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SLC26A4 genotype, but not cochlear radiologic structure, is correlated with hearing loss in ears with an enlarged vestibular aqueduct (EVA)

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Abstract

Objectives/Hypothesis—Identify correlations among *SLC26A4* genotype, cochlear structural anomalies, and hearing loss associated with enlargement of the vestibular aqueduct (EVA).

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Study Design—Prospective cohort survey, National Institutes of Health, Clinical Center, a federal biomedical research facility.

Methods—83 individuals, 11 months to 59 years of age, with EVA in at least one ear. Correlations among pure-tone hearing thresholds, number of mutant *SLC26A4* alleles, and the presence of cochlear anomalies detected by computed tomography or magnetic resonance imaging.

Results—Linear mixed-effect model indicates significantly poorer hearing in ears with EVA from individuals with two mutant alleles of *SLC26A4* than in those with EVA and a single mutant allele ($p = .012$) or no mutant alleles ($p = .007$) in this gene. There was no detectable relationship between degree of hearing loss and the presence of structural cochlear anomalies.

Conclusions—The number of mutant alleles of *SLC26A4*, but not the presence of cochlear anomalies, has a significant association with severity of hearing loss in ears with EVA. This information will be useful for prognostic counseling of patients and families with EVA.

Keywords

enlarged vestibular aqueduct; *SLC26A4*; hearing

Introduction

Enlargement of the vestibular aqueduct (EVA) is a common radiological malformation of the inner ear in children with early-onset sensorineural hearing loss (SNHL). It is identified by computed tomography (CT) or magnetic resonance imaging (MRI) 1–6. EVA can be unilateral or bilateral and either syndromic or nonsyndromic. It is the most penetrant feature of Pendred syndrome (PDS), an autosomal recessive disorder caused by biallelic mutations of the *SLC26A4* gene 7 (OMIM605646). PDS was originally defined as goiter and profound congenital SNHL, but is now known to comprise variable thyroid and auditory phenotypes 8. Nonsyndromic EVA can also be associated with *SLC26A4* mutations 9, 10. Approximately 25% of EVA patients have only one detectable mutant allele of *SLC26A4* and up to 50% of EVA patients have no mutations that we can detect in this gene 11, 12, further contributing to nosologic confusion.

The auditory phenotype of EVA can include fluctuations, progressive changes in hearing, or both 4, 13. The pathophysiological mechanism of the hearing loss is unknown. There is no correlation of either the degree of hearing loss or the occurrence of sudden hearing loss and the degree of enlargement of the vestibular aqueduct or its contents, the endolymphatic duct 3, 4. There are contradictory reports on the effect of the number of *SLC26A4* mutations on hearing 11, 14–18 and on inner ear radiologic findings. One study 11 suggested that biallelic mutations are associated with Mondini dysplasia (incomplete cochlear partition with a scala communis) more frequently than with EVA while another 15 reported a correlation of number of *SLC26A4* mutant alleles with the width of the vestibular aqueduct. However, other studies failed to demonstrate a significant relationship between the number of mutant *SLC26A4* alleles with the severity of the radiological phenotype 19, 5. The purpose of our study was to identify and characterize potential correlations among *SLC26A4* genotype, inner ear radiologic findings, and hearing in a cohort of patients with EVA.

Materials and Methods

Subjects

This study was approved by the Combined Neuroscience Institutional Review Board, National Institutes of Health (NIH), Bethesda, MD. We obtained written, informed consent

from adult subjects and parents of minor subjects. Audiological data were available for 83 of 86 subjects with nonsyndromic EVA or PDS who were previously described in reports on *SLC26A4* genotype and thyroid phenotype 12, 20, 21. Participants self-described race and ethnicity in accordance with our institutional designations (Table 1). All subjects had unilateral or bilateral EVA, defined as a diameter >1.5 mm at the midpoint of the course of the vestibular aqueduct between the posterior cranial fossa and the vestibule of the inner ear, or an otherwise grossly malformed morphology of the vestibular aqueduct 12.

SLC26A4 genotype

SLC26A4 genotypes for the subjects have been previously reported 12, 20. We categorized subjects according to number of detected mutant alleles of *SLC26A4*: zero (M0), one (M1), or two (M2). There are six hypofunctional variants (c.-60A>G, c.-3-2A>G, p.F335L, p.C565Y, p.L597S, and p.M775T) of uncertain pathogenicity within the cohort 20. Our preliminary analysis revealed no statistically significant effect of how we classified these variants (pathogenic versus nonpathogenic) upon comparisons of hearing (0.5/1/2/4-kHz pure-tone air-conduction threshold average) among M0, M1, and M2 subjects. Therefore, these hypofunctional variants were considered nonpathogenic for subsequent analyses, as in Madeo et al 21. Twenty (24%) subjects were classified as M2, 16 (19%) as M1, and 47 (57%) as M0.

Radiologic phenotype

We performed MRI of the inner ear, whenever possible, with fast induction employing steady state excitation (FIESTA), T2-weighted fast spin echo, or both on a 1.5-Tesla system at the NIH Clinical Center 12. Seventy of 83 subjects also had previous imaging of the inner ear either by CT (57), MRI (4), or both (9) available for review. All imaging studies were reviewed together at the completion of this study by a Neuroradiologist (J.A.B.) and an Otolaryngologist-Head and Neck Surgeon (A.J.G). We classified 146 ears with EVA into two groups: EVA, if no cochlear anomaly was identified, or EVA-plus-cochlear-anomaly, if an anomaly was identified. Potential cochlear anomalies included Michel deformity, cochlear aplasia or hypoplasia, common cavity, and incomplete cochlear partition. We did not classify hypoplastic modiolus as a cochlear anomaly due to its extremely high prevalence in ears with EVA 22. The cochlear structure of four ears was considered indeterminate due to insufficient technical quality of the images and these ears were excluded from further analysis involving radiologic data.

Audiologic phenotype

Three ears with cochlear implants were excluded from analysis. Thus, audiometric data for 143 ears with EVA from 83 subjects were evaluated (Table 1; age range = 11 months to 59 y, $\bar{x}$ = 11.3 y, SD = 12.0 y). Sixty-one subjects had an audiological evaluation at the NIH Clinical Center and 22 subjects provided outside audiological records for review.

Audiological evaluations at the NIH included, when possible, pure-tone air- (250 to 8000 Hz) and bone-conduction (250 to 4000 Hz) threshold tests using clinical audiometers in double-walled sound suites in accordance with ANSI standards 23, 24. Middle ear function was assessed by tympanometry (226-Hz probe tone). We attempted to obtain previous and subsequent audiograms performed at other facilities, which were included for analysis only if the reliability had been rated as good by the examining audiologist. For each subject, we defined the reference audiogram as the most recent and complete audiological evaluation conducted at the NIH, or elsewhere if the NIH evaluation was incomplete.

Type of hearing loss was based upon 0.5/1/2-kHz pure-tone averages for air- and bone-conduction thresholds and degree of hearing loss was classified according to the 0.5/1/2/4-

kHz pure-tone air-conduction threshold average as described previously 25. We also calculated low-(0.25 to 0.5 kHz), mid- (1 to 2 kHz), and high-frequency (4 to 8 kHz) pure-tone air-conduction threshold averages. The air-bone gap (ABG) was calculated for each octave frequency from 0.25 to 4 kHz and as an average for at 0.5, 1, and 2 kHz.

Seventy of 83 subjects had serial audiograms spanning at least one month for comparison to their earliest available (baseline) evaluation. We adapted the method of Madden et al 2 to categorize longitudinal pure-tone thresholds as stable, progressive, fluctuating, improving, fluctuating/improving, or fluctuating/progressive according to definitions in Table 2.

Statistical Analyses

Data from ears with evidence (i.e., abnormal tympanometric results) of middle ear pathology were removed from ABG and longitudinal analyses. We used one-way analysis of variance (ANOVA) to investigate possible relationships between the magnitude of the ABG and number of mutant alleles of *SLC26A4*. An independent-samples *t*-test was performed to search for associations of ABG size with cochlear radiological phenotype (EVA versus EVA-plus-cochlear-anomaly). We calculated Pearson's correlation coefficient for ABG magnitude and the vestibular aqueduct diameter on CT scans. We performed a one-way ANOVA to search for relationships between the size of the vestibular aqueduct measured by CT scan and the number of mutant alleles of *SLC264*.

Fisher's exact test was used to evaluate for associations between degree of hearing loss, number of mutant alleles of *SLC26A4* (M0, M1, M2), and cochlear radiological phenotype (EVA, EVA-plus-cochlear-anomaly). In order to avoid confounding effects between ear-specific data (degree of hearing loss and radiological phenotype) and person-specific data (genotype), we excluded 24 individuals (28 ears with EVA) for whom data from only one ear was available.

We analyzed the 143 ears with EVA using a linear mixed-effects model, which accounts for correlation structure due to inclusion of two ears from the same individual. We evaluated relationships between hearing (0.5/1/2/4-Hz, low-, mid- or high-frequency pure-tone threshold averages) and the following independent variables: age, gender, genotype, presence/absence of cochlear anomalies, and thyroid phenotype (goiter versus normal). We performed a similar mixed-effects analysis to model the interval change in 0.5/1/2/4-kHz pure-tone threshold average from baseline to final audiogram. In order to avoid ceiling effects, we did not include individuals with severe or profound hearing loss at baseline in this latter analysis.

All exploratory and modeling analyses were performed using SPSS software for Macintosh version 15 (SPSS Inc, Chicago, Illinois).

Results

Radiologic phenotype

Thirty-two (22.5%) of 142 ears with EVA with radiological data also had a cochlear anomaly. Thirty (21.1%) ears had an incomplete cochlear partition ("Mondini deformity"), one (0.7%) ear had a common cavity, and one (0.7%) ear had cochlear hypoplasia.

Audiologic phenotype

Ninety ears had sufficient data to classify the type of hearing loss. Sixty (67%) ears had SNHL, four (4%) had conductive loss, and 25 (28%) had mixed loss. One (1%) ear was

classified as normal based upon the 0.5/1/2-kHz pure-tone threshold average, although SNHL was present at 6000 Hz and 8000 Hz.

Degree of hearing loss could be defined in 139 ears (range = 10 to 125 dB HL, $\bar{x}$ = 70.63 dB HL, SD = 29.2 dB). It was mild in 18 (13%) ears, moderate in 43 (31%), severe in 36 (26%), and profound in 36 (26%). Six (4%) ears, all in subjects less than 14 years of age, were classified as having normal hearing based on the 0.5/1/2/4-kHz pure-tone air-conduction threshold average, although all six had hearing loss (>20 dB HL) for at least one frequency. We did not observe normal hearing in either ear of any subject with two mutant alleles of *SLC26A4*, although one subject (#1627 in Pryor et al 12) with unilateral hearing loss and EVA was classified as M1 in our analysis but would be M2 if p.F335L is pathogenic 20.

Fifty-seven ears had reference audiograms with both air- and bone-conduction threshold data and normal tympanometry. We detected air-bone gaps (ABGs) of 15 to 55 dB for at least one stimulus frequency from 0.25 to 4 kHz in 49 (86%) ears and for at least two frequencies in 37 (65%) ears. There was no significant (α = .05) relationship between the magnitude of the ABG at any frequency with the number of mutant alleles of *SLC26A4*, the presence or absence of a cochlear-anomaly, or the diameter of the vestibular aqueduct.

Relationships of hearing with genotype and cochlear structure

Figure 1a shows that mean air-conduction thresholds are greater in M2 ears than in M0 or M1 ears. Although there was a trend for poorer hearing in ears with EVA and a coinciding cochlear anomaly, a significant difference was not observed (Figure 1b).

There was a significant association of the number of mutant alleles of *SLC26A4* with the degree of hearing loss (Fisher's exact test, p = .002), and an association of the number of mutant alleles of *SLC26A4* with the presence of a cochlear anomaly that approached significance (p = .058). There was no significant association between degree of hearing loss and the presence of a cochlear anomaly (p = .82).

We sought to further explore these relationships and the potential influences of age, gender, and thyroid phenotype with a linear mixed-effects model of hearing loss on the number of mutant alleles of *SLC26A4* and the presence/absence of cochlear anomalies, including these factors as covariates. According to this model, hearing in the M2 group was 21.3 dB (p = .007) poorer than in the M0 group and 21.9 dB (p = .012) poorer than in the M1 group. Hearing in the M1 group was 5.9 dB poorer than in the M0 group, but this was not statistically significant (p = .50). Hearing in ears with cochlear anomalies was not significantly different (0.05 dB, p = .99) from ears without a cochlear anomaly. The model identified no effect of age on hearing, no significant difference in hearing between males and females, and no effect of thyroid phenotype. There was no significant (α = .05) relationship between the size of the vestibular aqueduct and the number of mutant *SLC26A4* alleles (p = .35).

Longitudinal hearing analyses

Twenty-eight (26%) ears had stable hearing over an average duration of 3.7 years of follow-up (Table 3). Thirty-three (30%) ears had progressive hearing loss when followed, on average, for 4.9 years, and eight (7%) ears had improvement in hearing when followed for an average of 2.7 years. The remaining three categories, all characterized by fluctuations (Table 2), included 40 (37%) ears. Twenty-three (58%) of 40 ears with fluctuations showed progressive loss of hearing.

We applied a linear mixed-effects model to longitudinal audiological data for ears with normal, mild, or moderate hearing loss at baseline. This revealed that duration of follow-up

had a small (0.96 dB/y) but significant (p = .046) relationship with hearing loss progression. There were no significant effects of number of mutant alleles of *SLC26A4* or the presence/absence of cochlear anomalies on longitudinal hearing.

Discussion

We observed that ears with EVA in individuals with two mutant alleles of *SLC26A4* have significantly greater hearing loss than those with one or no mutant alleles of *SLC26A4* (Figure 1a). This is consistent with reports 14, 15 of two different Caucasian-majority cohorts, but differs from those of some other studies 11, 16, 17, 18, 20. For example, there was no detectable relationship of hearing level with number of mutant (p.H723R) alleles of *SLC26A4* in a small Japanese cohort with probable ceiling effects on hearing levels 16, 17. Wu et al 18 detected no similar relationship in a Taiwanese cohort, but their metric for hearing was not defined. Azaiez et al 11 found no relationship between number of *SLC26A4* mutations and a binaural mean pure-tone threshold average in a Caucasian-majority cohort, but this discrepancy may reflect the misclassification of *SLC26A4* polymorphisms as mutations 20 or the use of a binaural measure of hearing in a cohort with a high prevalence of unilateral hearing loss. We cannot rule out potential associations of number of mutant *SLC26A4* alleles with sensory hearing because we had an insufficient number of audiograms with bone conduction data. This reflects, in part, a bias introduced by ceiling effects in individuals with severe and profound hearing loss in whom there is no response to bone-conduction stimuli at the limits of the test equipment.

We also observed that the presence of a cochlear anomaly in addition to EVA is not associated with more severe hearing loss when the contribution of other covariates (e.g., genotype) is taken into account. We are unable to directly compare our findings to those of a similar analysis by Wu et al 19 because they classified and grouped their ears with cochlear anomalies differently. Okumura et al 26 reported better hearing in 23 ears with additional cochlear anomalies among Japanese EVA patients, but they defined EVA as a midpoint diameter > 4mm and did not report their metric for hearing loss.

Although we detected a suggestive relationship between number of *SLC26A4* mutant alleles and cochlear radiological status, the relationship was not statistically significant. This is consistent with two 14, 18 previous independent reports finding no correlation of number of mutant *SLC26A4* alleles with pattern of inner ear malformations. In contrast, Azaiez et al 11 reported that individuals with biallelic *SLC26A4* mutations have a more severe radiological phenotype, but ambiguities in their genotypic and phenotypic classification preclude a direct conclusion or comparison 20. We did not observe a significant relationship between size of the EVA and number of mutant *SLC26A4* alleles, in contrast to the study of Madden et al 15 that more broadly defined EVA as a midpoint diameter >0.9 mm or an operculum diameter >1.8 mm. The strong inverse correlation of two mutant alleles of *SLC26A4* with a vestibular aqueduct midpoint diameter <1.5 mm 12 may account for their observed relationship and the apparent discrepancy with our conclusion.

Our data suggest that neither the number of mutant alleles of *SLC26A4* nor cochlear radiologic phenotype significantly affect longitudinal changes in hearing. Albert et al 14 reported a similar result. Although Madden et al 15 found that ears with monoallelic or biallelic *SLC26A4* mutations are more stable than those with no mutation, the difference was not significant among ears with >36 months of follow-up. Our correlation of number of mutant alleles with severity of hearing loss but not longitudinal hearing changes may reflect an inadequate duration of follow-up in our study. We indeed observed a small but significant effect of duration of follow-up on the amount of deterioration in hearing in ears with less

than severe hearing loss at baseline. It may also reflect the uncontrolled contribution of environmental factors (e.g., trauma) associated with changes in hearing in ears with EVA3.

We did not detect a significant relationship between the size of the air-bone gap (ABG) and either the number of *SLC26A4* mutations, the presence of a cochlear anomaly, or the size of the vestibular aqueduct. The frequent association of EVA with ABGs and normal tympanometry is thought to reflect the existence of a third mobile window in the labyrinth 27. This theory is supported by the observation of abnormally low thresholds for the vestibular-evoked myogenic potential (VEMP) in a subpopulation of children with EVA 28. The lack of correlation of VA diameter with ABG size does not invalidate this hypothesis because the midpoint diameter of the VA may not be mechanistically relevant to this model. We did not measure VEMP thresholds on a sufficient number of our subjects to test this hypothesis 28.

Conclusion

Our study indicates that the number of mutant alleles of *SLC26A4* has a significant association with severity of hearing loss in ears with EVA. Furthermore, the presence of structural anomalies of the cochlea is not associated with more severe hearing loss when other covariates (e.g., genotype) are taken into account. This information will be useful for prognostic counseling of patients and families with EVA.

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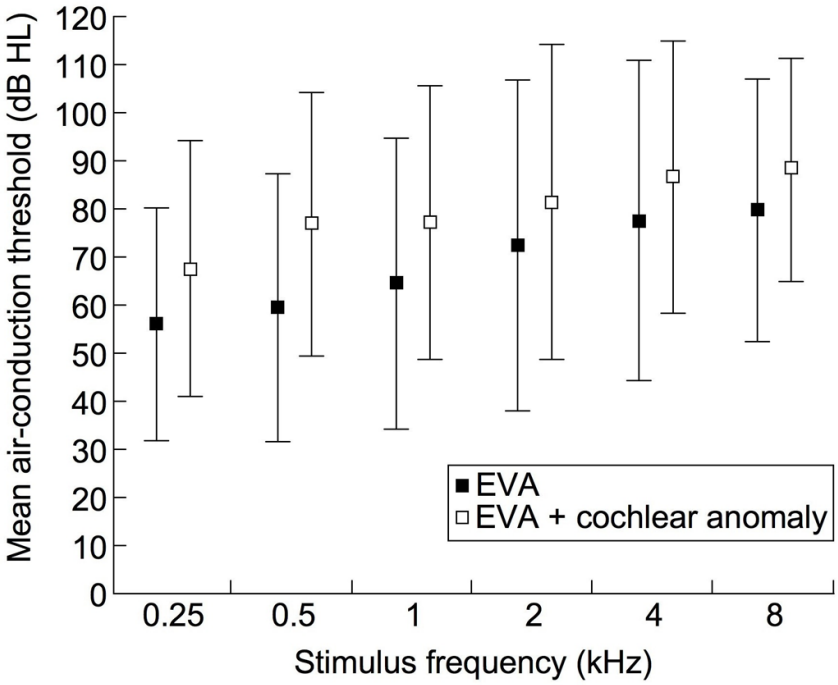

Figure 1.

Mean (+/− 1SD) pure-tone air-conduction thresholds in ears with EVA grouped according to (a) number of mutant alleles of *SLC26A4* and (b) presence or absence of cochlear anomalies.

Table I

Eighty-three EVA subjects analyzed in this study.

Variable	Classification	n (% of cohort)
Gender	Male	38 (46%)
	Female	45 (54%)
Ethnicity	White, non-Hispanic	72 (87%)
	Black, non-Hispanic	2 (2%)
	Hispanic	1 (1%)
	Other/unknown	8 (10%)
Number of mutant alleles of *SLC26A4*	0 (M0)	47 (57%)
	1 (M1)	16 (19%)
	2 (M2)	20 (24%)
EVA laterality	Bilateral	63 (76%)
	Unilateral	20 (24%)

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Table II

Longitudinal categorization of pure-tone threshold data.

Category	Definition
Stable	No significant fluctuation, improvement or progression as defined below
Improving	10-dB improvement at any three frequencies
	or 15-dB improvement at any two frequencies
	or ≥ 20-dB improvement at any one frequency
Progressive	10-dB decline at any three frequencies
	or 15-dB decline at any two frequencies
	or ≥ 20-dB decline at any one frequency
Fluctuating*	Interim audiogram shows 10-dB change at any three frequencies
	or 15-dB change at any two frequencies
	or ≥ 20-dB change at any one frequency
	and final audiogram does not show significant improvement or progression from baseline
Fluctuating/Improving*	Interim audiogram shows 10-dB change at any three frequencies
	or 15-dB change at any two frequencies
	or ≥ 20-dB change at any one frequency
	and final audiogram shows significant improvement from baseline
Fluctuating/Progressive*	Interim audiogram shows 10-dB change at any three frequencies
	or 15-dB change at any two frequencies
	or ≥ 20-dB change at any one frequency
	and final audiogram shows significant progression from baseline

* ≥ 3 audiograms required to assess.

Table III

Longitudinal hearing descriptive statistics.

	Stable	Progressive	Fluctuating- progressive	Fluctuating	Fluctuating-improving	Improving
	Number of ears					
Total	28	33	23	12	5	8
No mutations[+]	13	12	13	9	2	7
1 mutations[+]	6	9	6	2	1	1
2 mutations[+]	9	12	4	1	2	0
(−) Cochlear anomaly	20	27	19	5	5	8
(+) Cochlear anomaly	8	5	4	6	0	0
No data	0	1	0	1	0	0
	$\bar{x}$ (SD)					
Duration of follow-up (y)	3.7 (4.2)	4.9 (4.8)	8.5 (7.0)	3.2 (2.6)	9.9 (9.0)	2.7 (2.9)
Number of audiograms	3.4 (1.2)	4.6 (3.1)	9.4 (4.5)	8.0 (6.2)	6.2 (3.3)	3.5 (1.6)
Age (y) at baseline	11.2 (12.2)	11.4 (14.4)	5.2 (5.0)	3.8 (1.6)	5.6 (1.6)	6.4 (4.0)
Baseline PTA[*] (dB HL)	67.1 (32.2)	66.6 (31.6)	59.4 (26.4)	56.6 (19.2)	78.1 (27.0)	62.2 (36.4)
Final PTA[*] (dB HL)	61.6 (30.7)	76.5 (30.6)	78.1 (24.9)	55.3 (20.2)	64.8 (21.8)	52.3 (33.9)

[*] 0.5/1/2/4-kHz pure-tone average.

[+] Number of mutant *SLC26A4* alleles.