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Effect of Cochlear Implantation on Hearing Fluctuation in Patients with Biallelic *SLC26A4* Variants

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Keywords
SLC26A4 · Pendrin · DFNB4 · Cochlear implantation · Hearing fluctuation

Abstract
Introduction: Fluctuating hearing loss is a distinctive feature caused by *SLC26A4* variants. We investigated whether cochlear implantation had protective or deleterious effect on hearing fluctuation in patients with biallelic *SLC26A4* variants. ***Methods:*** Patients with biallelic *SLC26A4* variants (N = 16; age = 10.24 ± 9.20 years) who had unilateral cochlear implantation and consecutive postsurgical, bilateral pure-tone audiograms more than 3 times were selected. We retrospectively reviewed the patients' medical records from 2008 to 2019 obtained from a tertiary medical center and used the auditory threshold change (*Shift*) over time as a marker of hearing fluctuation. Fluctuation events were counted, and the *Shift* of the implanted and contralateral ears was compared using logistic regression with a generalized estimating equation and linear mixed model. A total of 178 values were included. ***Results:*** The odds of fluctuating hearing frequency were 11.185-fold higher in the unimplanted ears than in the implanted ears postoperatively (p = 0.001). The extent of fluctuation at 250 and 500 Hz was also significantly lower in the implanted ears than in the unimplanted ears after adjusting for every other effect (p = 0.003 and p < 0.001, respectively). Notably, higher residual hearing was rather associated with lesser fluctuation in frequency and the extent of fluctuation at 500 Hz, indicating residual hearing function is not the positive predictor for hearing fluctuation. ***Conclusion:*** In patients with biallelic *SLC26A4* variants, cochlear implantation may reduce the frequency and extent of hearing fluctuations.

Introduction

Fluctuating hearing loss is a distinctive feature caused by *SLC26A4* (OMIM 605646) variants and reported in 37–80% of patients bearing *SLC26A4* variants [King et al., 2010; Rose et al., 2017]. Clinically, affected individuals have residual hearing at birth, which worsens at the time of language acquisition. Stress, upper respiratory tract infections, and mild head injury cause precipitous hearing deterioration, and it is recovered slowly [Griffith and Wangemann, 2011]. Depending on the residual activity

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Table 1. Demographic and audiological outcomes

Total patients	16	Age, years	10.24±9.20
Sex, *n* (%)		Site, *n* (%)	
Male	10 (62.5)	Right	5 (68.8)
Female	6 (37.5)	Left	11 (31.3)
EVA, *n* (%)		Device, *n* (%)	
Bilateral	14 (87.5)	FLEX24 (Medel)	7 (43.8)
Unilateral	2 (12.5)	CI422 (Cochlear)	5 (31.3)
Cochlear anomaly, *n* (%)		CI24RE(CA) (Cochlear)	2 (12.5)
Anomaly	0 (0)	SONATA TI1000 (Medel)	1 (6.3)
Normal	16 (100)	Concerto (Medel)	1 (6.3)
Follow-up		Audiogram, *n*	6 (3–16)
Duration (months)	44.46±24.83		

PTA, dBHL	250 Hz					500 Hz				
	implanted		unimplanted		*p* value	implanted		unimplanted		*p* value
	mean	SD	mean	SD		mean	SD	mean	SD	
Preoperative (*n* = 14)	68.21	15.14	54.29	13.99	0.003[a]	84.29	12.38	65.00	10.74	0.001[b]
Postoperative; 1st (*n* = 18)	84.38	9.11	60.94	12.00	0.001[b]	96.88	10.14	66.25	8.85	<0.001[a]
Postoperative; last (*n* = 18)	85.94	7.35	59.06	14.86	<0.001[b]	93.44	5.69	66.88	15.04	0.001[a]
Hearing preservation, %	61.31		88.06			64.74		97.73		

EVA, enlarged vestibular aqueduct. [a] Indicates significance at $p < 0.05$ by the paired *t* test. [b] Indicates significance at $p < 0.05$ by the Wilcoxon signed-rank test.

of the pendrin protein encoded by the *SLC26A4* gene, the phenotype is classified into syndromic (Pendred syndrome [OMIM 274600]) and nonsyndromic (DFNB4 [OMIM 600791]) forms. Both present progressive hearing loss and episodic vertigo, with or without enlarged vestibular aqueduct (EVA) or Mondini dysplasia [Azaiez et al., 2007; Jung et al., 2016]. Although the *SLC26A4* gene shows an autosomal recessive inheritance pattern, patients with EVA who carry *SLC26A4* variant alleles from none to both express similar clinical phenotype, such as hearing fluctuation or episodic vertigo. In patients with EVA, the number of identified pathogenic variants in *SLC26A4*, size of the vestibular aqueduct, and presence of cochlear anomaly do not affect the hearing presentation or its natural course [King et al., 2010; Miyagawa et al., 2014; Rah et al., 2015].

Because gene therapy has not been commercialized yet for genetic hearing loss, the stabilization of hearing becomes a crucial therapeutic goal, especially for children. Steroid pulse therapy is first chosen as a temporary workaround when hearing fluctuations occur. However, once hearing deteriorates beyond serviceable hearing, cochlear implantation (CI) is one of the latter most solutions. As in other inner ear anomalies, EVA has an acceptable audiological outcome with CI [Buchman et al., 2004; Rachovitsas et al., 2012; Demir et al., 2019; Hall et al., 2019]. Even though we have already published notable results on CI in patients carrying *SLC26A4* variants [Roh et al., 2017], the effect of CI has not been evaluated in terms of the natural course of the disease over time. To investigate whether CI has a protective or deleterious effect on hearing fluctuation, we hypothesized that fluctuating hearing loss was attenuated in ears that underwent CI. This study aimed to assess whether differences are in hearing fluctuation between the implanted and contralateral unimplanted ears after CI. Furthermore, factors associated with changes in hearing fluctuation were evaluated.

Materials and Methods

Participants

This study was approved by the Severance Hospital Institutional Review Board, Seoul, Korea (4-2015-0659), and a retrospective medical record review was prosecuted at the tertiary medical center. Informed consent was obtained from all individual participants included in this study. Of the 161 patients with *SLC26A4* biallelic variants identified from 2008 to 2019, 60 patients underwent unilateral CI by a single surgeon and 23 patients underwent sequential CI on the other side. There were 19 patients with at least 3 postsurgical hearing thresholds recorded in both ears. In case of no residual hearing, hearing fluctuation occurrence and its extent

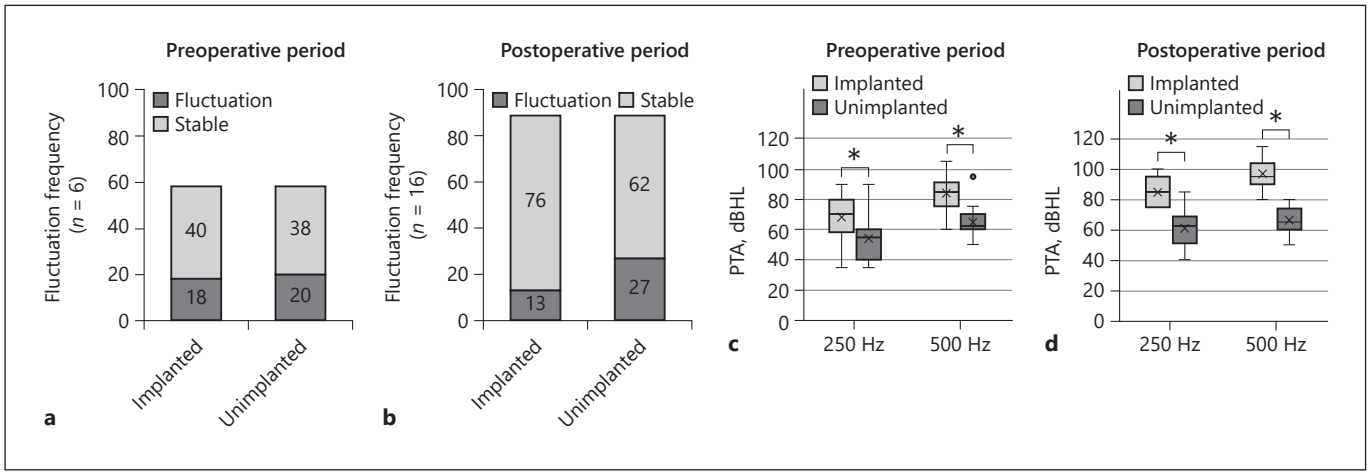

Fig. 1. Hearing threshold and fluctuation in patients with enlarged vestibular aqueducts. **a** The fluctuation frequency – the ratio of *fluctuation* and *stable* events – during the preoperative period between 128 audiograms (116 calculated values) measured in the unimplanted and implanted ears. **b** The fluctuation frequency after the CI in 210 audiograms (178 calculated values). **c** Baseline auditory thresholds prior to the surgery in implanted and unimplanted ears. **d** Postoperative hearing threshold. Regardless of surgery, there was significant difference in both ears at 250 and 500 Hz. None of the measurements was over the maximum output of the audiometer. CI, cochlear implantation.

might have been masked by a ceiling effect. Thus, we excluded 3 patients whose auditory threshold at 250 or 500 Hz was unmeasurable over the maximum output level of the audiometer even only once. Sixteen patients (10.24 ± 9.20 years, 10 males) were included in this study. Computed tomography and magnetic resonance imaging findings revealed bilateral EVA in 14 and unilateral EVA in 2 patients. The cochlear anomaly was not observed in all patients (Table 1).

Audiological Data Collection

Several types of behavioral audiometry such as play audiometry, visual reinforcement audiometry, or conventional pure-tone audiometry were carried out based on the age of the patients at least 1 week apart. The mean follow-up period was 44.46 ± 24.83 months, and the median number of postsurgical audiometry was 6 (3–16) times at each ear. The audiometry measured air conduction thresholds at 250 and 500 Hz, which were relatively well preserved after CI. Preoperative audiometry data were not available for 2 patients who were too young to be tested with behavioral audiometry at the time of surgery. Finally, we collected 210 audiograms of bilateral ears. We scored the hearing preservation rate after CI relying on each frequency by modifying Skarzynski's formula [Skarzynski et al., 2013].

Fluctuating Hearing

We defined *Shift* in each frequency as the shift of the hearing thresholds between the consecutive tests: the prior (PTA_{pre}) and the later one (PTA_{post}). The analysis included postoperative 178 calculated values (*Shift*) per frequency for both ears.

$$Shift = |PTA_{pre} - PTA_{post}|$$

We classified the threshold shift as *fluctuation* event in case of the following: (1) *Shift* by 15 dB at any one frequency or (2) *Shift* by 10 dB at 2 frequencies. When both conditions were not applicable, the threshold shift was defined as a *stable* event (Fig. 1a, b). The conditions were modified based on other published data [Brookhouser et al., 1994; Madden et al., 2003; King et al., 2010; Rose et al., 2017]. We considered changes in hearing, not hearing deterioration or improvement. As for the frequency of hearing fluctuation, we counted the "fluctuation event between 2 serial audiograms" on each ear. For instance, if a patient's condition, who was tested 3 times with pure-tone audiogram and hearing, was observed as deteriorating on 2 observations, the fluctuation was counted twice.

To sift the significant hearing fluctuations (*Sig.Fl*), we multiplied *Shift* by 1 for *fluctuation* or by 0 for *stable*. Hence, in case of *fluctuation*, *Sig.Fl* referred to *Shift;* in case of *stable*, *Sig.Fl* was zero. Lastly, we coded Δ to estimate the interaural difference of significant hearing fluctuations between implanted and contralateral unimplanted ears.

$$\Delta = Sig.Fl_{unimplanted} - Sig.Fl_{implanted}$$

We measured the residual hearing as the difference between the maximum output level of the audiometer (110 dBHL at 250 Hz; 120 dBHL at 500 Hz) and the hearing threshold.

DNA Sequencing and Evaluation of Variants

All the exons of *SLC26A4* were sequenced using direct sequencing for *SLC26A4* as described previously [Jung et al., 2017; Song et al., 2019]. In brief, peripheral blood was obtained from the affected individuals and genomic DNA was extracted using RBC Lysis Solution, Cell Lysis Solution, and Protein Precipitation Solution (iNtRon Biotechnology, Inc., Seongnam, South Korea). Variants were evaluated using Basic Variant Caller of CLC Workbench (https://www.qiagenbioinformatics.com/).

Table 2. Pathogenic[a] variants in *SLC26A4*

Patient	Sex	Age, years	Nucleotide change[b]	Protein change[c]	Location	Variant type
1	F	5	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.2168A>G	p.His723Arg	Exon 19	Missense
2	M	4	c.919-2A>G	Splicing	Intron 7	Splicing variant
			c.916dupG	p.Val306Glyfs*24	Exon 7	Insertion
3	F	5	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.919-2A>G	Splicing	Intron 7	Splicing variant
4	M	9	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.919-2A>G	Splicing	Intron 7	Splicing variant
5	F	18	c.919-2A>G	Splicing	Intron 7	Splicing variant
			c.2027T>A	p.Leu676Gln	Exon 17	Missense
6	M	4	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.919-2A>G	Splicing	Intron 7	Splicing variant
7	M	13	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.2168A>G	p.His723Arg	Exon 19	Missense
8	M	18	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.919-2A>G	Splicing	Intron 7	Splicing variant
9	F	3	c.919-2A>G	Splicing	Intron 7	Splicing variant
			c.589G>A	p.Gly197Arg	Exon 5	Missense
10	F	2	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.2168A>G	p.His723Arg	Exon 19	Missense
11	F	24	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.1262A>C	p.Gln421Pro	Exon 10	Missense
12	M	19	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.439A>G	p.Met147Val	Exon 5	Missense
13	M	2	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.439A>G	p.Met147Val	Exon 5	Missense
14	M	5	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.412G>C	p.Val138Leu	Exon 4	Missense
15	M	3	c.2168A>G	p.His723Arg	Exon 19	Missense
			c.439A>G	p.Met147Val	Exon 5	Missense
16	M	32	c.919-2A>G	Splicing	Intron 7	Splicing variant
			c.1262A>C	p.Gln421Pro	Exon 10	Missense

[a] All variants were reported as pathogenic or likely pathogenic in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), and Deafness Variation Database (http://deafnessvariationdatabase.org/). [b] cDNA variants are numbered according to human cDNA reference sequence NM_000441.2 (SLC26A4); +1 corresponds to the A of ATG, translation initiation codon. [c] Protein changes are numbered according to human protein reference sequence NP_000432.1.

Table 3. Logistic regression model with the GEE for the frequency of hearing fluctuation

Parameters	*B*	Exp(*B*)	95% confidence interval		
			lower bound	upper bound	*p* value
Intercept	−0.598	0.550	0.047	6.423	0.634
Group; unimplanted ear (ref = implanted ear)	2.415	11.185	2.563	48.802	0.001[a]
Time (months)	−0.016	0.984	0.953	1.017	0.334
c.[919-2A>G] (ref = c.[2168A>G];[2168A>G])	1.129	3.094	0.730	13.103	0.125
c.[2168A>G] (ref = c.[2168A>G];[2168A>G])	1.279	3.593	0.746	17.316	0.111
c.[919-2A>G];[2168A>G] (ref = c.[2168A>G];[2168A>G])	0.111	1.117	0.365	3.418	0.846
Sex; male (ref = female)	1.580	4.854	0.900	26.173	0.066
Age	−0.127	0.881	0.821	0.945	<0.001[a]
Residual hearing	−0.058	0.944	0.912	0.976	0.001[a]

[a] Indicates significance at $p < 0.05$.

Statistical Analysis

We compared (1) how frequent the fluctuation was and (2) how much the fluctuation ranged among the implanted ear and the contralateral ear. First, to analyze the longitudinal data of either *fluctuation* or *stable*, we performed a logistic regression model with the generalized estimating equation (GEE) and specified the covariance with the exchangeable correlation structure. The frequency of *fluctuation* was counted as the number of *fluctuation* events (Fig. 1a, b). For instance, if hearing levels kept changing (worsening or improving) in 3 consecutive tests and were compatible with the criteria in both ears, fluctuation frequency was counted twice in each ear. We assessed the interaction effect among repeated events of *fluctuation* over time that nested within each ear. In addition, allelic variants – c.[2168A>G];[2168A>G], c.[2168A>G];[919-2A>G] – compound heterozygotes including c.2168A>G or c.919-2A>G, sex, age, residual hearing, and postoperative time were selected as factors and covariates in predicting the dependent variable (i.e., the group of the implanted ear or unimplanted ear).

Second, linear mixed-effects model (LMM) with repeated measures was used to estimate the range of significant fluctuation over time in each of the frequencies. The same covariates as of GEE were included in the LMM. The patient effect was analyzed with a random-effects model, and every covariate was analyzed with a fixed-effects model. The operation and time were the within-subject variables; therefore, those have been specified as repeated effects, with a compound symmetry covariance structure. The independent variables were entered into the LMM model designed to minimize the Akaike information criteria.

Significance was set at $p < 0.05$ for all comparisons. SPSS Statistics version 25.0 (IBM, Inc., Armonk, NY, USA) software package was used for all analyses.

Results

Clinical and Genetic Analyses in Patients with EVA Caused by SLC26A4 *Variants*

The baseline auditory threshold in the implanted ear was significantly higher than that in the unimplanted ear before (Fig. 1c) and after CI (Fig. 1d). After surgery, there was no significant deterioration depending on the first and the last test on both sides of the ears. Modifying Skarzynski's formula, the postoperative hearing preservation rate at 250 and 500 Hz was approximately 61.31 and 64.74% in the implanted ears and 88.06 and 97.73% in the unimplanted ears, respectively (Table 1).

All the enrolled 16 patients had biallelic variants in *SLC26A4* (Table 2). The most common type of variant was c.2168A>G accounting for 43.75%, and the second was c.919-2A>G found in 28.13%. Three patients carried c.2168A>G homozygotes. c.[2168A>G];[919-2A>G] was the most common compound heterozygote in 4 patients. All the others carried one c.2168A>G or c.919-2A>G variant.

Comparison of Hearing Fluctuation after Cochlear Implantation

Among the 16 enrolled patients, 6 patients' preoperative consecutive audiograms were available more than 3 times. The number of audiograms was 128, and the number of the calculated *shifts* was 116 on bilateral ears (Fig. 1a). The median follow-up duration was 34.4 (2.2–78.03) months; the number of tests was 6.5 (3–30) until the operation. There was no significant interaction effect between time and repeated tests for ears. Given the fluctuating pattern of frequency and the extent of fluctuation at 250 and 500 Hz, there was no significant difference between 2 ears preoperatively ($p = 0.648$ by GEE, $p = 0.817$, and $p = 0.13$ by LMM, respectively).

Among the postoperative 178 calculated values in 16 patients – categorized as *fluctuation* or *stable* depending on the shift by each frequency – *fluctuation* event was in-

Table 4. LMM model for the magnitude of significant fluctuating hearing

	B	SE	*t*	95% confidence interval		
				lower bound	upper bound	*p* value
250 Hz						
Intercept	2.477	3.029	0.818	−3.762	8.716	0.421
Group; unimplanted ear (ref = implanted ear)	5.147	1.703	3.023	1.785	8.508	0.003[a]
Time (months)	−0.018	0.032	−0.547	−0.081	0.046	0.585
c.[919-2A>G] (ref = c.[2168A>G];[2168A>G])	3.681	2.473	1.488	−2.142	9.503	0.179
c.[2168A>G] (ref = c.[2168A>G];[2168A>G])	3.085	2.644	1.167	−3.414	9.583	0.288
c.[919-2A>G];[2168A>G] (ref = c.[2168A>G];[2168A>G])	2.158	2.440	0.885	−3.945	8.262	0.413
Sex; male (ref = female)	4.050	2.134	1.898	−0.694	8.794	0.086
Age	−0.236	0.100	−2.369	−0.467	−0.005	0.046[a]
Residual hearing	−0.097	0.060	−1.605	−0.215	0.022	0.111
500 Hz						
Intercept	7.777	2.582	3.012	2.677	12.878	0.003[a]
Group; unimplanted ear (ref = implanted ear)	10.315	1.754	5.882	6.853	13.777	<0.001[a]
Time (months)	−0.021	0.029	−0.713	−0.079	0.037	0.477
c.[919-2A>G] (ref = c.[2168A>G];[2168A>G])	−0.831	1.812	−0.459	−4.413	2.751	0.647
c.[2168A>G] (ref = c.[2168A>G];[2168A>G])	2.524	1.767	1.429	−0.970	6.018	0.155
c.[919-2A>G];[2168A>G] (ref = c.[2168A>G];[2168A>G])	−0.829	1.549	−0.535	−3.894	2.236	0.593
Sex; male (ref = female)	1.584	1.680	0.943	−1.734	4.903	0.347
Age	−0.139	0.076	−1.830	−0.289	0.011	0.069
Residual hearing	−0.226	0.054	−4.210	−0.331	−0.120	<0.001[a]

[a] Indicates significance at $p < 0.05$.

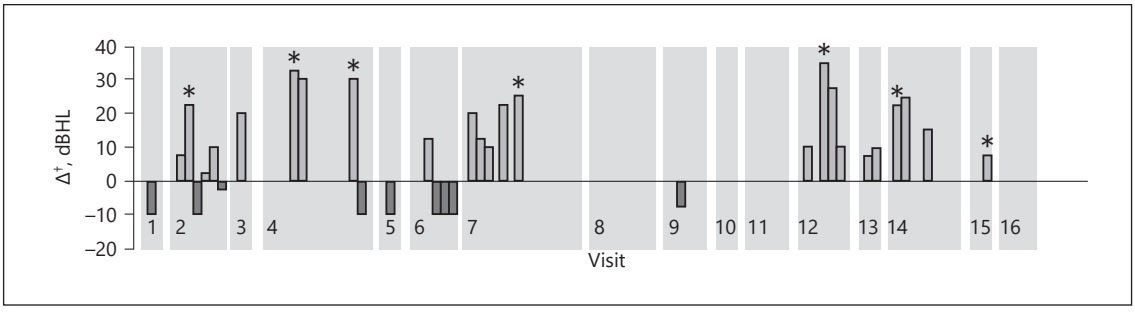

Fig. 2. Hearing fluctuation pattern in patients. Interaural comparison of hearing fluctuation after cochlear implantation. The data of 16 patients were arranged horizontally according to the number of visits on the *x* axis, and the gray shaded box separated each patient. The *y* axis represented the interaural difference (Δ, unimplanted ear − implanted ear) of the change in the hearing threshold between serial tests. $\Delta > 0$ meant that hearing fluctuation in the unimplanted ear was larger, and $\Delta < 0$ meant that hearing fluctuation in the implanted ear was larger. If Δ was zero, the hearing might fluctuate bilaterally or be stable. The hearing fluctuation in the unimplanted ear (gray bars, $\Delta > 0$) was more dominant than that in the implanted ear (striped bars, $\Delta < 0$). The asterisk indicates the point when a patient suffered sudden hearing loss subjectively and was treated with steroids at our outpatient clinic. †$\Delta = Sig.Fl_{\text{unimplanted}} - Sig.Fl_{\text{implanted}}$.

cluded in the logistic regression model with GEE (Table 3). No statistical significance was observed for the interaction effect among repeated *fluctuation* events. In the interaural comparison of hearing fluctuation frequency, the odds were 11.185-fold higher in the unimplanted ear compared with the implanted ear ($p = 0.001$). Notably, the frequency of hearing fluctuation decreased slightly as the residual hearing function improved ($p = 0.001$). As the age at operation increased, the fluctuation frequency tended to decrease ($p < 0.001$). Sex, postoperative time,

and allelic variants did not affect the frequency of hearing fluctuation.

Meanwhile, the magnitude of hearing fluctuation after CI was analyzed with the LMM model (Table 4). At both 250 and 500 Hz, the extent of fluctuation was significantly lower in the implanted ear than in the unimplanted ear after adjusting for every other effect ($p = 0.003$ and $p < 0.001$, respectively). At 500 Hz, as the residual hearing was higher, the extent of fluctuation was smaller. At 250 Hz, age at operation was a factor that decreased the extent of fluctuation ($p = 0.046$).

We also individually calculated the biaural differences (unimplanted ear − implanted ear) for changes in the hearing threshold at averages of 250 and 500 Hz between consecutive audiological tests – delta (Δ) (Fig. 2). $\Delta > 0$ meant that hearing fluctuation in the unimplanted ear was larger, and $\Delta < 0$ meant that hearing fluctuation in the implanted ear was larger. For example, if Δ was zero, the hearing might fluctuate bilaterally or be stable. For visualization, the data on 16 patients were arranged horizontally according to the number of visits on the *x*-axis being each subject marked off by the shaded box. The hearing fluctuation in the unimplanted ear ($\Delta > 0$) was more dominant than that in the implanted ear ($\Delta < 0$). Although patient 1, 5, and 9 showed dominant fluctuation toward the implanted ear, the degree was lower than 15 dB. The unimplanted ear was the main side, where patients experienced subjective hearing fluctuations.

Discussion

In the present study, we performed longitudinal analyses of audiological data in biallelic *SLC26A4* variants. Our results are the first reports evaluating whether fluctuation pattern is changed after CI [Brookhouser et al., 1994; Lee et al., 2014; Miyagawa et al., 2014; Gratacap et al., 2015]. We considered not only CI as predictors of hearing fluctuation but also the sex, age at operation, postoperative time, allelic variants, and residual hearing. CI and better residual hearing were associated with smaller frequency and range of hearing fluctuation. Our previous study reported that c.2168A>G homozygotes were associated with worse hearing and less fluctuation [Lee et al., 2014]. However, with longitudinal data, we could not verify significant differences among genotypes.

The reason why we initially excluded 3 patients was that their hearing fluctuation may have been underestimated below their hearing capacity. For instance, one of the patients' preoperative hearing in the implanted ear was 55 dB at 250 Hz and 60 dB at 500 Hz. The hearing on the contralateral ear was 65 and 70 dB, respectively. The patient had experienced frequent hearing fluctuation on the implanted ear before she came to our hospital, and her hearing at high frequency was worse than that of the unimplanted ear. After CI, the hearing of the implanted ear was unmeasurable in 3 sequential tests, whereas the hearing of the contralateral ear showed fluctuation once. In this case, we were unable to measure the extent and frequency of hearing fluctuation in the dead cochlea and were hardly able to compare bilateral ears without any bias in interpretation.

In addition, we analyzed the effect of residual hearing in terms of not only the extent but also the frequency of hearing fluctuation to minimize the concern about a ceiling effect. Subsequently, we found that the frequency of hearing fluctuation was higher in the unimplanted ears than in the implanted ears regardless of the extent of postoperative residual hearing according to the multivariable analysis in the logistic regression model with the GEE (Table 3; OR = 2.415, $p = 0.001$). Interestingly, the frequency of hearing fluctuation decreased slightly as the residual hearing function improved (Table 3; OR = 0.944, $p = 0.001$); this finding is consistent with the finding that the magnitude of hearing fluctuation decreased as residual hearing improved (Table 4; $B = -0.226$, $p < 0.001$). Several considerations should be explored. For example, our findings imply that hearing fluctuation may happen more frequently and severely in the dead cochlea. Otherwise, the cochlea may become more degenerated because of frequent and severe hearing fluctuation due to pendrin dysfunction. With these efforts, we effectively minimized the ceiling effect of the hearing threshold and evaluated the effect of implantation on the hearing fluctuation without biases.

We can infer the mechanism of fluctuation in patients with *SLC26A4* variants through animal models. *SLC26A4* encodes a chloride/bicarbonate (Cl^-/HCO_3^-) exchanger called pendrin, which is expressed in the cochlea, saccule, utricle, endolymphatic sac, and duct [Jung et al., 2016]. Pendrin dysfunction causes HCO_3^- accumulation and alkalization of the intrastrial spaces, inducing oxidative stress. Moreover, the enlargement of cochlear lumens due to reduced fluid absorption by the endolymphatic sac causes an increase in the diffusional distance of epithelial cells and disrupts cell-to-cell signaling between gap junctions in early postnatal development. Reduced KCNJ10 channel expression (sensitive to reactive oxygen species [ROS], located in the intermediate cell of stria vascularis, and releasing K^+ into the intrastrial space) compromises

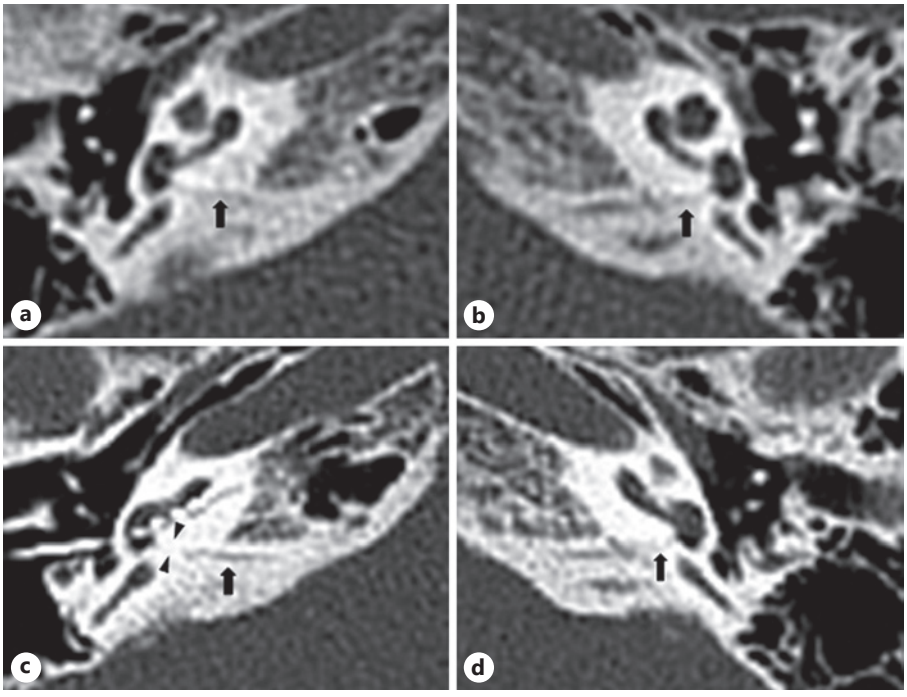

Fig. 3. Stenosis in the cochlear aqueduct after CI. Comparing preoperative and postoperative temporal bone CT in patient 4, distinct stenosis of the cochlear aqueduct (arrowhead) of the ipsilateral side was observed after surgery. Ipsilateral side before surgery (**a**), contralateral side before surgery (**b**), ipsilateral side after surgery (**c**), and contralateral side after surgery (**d**). The arrow points to the cochlear aqueduct. CI, cochlear implantation.

endocochlear potential stability [Choi et al., 2020]. Normally, ROS is generated when marginal cells secrete K^+ to endolymphatic space and attenuates KCNJ10 channel expression. Thereafter, if the K^+ supply to marginal cells decreases, ROS production will be negatively controlled and KCNJ10 can be restored. As for the *SLC26A4*$^{-/-}$ mouse, enlarged scala media demands increased K^+ flux for maintaining endolymphatic K^+ concentration. As such, the oscillation of the negative feedback loop of ROS can cause fluctuating hearing loss [Wangemann et al., 2004; Kim and Wangemann, 2010].

Clinically, sudden hearing loss in patients with EVA often occurs after exercise or minor head trauma. Moreover, intraoperative perilymphatic gusher during CI could be followed by sudden postoperative sensorineural hearing loss even in ipsilateral or contralateral ears [Walsted, 2000; Vaisbuch et al., 2019]; it is regarded as a result of the communication of fluid pressures through the CSF, perilymph, and endolymph [Satzer and Guillaume, 2016]. The sudden intracranial compartment pressure change can be transmitted through the cochlear aqueduct to the inner ear [Densert et al., 1986; Takeuchi et al., 1990; Thalen et al., 2001]. Carlborg et al. observed the perilymph pressure in a cat with a patent cochlear aqueduct changed at 1.5 s after a change in CSF pressure. However, with an obstructed cochlear aqueduct, the perilymph pressure change occurred only 21% of the time, and the mean lag time was 2 min. The authors argued that delayed pressure transmission occurred through the endolymphatic sac with cochlear aqueduct obstruction [Carlborg and Farmer, 1983; Carlborg et al., 1992].

Structural and anatomical changes by the CI can transform the delivery of the traveling wave. Physiologically, the sound pressure enters from the scala vestibuli and exits the round window getting through the low impedance of scala tympani, which causes a traveling wave. Choi and Oghalai [2005] reported that the damping caused by sinus tympani fibrosis predominantly affected the tuning in the cochlear apex in the postimplant traumatized cochlear microphonic model. Also, even the stiffened round window reduces residual hearing by about 20 dB below 1 kHz after CI [Elliott et al., 2016]. Quesnel et al. [2016] reported fibrosis and osteoid deposition at the round window, cochlear aqueduct, scala tympani, and scala vestibuli in the postmortem pathology of patients with unilateral cochlear implants.

Disruption of cell-to-cell signaling and atrophy of stria vascularis in patients carrying *SLC26A4* variants may cause oscillation in endocochlear potential and driving force. Besides, the basilar membrane and Reissner's membrane are fragile due to hydrops. The intracranial compartment pressure is transmitted with ease to the scala media through the cochlear aqueduct and vestibular aqueduct, which were significantly enlarged [Kim et al.,

2013]. However, after CI, stenosis of the cochlear aqueduct and the scala tympani of basal turn might compensate this oscillation by decreasing pressure transmission from the intracranial compartment (Fig. 3). Even after CI, though the pressure transmission could result in fluctuating hearing loss under the influence of EVA, it is less frequent than that of the unimplanted ear. Elliott et al. created a round window stiffness model to study the effects of residual hearing loss after CI. The size of the endolymphatic ducts and cochlear aqueducts influenced the reverse prediction of hearing loss at low frequencies below 1 kHz [Elliott et al., 2016]. Although our study did not analyze the sizes of the endolymphatic ducts and cochlear aqueducts, it is noteworthy to consider it as another factor.

Our study has some limitations. First, our study is retrospective and the timing of repeated measures varies among the limited number of patients. Therefore, the data of analysis are unbalanced, and the results can be biased. Collecting sufficient data from the real world is demanding because patients who were well educated about their disease visited the nearest clinic rather than the distant tertiary center; therefore, audiometric testing was not collected whenever fluctuations occurred. Second, we did not compare the least-affected frequency of 125 Hz after CI, resulting in an incomplete evaluation of low-tone fluctuation. An audiogram analysis at 125 Hz could have yielded a detailed pattern of fluctuations. Third, bone conduction threshold was not measured in our study. The hearing loss increases due to the third window effect caused by the widened cochlear and vestibular aqueducts. However, the third window effect cannot be taken into account in this study. Fourth, though hearing fluctuation is more prominent at the time of language acquisition, children and adults were analyzed at the same time in our study, causing a potential bias. However, this study reports that in patients with biallelic *SLC26A4* variants, CI may reduce the frequency and range of hearing fluctuation.

Statement of Ethics

The current study was conducted with the approval of the Ethics Committee of our hospital (4-2015-0659) and performed following the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients provided written informed consent before participating. All procedures involving human participants were under the ethical standards of our institutional research committee.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All authors contributed equally to this work. Conceptualization: J.J. and J.Y.C. Data curation: G.N. and Y.J. Funding acquisition: J.J. Formal analysis: H.J.L. Methodology: J.M.L. Project administration: G.N. Visualization: J.J. Writing – original draft: G.N. Writing – review & editing: J.J. and J.Y.C.

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