*The Laryngoscope*


# Subgroups of Enlarged Vestibular Aqueduct in Relation to *SLC26A4* Mutations and Hearing Loss

Yasuhide Okamoto, MD; Hideki Mutai, DVM, PhD; Atsuko Nakano, MD, PhD; Yukiko Arimoto, MD, PhD; Tomoko Sugiuchi, MD; Sawako Masuda, MD; Noriko Morimoto, MD, PhD; Hirokazu Sakamoto, MD, PhD; Noboru Ogahara, MD, PhD; Akira Takagi, MD; Hidenobu Taiji, MD, PhD; Kimitaka Kaga, MD, PhD; Kaoru Ogawa, MD, PhD; Tatsuo Matsunaga, MD, PhD

**Objectives/Hypothesis:** To investigate possible association of hearing loss and *SLC26A4* mutations with the subgroups of enlarged vestibular aqueduct (EVA) morphology in Japanese subjects with hearing loss.

**Study Design:** Retrospective multicenter study.

**Methods:** Forty-seven subjects who had vestibular aqueduct with midpoint diameter >1 mm by computed tomography of the temporal bone were enrolled at multiple sites across Japan, and DNA samples and clinical data were collected. EVA morphology was classified into four subgroups by the pattern of enlargement: aperture, aperture and midpoint, midpoint, and borderline enlargement. Venous blood DNA samples were subjected to polymerase chain reaction–based direct sequencing of all exons and exon–intron boundaries of the *SLC26A4*.

**Results:** Four novel *SLC26A4* mutations were identified in the present study. *SLC26A4* mutations were detected in almost all subjects with aperture, aperture and midpoint, and midpoint enlargement. In contrast, 71% of subjects with borderline enlargement had no *SLC26A4* mutation. No significant difference was found in the distribution of truncating and nontruncating *SLC26A4* mutations between the EVA subgroups. In addition, no significant correlation was observed between the EVA subgroups and hearing levels, incidence of hearing fluctuation, or progression of hearing loss.

**Conclusions:** Subgroups of EVA morphology were significantly correlated with the presence or absence of *SLC26A4* mutation. In a subgroup analysis of subjects with *SLC26A4* mutations, however, differences in the EVA subgroups were not correlated with *SLC26A4* genotypes or characteristics of hearing loss.

**Key Words:** Enlarged vestibular aqueduct, Pendred syndrome, DFNB4, *SLC26A4*, computed tomography, hearing loss.

**Level of Evidence:** NA

***Laryngoscope*, 124:E134–E140, 2014**

## INTRODUCTION

Enlarged vestibular aqueduct (EVA) is one of the most common inner ear deformities, often identified by computed tomography (CT) in subjects with hearing loss.[1–5] The shape and size of the EVA differ between subjects. As such, a variety of radiographic criteria to define EVA have been published. Valvassori and Clemis[6] defined EVA as a vestibular aqueduct ≥1.5 mm at the midpoint diameter. Jackler and De La Cruz[7] developed a criterion of a midpoint diameter >2.0 mm, whereas Levenson and colleagues[8] proposed a cutoff of 2.0 mm at the external aperture diameter. Okumura et al.[9] suggested an external aperture diameter >4.0 mm. Madden et al.[1] considered external aperture diameter >2.0 mm and midpoint diameter >1.5 mm as definitive, and midpoint diameter of 1.0 to 1.5 mm as borderline enlargement. Vijayasekaran et al.[10] advocated the criteria of 0.9 mm midpoint diameter or 1.9 mm external aperture diameter.

Mutations in the *SLC26A4* have been identified as a major cause of vestibular aqueduct anomalies. *SLC26A4* mutations are known to cause Pendred syndrome (Mendelian Inheritance in Man [MIM] #274600) and nonsyndromic sensorineural deafness autosomal recessive type 4 (DFNB4, MIM #600791).[11–14] Some researchers have identified a correlation between *SLC26A4* mutations, EVA, and hearing loss, whereas others report no significant relationship among *SLC26A4* genotype and these phenotypes.[15] Previous

From the Department of Otolaryngology, National Hospital Organization Tokyo Medical Center, Tokyo (Y.O., H.M., K.K., T.M.); and Department of Otorhinolaryngology, Inagi Municipal Hospital, Tokyo (Y.O.); Department of Otolaryngology, Keio University Hospital, Tokyo (Y.O., K.O.); Division of Otorhinolaryngology, Chiba Children's Hospital, Chiba (A.N., Y.A.); Department of Otolaryngology, Kanto Rosai Hospital, Kanagawa (T.S.); Department of Otorhinolaryngology, National Mie Hospital, Mie (S.M.); Department of Otorhinolaryngology, National Center for Children and Development, Tokyo (N.M., H.T.); Department of Otorhinolaryngology, Kobe Children's Hospital, Hyogo (H.S.); Department of Otorhinolaryngology, Kanagawa Children's Medical Center, Kanagawa (N.O.); Department of Otorhinolaryngology, Shizuoka General Hospital, Shizuoka (A.T.), Japan

Editor's Note: This Manuscript was accepted for publication June 27, 2013.

Supported by Health and Labor Sciences Research (Research on Rare and Intractable Diseases) grants 2009-187, 2010-205, and 2011-092, and a Grant-in-Aid for Clinical Research from the National Hospital Organization.

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to Tatsuo Matsunaga, MD, PhD, Department of Otolaryngology, Laboratory of Auditory Disorders, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro, Tokyo, 152-8902, Japan. E-mail: matsunagatatsuo@kankakuki.go.jp

**DOI: 10.1002/lary.24368**

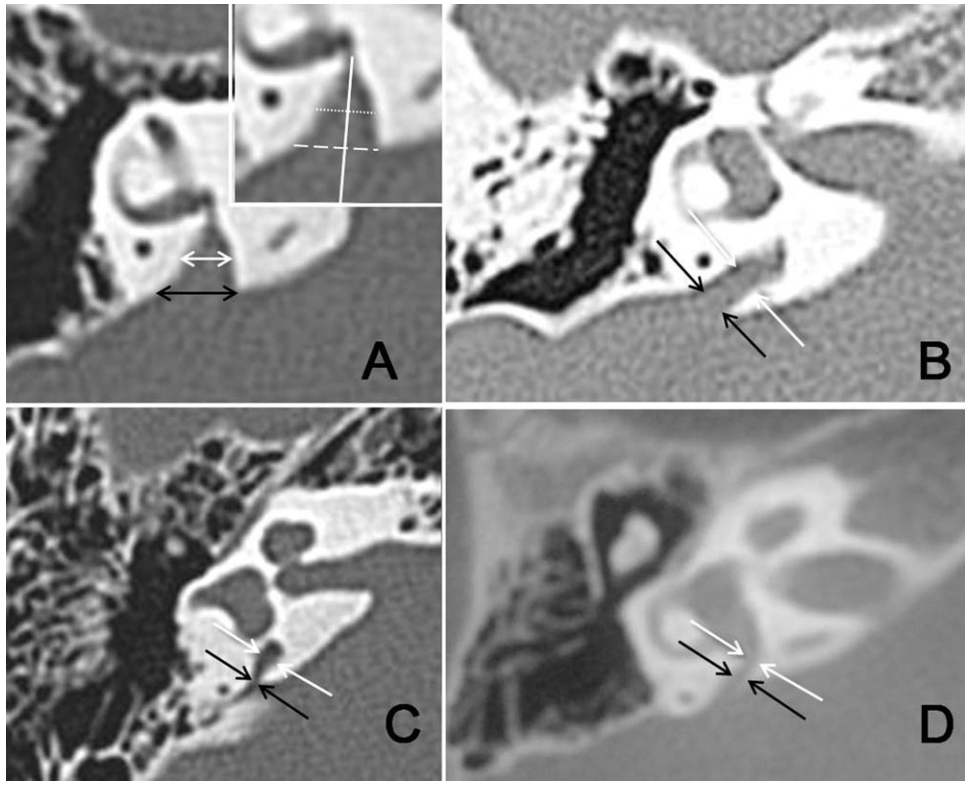


Fig. 1. Typical temporal bone computed tomographic images of the enlarged vestibular aqueduct subgroups. (A) Aperture enlargement. (B) Aperture and midpoint enlargement. (C) Midpoint enlargement. (D) Borderline enlargement. The midpoint and external aperture of the vestibular aqueduct are indicated by white and black arrows, respectively. As shown in the inset of A, the midpoint diameter (dotted line) and aperture diameter (dashed line) were measured perpendicular to the long axis (solid line) of the vestibular aqueduct.

studies have not evaluated the relationship between *SLC26A4* mutations and clinical features of hearing loss taking into consideration morphologic variations of the EVA. We conducted a multicenter study and differentiated subjects into subgroups according to vestibular aqueduct midpoint and external aperture diameters to examine a possible relationship between subgroups of EVA morphology, *SLC26A4* mutations, and hearing loss.

## MATERIALS AND METHODS

We enrolled 47 bilateral EVA subjects with unilateral or bilateral sensorineural hearing loss of unknown causes (mean age = 13.5 years, range = 0–56 years; 33 children and 14 adults; 17 males and 30 females), and collected DNA samples and clinical data. Specifically, subjects whose bilateral vestibular aqueduct midpoint diameter was $\geq$1 mm on temporal bone CT scans were included. The midpoint and external aperture diameters were measured perpendicular to the long axis of the vestibular aqueduct on the transverse plane, as shown in the upper right-hand inset in Figure 1A. Subjects were classified into the following four subgroups based on the morphologic characteristics of the vestibular aqueduct according to the criteria in Table I: aperture enlargement, aperture and midpoint enlargement, midpoint enlargement, and borderline enlargement.

For mutation analysis, genomic DNA was extracted from venous blood and subjected to polymerase chain reaction–based direct sequencing of the exons and exon–intron boundaries of the *SLC26A4* (GenBank NG_008489). For the purpose of this study, frameshift, splice site, and nonsense mutations were categorized as "truncating," and missense mutations as "nontruncating" mutations. Novel variants were defined as pathogenic if they 1) were nonsynonymous; 2) demonstrated low carrier rates (<1%) in 96 normal control Japanese subjects, absence in database Exome Variant Server[16] and dbSNP,[17] and high amino acid conservation among various mammalian species; and 3) were detected as heterozygous in association with the other allele with another heterozygous mutation already reported as pathogenic. Alteration of splice site was predicted by NNSPLICE.[18] Subjects with *SLC26A4* mutations were analyzed for degree of hearing loss, fluctuations in hearing acuity, and progression of hearing loss to assess the relationship between these hearing parameters and EVA subgroups. Subjects underwent conditioned orientation reflex or conventional pure-tone audiometry, depending on their ages. Auditory steady-state response measurements were utilized for five subjects who did not receive any of these audiometric tests.

TABLE I.
Criteria for the Subgroups of Enlarged Vestibular Aqueduct.

| Enlarged Vestibular Aqueduct Subgroup | Midpoint Diameter | External Aperture Diameter |
|---|---|---|
| Aperture enlargement | $\geq$1.5 mm | Wider than midpoint |
| Aperture and midpoint enlargement | $\geq$1.5 mm | Equal to midpoint |
| Midpoint enlargement | $\geq$1.5 mm | Narrower than midpoint |
| Borderline enlargement | 1.0 mm to <1.5mm | 1.0 mm to <1.5 mm |

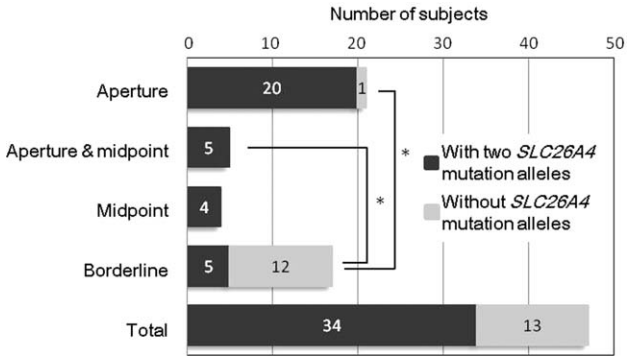


Fig. 2. Number of subjects with or without *SLC26A4* mutation alleles in each enlarged vestibular aqueduct subgroup. *Significant difference ($P < .0125$).

Hearing level was evaluated based on averages at 500, 1,000, 2,000, and 4,000 Hz (slight, 26–40 dB; moderate, 41–60 dB; severe, 61–80 dB; profound, ≥81 dB) according to the World Health Organization Grades of Hearing Impairment.[19] Subjects were considered to have fluctuating hearing loss if they had at least one bout of aggravation of hearing loss and recovery (at least 15 dB in one frequency). Subjects were considered to have progressive hearing loss if they showed aggravation of hearing loss by 10 dB or more at one or more frequencies within a 10-year interval. Statistical significance was assessed using the Fisher exact test.

All procedures were approved by the Ethics Review Committee of National Hospital Organization Tokyo Medical Center, Japan and other participating institutions, and were conducted only after written informed consent had been obtained from each subject or from the parents of the subjects.

## RESULTS

### *Subgrouping of EVA and Its Association With* SLC26A4 *Mutations*

Figure 1 shows typical CT findings in subjects with aperture enlargement (Fig. 1A), aperture and midpoint enlargement (Fig. 1B), midpoint enlargement (Fig. 1C), and borderline enlargement (Fig. 1D). Among 47 subjects, 21 (44%) were classified with aperture enlargement, 17 (36%) with borderline enlargement, five (11%) with aperture and midpoint enlargement, and four (9%) with midpoint enlargement (Fig. 2). All subjects had the same subgroup of enlargement bilaterally.

Genetic analysis of the 47 subjects showed that 34 (72%) had two *SLC26A4* mutation alleles (Table II), and the other 13 (28%) had no *SLC26A4* mutation alleles. None had a single *SLC26A4* mutation allele. The 34 subjects with two *SLC26A4* mutation alleles were diagnosed with Pendred syndrome or DFNB4. The majority of these subjects had aperture enlargement (n = 20, 59%), followed by aperture and midpoint enlargement (n = 5, 14%), borderline enlargement (n = 5, 14%), and midpoint enlargement (n = 4, 12%; Fig. 2). Conversely, most of the subjects without *SLC26A4* mutation alleles had borderline enlargement (n = 12, 91%), whereas the one remaining subject (8%) had aperture enlargement. The frequency of subjects without *SLC26A4* mutation alleles in the borderline enlargement subgroup was significantly higher than in the aperture enlargement and aperture and midpoint enlargement subgroups ($P < .0125$). It tended to be higher than in the midpoint enlargement subgroup, but this difference was not statistically significant ($P = .021$), probably due to the small number of subjects in the midpoint enlargement subgroup (n = 4).

### SLC26A4 *Mutations and Genotypes in Association With EVA Morphology in Subjects With Pendred Syndrome or DFNB4*

The types and locations of all the *SLC26A4* mutations in 34 subjects with Pendred syndrome or DFNB4 are shown in Table II and Figure 3. Five splice site mutations (c.601-1G>A [intron 5], c.919-2A>G [intron 7], c.1614+1G>A [intron 14], c.1708-32_1708-16del [intron 15], c.1707+5G>A [intron 15]), one nonsense mutation (p.L743X), two insertion/deletion mutations (p.S551Ffs13, p.Q705Wfs18), and 14 missense mutations (p.S28G, p.P76S, p.A372V, p.N392Y, p.R409H, p.T410M, p.T527P, p.I529S, p.Y556C, p.V659L, p.D669E, p.F692L, p.T721M, p.H723R) were detected. These included four novel mutations, p.S28G (c.82A>G), p.D669E (c.2007C>A), p.F692L (c.2074T>C), and c.1708-32_1708-16del (marked with ** in Table II), based on the criteria for novel mutations in the present study (described in Materials and Methods). Electropherograms of the novel mutations and conservation of the amino acid residues among various species are shown in Figure 3B and C. NNSPLICE predicted c.1708-32_1708-16del to decrease the probability of an acceptor site at exon 16 from 0.49 (for a normal allele) to 0.19 (for a mutation allele), which is likely to cause aberrant splicing (Fig. 3C).

The list of subjects with two *SLC26A4* mutation alleles is shown in Table II. Analysis of genotypes of *SLC26A4* mutation alleles in these subjects showed that 20 (59%) had nontruncating/nontruncating genotypes, 13 (38%) had nontruncating/truncating genotypes, and 1 (3%) had truncating/truncating genotypes (Fig. 4A). Comparison of the incidence of each genotype found no significant statistical difference between the subgroups of EVA morphology ($P = 1.000$).

### *Characteristics of Hearing Loss in Association With EVA Morphology in Subjects With Pendred Syndrome or DFNB4*

The hearing levels, incidence of hearing fluctuation, and progression of hearing loss in subjects with two *SLC26A4* mutation alleles are shown in Table II. The relation between the hearing level and EVA morphology was examined in the ears of 34 subjects (68 ears; Fig. 4B). Thirty-four ears (50%) had profound hearing loss in total. No significant differences in the hearing levels were detected between the subgroups of EVA morphology ($P = .462$). To exclude the effect of aging in this analysis, we also stratified the subjects into two groups (age 0–9 and ≥10 years) and conducted the same analysis. These analyses also demonstrated the same results, indicating that the difference in ages among subgroups did

TABLE II.
Types of *SLC26A4* Mutations and Characteristics of Hearing Loss in 34 Subjects With Pendred Syndrome or DFNB4 by EVA Subgroups.

| | | | Allele 1 | | | Allele 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EVA Morphology | Age at Deafness Diagnosis, yr | Age, yr | Exon/Intron | DNA Change | Amino Acid Change or Splicing Mutation | Exon/Intron | DNA Change | Amino Acid Change or Splicing Mutation | T/N | Hearing Level, R/L, dBHL* | Fluctuation of Hearing | Progression of Hearing Loss |
| Aperture enlargement | 0 | 1 | Intron 15 | c.1707+5G>A | Splice site mutation | 19 | c.2106–2110dup5 | p.Q705Wfs18 | T/T | 90/70† | – | + |
| | 0 | 33 | 15 | c.1652insT | p.S551Ffs13 | 19 | c.2168A>G | p.H723R | T/N | 95/95 | – | + |
| | 2 | 6 | Intron 7 | c.919-2A>G | Splice site mutation | 19 | c.2168A>G | p.H723R | T/N | 53.75/63.75 | – | + |
| | 0 | 27 | Intron 7 | c.919-2A>G | Splice site mutation | 19 | c.2168A>G | p.H723R | T/N | 98.75/100 | + | + |
| | 0 | 1 | Intron 15 | c.1707+5G>A | Splice site mutation | 19 | c.2168A>G | p.H723R | T/N | 85‡ | Unknown | + |
| | 0 | 31 | Intron 5 | c.601-1G>A | Splice site mutation | 19 | c.2168A>G | p.H723R | T/N | 73.75/60 | + | + |
| | 3 | 11 | Intron 5 | c.601-1G>A | Splice site mutation | 19 | c.2168A>G | p.H723R | T/N | 70/87.5 | + | + |
| | 0 | 4 | Intron 15 | c.1707+5G>A | Splice site mutation | 2 | c.82A>G** | p.S28G** | T/N | 61.25/61.25 | – | Unknown |
| | 0 | 35 | Intron 5 | c.601-1G>A | Splice site mutation | 10 | c.1229C>T | p.T410M | T/N | 80/73.75 | + | + |
| | 3 | 12 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 82.5/106.25 | + | + |
| | 3 | 3 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 62.5/73.75 | – | – |
| | 0 | 4 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 55/70 | + | – |
| | 0 | 2 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 37.5‡ | Unknown | Unknown |
| | 0 | 1 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 102.5/115§ | – | – |
| | 0 | 0.5 | 10 | c.1229C>T | p.T410M | 19 | c.2228T>A | p.L743X | N/N | 73.75‡ | Unknown | Unknown |
| | 0 | 1 | 9 | c.1115C>T | p.A372V | 10 | c.1226G>A | p.R409H | N/N | 92.5‡ | – | – |
| | 0 | 20 | 19 | c.2168A>G | p.H723R | 14 | c.1579A>C | p.T527P | N/N | 97.5/101.25 | – | – |
| | 0 | 4 | 15 | c.1667A>G | p.Y556C | 14 | c.1579A>C | p.T527P | N/N | 77.5/75 | – | + |
| | 0 | 6 | 3 | c.266C>T | p.P76S | 14 | c.1579A>C | p.T527P | N/N | 17.5/93.75 | – | + |
| | 0 | 9 | 10 | c.1174A>T | p.N392Y | 19 | c.2162C>T | p.T721M | N/N | 103.75/110 | + | + |
| Aperture and midpoint enlargement | 0 | 15 | Intron 15 | c.1708-32_1708-16del** | Splice site mutation** | 19 | c.2168A>G | p.H723R | T/N | 76.25/91.25 | + | + |
| | 0 | 9 | Intron 7 | c.919-2A>G | Splice site mutation | 17 | c.2007C>A** | p.D669E** | T/N | 100/100 | + | – |
| | 0 | 1 | 19 | c.2168A>G | p.H723R | 14 | c.1579A>C | p.T527P | N/N | 115‡ | – | – |
| | 5 | 6 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 47.5/62.5 | – | – |
| | 1 | 2 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 105/93.75§ | Unknown | Unknown |
| Midpoint enlargement | 0 | 3 | Intron 7 | c.919-2A>G | Splice site mutation | 17 | c.2007C>A** | p.D669E** | T/N | 82.5/93.75# | + | + |
| | 0 | 8 | 19 | c.2168A>G | p.H723R | 18 | c.2074T>C** | p.F692L** | N/N | 75/115 | + | + |
| | 7 | 10 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 60/15 | + | + |
| | 0 | 35 | 10 | c.1229C>T | p.T410M | 17 | c.1975G>C | p.V659L | N/N | 97.5/87.5 | + | + |

TABLE II.
(*Continued*)

| EVA Morphology | Age at Deafness Diagnosis, yr | Age, yr | Allele 1 | | | Allele 2 | | | | Hearing Level, R/L, dBHL* | Fluctuation of Hearing | Progression of Hearing Loss |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Exon/Intron | DNA Change | Amino Acid Change or Splicing Mutation | Exon/Intron | DNA Change | Amino Acid Change or Splicing Mutation | T/N | | | |
| Borderline enlargement | 0 | 5 | Intron 7 | c.919-2A>G | Splice site mutation | 19 | c.2168A>G | p.H723R | T/N | 73.75/77.5 | + | + |
| | 2 | 2 | Intron 14 | c.1614+1G>A | Splice site mutation | 10 | c.1229C>T | p.T410M | T/N | 55‡ | Unknown | Unknown |
| | 4 | 4 | 19 | c.2168A>G | p.H723R | 19 | c.2168A>G | p.H723R | N/N | 106.25/88.75# | Unknown | + |
| | 0 | 6 | 14 | c.1586T>G | p.I529S | 19 | c.2168A>G | p.H723R | N/N | 80/66.25 | – | – |
| | 4 | 14 | 10 | c.1229C>T | p.T410M | 19 | c.2168A>G | p.H723R | N/N | 118.75/58.75 | + | + |

*Value without slash indicates binaural stimulus.
†Auditory brainstem response.
‡Conditioned Orienting Response.
**Candidate novel mutation.
§Auditory steady state response.
#Conditioned Play Audiometry.
EVA = enlarged vestibular aqueduct; L = left; N = nontruncating; R = right; T = truncating.

not affect distribution of subjects among different hearing levels (data not shown). Next, the relation between hearing fluctuation and EVA morphology was investigated in 28 subjects for whom relevant audiometric data were available (Fig. 4C). Hearing fluctuations were detected in 15 subjects (54%) in total, and no significant differences were noted in the incidence of hearing fluctuations between the subgroups of EVA morphology ($P = .209$). Lastly, the relation between progression of hearing loss and EVA morphology was analyzed in 29 subjects for whom relevant clinical data were available (Fig. 4D). Twenty subjects (69%) had progressive hearing loss in total, and the results showed no significant differences in the incidence of progressive hearing loss between the subgroups of EVA morphology ($P = .207$).

## DISCUSSION

Although a variety of EVA criteria using the midpoint and aperture diameters of the vestibular aqueduct have been proposed to date,[1,6–10] our study is the first attempt to divide EVA into subgroups based on the shape and size of the vestibular aqueduct, and the first to investigate the possible relationship of these subgroups with genotypes and audiometric findings. *SLC26A4* mutations were detected in 72% of the Japanese subjects with bilateral EVA. Among these *SLC26A4* mutations, four mutations were novel. The discovery of these novel mutations would expand the *SLC26A4* mutation spectrum, thereby contributing to a more accurate gene-based diagnosis of hearing loss with EVA.

Nearly all subjects with aperture, aperture and midpoint, and midpoint enlargement presented *SLC26A4* mutations, suggesting that subjects with these EVA subgroups are most likely to be diagnosed with Pendred syndrome or DFNB4. Conversely, only approximately 30% of subjects with borderline enlargement had *SLC26A4* mutation, which suggests that the majority of subjects in this EVA subgroup have a pathological mechanism other than Pendred syndrome or DFNB4.

None of the 47 EVA subjects enrolled in the present study had only a single *SLC26A4* mutation allele. This finding is in striking contrast with previous research reporting single *SLC26A4* mutation alleles in approximately one third of Caucasian subjects with EVA.[3,4,20–22] This discrepancy might be associated with Japanese subjects, who were reported to have a spectrum of *SLC26A4* mutations distinct from that of Caucasian subjects.[22] One possible explanation is that the development of EVA in the Caucasian population may more frequently involve mutations in the introns or promoter regions of the *SLC26A4* than that in the Japanese population. Another possibility is that the Caucasian population may have higher mutation frequencies in genes than the Japanese population, causing digenic hearing loss in association with heterozygous *SLC26A4* mutations (e.g., *KCNJ10* and *FOXI1*).[23–25] The other possible explanation for the discrepancy is that the present study registered only subjects with bilateral EVA, whereas previous studies included those with unilateral hearing loss or unilateral EVA. This implicates the hypothesis that

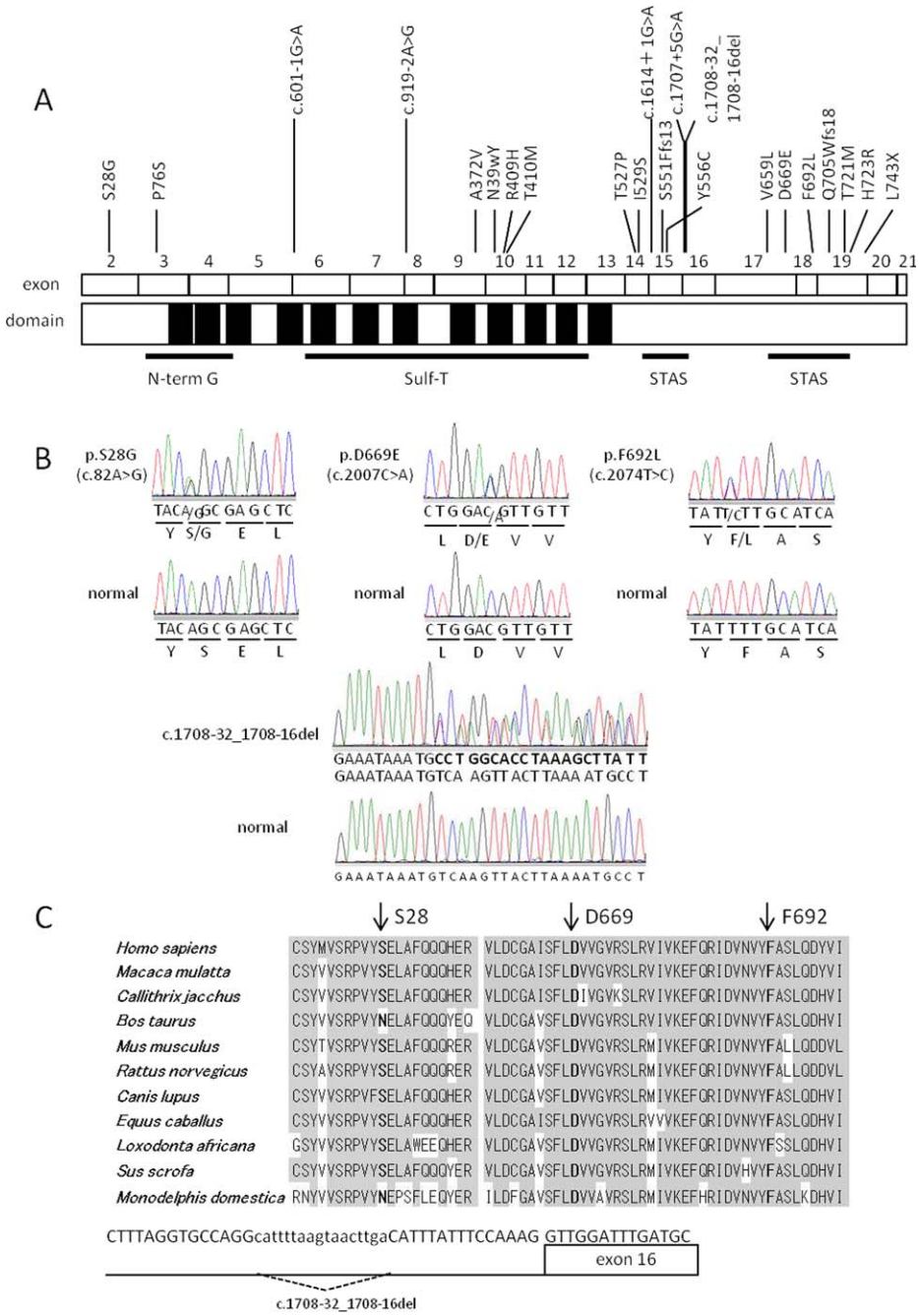


Fig. 3. The location of each mutation in *SLC26A4*, the evolutionary conservation of the amino acids, and nucleotides affected by the novel missense and splice site mutations. (A) Location of the *SLC26A4* mutations found in this study. Putative transmembrane regions are shown in black. N-term G = sulfate transporter N-terminal domain with Gly motif; STAS = sulfate transporter and anti–sigma factor antagonist domain; Sulf-T = sulfate transporter family domain. (B) electropherograms of the novel mutations and the corresponding sequence from normal alleles. Note that the nucleotide sequence of c.1708-32_1708-16del is shown reverse complementary. (C) Upper: multiple alignments of SLC26A4 protein orthologues at two noncontiguous regions. Arrows indicate affected amino acids. Conserved amino acids are shaded in gray. Lower: boundaries between intron 15 and exon 16 and deleted nucleotides are indicated at the bottom. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

biallelic mutations of *SLC26A4* are more strongly associated with bilateral EVA.

Our analysis of subjects with *SLC26A4* mutations revealed no significant difference in the proportion of truncating and nontruncating *SLC26A4* mutations between subgroups of EVA morphology. This suggests that, in addition to malfunction of the *SLC26A4* protein, environmental factors or genes other than *SLC26A4* may contribute to variations in vestibular aqueduct morphology.

Some researchers argue that there is no significant relationship between the degree of the EVA and the severity and progression of hearing loss and hearing fluctuations, whereas others propose that there is a significant relationship.[26] In the present study, no significant differences were detected in the level, fluctuation, and progression of hearing loss between the subgroups of EVA morphology, indicating that characteristics of hearing loss cannot be predicted based on the EVA morphology in subjects with Pendred syndrome or DFNB4.

## CONCLUSION

Almost all the subjects with aperture, aperture and midpoint, and midpoint enlargement of EVA had two *SLC26A4* mutation alleles, whereas more than two thirds

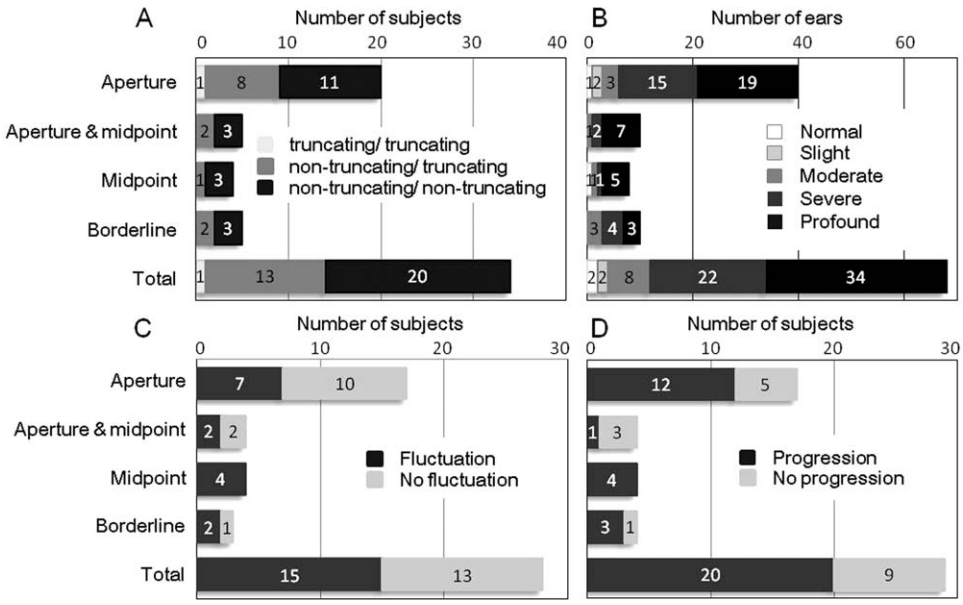


Fig. 4. Association of enlarged vestibular aqueduct (EVA) subgroups with *SLC26A4* genotypes or characteristics of hearing loss in subjects with biallelic *SLC26A4* mutations. (A) Proportion of *SLC26A4* genotypes in subjects of each EVA subgroup. (B) Proportion of different hearing levels in ears of each EVA subgroup. (C) Prevalence of fluctuating hearing loss in subjects of each EVA subgroup. (D) Prevalence of progressive hearing loss in subjects of each EVA subgroup.

of subjects with borderline enlargement of EVA had no *SLC26A4* mutation alleles. Analysis of subjects with two *SLC26A4* mutation alleles revealed no significant correlation between the morphologic subgroups of EVA and *SLC26A4* genotypes or characteristics of hearing loss, suggesting that the subgroups of EVA morphology may be associated with factors other than genotypes of *SLC26A4* mutations and that the subgroups of EVA morphology are not a predictive factor for characteristics of hearing loss.

## BIBLIOGRAPHY


1. Madden C, Halsted M, Benton C, Greinwald J, Choo D. Enlarged vestibular aqueduct syndrome in the pediatric population. *Otol Neurotol* 2003; 24:625–632.
2. Madden C, Halsted M, Meinzen-Derr J, et al. The influence of mutations in the SLC26A4 gene on the temporal bone in a population with enlarged vestibular aqueduct. *Arch Otolaryngol Head Neck Surg* 2007; 133:162–168.
3. Pryor SP, Madeo AC, Reynolds JC, et al. SLC26A4/PDS genotype-phenotype correlation in hearing loss with enlarged vestibular aqueduct (EVA): evidence that Pendred syndrome and non-syndromic EVA are distinct clinical and genetic entities. *J Med Genet* 2005;42:159–165.
4. Azaiez H, Yang T, Prasad S, et al. Genotype-phenotype correlations for SLC26A4-related deafness. *Hum Genet* 2007;122:451–457.
5. Choi BY, Stewart AK, Madeo AC, et al. Hypo-functional SLC26A4 variants associated with nonsyndromic hearing loss and enlarged vestibular aqueduct: genotype-phenotype correlation or coincidental polymorphisms? *Hum Mutat* 2009;30:599–608.
6. Valvassori GE, Clemis JD. The large vestibular aqueduct syndrome. *Laryngoscope* 1978;88:723–728.
7. Jackler RK, De La Cruz A. The large vestibular aqueduct syndrome. *Laryngoscope* 1989;99:1238–1242.
8. Levenson MJ, Parisier SC, Jacobs M, Edelstein DR. The large vestibular aqueduct syndrome in children. A review of 12 cases and the description of a new clinical entity. *Arch Otolaryngol Head Neck Surg* 1989; 115:54–58.
9. Okumura T, Takahashi H, Honjo I, Takagi A, Mitamura K. Sensorineural hearing loss in patients with large vestibular aqueduct. *Laryngoscope* 1995;105:289–293.
10. Vijayasekaran S, Halsted MJ, Boston M, et al. When is the vestibular aqueduct enlarged? A statistical analysis of the normative distribution of vestibular aqueduct size. *Am J Neuroradiol* 2007;28:1133–1138.
11. Smith SD, Harker LA. Single gene influences on radiologically-detectable malformations of the inner ear. *J Commun Disord* 1998;31:391–408; quiz 409–410.
12. 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.
13. Phelps PD, Coffey RA, Trembath RC, et al. Radiological malformations of the ear in Pendred syndrome. *Clin Radiol* 1998;53:268–273.
14. 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.
15. Reyes S, Wang G, Ouyang X, et al. Mutation analysis of SLC26A4 in mainland Chinese patients with enlarged vestibular aqueduct. *Am Acad Otolaryngol Head Neck Surg* 2009;141:502–508.
16. National Heart, Lung, and Blood Institute Exome Sequencing Project. Exome variant server. Available at: http://evs.gs.washington.edu/EVS/. [Visited May 13, 2013].
17. National Center for Biotechnology Information. dbSNP. Available at: http://www.ncbi.nlm.nih.gov/snp/. [Visited May 13, 2013].
18. Reese MG, Eeckman, FH, Kulp, D, Haussler, D. Improved splice site detection in Genie. *J Comp Biol* 1997;4:311–323.
19. World Health Organization. Grades of hearing impairment. Available at: http://www.who.int/pbd/deafness/hearing_impairment_grades/en/ index.html. [Visited May 13, 2013].
20. Albert S, Blons H, Jonard L, et al. SLC26A4 gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations. *Eur J Hum Genet* 2006;14:773–779.
21. 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.
22. 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.
23. Madeo AC, Manichaikul A, Reynolds JC, et al. Evaluation of the thyroid in patients with hearing loss and enlarged vestibular aqueducts. *Arch Otolaryngol Head Neck Surg* 2009;135:670–676.
24. Yang T, Vidarsson H, Rodrigo-Blomqvist S, Rosengren SS, Enerback S, Smith RJ. Transcriptional control of SLC26A4 is involved in Pendred syndrome and nonsyndromic enlargement of vestibular aqueduct (DFNB4). *Am J Hum Genet* 2007;80:1055–1063.
25. Yang T, Gurrola JG Jr, Wu H, et al. Mutations of KCNJ10 together with mutations of SLC26A4 cause digenic nonsyndromic hearing loss associated with enlarged vestibular aqueduct syndrome. *Am J Hum Genet* 2009;84:651–657.
26. Gopen Q, Zhou G, Whittemore K, Kenna M. Enlarged vestibular aqueduct: review of controversial aspects. *Laryngoscope* 2011;121:1971–1978.