The Influence of Mutations in the SLC26A4 Gene on the Temporal Bone in a Population With Enlarged Vestibular Aqueduct

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Objective: To correlate genetic and audiometric findings with a detailed radiologic analysis of the temporal bone in patients with enlarged vestibular aqueduct (EVA) to ascertain the contribution of SLC26A4 gene mutations to this phenotype.

Design: A retrospective review of patients with EVA identified in a database of pediatric hearing-impaired patients.

Setting: A tertiary care pediatric referral center.

Patients: Seventy-one children with EVA and screening results for SLC26A4 mutations.

Main Outcome Measures: Genetic screening results, audiometric thresholds, and radiographic temporal bone measurements.

Results: Seventy-one children with EVA were screened for SLC26A4 mutations. Mutations were found in 27% of children overall, while only 8% had biallelic mutations. The mean initial pure-tone average (PTA) was 59 dB; the mean final PTA was 67 dB. A bilateral EVA was found in 48 (67%) of the children; a unilateral EVA was found in 23 (33%). Progressive hearing loss (in at least 1 ear) was seen in 29 (41%) of the patients. The strongest genotype-phenotype interaction was seen in children with a bilateral EVA. Among children with SLC26A4 mutations, there was a significantly wider vestibular aqueduct at the midpoint and a wider vestibule width (P<.05) than in children without the mutation. Among patients with a bilateral EVA, children with any SLC26A4 mutation were more likely to have a more severe final PTA (64 dB vs 32 dB), larger midpoint measurement (2.1 vs 1.1 mm), and larger operculum measurement (3.0 vs 2.0 mm) than those without the mutation in their better-hearing ear (P<.05).

Conclusions: In a population of pediatric patients with an EVA and hearing loss, SLC26A4 mutations are a contributor to the phenotype. Our data suggest that other genetic factors also have important contributions to this phenotype. The presence of an abnormal SLC26A4 allele, even in the heterozygous state, was associated with greater enlargement of the vestibular aqueduct, abnormal development of the vestibule, and possibly a stable hearing outcome.

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Pendred Syndrome is an autosomal recessive disorder characterized by sensorineural hearing loss (SNHL), goiter, and enlarged vestibular aqueduct (EVA) on radiologic assessment. The genetic mutation in Pendred syndrome is found on chromosome 7q31 in the gene SLC26A4 (also known as PDS). Animal studies have shown that SLC26A4 is expressed in the endolympathic sac and duct, in discrete areas adjacent to the maculae of the utricle and saccule, and in the spiral prominence region within the scala media of the cochlea. The protein called pendrin is believed to play a role in endolymph homeostasis and maintenance of the endocochlear potential. An EVA is the most common radiologic abnormality seen in children with SNHL. A number of mutations in the SLC26A4 gene have been described in patients with syndromic and nonsyndromic EVA. It is possible that mutations in different regions of the SLC26A4 gene can cause different phenotypes; therefore, mutations in the SLC26A4 gene do not necessarily result in the full Pendred syndrome but can cause EVA and cochleovestibular anomalies without the associated goiter. Biallelic mutations in the SLC26A4 gene have been postulated as the cause of Pendred syndrome. A variety of cochlear and vestibular malformations has been described in the radiologic assessment of children with EVA. Subtle abnormalities may be missed by the clinician on analysis of the temporal bone scan. A series of radiologic measurements of normative data for
the temporal bone would help demonstrate these abnormalities.\textsuperscript{19} The use of picture archiving and communication systems enables the precise digital measurements of the cochleovestibular system. It also allows for standardization of the classification and description of temporal bone anomalies in patients with SNHL.\textsuperscript{20}

Our aim was to examine the clinical, audiologic, and radiologic measurements in a pediatric population with EVA who had available results from an SLC26A4 mutation screen. A large control group of children without SNHL had their temporal bone scans analyzed for the purpose of obtaining normative data to aid in identifying abnormalities in the temporal bone. We hypothesize that the presence of an SLC26A4 genotype will be associated with a more severe hearing loss, an increased risk of a progressive hearing loss, greater enlargement of the vestibular aqueduct, and decreased size of the modiolus.

**METHODS**

**PATIENTS**

Ninety-six children (192 temporal bone scans of the ear) served as radiologic controls. The scans in these control patients were performed on children without SNHL but with a history of trauma, chronic otitis media, facial nerve anomalies, cholesteatoma, mastoidectomy follow-up, headache, otalgia, or external otitis. The presence of radiologic abnormalities of the temporal bone in our population with an EVA was based on the measurements from this normative data. An EVA was considered present when the midpoint width and/or the operculum width equaled or exceeded 0.9 mm and 1.8 mm, respectively, in one or both ears.

An otologic database consisting of 1985 children diagnosed as having permanent SNHL was searched for children with an EVA diagnosed on computed tomographic (CT) evaluation. Twelve hundred and eleven children had temporal bone CT scans performed as part of their SNHL workup, and 204 of these children were found to have an EVA. Seventy-one children with an EVA had an SLC26A4 and a GJB2 mutation genetic screen. The medical charts of these 71 children were then reviewed for additional clinical information, including presenting history, neonatal and medical histories, family and social histories, imaging data, laboratory findings, and clinical examination findings.

**AUDIOLOGIC TESTING**

Behavioral audimetric testing was performed on all children included in this report. The test protocol was selected according to the developmental age of the child: (1) soundfield or ear- phone visual reinforcement audiometry; (2) play audiometry; or (3) conventional audimetric testing from 250 to 8000 Hz. Tympanometry was also used to assess middle ear status. A pure-tone average (PTA) was derived by averaging the audiometric thresholds at 500, 1000, 2000, and 4000 Hz. Hearing outcome was defined as stable, fluctuating, or progressive. Fluctuation of hearing was defined as a change in threshold of 15 dB or more at any frequency or a 20-dB threshold change in the auditory brainstem response, with a follow-up of at least 1 month. Progression was defined as a decline in the audiometric thresholds of 10 dB at 3 frequencies, 15 dB at 2 frequencies, and/or 20 dB at 1 frequency over a follow-up period of at least 1 month. These definitions were based on modifications to previously reported criteria.\textsuperscript{21,22} The slope or configuration of the audiogram was categorized as flat, down-sloping, up-sloping, or cookie-bite shaped.

**RADIOLOGIC PROTOCOL**

Computed tomographic scans of the temporal bones were performed in the axial projection at 1-mm intervals. Using the Medical Systems PathSpeed Workstation, version 8.1 (GE Healthcare, Slough, England), we obtained 7 measurements of each temporal bone: (1) vestibular aqueduct midpoint; (2) operculum; (3) vestibule width, (4) length, and (5) area; (6) modiolus; and (7) degree of cochlear partitioning. Details of our method of radiologic measurements and assessments can be found in 2 previous articles.\textsuperscript{11,21}

**SLC26A4 MUTATION SCREENING**

After extracting DNA from whole blood using standard procedures, mutation screening was completed by single-stranded conformational polymorphism and direct sequencing of the SLC26A4 coding region.\textsuperscript{14} In brief, polymerase chain reaction was performed with 40 ng of genomic DNA in a 10-µL reaction containing 1 µL of buffer (160mM ammonium sulfate, 670mM Tris-hydrochloride [pH 8.8], and 0.1% Tween 20); 1.65mM magnesium chloride; 0.4 µL of 2.5mM each of dATP, dCTP, dTTP, and GTP; 1 pmol each of forward and reverse primer; 5% wt/vol glycerol; and 0.25 U of Taq polymerase (primer sequences available on request). Usual amplification conditions were 94°C for 1 minute followed by 3 sets of 13 cycles each at 94°C for 30 seconds, 56°C (55°C, set 2; 54°C, set 3) for 30 seconds, and 72°C for 30 seconds, ending with an extension cycle of 72°C for 10 minutes, although for some primer pairs, adjustments were made to optimize the polymerase chain reaction. Reaction products were resolved on either MDE gel (FMC Bioproducts, Rockland, Me) or 6% nondenaturing polyacrylamide, 10% wt/vol glycerol gels, and visualized by silver staining. Sequencing was completed on a Model 373 (Applied Biosystems Inc, Foster City, Calif) automated sequencer. Sequence data were compared with published sequences for SLC26A4 using the Sequencer 3.1 software program package (Gene Codes, Ann Arbor, Mich).

**STATISTICAL ANALYSIS**

Associations among Pendrin genotype groups (biallelic, heterozygous, and none) and continuous variables (ie, PTA thresholds, vestibular aqueduct at midpoint and operculum, and modiolus size) were determined using the analysis of variance procedure and the Kruskal-Wallis procedure for nonparametric data, as appropriate. Associations among Pendrin genotype groups and categorical variables (ie, hearing loss progression and outcome and cochlear partitioning) were determined using the Pearson χ² and Fisher exact test, as appropriate. Post hoc comparisons were conducted when differences among groups were statistically significant at P= .05. All necessary post hoc comparisons were conducted using the Wilcoxon rank sum test (for continuous data) and Fisher exact test (for categorical data) with a Bonferroni correction for multiple comparisons to adjust for increased type 1 error. All statistical analyses were carried out using SAS software, version 9.1 for Windows (SAS Institute Inc., Cary, NC).

**RESULTS**

**STUDY POPULATION**

Seventy-one children were identified with an EVA who had subsequently undergone an SLC26A4 mutation screening. This population was 80% white (n=57), 19%...
African American (n=13), and 1% Asian (n=1). At the time of this study, the median age of our patient population was 8.7 years, ranging from 1.2 to 21.7 years. A family history of SNHL was present in 13 (18%) of the children. Among these cases with a positive family history of hearing loss, 1 family showed an autosomal recessive pattern of SNHL, with 2 siblings affected with an EVA. One child in this group with a positive family history of SNHL was clinically diagnosed as having Waardenburg syndrome (WS) type 2. The remaining 10 cases showed varying, nonspecific patterns of SNHL inheritance.

The overall female-male ratio was 1.2:1 (38 girls to 33 boys). The median age at diagnosis of SNHL was 57 months (age range, 0-204 months). The median duration of audiometric follow-up was 14 months (range, 0-140 months). None of our children had developed a goiter. Perchlorate discharge tests were not performed.

**AUDIOLOGIC FINDINGS**

A bilateral EVA was found in 48 (67%) of the children; a unilateral EVA was found in 23 (33%). The total number of ears with an EVA in our study was 119. Unilateral hearing loss was found in 16 children (22%). There was a match between unilateral SNHL and unilateral EVA in only 10 of these children (14 children with unilateral EVA had bilateral SNHL, and 6 children with unilateral SNHL had bilateral EVA). Among the 119 EVA ears, the most common audiometric configurations were flat in 62 ears (52%) and down-sloping in 37 ears (31%). A reverse cookie-bite shape (combination of low- and high-frequency loss with relatively normal mid-frequency thresholds) was found in 10 ears (8%), and an upsloping configuration was found in 10 ears (8%).

Of the 142 ears assessed for this study (from 71 children), 2 subjects (4 ears) had no audiologic follow-up. Of the 138 ears with audiologic follow-up, 69 ears (50%) continued to have a stable audiogram; 31 ears (22%) showed a fluctuating loss; and 38 ears (28%) had a progressive loss. The median initial PTA was 43 dB, with a range of 0 to 130 dB. The median final PTA was 55.00 dB (range, 1.25-121.25 dB). Two children with progressive and fluctuating hearing loss had slight improvement in PTA thresholds at the final audiologic assessment. Among the ears with progressive SNHL, the mean increase in PTA thresholds was 17.50 dB, ranging from 1.25 to 58.75 dB. Twenty-one (58%) of these ears had a progressive change in their PTA thresholds of less than 20 dB and those that had a progressive change 20 dB or more (33 vs 27 months, respectively; P=.98).

**LABORATORY FINDINGS**


Of the 16 children with unilateral SNHL, biallelic mutations in the SLC26A4 gene were seen in 2 (12%), heterozygous mutations in 3 (19%), and no SLC26A4 mutations were seen in the other 11 children (69%). Of the 23 children with unilateral EVA, biallelic mutations in the SLC26A4 gene were seen in 2 (9%), a heterozygous mutation in 1 child (4%), and the remaining 20 children (87%) had no SLC26A4 mutations.

A GJB2 screening was also performed in these 71 children, and the findings were positive in 5 cases (7%). One child had a homozygous 35delG/35delG mutation, and 4 children had a heterozygous mutation (T189M, M34T [twice], and S139N). One of the children with a biallelic mutation in the SLC26A4 gene (A189S/N324Y) had an M34T mutation in GJB2. There were no other children with mutations in both genes.

**RADIOLOGIC MEASUREMENTS**

A CT scan of the temporal bones was obtained in the 71 children with an EVA. All the data points were not available for each ear owing to limitations in scan quality, accounting for some of the variations in the following numbers. The median vestibular aqueduct size at the midpoint was 1.4 mm (range, 0.0-3.9 mm). The median vestibular aqueduct size at the operculum was 2.1 mm (range, 0.0-6.5 mm). The median modiolus size was 3.8 mm² (range, 1.0-6.9 mm²). Cochlear partitioning was demonstrated in 134 ears. It was clearly seen in 63 ears (47%), probably visible in 18 ears (13%), possibly visible in 27 ears (20%), and not present in 26 ears (19%). The length of the vestibule was measurable in 70 children (140 ears). The median length was 5.8 mm (range, 2.2-7.0 mm). The width of the vestibule was measurable in 70 children (139 ears). The median width was 3.2 mm (range, 2.3-5.6 mm). The area of the vestibule was measurable in 71 children (141 ears). The median area was 18.2 mm² (range, 12.5-27.3 mm²).

We defined abnormal temporal bone measurements by comparing them with the measurement range in a control group of 192 non-SNHL ears that had undergone temporal bone CT studies. The lack of SNHL in the control ears was confirmed by audiogram and obtaining scans for other otologic reasons (ie, cholesteatomas and facial nerve disorders). The resulting normal ranges, defined as values equal to or below the 95th percentile (2 standard deviations from the mean) were determined to be as follows: operculum 0.0 to 1.7 mm; midpoint, 0.0 to 0.8 mm; and modiolus, 3.4 to 7.4 mm.

**AUDIOMETRIC, GENETIC, AND RADIOLOGIC CORRELATIONS**

When including all the ears (n=142) in our study, the presence of SLC26A4 mutations was significantly associated with a wider vestibular aqueduct at the midpoint (P=.01) (Table 1). There was a trend toward hearing outcomes being similar in the ears of children with het-
Table 1. Genotype-Phenotype Analysis for All Studied Ears*

<table>
<thead>
<tr>
<th>Phenotypic Characteristic</th>
<th>Genotype (n = 142)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type (n = 104)</td>
<td>Heterozygous Mutation (n = 26)</td>
</tr>
<tr>
<td>PTA, dB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>36.25 (0.00-130.00)</td>
<td>51.25 (5.00-117.50)</td>
</tr>
<tr>
<td>Final</td>
<td>43.75 (1.25-121.25)</td>
<td>64.38 (11.25-117.50)</td>
</tr>
<tr>
<td>Ear measurement, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midpoint, mm‡</td>
<td>1.10 (0.00-3.50)</td>
<td>1.90 (0.00-3.90)</td>
</tr>
<tr>
<td>Operculum, mm</td>
<td>2.00 (0.00-6.50)</td>
<td>2.65 (0.70-4.60)</td>
</tr>
<tr>
<td>Vestibular width, mm</td>
<td>3.20 (2.50-4.20)</td>
<td>3.15 (2.30-4.20)</td>
</tr>
<tr>
<td>Vestibular length, mm</td>
<td>5.75 (4.90-7.00)</td>
<td>5.65 (4.90-6.50)</td>
</tr>
<tr>
<td>Vestibular area, mm²</td>
<td>18.24 (14.00-24.36)</td>
<td>18.92 (12.50-27.30)</td>
</tr>
<tr>
<td>Modiolus, mm²</td>
<td>3.90 (1.20-6.60)</td>
<td>3.25 (1.40-6.50)</td>
</tr>
<tr>
<td>Hearing outcome</td>
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</tr>
<tr>
<td>Stable</td>
<td>47 (48)</td>
<td>54 (14)</td>
</tr>
<tr>
<td>Fluctuating</td>
<td>27 (28)</td>
<td>11 (3)</td>
</tr>
<tr>
<td>Progressive</td>
<td>26 (26)</td>
<td>35 (9)</td>
</tr>
<tr>
<td>Cochlea partitioning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearly</td>
<td>49 (51)</td>
<td>38 (10)</td>
</tr>
<tr>
<td>Probably</td>
<td>11 (11)</td>
<td>15 (4)</td>
</tr>
<tr>
<td>Possibly</td>
<td>21 (22)</td>
<td>15 (4)</td>
</tr>
<tr>
<td>Not</td>
<td>15 (16)</td>
<td>23 (6)</td>
</tr>
<tr>
<td>NA</td>
<td>4 (4)</td>
<td>8 (2)</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; PTA, pure-tone average.

*P value comparison of 3 groups using Wilcoxon rank sum test or Fisher exact test.
†With Bonferroni adjustment, statistical differences remain between wild-type and heterozygous mutation groups (P=.02). No statistical differences between heterozygous mutation and biallelic mutation or biallelic mutation and wild-type groups, although the heterozygous mutation and biallelic mutation groups look similar.

The presence of any SLC26A4 mutation demonstrated a significant difference in measurements at the midpoint and operculum size and in hearing outcomes for the better-hearing ear among children with bilateral EVA when those were compared with combined biallelic and heterozygous mutations vs wild-type (Table 2). Children with SLC26A4 mutations had more severe final PTA than patients with no mutations (63.75 dB vs 31.88 dB, respectively) (P=.04); the midpoint measurements were significantly larger (2.15 mm vs 1.10 mm) (P=.003); and the operculum measurements were significantly larger (3.05 vs 2.00)(P=.03).

An EVA with enlargement of the endolymphatic sac is commonly found in Pendred syndrome. Mutations in SLC26A4 can cause a phenotype ranging from EVA to a severe Mondini anomaly. Thyromegaly and alterations of thyroid hormone status, a classic feature of Pendred syndrome, has not yet been seen in our patient population. Because these indications may not manifest until adolescence or adulthood, it may be too early for this feature to become clinically evident in some of our pediatric population. Mutations in SLC26A4 were seen in only...
**Table 2. Genotype-Phenotype Analysis for Bilateral EVA Ears**

<table>
<thead>
<tr>
<th>Phenotypic Characteristic</th>
<th>Wild-type (n = 64)</th>
<th>Heterozygous Mutation (n = 24)</th>
<th>Biallelic Mutation (n = 8)</th>
<th>P Value†</th>
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<tbody>
<tr>
<td><strong>PTA, dB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>43.13 (6.25-120.0)</td>
<td>51.25 (5.00-102.50)</td>
<td>40.00 (20.00-100.00)</td>
<td>.88</td>
</tr>
<tr>
<td>Final</td>
<td>49.38 (10.00-111.25)</td>
<td>64.38 (13.75-113.75)</td>
<td>80.63 (12.50-105.00)</td>
<td>.25</td>
</tr>
<tr>
<td><strong>Ear measurement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midpoint, mm‡</td>
<td>1.50 (0.00-3.50)</td>
<td>1.95 (0.00-3.90)</td>
<td>2.50 (0.80-3.60)</td>
<td>.006</td>
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<tr>
<td>Operculum, mm</td>
<td>2.30 (0.00-6.50)</td>
<td>2.90 (0.80-4.60)</td>
<td>3.00 (1.70-4.20)</td>
<td>.20</td>
</tr>
<tr>
<td>Vestibular width, mm§</td>
<td>3.15 (2.60-4.20)</td>
<td>3.20 (2.30-4.20)</td>
<td>3.60 (2.30-5.60)</td>
<td>.03</td>
</tr>
<tr>
<td>Vestibular length, mm</td>
<td>5.65 (4.90-7.00)</td>
<td>5.85 (4.90-6.50)</td>
<td>5.95 (2.20-6.20)</td>
<td>.86</td>
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<tr>
<td>Vestibular area, mm²</td>
<td>17.97 (14.50-24.36)</td>
<td>19.18 (12.74-27.30)</td>
<td>17.71 (0.00-24.60)</td>
<td>.80</td>
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<tr>
<td>Modiolus, mm²</td>
<td>3.60 (1.20-6.80)</td>
<td>3.10 (1.40-6.50)</td>
<td>2.0 (1.00-6.90)</td>
<td>.28</td>
</tr>
<tr>
<td><strong>Hearing outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td>.03</td>
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<tr>
<td>Stable</td>
<td>47 (30)</td>
<td>50 (12)</td>
<td>63 (5)</td>
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<tr>
<td>Fluctuating</td>
<td>36 (23)</td>
<td>12 (3)</td>
<td>0</td>
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<tr>
<td>Progressive</td>
<td>17 (11)</td>
<td>38 (9)</td>
<td>37 (3)</td>
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<td><strong>Cochlea partitioning</strong></td>
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<td></td>
<td></td>
<td>.13</td>
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<tr>
<td>Clearly</td>
<td>42 (27)</td>
<td>42 (10)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Probably</td>
<td>6 (6)</td>
<td>8 (2)</td>
<td>25 (2)</td>
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<tr>
<td>Possibly</td>
<td>24 (15)</td>
<td>17 (4)</td>
<td>12 (1)</td>
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<tr>
<td>Not</td>
<td>22 (14)</td>
<td>25 (6)</td>
<td>38 (3)</td>
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<tr>
<td>NA</td>
<td>6 (4)</td>
<td>8 (2)</td>
<td>25 (2)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** EVA, enlarged vestibular aqueduct; NA, not applicable; PTA, pure-tone average.

*Unless otherwise indicated, data are reported as mean (range) value or number (percentage) of subjects.

†P value comparison of 3 groups using Wilcoxon rank sum test or Fisher exact test.

‡With Bonferroni adjustment, statistical differences remain between the wild-type and heterozygous mutation groups (P = .03); marginal statistical difference between wild-type and biallelic mutation groups (P = .058); and no statistical differences between heterozygous mutation and biallelic mutation groups.

§With Bonferroni adjustment, statistical differences remain between the wild-type and biallelic mutation groups (P = .03) and marginal statistical difference between biallelic mutation and heterozygous mutation groups (P = .09).

**Table 3. Genotype-Phenotype Analysis of the Better-Hearing Ear in Children With Bilateral EVA**

<table>
<thead>
<tr>
<th>Phenotypic Characteristic</th>
<th>Wild-type (n = 32)</th>
<th>Heterozygous Mutation (n = 12)</th>
<th>Biallelic Mutation (n = 4)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PTA, dB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>32.50 (6.25-120.00)</td>
<td>45.63 (5.00-102.50)</td>
<td>40.00 (20.00-95.00)</td>
<td>.65</td>
</tr>
<tr>
<td>Final</td>
<td>31.88 (10.00-106.25)</td>
<td>58.75 (13.75-83.75)</td>
<td>80.60 (63.75-100.00)</td>
<td>.048</td>
</tr>
<tr>
<td><strong>Ear measurement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midpoint, mm</td>
<td>1.10 (0.00-3.50)</td>
<td>2.05 (0.30-3.90)</td>
<td>2.50 (1.40-3.20)</td>
<td>.01</td>
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<td>Operculum, mm</td>
<td>2.00 (0.00-6.50)</td>
<td>3.00 (1.00-4.60)</td>
<td>3.55 (2.00-4.20)</td>
<td>.05</td>
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<tr>
<td>Vestibular width, mm</td>
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<td>3.30 (2.30-4.00)</td>
<td>3.70 (3.40-5.60)</td>
<td>.10</td>
</tr>
<tr>
<td>Vestibular length, mm</td>
<td>5.70 (4.90-6.90)</td>
<td>5.95 (5.00-6.50)</td>
<td>5.90 (2.20-6.00)</td>
<td>.56</td>
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<tr>
<td>Vestibular area, mm²</td>
<td>17.40 (14.50-24.36)</td>
<td>19.55 (14.03-26.00)</td>
<td>16.19 (0.00-22.20)</td>
<td>.54</td>
</tr>
<tr>
<td>Modiolus, mm²</td>
<td>3.60 (1.20-6.60)</td>
<td>3.00 (1.40-6.50)</td>
<td>2.10 (1.00-5.50)</td>
<td>.45</td>
</tr>
<tr>
<td><strong>Hearing outcome</strong></td>
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<td></td>
<td>.35</td>
</tr>
<tr>
<td>Stable</td>
<td>44 (14)</td>
<td>50 (6)</td>
<td>50 (2)</td>
<td></td>
</tr>
<tr>
<td>Fluctuating</td>
<td>37 (12)</td>
<td>17 (2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Progressive</td>
<td>19 (6)</td>
<td>33 (4)</td>
<td>33 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Cochlea partitioning</strong></td>
<td></td>
<td></td>
<td></td>
<td>.40</td>
</tr>
<tr>
<td>Clearly</td>
<td>44 (14)</td>
<td>25 (3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Probably</td>
<td>9 (3)</td>
<td>17 (2)</td>
<td>25 (1)</td>
<td></td>
</tr>
<tr>
<td>Possibly</td>
<td>19 (6)</td>
<td>25 (3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Not</td>
<td>22 (7)</td>
<td>25 (3)</td>
<td>50 (2)</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>6 (2)</td>
<td>8 (1)</td>
<td>25 (1)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** EVA, enlarged vestibular aqueduct; NA, not applicable; PTA, pure-tone average.

*Unless otherwise indicated, data are reported as mean (range) value or number (percentage) of subjects.

†P value comparison of 3 groups using Wilcoxon rank sum test or Fisher exact test.
27% of our patient population. We speculate that other genes might play roles as modifiers to the SLC26A4 gene, or they might act independently to produce the EVA phenotype. The low level of mutations found in our study may be related to the low level of affected families in our cohort. It may also reflect the limitations of current mutation detection. An additional screening in these children for FOXI1 gene mutations did not show any mutations in this gene.15,24

Other genetic factors, such as WS and branchio-oto-renal syndrome (BOR), can also account for an EVA phenotype. Our group has previously reported that approximately 10% of an unselected group of patients with EVA had WS.23 Our present cohort is a selected group for “non-syndromic” EVA (ie, no obvious features of WS or BOR); therefore, the incidence of WS is low. The single patient with WS in our cohort was diagnosed as having type 2 WS after his SLC26A4 testing was complete. This was owing to the subtlety of the phenotype. Careful physical examination in the rest of our patients has failed to discover WS or BOR to date.

A difference in mutations of the SLC26A4 gene in different ethnic populations has been observed between Western and Southeast Asian studies of patients with Pendred syndrome.25,26 We cannot comment on this ethnic variation because no patients from this ethnic background took part in our study. There was, however, a trend toward better hearing thresholds, smaller midpoint size, and smaller vestibular area in our African American population with biallelic SLC26A4 mutations, suggesting a potential gene-gene interaction with race. We also present the F35S and E622K mutations as novel SLC26A4 mutations that have not been present in previous control samples.

The presence of positive GJB2 mutations in our EVA population has been previously described.11 Together with our current data, there were 3 heterozygous mutations and 1 biallelic GJB2 mutation seen in children with an EVA who did not have a positive finding on SLC26A4 mutation screening. One child with a biallelic SLC26A4 mutation also had a heterozygous GJB2 mutation. This child also had a unilateral EVA on her temporal bone analysis. The presence of heterozygous GJB2 mutations in the present population is unlikely to affect the hearing thresholds and outcomes described. In the child with a biallelic GJB2 mutation, her audiometric data may reflect the presence of an EVA as well as her GJB2 genotype. Her risk of progression and the presence of an EVA in her case were included in the analysis because it was assumed to be independent of her GJB2 genotype.

One limitation of the present study is the small size of the biallelic mutation group. Other interesting yet confounding issues were the presence of biallelic mutations in children with unilateral SNHL, no SNHL, and with a unilateral EVA. These children would be classified as having Pendred syndrome by the criteria proposed by Pryor et al12; however, unilateral EVA and SNHL have not been described in children with Pendred syndrome in the literature previously.

We did find a significant association with enlargement of the vestibular aqueduct at the midpoint and the vestibule width in our children with an EVA and a positive SLC26A4 finding. These children were also more likely to have a stable hearing outcome. Further recruitment of children with an EVA and subsequent genetic analysis will help to clarify some of the trends and associations seen in these groups and provide more conclusive evidence of this relationship. The use of a standardized system for the analysis and reporting of CT and magnetic resonance imaging scans of the temporal bone will improve the identification of inner-ear anomalies.

In conclusion, children with an EVA have a wide variety of audiometric thresholds and radiologic anomalies of the temporal bone. Mutations in the SLC26A4 gene were seen in 27% of children with an EVA. Progression of hearing loss was seen in 28% of the ears in our study. SLC26A4 mutations in children with an EVA are associated with specific abnormalities of the temporal bone and a stable hearing outcome. A heterozygous SLC26A4 genotype appears to have a mixed phenotype compared with patients with a biallelic and wild-type genotype in regard to audiometric and radiologic measurements.

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Author Contributions: Dr Greinwald had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Madden, Halsted, Smith, Choo, and Greinwald. Acquisition of data: Madden, Halsted, Bardo, Boston, Nishimura, Yang, Benton, Smith, and Greinwald. Analysis and interpretation of data: Madden, Halsted, Meinzen-Derr, Arjmand, Das, Choo, and Greinwald. Drafting of the manuscript: Madden, Meinzen-Derr, Yang, Smith, Choo, and Greinwald. Critical revision of the manuscript for important intellectual content: Madden, Halsted, Meinzen-Derr, Boston, Arjmand, Benton, Das, Smith, Choo, and Greinwald. Statistical analysis: Meinzen-Derr. Obtained funding: Smith and Greinwald. Administrative, technical, and material support: Halsted, Boston, Yang, Smith, and Greinwald. Study supervision: Halsted, Yang, Smith, Choo, and Greinwald. Financial Disclosure: None reported.

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REFERENCES


**Correction**

**Typographic Errors in Genotype Terms.** In the Original Article titled “The Influence of Mutations in the SLC26A4 Gene on the Temporal Bone in a Population With Enlarged Vestibular Aqueduct,” published in the February issue of the *Archives* (2007;133:162-168), 5 typographic errors were published in the genotype terms in the first paragraph of the “Laboratory Findings” subsection of the “Results” section. The correct terms appear in bold in the following paragraph: