Laryngoscope Investigative Otolaryngology

Elongated EABR Wave Latencies Observed in Patients With Auditory Neuropathy Caused by *OTOF* Mutation

Makoto Hosoya, MD, PhD; Shujiro B. Minami, MD, PhD; Chieko Enomoto; Tatsuo Matsunaga, MD, PhD; Kimitaka Kaga, MD, PhD

Objectives: We sought to determine how the pathology altered electrically evoked auditory brainstem responses (EABRs) in patients with hearing loss by evaluating EABRs in auditory neuropathy patients with *OTOF* mutations comparing with various types of congenital deafness.

Methods: We included 15 patients with congenital hearing loss, grouped according to pathology: *OTOF* mutations (n = 4), *GJB2* mutations (n = 4), *SLC26A4* mutations (n = 4), or cytomegalovirus infections (n = 3). EABRs were recorded when patients underwent cochlear implantation surgery. We evaluated the latencies and amplitudes of the recorded EABRs and compared them statistically between four groups.

Results: The EABR latencies of Wave III and Wave V, and of the interval between them, were significantly longer in the *OTOF* mutation group than in the *GJB2* and *SLC26A4* mutation groups (Wave III) and in all three other groups (Wave V and Wave III-V latency); amplitudes were not significantly different between groups.

Conclusions: Our results suggest *OTOF* mutations cause delayed (or slowed) postsynaptic neurotransmission, although the presumed mechanism involved reduced presynaptic transmission between hair cells and spiral ganglion neurons.

Level of Evidence: Mainly a case report

Key Words: Auditory neuropathy, *OTOF*, electrically evoked auditory brainstem responses.

INTRODUCTION

Auditory neuropathy (AN) is a disease characterized by absent or abnormal auditory nerve function with normal outer hair cell function. Clinically, AN presents as sensorineural hearing loss with accompanying impaired speech discrimination; diagnostic data are characterized by preserved otoacoustic emissions (OAE) or cochlear microphonic (CM) and a disturbed auditory brain stem response (ABR).[1,2]

A part of AN is known to be caused by genetic mutations including *OTOF*,[3] *OPA1*,[4] and *PJVK* mutations.[5] In fact, an *OTOF* mutation was first reported as a genetic cause of DFNB9.[6] *OTOF* mutations account for 1.4% to 5% of cases of autosomal recessive non-syndromic hearing impairment.[7–14] The majority of patients carrying two mutant alleles of *OTOF* show severe-to-profound congenital hearing loss. In Japanese patients with AN, *OTOF* mutations accounted approximately 60% of the cases.[15] Within at least the first one or two years after birth they show preserved OAE or CM without an ABR response,[16] so they are diagnosed with AN. The trans-membrane protein OTOFERLIN, encoded by the *OTOF* gene, is expressed in the inner and outer hair cells of the rodent cochlea.[6,17] This protein is a critical regulator of vesicle fusion with the plasma membrane following glutamate release or during the need for vesicle replenishment at the afferent ribbon synapses between inner hair cells and spiral ganglion neurons.[18] Thus, AN caused by *OTOF* mutation is thought to be caused by disrupted synaptic function (an auditory synaptopathy) at synapses between the inner hair cells and spiral ganglion neuron.[19]

Treatment of profound-to-severe hearing loss in patients with AN requires cochlear implantation (CI); however, the efficacy of CI in such cases is controversial.[20–24] On the other hand, AN caused by *OTOF* mutations is thought to be a better candidate for cochlear implantation because the electrode can stimulate the auditory nerves directly, thus bypassing impaired synapses. Several reports have demonstrated an adequate level of cochlear implant performance in patients with *OTOF* mutations.[25–27] However, precise evaluations of postsynaptic functions in this disease are lacking.

Electrically evoked auditory brainstem responses (EABRs) can be used for measuring neuronal activity in the

From the the National Institute of Sensory Organs (M.H., S.B.M., C.E., K.K.); the Laboratory of Auditory Disorders and Division of Hearing and Balance Research (T.M.); the Medical Genetics Center (T.M.); National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan; the Center for Speech and Hearing Disorders (K.K.)International University of Health and Welfare, Tokyo, Japan

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript and have approved this submission.

Conflict of Interest: The authors declare that they have no competing interests.

Financial Disclosure: This work was supported by JSPS KAKENHI (Grant Number 16K11205), and research grants from Pfizer health Research Foundation and GSK Japan

Author Contributions: MH, SM, and KK designed the study and wrote the manuscript. MH, SM, CE, TM, and KK analyzed the data.

Co-corresponding authors Send correspondence to Shujiro B. Minami, 2-5-1, Higashigaoka, Meguro-Ku, Tokyo, Japan. Email: shujirominami@me.com

DOI: 10.1002/lio2.210

cochlear nerve after CI implantation; in addition, the clinical usefulness of EABR analysis has been reported.[28,29] Thus far, electrophysiological investigations were reported using EABR analysis in AN patient.[30–33] Runge et al. showed reduced wave V supra-threshold amplitude with AN and indicated residual dys-synchronous neuronal activity in the central auditory pathway.[31]

Given the heterogeneity of potential cause of AN, grouping the patient by the cause is needed. In this report we focused on AN caused by *OTOF* mutations. To clarify the postsynaptic neuronal physiological changes in auditory neuropathy caused by *OTOF* mutations, we compared the EABR responses of patients who underwent CI to treat one of several forms of congenital hearing loss. We also compared the physiological characteristics of EABRs after long-term or short-term cochlear implantation for patients with *OTOF* mutations.

MATERIALS AND METHODS

Enrolled Patients

We retrospectively analyzed the EABR results of patients who had undergone CI from December 2008 to November 2016, with implants manufactured by MED-EL or Advanced Bionics; we had to exclude implants manufactured by Cochlear for technical reasons. We included patients who were diagnosed with *OTOF, GJB2*, or *SLC26A4* mutations by genetic testing, and who were diagnosed with maternal CMV infections by umbilical cord inspection. We enrolled 15 patients, including four each with *OTOF, GJB2*, or *SLC26A4* mutations; and three patients with previous maternal CMV infections. Among these patients, four patients had undergone bilateral CI. We analyzed EABR wave forms obtained from six ears with *OTOF* mutations, six with *GJB2* mutations, four with *SLC26A4* mutations, and four from patients with CMV infections (Table I).

Deafness genes were tested at the Laboratory of Auditory Disorders, National Institute of Sensory Organs, National Tokyo Medical Center by the Sanger method using the genomic DNA extracted from patients' peripheral blood cells when their deafness genes have not been clarified by previous genetic testing performed by BML supported by National Health Insurance. We performed the Sanger methods as previously reported.[15,34,35] Genes and mutations of the enrolled patients are shown in Supplementary Table I.

While c.3256G>A (p.G1086R) mutation of *OTOF* gene has not been reported as a pathogenic mutation, according to the ACMG guideline,[36] we concluded it as a likely pathogenic mutation because this variant fulfilled PM2, PM3, PP3, and PP4. Similarly, c.1264-2A>G mutation of *SLC26A4* gene also has not been reported as a pathogenic mutation, but we concluded it as a pathogenic mutation because this variant fulfilled PVS1, PM2, PM3, and PP4.

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.

Measurement of EABR

EABRs were recorded as described previously.[28] In brief, they were recorded by stimulating each electrode of cochlea implant in the cochlea using the Neuropack Σ (Nihon Koden Co., Tokyo, Japan) electrodiagnostic system, which was triggered externally by the stimulus output of the proprietary MED-EL or Advanced Bionics software and interface unit. The interface unit was also connected to a stock speech processor, which transmitted the stimulus signal across the skin to the implanted device. The electrically evoked brainstem potentials were recorded by using needle electrodes placed on the forehead (different electrode) and nape (indifferent electrode), and the reference electrode was placed on the contralateral shoulder. The recording of electrical activity included two or three replications of 1000 sweeps at each stimulus level, with a time window of 10 ms for each stimulus condition. Frequency cut-offs of 100 and 1000 Hz were used. The pulse duration was set to 30 μs and the stimulation amplitude for a single recording fell from 1200 current unit (cu) to 200 cu at 200 cu intervals for MED-EL and 600 cu to 200 cu at 100 cu intervals for Advanced Bionics. If no response was detected, pulse duration was increased up to 100 μsec.

Categories of Auditory Performance (CAP) Scale

For accessing speech perception, we used CAP scale, which comprises a hierarchical scale of auditory perceptive ability. In this scale, the lowest level (0) describe no awareness of environmental sounds and the level (7) is presented by the ability to use a telephone with a known speaker.[37,38]

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, New York, USA). For multiple comparisons, we used the Tukey–Kramer method. The results of multiple experiments are presented as the mean ± standard deviation. All tests used a P value of .05 as the threshold for significance.

RESULTS

Representative EABR waveforms obtained from each group, and the aspects of the waveforms that were analyzed, are shown in Figure 1. We compared latencies and amplitudes of Wave III and Wave V in this study. The Wave V latencies were significantly longer in the group with *OTOF* mutations than in any other group (Fig. 2A); however, no difference was observed in amplitudes (Fig. 2B). When we compared Wave III latencies and amplitudes, the latencies were significantly longer in the *OTOF* mutation group relative to the *GJB2* and *SLC26A4* mutation groups, but not the CMV group (Fig. 3A). No significant differences were observed in Wave III amplitudes (Fig. 3B). Amplitudes in all groups were found to have very high standard deviations. We also analyzed the latency difference between Wave III and Wave V across groups. The Wave III–Wave V latencies were also significantly longer in the *OTOF* group than in all other groups (Fig. 4).

Finally, in order to evaluate the clinical relevant of this elongation of EABR wave form, we compare the patients' speech perception between the groups evaluated by CAP score. There was no significant difference between the groups (Fig. 5A). Moreover, there was no significant relationship between CAP score and EABR latency (Fig. 5B).

DISCUSSION

Our EABR analyses revealed what appears to be delayed postsynaptic neurotransmission in AN caused by

TABLE I.
Enrolled Patients, Their Demographic Characteristics, and Their Pathological Findings

Patient	Cause of Deafness	Sex	Operation Age	Operation side	Implanted CI model	Imaging Findings	Wave III Latency (mSec)	Wave V Latency (mSec)
#1	*OTOF*	Male	3Y4M	rt	MED-EL PULSAR FLEX soft	no inner ear malformation	2.24	4.65
#2	*OTOF*	Female	1Y9M	rt	MED-EL CONCERTO flex28	no inner ear malformation	2.66	5.60
#2-2nd	*OTOF*	Female	3Y0M	lt	MED-EL CONCERTO flex28	no inner ear malformation	2.70	5.49
#3	*OTOF*	Female	2Y3M	rt	Advanced Bionics Hifocus MS	no inner ear malformation	2.34	5.28
#3-2nd	*OTOF*	Female	2Y10M	lt	Advanced Bionics Hifocus MS	no inner ear malformation	2.34	5.28
#4	*OTOF*	Female	1Y11M	rt	Advanced Bionics Hifocus MS	no inner ear malformation	2.44	4.46
#5	*GJB2*	Male	2Y1M	rt	MED-EL PULSAR FLEX soft	no inner ear malformation	2.36	4.19
#5-2nd	*GJB2*	Male	5Y5M	lt	MED-EL CONCERTO flex28	no inner ear malformation	2.00	4.30
#6	*GJB2*	Male	1Y6M	rt	MED-EL CONCERTO MI1000PIN flex soft	no inner ear malformation	2.13	4.34
#6-2nd	*GJB2*	Male	2Y1M	lt	MED-EL CONCERTO flex28	no inner ear malformation	2.20	4.06
#7	*GJB2*	Male	2Y7M	rt	MED-EL CONCERTO flex28	no inner ear malformation	2.02	4.39
#8	*GJB2*	Male	3Y6M	rt	MED-EL CONCERTO Flex28	no inner ear malformation	2.22	4.08
#9	*SLC26A4*	Male	1Y11M	rt	MED-EL CONCERTO Mi100 Flex soft	large vestibular aqueduct	2.12	3.97
#10	*SLC26A4*	Female	3Y5M	rt	MED-EL CONCERTO Mi100 Flex soft	large vestibular aqueduct	2.31	4.07
#11	*SLC26A4*	Female	4Y0M	rt	Advanced Bionics Mid Scala	large vestibular aqueduct	2.20	4.00
#12	*SLC26A4*	Female	3Y10M	rt	MED-EL CONCERTO flex28	large vestibular aqueduct	2.08	3.86
#13	CMV	Female	3Y8M	lt	MED-EL CONCERTO flex28	no inner ear malformation	2.38	4.26
#14	CMV	Male	3Y8M	lt	MED-EL CONCERTO flex28	no inner ear malformation	2.06	4.17
#15	CMV	Female	1Y6M	lt	MED-EL CONCERTO flex28	no inner ear malformation	2.21	4.15
#15-2nd	CMV	Female	2Y1M	rt	MED-EL CONCERTO flex28	no inner ear malformation	2.21	4.37

Notes: * The note "2nd" in the Patient column represents the second operation undergone by that patient. CI = cochlear implant; CMV = cytomegalovirus.

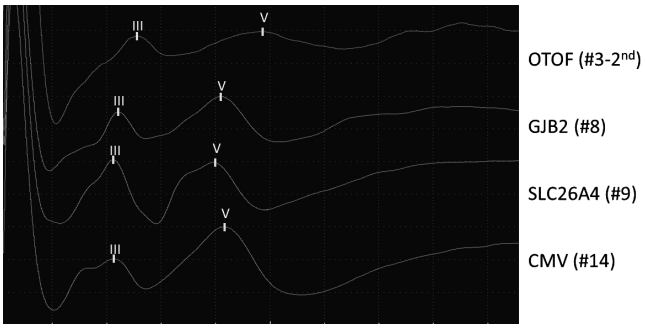

Fig. 1. Representative evoked auditory brainstem response wave forms for each group.
Groups were composed according to the identified pathology (*OTOF, GJB2*, or *SLC26A4* mutations or cytomegalovirus [CMV] infection).

OTOF mutations. We also noted that these results indicated that postsynaptic activity was still disturbed after CI implantation. However, *OTOF* mutations induce presynaptic insufficiency at the synaptic junctions of the hair cells and spiral ganglion neurons. Given these observations, we suggest that cochlear nerve synchronies were reduced in the *OTOF* group, whereas primarily neuronal conduction was preserved. Nerve development and nervous system maturation resulting in firing synchrony develops through increasing electrical pre- and postsynaptic stimulation.[39,40] The disturbed synchronies also observed with *OTOF* mutations could be caused by insufficient presynaptic stimulation, and/or the delay of nervous system maturation including the pre- and postsynaptic neural network.

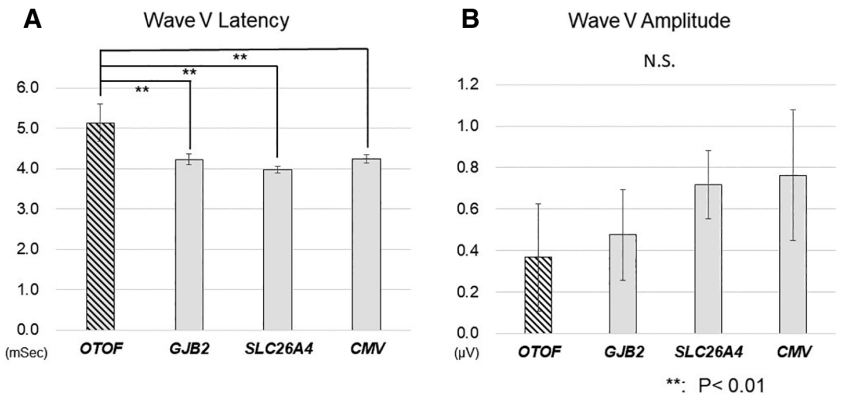

Fig. 2. Comparison of evoked auditory brainstem response (EABR) Wave V latencies (A) and amplitudes (B) between pathology groups.
EABR Wave V latency was significantly longer in patients with *OTOF* mutations than those in all other groups; no significant changes were observed in Wave V amplitudes between the groups. ** $P < .01$

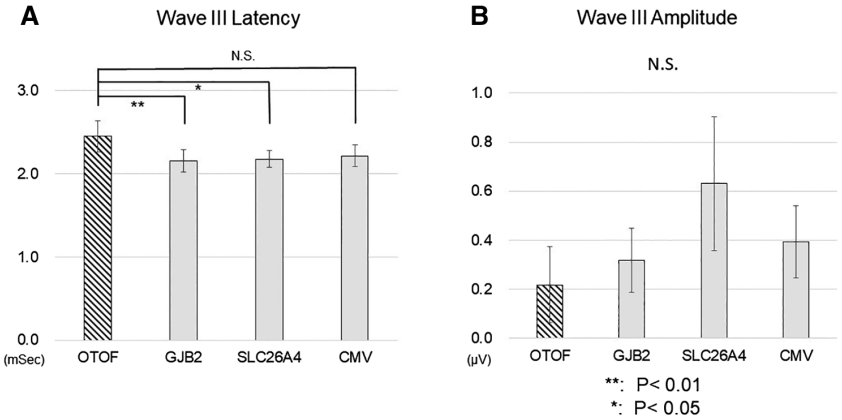

Fig. 3. Comparison of Wave III latencies (A) and amplitudes (B) between groups.
The evoked auditory brainstem response Wave III latency was significantly longer in patients with *OTOF* mutations than in those with *GJB2* and *SLC26A4* mutations, but not in those with CMV infection; no significant changes were observed in wave III amplitudes between the groups. ** $P < .01$, * $P < .05$

It is also possible that the *OTOF* mutation disturbs neurotransmission in not only hair cell-spiral ganglion synapses, but also in the cochlear nucleus or other more central aspects of the auditory pathway. Thus far, *OTOF* expression in the central auditory pathway includes spiral ganglion neurons has not been reported in the adult rodent. However, its expression was reported in other parts of the central nervous system, including the cerebellum, in the rat.[17] Our results suggest that otoferlin is important in normal neurotransmission in the human central auditory pathway, although there are no reports on otoferlin expression in primate or human. A more detailed expression study of the auditory pathway in primates will need to be carried out in near future.

Our results showed that the latencies of Wave III and Wave V in patients with AN due to *OTOF* mutations were longer than those in patients with AN due to other mutations or CMV. Thus far, Runge et al. documented poor post-synaptic ECAP response in one of two patients with an *OTOF* mutation.[41] Our observation with EABR analysis is compatible with their report. Some reports have mentioned that postoperative speech and hearing ability is affected by the EABR latencies, and that longer latencies (greater delays) might predict poorer outcomes.[42,43] Whereas patients with *OTOF* mutations generally respond well to CI and actually we could not

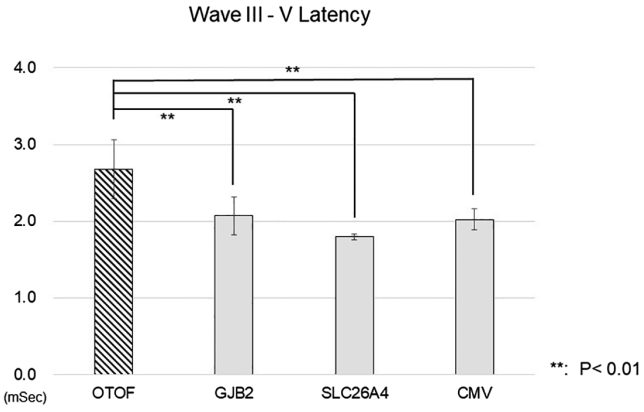

Fig. 4. Comparison of the latencies from Wave III to Wave V between groups.
The latency between evoked auditory brainstem response Wave III and Wave V was significantly longer in patients with *OTOF* mutations than in all other groups. ** $P < .01$, * $P < .05$

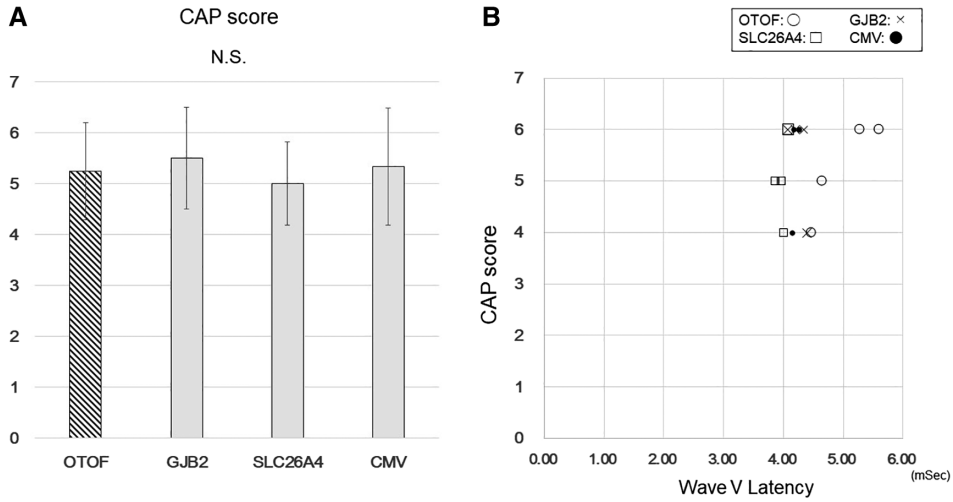

Fig. 5. Comparison of CAP score between the groups.
There was no significant difference between the groups (A). No significant relationship was detected between CAP score and EABR latency (B).

point out clinical relevant with this elongation of EABR wave form by CAP score (Fig. 5A,B), our result suggest there is a hidden negative effect on neurotransmission between the cochlear implant and the brain which cannot be detected by CAP score. Our results suggest the need for more careful follow-up of the effects of CI implantation in patients with AN caused by *OTOF* mutations. Moreover, it is possible that neuronal maturation mediated by CI would be observed. A larger-scale study with follow-up analysis will need to be carried out in near future.

In conclusion, we unveiled a novel pathophysiology of auditory neuropathies caused by *OTOF* mutations which affect more central auditory pathway beyond the synapse between the hair cells and spiral ganglion neurons. We also found that EABRs are useful for clarifying the pathophysiology of congenital hearing loss with cochlear implants.

BIBLIOGRAPHY

1. Starr A, Picton TW, Sininger Y, Hood LJ, Berlin CI. Auditory neuropathy. *Brain* 1996;119:741–753.
2. Kaga K, Nakamura M, Shinogami M, Tsuzuku T, Yamada K, Shindo M. Auditory nerve disease of both ears revealed by auditory brainstem responses, electrocochleography and otoacoustic emissions. *Scand Audiol* 1996;25:233–238.
3. Varga R, Kelley PM, Keats BJ, et al. Non-syndromic recessive auditory neuropathy is the result of mutations in the otoferlin (OTOF) gene. *J Med Genet* 2003;40:45–50.
4. Delettre C, Lenaers G, Griffoin JM, et al. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 2000;26:207–210.
5. Delmaghani S, del Castillo FJ, Michel V, et al. Mutations in the gene encoding pejvakin, a newly identified protein of the afferent auditory pathway, cause DFNB59 auditory neuropathy. *Nat Genet* 2006;38:770–778.
6. Yasunaga S, Grati M, Cohen-Salmon M, et al. A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. *Nat Genet* 1999;21:363–369.
7. Choi BY, Ahmed ZM, Riazuddin S, et al. Identities and frequencies of mutations of the otoferlin gene (OTOF) causing DFNB9 deafness in Pakistan. *Clin Genet* 2009;75:237–243.
8. Duman D, Sirmaci A, Cengiz FB, Ozdag H, Tekin M. Screening of 38 genes identifies mutations in 62% of families with nonsyndromic deafness in Turkey. *Genet Test Mol Biomarkers* 2011;15:29–33.
9. Hutchin T, Coy NN, Conlon H, et al. Assessment of the genetic causes of recessive childhood non-syndromic deafness in the UK—implications for genetic testing. *Clin Genet* 2005;68:506–512.
10. Iwasa Y, Nishio SY, Yoshimura H, et al. OTOF mutation screening in Japanese severe to profound recessive hearing loss patients. *BMC Med Genet* 2013;14:95.
11. Jin YJ, Park J, Kim AR, Rah YC, Choi BY. Identification of a novel splice site variant of OTOF in the Korean nonsyndromic hearing loss population with low prevalence of the OTOF mutations. *Int J Pediatr Otorhinolaryngol* 2014;78:1030–1035.
12. Mahdieh N, Shirkavand A, Rabbani B, et al. Screening of OTOF mutations in Iran: A novel mutation and review. *Int J Pediatr Otorhinolaryngol* 2012;76:1610–1615.
13. Rodriguez-Ballesteros M, Reynoso R, Olarte M, et al. A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (OTOF) in subjects with nonsyndromic hearing impairment and auditory neuropathy. *Hum Mutat* 2008;29:823–831.
14. Romanos J, Kimura L, Favero ML, et al. Novel OTOF mutations in Brazilian patients with auditory neuropathy. *J Hum Genet* 2009;54:382–385.
15. Matsunaga T, Mutai H, Kunishima S, et al. A prevalent founder mutation and genotype-phenotype correlations of OTOF in Japanese patients with auditory neuropathy. *Clin Genet* 2012;82:425–432.
16. Kaga K. Auditory nerve disease and auditory neuropathy spectrum disorders. *Auris Nasus Larynx* 2016;43:10–20.
17. Schug N, Braig C, Zimmermann U, et al. Differential expression of otoferlin in brain, vestibular system, immature and mature cochlea of the rat. *Eur J Neurosci* 2006;24:3372–3380.
18. Roux I, Safieddine S, Nouvian R, et al. Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* 2006;127:277–289.
19. Moser T, Starr A. Auditory neuropathy—neural and synaptic mechanisms. *Nat Rev Neurol* 2016;12:135–149.
20. Teagle HF, Roush PA, Woodard JS, et al. Cochlear implantation in children with auditory neuropathy spectrum disorder. *Ear Hear* 2010;31:325–335.
21. Kontorinis G, Lloyd SK, Henderson L, et al. Cochlear implantation in children with auditory neuropathy spectrum disorders. *Cochlear Implants Int* 2014;15(Suppl 1):S51–S54.
22. Humphriss R, Hall A, Maddocks J, Macleod J, Sawaya K, Midgley E. Does cochlear implantation improve speech recognition in children with auditory neuropathy spectrum disorder? A systematic review. *Int J Audiol* 2013;52:442–454.
23. Ji F, Li J, Hong M, et al. Determination of benefits of cochlear implantation in children with auditory neuropathy. *PLoS One* 2015;10:e0127566.
24. Harrison RV, Gordon KA, Papsin BC, Negandhi J, James AL. Auditory neuropathy spectrum disorder (ANSD) and cochlear implantation. *Int J Pediatr Otorhinolaryngol* 2015;79:1980–1987.
25. Rouillon I, Marcolla A, Roux I, et al. Results of cochlear implantation in two children with mutations in the OTOF gene. *Int J Pediatr Otorhinolaryngol* 2006;70:689–696.
26. Wu CC, Hsu CJ, Huang FL, et al. Timing of cochlear implantation in auditory neuropathy patients with OTOF mutations: Our experience with 10 patients. *Clin Otolaryngol* 2018;43:352–357.
27. Rodriguez-Ballesteros M, del Castillo FJ, Martin Y, et al. Auditory neuropathy in patients carrying mutations in the otoferlin gene (OTOF). *Hum Mutat* 2003;22:451–456.
28. Minami SB, Takegoshi H, Shinjo Y, Enomoto C, Kaga K. Usefulness of measuring electrically evoked auditory brainstem responses in children with inner ear malformations during cochlear implantation. *Acta Otolaryngol* 2015;135:1007–1015.

29. Bierer JA, Faulkner KF, Tremblay KL. Identifying cochlear implant channels with poor electrode-neuron interfaces: Electrically evoked auditory brain stem responses measured with the partial tripolar configuration. *Ear Hear* 2011;32:436–444.
30. Buss E, Labadie RF, Brown CJ, Gross AJ, Grose JH, Pillsbury HC. Outcome of cochlear implantation in pediatric auditory neuropathy. *Otol Neurotol* 2002;23:328–332.
31. Runge-Samuelson CL, Drake S, Wackym PA. Quantitative analysis of electrically evoked auditory brainstem responses in implanted children with auditory neuropathy/dyssynchrony. *Otol Neurotol* 2008;29: 174–178.
32. Shallop JK, Peterson A, Facer GW, Fabry LB, Driscoll CL. Cochlear implants in five cases of auditory neuropathy: Postoperative findings and progress. *Laryngoscope* 2001;111:555–562.
33. Jeong SW, Kim LS, Kim BY, Bae WY, Kim JR. Cochlear implantation in children with auditory neuropathy: Outcomes and rationale. *Acta Otolaryngol* 2007;127:36–43.
34. Okamoto Y, Mutai H, Nakano A, et al. Subgroups of enlarged vestibular aqueduct in relation to SLC26A4 mutations and hearing loss. *Laryngoscope* 2014;124:E134–E140.
35. Yamamoto N, Mutai H, Namba K, et al. Prevalence of TECTA mutation in patients with mid-frequency sensorineural hearing loss. *Orphanet J Rare Dis* 2017;12:157.
36. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–424.
37. Archbold S, Lutman ME, Marshall DH. Categories of auditory performance. *Ann Otol Rhinol Laryngol Suppl* 1995;166:312–314.
38. Archbold S, Lutman ME, Nikolopoulos T. Categories of auditory performance: Inter-user reliability. *Br J Audiol* 1998;32:7–12.
39. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. *Science* 1996;274:1123–1133.
40. Yamada A, Uesaka N, Hayano Y, Tabata T, Kano M, Yamamoto N. Role of pre- and postsynaptic activity in thalamocortical axon branching. *Proc Natl Acad Sci U S A* 2010;107:7562–7567.
41. Runge CL, Erbe CB, McNally MT, et al. A novel otoferlin splice-site mutation in siblings with auditory neuropathy spectrum disorder. *Audiol Neurootol* 2013;18:374–382.
42. Groenen PA, Makhdoum M, van den Brink JL, Stollman MH, Snik AF, van den Broek P. The relation between electric auditory brain stem and cognitive responses and speech perception in cochlear implant users. *Acta Otolaryngol* 1996;116:785–790.
43. Wang Y, Pan T, Deshpande SB, Ma F. The relationship between EABR and auditory performance and speech intelligibility outcomes in pediatric cochlear implant recipients. *Am J Audiol* 2015;24:226–234.