotype were compared with the genotypes. We defined nonsense or frame shift mutations as truncating (T) and missense mutations as non-truncating (NT) and classified the genotypes as truncating/truncating (T/T), truncating/non-truncating (T/NT), or non-truncating/non-truncating (NT/NT). However, statistical differences were not found between the groups in any of the clinical features ( $\chi^2$  tests,  $P > 0.05$ ; Figure 1).

Concerning the relationship between the severity of hearing loss and individual *SLC26A4* mutations, several functional studies have demonstrated the property of transporter function.<sup>16-18</sup> Furthermore,

**Table 2** Phenotypes and genotypes of affected EVA subjects

ID	Age	Mutation allele 1/allele 2	Age of							Threshold (Rt) (dB) <sup>a</sup>	Threshold (Lt) (dB) <sup>a</sup>	Hearing level in the low frequencies <sup>b</sup>
			awareness	Progression	Fluctuation	Tinnitus	Vertigo	Goiter				
77	12	p. [917delT];[=]	12	+	+	+	+	-	58.75	45	49.375	
237	7	p. [T721M];[H723R]	0	+	-	-	+	-	112.5	68.75	83.75	
334	23	p. [A372V];[H723R]	0	NA	NA	+	NA	NA	96.25	83.75	81.9	
695	4	p. [K369E];[H723R]	0	+	-	NA	NA	-	100	90	89.4	
752	18	p. [1652insT];[=]	1	-	-	+	+	+	98.75	102.5	96.3	
1045	25	p. [H723R];[H723R]	0	+	NA	-	+	+	78.75	90	85.6	
1306	3	p. [919-2A>G];[H723R]	0	NA	NA	NA	NA	NA	NA	NA	NA	
1365	20	p. [T721M];[=]	2	NA	NA	NA	NA	NA	96.25	105	96.9	
1379	10	p. [1001 + 1G>A];[H723R]	0	+	+	-	-	NA	66.25	46.25	57.5	
1432	6	p. [H723R];[=]	0	+	-	-	-	NA	102.5	105	100.0	
1625	16	p. [919-2A>G];[H723R]	0	+	+	NA	+	NA	100	95	88.1	
1795	NA	p. [H723R];[=]	NA	NA	NA	NA	NA	NA	NA	NA	NA	
1820	12	p. [H723R];[H723R]	5	+	+	-	-	NA	72.5	73.75	61.3	
1957	7	p. [S666F];[H723R]	3	+	+	NA	NA	-	95	101.25	93.8	
1961	12	p. [C565Y];[H723R]	0	+	N/A	NA	NA	NA	108.75	110	103.8	
2010	12	p. [416-1G>A];[H723R]	9	+	+	-	-	+	80	91.25	81.3	
2202	4	p. [P297Q];[T527P]	3	+	-	-	-	-	77.5	76.25	73.8	
2331	31	p. [H723R];[H723R]	0	+	+	+	+	+	90	100	87.5	
2449	1	p. [139insC];[322delC]	0	NA	NA	-	+	-	100	85	92.5	
2462	52	p. [M147V];[H723R]	2	+	+	-	-	-	98.75	95	88.1	
2498	0	p. [919-2A>G]; [1001 + 1G>A]	0	+	+	NA	-	-	86.25	86.25	83.8	
2538	10	p. [H723R];[H723R]	3	+	+	-	-	+	81.25	55	66.9	
2621	3	p. [R581S];[H723R]	0	+	+	-	-	-	91.25	91.25	90.0	
2695	13	p. [T527P];[H723R]	2	+	+	+	+	-	62.5	61.25	63.1	
2728	3	p. [919-2A>G];[H723R]	1	+	+	-	-	-	97.5	97.5	93.8	
2798	15	p. [H723R];[H723R]	4	+	+	NA	+	+	52.5	96.25	66.3	
2804	2	p. [1707 + 5G>A];[H723R]	0	+	+	-	-	-	78.75	78.75	82.5	
3072	44	p. [G439R];[H723R]	6	+	+	+	+	-	110	108.75	105.0	
3074	21	p. [H723R]; [ = ]	2	+	+	+	+	+	105	106.25	99.4	
3298	6	p. [919-2A>G];[H723R]	0	+	+	+	+	-	73.75	110	86.9	
3301	4	p. [416-1G>A];[H723R]	0	+	+	+	+	-	65	72.5	68.1	
3442	6	p. [919-2A>G];[H723R]	NA	+	NA	+	+	-	81.25	50	60.0	
3450	14	p. [H723R];[H723R]	0	+	+	+	+	-	110	73.75	87.5	
3561	6	p. [H723Y];[H723Y]	4	NA	NA	NA	NA	NA	83.75	65	71.3	
3994	59	p. [601-1G>A];[H723R]	10	+	+	+	+	+	96.0	94	91.3	
3996	8	p. [H723R];[1652insT]	0	+	-	+	-	-	100	110	98.1	
3999	8	p. [H723R];[1652insT]	0	+	+	-	+	-	30	50	40.0	
4050	5	p. [M147V];[H723R]	1	+	+	+	+	-	107.5	85	93.8	
4097	3	p. [N392Y];[1002-9A>G]	0	-	-	-	-	-	106.25	85	93.1	
4098	26	p. [N392Y];[919-2A>G]	2	-	+	+	+	-	110	37.5	71.3	
4102	5	p. [N392Y];[H723R]	0	+	+	+	+	-	95	78.75	83.1	
4131	10	p. [H723R];[=]	8	+	+	-	-	-	81.25	60	70.6	
4144	21	p. [H723R];[H723R]	4	+	NA	+	+	-	93.75	105	95.6	
4232	15	p. [V659L];[H723R]	NA	-	+	+	+	-	60	92.5	69.4	
4299	4	p. [S532I];[2111ins GCTGG]	3	-	+	-	+	-	17.5	70	42.5	
4305	14	p. [A434T];[=]	0	+	-	+	-	-	110	110	105.0	
4320	10	p. [G396E];[S532I]	NA	+	+	+	-	-	72.5	80	72.5	
4338	6	p. [R581S];[H723R]	0	+	+	+	+	-	78.75	52.5	64.4	
4380	10	p. [1707 + 5G>A];[H723R]	2	+	+	-	-	-	96.25	81.25	84.4	
4386	21	p. [H723R];[H723R]	NA	+	+	+	+	+	77.5	93.75	85.0	
4398	4	p. [1652insT];[H723R]	2	+	+	+	+	-	70	97.5	86.9	
4434	8	p. [T410M];[1707 + 5G>A]	1	+	+	-	+	-	92.5	100	91.3	
4469	11	p. [H723R]; [ = ]	0	+	NA	-	-	-	20	21.25	16.9	
4485	40	p. [H723R]; [ = ]	10	+	+	+	+	-	56.25	65	58.8	

Table 2 (Continued)

ID	Age	Mutation allele 1/allele 2	Age of awareness	Progression	Fluctuation	Tinnitus	Vertigo	Goiter	Threshold (Rt) (dB) <sup>a</sup>	Threshold (Lt) (dB) <sup>a</sup>	Hearing level in the low frequencies <sup>b</sup>
4486	20	p. [1707 + 5G>A]; [1707 + 5G>A]	4	+	+	+	+	+	72.5	95	78.1
4490	25	p. [T410M];[T410M]	0	-	-	+	+	+	87.5	92.5	90.0
4508	29	p. [H723R];[H723R]	5	+	+	-	-	-	85	110	91.9
4518	26	p. [H723R];[919-2A>G]	0	+	+	+	+	-	105	97.5	98.1
4530	5	p. [H723R];[919-2A>G]	0	+	+	-	+	-	67.5	86.25	71.9
4545	12	p. [1707 + 5G>A];[H723R]	4	+	+	+	+	+	86.25	28.75	53.1
4549	13	p. [V659L];[1219delCT]	NA	+	+	+	+	-	38.75	50	38.1
4663	0	p. [1707 + 5G>A];[H723R]	0	-	+	NA	NA	-	68.75	68.75	99.2
4696	0	p. [V659L];[H723R]	0	+	-	NA	NA	-	NA	NA	97.5
4362	26	p. [H723R]; [=]	6	+	-	-	-	-	70	68.75	63.8
4513	34	p. [H723R]; [=]	NA	+	+	+	NA	-	71.25	53.75	61.3
4645	23	p. [919-2A>G]; [=]	14	+	-	+	-	-	96.25	105	93.8
723	NA	p. [H723R]; [=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
724	NA	p. [2111ins5bp]; [=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
742	NA	p. [H723R]; [=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
1975	3	p. [H723R];[H723R]	0	NA	NA	NA	NA	NA	80	70	62.5
2082	2	p. [H723R];[H723R]	0	-	-	-	-	-	NA	NA	NA
4735	9	p. [H723R];[919-2A>G]	0	+	+	+	+	-	107.5	110	103.8
195	20	p. [=];[=]	2	+	+	+	+	-	83.75	83.75	81.9
670	8	p. [=];[=]	3	+	-	+	-	-	26.25	107.5	62.5
1755	16	p. [=];[=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
2607	5	p. [=];[=]	0	-	+	-	-	-	97.5	105	98.8
3851	33	p. [=];[=]	0	+	+	+	-	+	103.75	103.75	100.6
4194	11	p. [=];[=]	NA	+	+	-	-	-	67.5	80	76.3
4215	5	p. [=];[=]	0	+	+	-	-	-	98.75	93.75	93.8
4216	55	p. [=];[=]	NA	+	+	+	+	NA	51.25	78.75	68.8
4258	30	p. [=];[=]	28	NA	-	+	-	-	17.5	7.5	13.8
4281	6	p. [=];[=]	2	-	-	-	-	-	57.5	61.25	63.1
4324	37	p. [=];[=]	6	-	-	-	-	-	10	27.5	22.5
4352	3	p. [=];[=]	0	+	+	-	-	-	86.25	88.75	88.1
4357	6	p. [=];[=]	4	+	+	+	-	-	71.25	72.5	67.5
4397	5	p. [=];[=]	0	-	-	-	-	-	102.5	105	100.6
4402	8	p. [=];[=]	0	+	-	-	-	-	100	90	88.8
4450	12	p. [=];[=]	NA	+	+	+	-	-	NA	NA	NA
4462	8	p. [=];[=]	7	+	-	+	-	-	63.75	20	41.3
4488	1	p. [=];[=]	0	-	-	NA	-	-	97.5	97.5	95.0
4671	2	p. [H723R];[600 + 1G>T]	0	+	-	-	+	-	NA	NA	NA
3253	NA	p. [I529S];[H723R]	NA	NA	NA	NA	NA	NA	NA	NA	NA
4949	0	p. [L727X];[H723R]	0	+	-	-	-	-	NA	NA	51.7
J27	NA	p. [H723R];[S448L]	NA	NA	NA	NA	NA	NA	NA	NA	90.6
3309	5	p. [919-2A>G];[P76S]	0	+	+	+	+	-	106.25	106.25	101.3
J15	0	p. [P123S];[H723R]	0	NA	NA	NA	NA	NA	NA	NA	NA
FUK2004	1	p. [H723R];[T94I]	0	NA	NA	NA	NA	NA	NA	NA	85.0
1299	NA	p. [S610X];[S657N]	0	NA	NA	NA	NA	NA	NA	NA	NA
SNS5500	42	p. [919-2A>G];[919-2A>G]	4	+	+	+	+	+	70	81.3	64
SNS5503	37	p. [H723R];[1707 + 5G>A]	5	+	+	+	+	+	67.5	70	NA

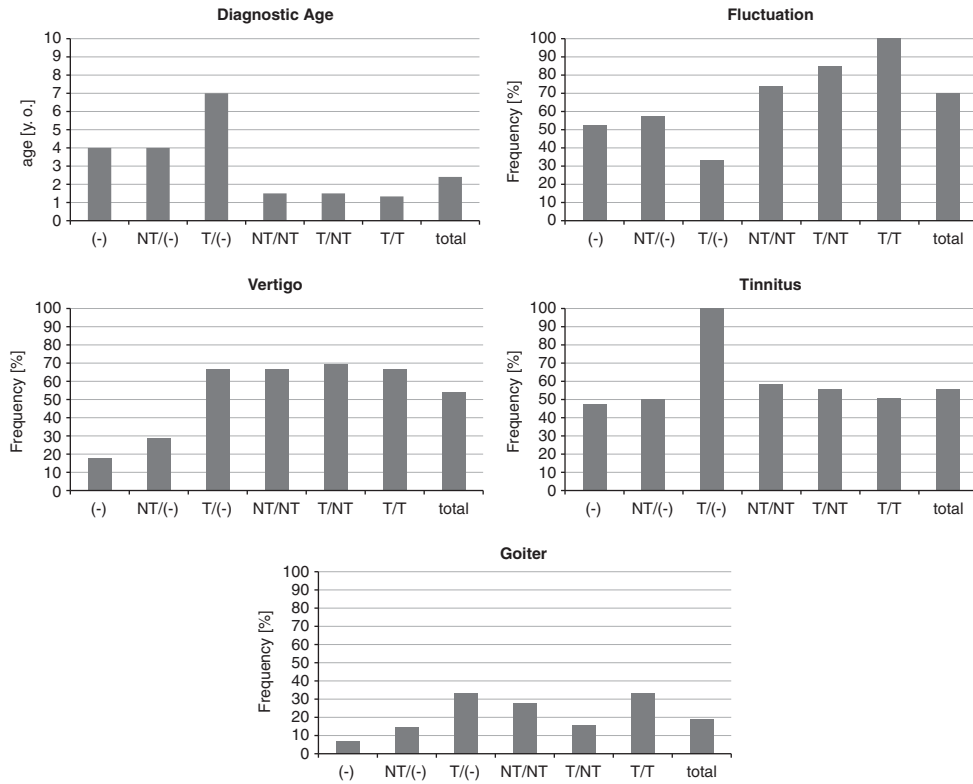
Abbreviation: EVA, enlarged vestibular aqueduct; Lt, left; NA, not available; Rt, right.

<sup>a</sup>Average of 500, 1000, 2000 and 4000 Hz.

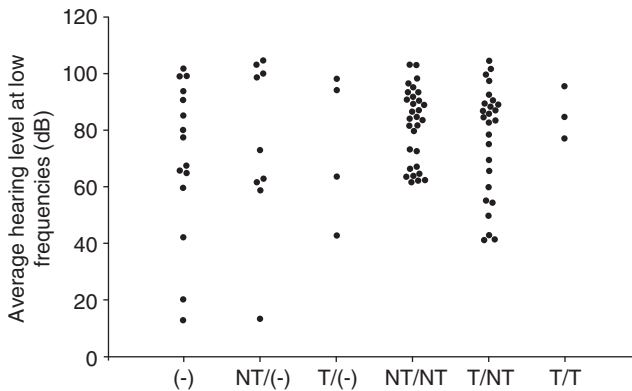
<sup>b</sup>Average of 125, 250 and 500 Hz.

retention of improperly folded Pendrin mutants in the endoplasmic reticulum has been suggested as the major pathological mechanism for Pendred syndrome.<sup>19,20</sup> In this study, we compared not only the difference between the T and NT mutations, but also compared the individual mutations and severity of hearing. However, there were no

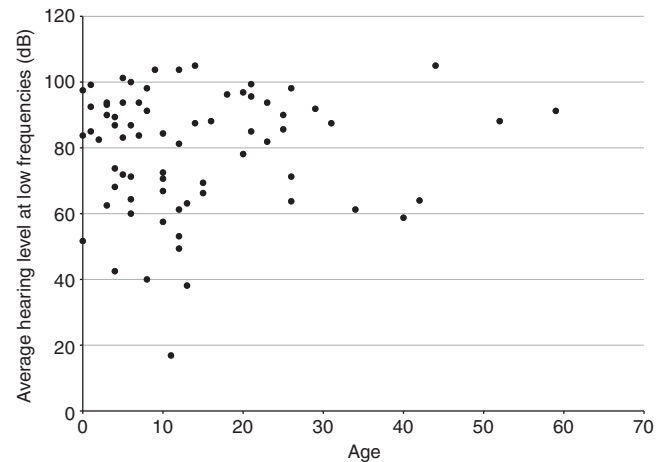
correlations (data not shown). Indeed, there was great variation regarding hearing loss severity even with the same mutations. For example, in the patients homozygous for the most prevalent mutation, p.H723R, hearing level at low frequency varied from 61 to 99 dB (Table 2). In addition, many reports have described intrafamilial



**Figure 1** Genotypes and phenotypes (diagnostic age, fluctuation, vertigo, tinnitus and goiter) in the current study. NT/(-), heterozygote of nontruncating mutation; NT/NT, nontruncating/nontruncating; NT/T, nontruncating/truncating; T/(-), heterozygote of truncating mutation; T/T, truncating/truncating; (-), wild type.



**Figure 2** The relationship between hearing level at the lower frequencies and genotype. Hearing level was the average of 125, 250 and 500 Hz. NT/(-), heterozygote of nontruncating mutation; NT/NT, nontruncating/nontruncating; NT/T, nontruncating/truncating; T/(-), heterozygote of truncating mutation; T/T, truncating/truncating; (-), wild type.



**Figure 3** The relationship between hearing level and age in subjects with biallelic *SLC26A4* mutations. Hearing level was calculated as the average of 250, 500, 1000 and 2000 Hz in both sides.

phenotypic variation.<sup>8-12</sup> Therefore, phenotype may be determined not only by *SLC26A4* mutations but also other factors (genetic as well as environmental), contributing to such variability (Figure 2).

Unlike in the case of *GJB2*, phenotype cannot be predicted from the genotype;<sup>6</sup> however, the clarification of clinical features will enable more appropriate genetic counseling and proper medical management for these patients.

The present study confirmed clinical characteristics of 66 patients with EVA caused by biallelic *SLC26A4* mutations. These included

congenital (5/63, 7.9%), fluctuated (42/52, 80.8%) and progressive (49/56, 87.5%) hearing loss usually associated with vertigo (35/52, 67.3%) and/or goiter (12/53, 22.6%) during long-term follow-up, in accordance with our previous study.<sup>6</sup> It is known that goiter sometimes becomes apparent between 10 and 20 years of age. The present cohort included young children, and therefore the frequency of goiter may be underestimated. As seen in Figure 3, in 66 patients with biallelic mutations for whom data were available, onset of hearing loss was likely to be early onset, and progressive with age.



## CONCLUSIONS

Pendred syndrome and nonsyndromic hearing loss associated with EVA are a continuum of disease characterized as being associated with congenital, fluctuating and progressive hearing loss, and most patients have vertigo and/or goiter. However, in the present study, no genotype–phenotype correlation was found. The results obtained from the present study will facilitate accurate molecular diagnosis and better genetic counseling.

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