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

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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 PJKV 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
performed the Sanger methods as previously reported.15,34,35 We
ness genes have not been clari
ted Clinical and Experimental Hearing Research, National Children’s Medical Center by the Sanger method using the genomic DNA from patients with CMV infections (Table I).

Disorders, National Institute of Sensory Organs, National Tokyo Medical Center by the Sanger method using the genomic DNA extracted from 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 had undergone bilateral CI. We analyzed EABR waveforms obtained from six ears with OTOF mutations, six with GJB2 mutations, four with SLC26A4 mutations, and four from patients with CMV infections (Table I).

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) describes 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 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 had undergone bilateral CI. We analyzed EABR waveforms obtained from six ears with OTOF mutations, six with GJB2 mutations, four with SLC26A4 mutations, and four from patients with CMV infections (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. Similar-ly, 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 Neurepack Σ (Nihon Koden Co., Tokyo, Japan) electrodagnostic 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 (μA) to 200 μA at 200 μA intervals for MED-EL and 600 μA to 200 μA at 100 μA intervals for Advanced Bionics. If no response was detected, pulse duration was increased up to 100 μsec.

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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. 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.

| Patient | Cause of Deafness | Sex | Operation Age | Operation side | Implant 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 M1000PIN 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 M100 Flex soft | large vestibular aqueduct | 2.12 | 3.97 |
| #10     | SLC26A4           | Female | 3Y5M | rt | MED-EL CONCERTO M100 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.
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. 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 in 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

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
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.

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