Detailed Clinical Features and Genotype-Phenotype Correlation in an OTOF-Related Hearing Loss Cohort in Japan

Yoh-ichiro Iwasa
Shinshu Daigaku

Shin-ya Nishio
Shinshu Daigaku

Hidekane Yoishimura
Shinshu Daigaku

Akiko Sugaya
Okayama University: Okayama Daigaku

Yuko Kataoka
Okayama University: Okayama Daigaku

Yukihide Maeda
Okayama University: Okayama Daigaku

Yukihiko Kanda
Kanda ENT Clinic, Nagasaki Bell Hearing Center

Kyoko Nagai
TAKASAKI Ear Nose & Throat Clinic

Yasushi Naito
Kobe City Medical Center General Hospital: Kobe Shiritsu Iryo Center Chuo Shimin Byoin

Hiroshi Yamazaki
Kobe City Medical Center General Hospital: Kobe Shiritsu Iryo Center Chuo Shimin Byoin

Tetsuo Ikezono
Saitama Medical University: Saitama Ika Daigaku

Han Matsuda
Saitama Medical University: Saitama Ika Daigaku

Masako Nakai
Shiga medical center for children

Risa Tona
Shiga medical center for children

Yuika Sakurai
Jikei University School of Medicine: Tokyo Jikeikai Ika Daigaku

Remi Motegi
Juntendo University: Juntendo Daigaku
Hidehiko Takeda
Toranomon Hospital: Toranomon Byoin

Marina Kobayashi
Toranomon Hospital: Toranomon Byoin

Chiharu Kihara
Nagasaki University: Nagasaki Daigaku

Takahiro Ishino
Hiroshima University: Hiroshima Daigaku

Shin-ya Morita
Hokkaido University: Hokkaido Daigaku

Satoshi Iwasaki
IUHW Mita Hospital: Kokusai Iryo Fukushi Daigaku Mita Byoin

Masahiro Takahashi
IUHW Mita Hospital: Kokusai Iryo Fukushi Daigaku Mita Byoin

Sakiko Furutate
IUHW Mita Hospital: Kokusai Iryo Fukushi Daigaku Mita Byoin

Shin-ichiro Oka
IUHW Mita Hospital: Kokusai Iryo Fukushi Daigaku Mita Byoin

Toshinori Kubota
Yamagata University: Yamagata Daigaku

Yasuohiro Arai
Yokohama City University: Yokohama Shiritsu Daigaku

Yumiko Kobayashi
Iwate Medical University: Iwate Ika Daigaku

Daisuke Kikuchi
Fukushima medical university

Tomoko Shintani
Sapporo Medical University: Sapporo Ika Daigaku

Noriko Ogasawara
Sapporo Medical University: Sapporo Ika Daigaku

Yohei Honkura
Tohoku Daigaku

Shuji Izumi
Niigata University: Niigata Daigaku

Misako Hyogo
Kyoto Prefectural University of Medicine: Kyoto Furitsu Ika Daigaku

Yuzuru Ninoyu
Kyoto Prefectural University of Medicine: Kyoto Furitsu Ika Daigaku
Keywords: OTOF, hearing loss, auditory neuropathy spectrum disorder, massively parallel DNA sequencing, OAE, cochlear implant

DOI: https://doi.org/10.21203/rs.3.rs-588334/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

OTOF is one of the most frequent causes of hereditary hearing loss and a main cause of auditory neuropathy spectrum disorder (ANSD). Although it is reported that most of the patients with OTOF mutations have stable, congenital or prelingual onset severe-to-profound hearing loss, some patients show atypical clinical phenotypes, and the genotype-phenotype correlation in the patients with OTOF mutations is not yet fully understood. In this study, we aimed to reveal detailed clinical characteristics of OTOF-related hearing loss patients and the genotype-phenotype correlation. Detailed clinical information was available for 65 patients in our database who were diagnosed with OTOF-related hearing loss. As reported previously, most of the patients (90.8%) showed a “typical” phenotype, prelingual and severe-to-profound hearing loss. Forty-seven patients (72.3%) underwent cochlear implantation surgery and showed successful outcomes; approximately 85-90% of the patients showed a hearing level of 20-39dB with cochlear implant and a CAP scale (Categories of Auditory Performance) level 6 or better. Although truncating mutations and p.R1939Q were clearly related to severe phenotype, almost half of patients with one or more non-truncating mutations showed mild-to-moderate hearing loss. Notably, patients with p.H513R, p.I1573T and p.E1910K showed “true” auditory neuropathy-like clinical characteristics. In this study, we have clarified genotype-phenotype correlation and efficacy of cochlear implantation for OTOF-related hearing loss patients in the biggest cohort studied to date. We believe that the clinical characteristics and genotype-phenotype correlation found in this study will support preoperative counseling and appropriate intervention for OTOF-related hearing loss patients.

Introduction

Hearing loss is one of the most common sensory disorders, with around 466 million people suffering hearing loss (WHO Deafness and hearing loss. 2021). It is reported that 1 out of 500 newborns has bilateral hearing loss and 50–60% of them are due to genetic etiologies (Smith et al. 2005). OTOF is reported to be the causative gene of DFNB9 and one of the frequent causes of non-syndromic recessive sensorineural hearing loss. The prevalence of OTOF mutations has been reported to be 1.4–8.3% of non-syndromic hearing loss cases and 1.72% in Japan (Iwasa et al. 2019). To date, more than 220 mutations in OTOF have been reported (Vona et al. 2020). Although it is reported that most of the patients with OTOF mutations have stable, congenital or prelingual onset severe-to-profound hearing loss, some patients show atypical clinical phenotypes, such as mild-to-moderate progressive cases (Chiu et al. 2010). The genotype-phenotype correlation in the patients with OTOF mutations is, subsequently, not yet fully understood.

OTOF is also reported to be a main cause of auditory neuropathy spectrum disorder (ANSD), which is a specific form of hearing loss with an abnormal auditory brainstem response (ABR) and the presence of otoacoustic emissions (OAE) (A Starr, T W Picton, Y Sininger, L J Hood 1996). Both genetic and environmental factors cause ANSD, such as hyperbilirubinemia, thiamine deficiency, hypoxia, and noise-induced and age-related hearing loss (Shearer 2019). OTOF-related ANSD is the most prevalent form of ANSD, and it is reported that 41.3–90.9% of pediatric cases of ANSD are caused by OTOF mutations.
(Matsunaga et al. 2012; Zhang et al. 2016; Kim et al. 2018a). OTOF is mainly expressed in the inner hair cells (IHCs), and mutations in the OTOF gene cause dysfunction of synaptic exocytosis at the ribbon synapse (Roux et al. 2006). OTOF-related ANSD is also known as auditory synaptopathy, presenting with impaired synaptic function between inner hair cells and spiral ganglion neurons, to differentiate it from auditory neuropathy (i.e., “true” auditory neuropathy) (Moser and Starr 2016). Due to the presence of an OAE response, there are some clinical problems with ANSD patients; they inappropriately pass the newborn hearing screening (NBHS) in countries where OAE is used as a screening tool, resulting in the late diagnosis of hearing loss. As early intervention by hearing aid (HA), cochlear implant (CI) and language rehabilitation is necessary for patients with hearing impairment to develop their speech skills, late diagnosis is disadvantage for such hearing loss patients. Moreover, the presence of an OAE response makes it difficult for clinicians to decide the appropriate timing of cochlear implantation, as OAE positivity means the outer hair cells (OHCs) are functional and the efficacy of CI could be limited in case the patient is “true” auditory neuropathy. Therefore, the genetic diagnosis of OTOF-related hearing loss in ANSD patients can encourage clinicians to recommend cochlear implantation for the patients as the performance of CI for OTOF-related ANSD is reported to be excellent (Zheng and Liu 2020). However, each report was based on only a limited number of patients, and different evaluation tools were used for assessing the performance of CIs in each report.

In Japan, social health insurance-based genetic testing for the patients with hereditary hearing loss was approved in 2012 and, from 2015, massively parallel DNA sequencing technology was combined with genetic testing, so that magnificent mutation data from Japanese hearing loss patients has been accumulated over the years in the whole-Japanese database. In this study, we investigated the clinical data of the patients diagnosed with OTOF-related hearing loss registered in our database, and aimed to reveal detailed clinical characteristics of OTOF-related hearing loss patients and the genotype-phenotype correlation in the patients with OTOF mutations.

**Subjects And Methods**

**Subjects**

A total of 12,137 patients were registered in our database between February 2012 and December 2020 from 96 otolaryngology departments from across Japan. Seventy-four patients had two or more mutations in the OTOF gene. Detailed clinical information was available for 67 of 74 patients, and their clinical characteristics were analyzed retrospectively through review of their medical records, and 2 of them were excluded because the clinical phenotype was completely incompatible with OTOF-related hearing loss (Late-onset ski-slope hearing loss and acute-onset unilateral mild hearing loss). Hearing level was evaluated using pure-tone audiometry (PTA) classified by a pure-tone average over 500, 1000, 2000 and 4000 Hz in the better hearing ears. For children who could not undergo PTA, we used an average over 500, 1000, 2000 and 4000 Hz in conditioned oriented reflex audiometry (COR). Severity of hearing loss was classified as follows; normal hearing, <25dB; mild hearing loss, 25-39dB; moderate hearing loss, 40-69dB; severe hearing loss, 70-89dB; profound hearing loss, greater than 90dB. Written informed consent
was obtained from all subjects (or from their next of kin, caretaker, or guardian on the behalf of minors/children) prior to enrollment in the project. This study was approved by the ethical committees of Shinshu University and each of the other participating institutions.

MPS sequencing

Amplicon resequencing with MPS

Amplicon libraries were prepared using an Ion AmpliSeq Custom Panel (Applied Biosystems, Life Technologies), in accordance with the manufacturer's instructions, for 68 genes reported to cause non-syndromic hereditary HL. The detailed sample preparation protocol has been described elsewhere (Iwasa et al. 2019). Sequencing was performed in accordance with the manufacturer’s instructions. Massively Parallel Sequencing (MPS) was performed with an Ion Torrent Personal Genome Machine (PGM) system, Ion Proton System or Ion S5 system using an Ion PGM 200 Sequencing Kit with an Ion 318 Chip (Life Technologies) or Ion HiQ Chef Kit with an Ion P1 chip or Ion 540 Chip kit-Chef. The sequence data were mapped against the human genome sequence (build GRCh37/ hg19) with a Torrent Mapping Alignment Program. After sequence mapping, the DNA variants were detected with Torrent Variant Caller plug-in software. After variant detection, their effects were analyzed using ANNOVAR software (Wang et al. 2010). The missense, nonsense, insertion/deletion, and splicing variants were selected from among the identified variants. Variants were further selected as less than 1% of (1) the 1000 genome database, (2) the 6500 exome variants, (3) the Human Genetic Variation Database (dataset for 1208 Japanese exome variants), and (4) the 333 in-house Japanese normal hearing controls by using our database software. All the mutations found in this study were confirmed by Sanger sequencing using exon-specific custom primers. The pathogenicity of the candidate variants was interpreted based on the standards and guidelines of the American College of Medical Genetics (ACMG) and ClinGen HL-CDWG expert specification (Oza et al. 2018; Brandt et al. 2019).

Results

Mutation analysis

Detailed mutation information detected in 65 patients whose clinical information was available is shown in Table 1. Twenty-seven (41.5%) of the 65 patients with biallelic OTOF mutations had homozygous OTOF:NM_001287489:c.5816G > A:p.R1939Q mutations. Thirty (46.2%) were compound heterozygote with c.5816G > A and another mutation. Eight patients (12.3%) had other non-c.5816G > A mutations. The pathogenicity of mutations found in the 65 patients were categorized in accordance with the ACMG criteria (Suppl. Table 1).

Clinical characteristics

A summary of clinical information is shown in Fig. 1. Hearing level information was as follows; Fifty-one patients (78.4%) showed profound hearing loss, 8 patients (12.3%) showed severe hearing loss and 5
patients (8.2%) showed moderate hearing loss (Fig. 1a). Although most of the patients with OTOF-related hearing loss show congenital severe-to-profound hearing loss, NBHS only detects 43.1% of these cases of hearing loss (Fig. 1b). The rest of them were found to have hearing loss due to a lack of response to sound (24.6%), delayed language development (9.2%) or other reasons. Forty-seven patients (72.3%) underwent CI surgery; 9 patients (13.8%) were implanted simultaneously in both ears, 24 of them (36.9%) were implanted sequentially and 14 of them (21.5%) were implanted unilaterally (Fig. 1c). Figure 2 shows the detailed information for NBHS; 28 patients (43.1%) were for referred NBHS, 14 (21.5%) passed, 12 (18.5%) did not undergo NBHS (Fig. 2a). Among the 44 patients who underwent NBHS, AABR and OAE was performed in 32 and 12 patients, respectively. All of the 12 patients examined by OAE improperly passed NBHS, while 3 out of 32 patients (9.4%) passed AABR screening (Fig. 2b, c).

**Performance of cochlear implantation**

In this study, the performance of cochlear implantation was evaluated using PTA and the Categories of Auditory Performance (CAP) scale for 36 patients who had used CI for over 2 years after the initial implantation. Hearing threshold data with CI in the better hearing ear were available for all of 36 patients and are shown in Fig. 3a: 20-29dB in 17 patients (47.2%), 30-39dB in 15 patients (41.6%) and 40-49dB in 4 patients (11.1%). The CAP scale data were available for 33 patients and are shown in Fig. 3b: CAP scale level 7 in 2 patients (6.1%), level 6 in 26 patients (87.8%), level 5 in 3 patients (9.1%), level 4 in 1 patient (3%) and level 3 in 1 patient (3%).

**Disappearance of OAE**

Figure 4A shows the timing of the disappearance of OAE response in the ear in which the OAE response remained longer; 20 patients (30.8%) lost OAE response at CI implantation, 22 patients (33.8%) were tested only once and showed OAE, 6 patients (9.2%) showed no response at the first visit and 2 patients (3.1%) still had a positive OAE at the time of this survey. Accurate timing of OAE disappearance was available only in one patient (between two and half years to three years old). Figure 4b shows the OAE results for the ears in which OAE response remained longer at the last OAE testing. No patients showed OAE passed 5 years of age.

**Discussion**

In this study, we investigated the clinical characteristics of 65 patients who were diagnosed with OTOF-related hearing loss. One of the main aims was to identify genotype-phenotype correlations. As reported previously, most of the patients (90.8%) participating in this study show a “typical” phenotype, congenital or prelingual onset and severe-to-profound hearing loss (Fig. 1). All of the patients who revealed a homozygous c.5816G > A (p.R1939Q) and compound heterozygote with p.R1939Q and a truncating mutation, and 1 patient with two truncating mutations (c.1422T > A (p.Y474X) and c.885+5G > A) showed profound hearing loss. Therefore, it is plausible that p.R1939Q and a truncating mutation are related to severe-to-profound hearing loss based on these results. On the other hand, the phenotype of patients with non-truncating mutations is more complicated. Figure 5a shows a summary of genotype-
phenotype correlations in this and previous studies, in which the patients’ hearing level and mutation information were available (Tekin et al. 2005; Varga et al. 2006; Rodríguez-Ballesteros et al. 2008; Santarelli et al. 2009; Chiu et al. 2010; Zadro et al. 2010; Mahdieh et al. 2012; Matsunaga et al. 2012; Yildirim-Baylan et al. 2014a; Zhang et al. 2016; Kim et al. 2018b; Wang et al. 2018). There is a clear relationship between p.R1939Q and a severe phenotype, as well as truncating mutations including c.2485C > T (p.Q829X). On the other hand, when patients have one or more non-truncating mutations, almost half of them showed mild-to-moderate hearing loss (Fig. 5c, d). Based on the results, patients with non-truncating mutations could show a milder phenotype even when they have p.R1939Q or a truncating mutation.

Among the moderate cases in our cohort, there were representative 3 cases who showed “true” auditory neuropathy-like clinical characteristics (AK6640, AL8610 and AH0083 were compound heterozygote with c.[5816G > A]; [1538A > G] (p.[R1939Q]);[H513R], c.[5815C > T];[5728G > A](p.[R1939W];[E1910K]) and c. [4718T > C];[4129_4138del](p.[I1573T];[A1377Rfs*142], respectively)); the PTA of all patients showed moderate hearing loss while their ABR showed no response. p.H513R is located in the C2C domain, which also contains the p.I515T and p.G541S mutations reported as mutations related to temperature-sensitive auditory neuropathy (TS-AN) and milder hearing loss in a non-febrile state (Varga et al. 2006; Matsunaga et al. 2012). p.I1573T was reported as the cause of ANSD with mild-to-moderate hearing loss in a previous report, which is compatible to the present case (Yildirim-Baylan et al. 2014b). We consider that p.H513R and p.I1573T are related to a milder phenotype. p.R1939W was reported previously, and the patient with homozygous p.R1939W showed severe-to-profound hearing loss (Choi et al. 2009). p.E1910K is novel mutation. As p.R1939W causes the same amino acid change as p.R1939Q, p.E1910K might cause moderate hearing loss. Other than these mutations, it has been reported that p.E1700Q was related to a progressive and milder phenotype (Chiu et al. 2010), and p.R1607W and p.G1804del were related to TS-AN (Marlin et al. 2010; Wang et al. 2010). Interestingly, all of the patients with the aforementioned mutations related to a milder and non-typical phenotype showed no ABR response, just like “true” auditory neuropathy. Electrophysiological testing, such as ABR, is important, especially when estimating the hearing threshold of very young children. However, when determining the hearing level for OTOF-related hearing loss, the results of audiological testing should be carefully interpreted, considering that there is a discrepancy between PTA threshold and ABR response, especially when the PTA thresholds are mild-to-moderate.

This study revealed that hearing loss was detected by NBHS in only 43.1% of the patients. The presence of OAE is main reason for missing OTOF-related ANSD at NBHS. Similar to our result, it was reported that 75% of OTOF-related patients improperly passed NBHS in a previous study (Wu et al. 2019). The risk of using OAE for NBHS has been discussed from the point of view of its inability in detecting ANSD. Nevertheless, OAE is still used for NBHS in most countries and there are only a few countries where both OAE and AABR are used for NBHS (Wroblewska-Seniuk et al. 2017). In Japan, the screening method differs depending on the clinic and prefecture. All of the patients in this study screened by OAE passed NBHS improperly, and their hearing loss was detected by their unawareness to sound or delayed language development. For better language development, early detection of HL is important. We strongly
believe that NBHS should be performed by AABR or combination of OAE and AABR for reliable detection of ANSD.

The efficacy of cochlear implantation for ANSD has been controversial due to the heterogenicity of ANSD in terms of its etiology: ANSD is a disorder which includes auditory synaptopathy and neuropathy. Generally, prior to cochlear implantation, genetic testing should be considered in order to clarify which part of the auditory pathway is impaired and in order to be able to predict the outcomes of CI (Miyagawa et al. 2016). Once a patient is diagnosed with OTOF-related ANSD, a good CI outcome is expected because the auditory nerve remains intact. Indeed, past reports suggested that patients with OTOF-related ANSD are good candidates for CI (Zheng and Liu 2020). In this study, we investigated the efficacy of CI for OTOF-related hearing loss in the biggest cohort studied to date. Most of the patients who underwent cochlear implantation showed successful outcomes: approximately 85–90% of patients showed a hearing level of 20-39dB with CI and a CAP scale level 6 or better, which means the OTOF-related hearing loss patients who underwent CI can understand conversation without lip-reading. This data will support preoperative counseling for OTOF-related ANSD patients who are considered to be CI candidates.

In this study, we also investigated the timing of OAE disappearance. Although the exact timing of OAE disappearance was not available for most of the patients, some patients showed a positive OAE even at 3–4 years of age (Fig. 3b). Given no patients showed a positive OAE passed 5 years of age, we presume that the OAE response in OTOF-related ANSD disappears by 4–5 years of age at most, a little longer than previously thought. Related to the disappearance of OAE, some reports suggest that the disappearance of OAE could occur due to OHC damage originating from hearing aid use (Rouillon et al. 2006; Vona et al. 2020). On the other hand, it is also suggested that the use of hearing aids is not a definitive factor for deterioration of OAE (Kitao et al. 2019). In our study, some patients lost OAE regardless of HA use, and some kept their OAE responses even after wearing HAs until CI surgery; therefore, it is unclear whether the disappearance of OAE is related to wearing HAs or is part of the natural course of OTOF-related hearing loss. As early intervention of HA for deaf patients is important for language development, there is no evidence to not recommend HAs for patients with OTOF-related ANSD with the aim of maintaining OAE response.

Although CIs provide excellent hearing performance in cases of OTOF-related ANSD, it does not reach normal hearing level. Therefore, there has been a strong desire for a curative therapy, such as gene therapy. Recently, two reports have shown that cochlear gene therapy mediated by adeno-associated virus (AAV) successfully improved the prognosis of hearing impairment in Otof−/− mice (Al-Moyed et al. 2018; Akil et al. 2019). AAV-based gene therapy is considered to be a promising tool for actual human gene therapy due to its low immunogenicity, long-lasting transgene expression and ability to infect both dividing and non-dividing cells (Chandran et al. 2017). At present, a few companies around the world are preparing for clinical trials using AAV (Akouos, Boston, MA, USA; Decibel Therapeutics, Boston, MA, USA; Sensorion, Montpellier, France) (Vona et al. 2020). A new era of next-generation treatments for hereditary hearing loss, such as gene therapy, is certainly approaching; however, we should not forget that accurate treatment is based on accurate diagnosis and understanding of the clinical course. With the
improvements in molecular biological diagnostic method, the etiologies of hereditary hearing loss have gradually become better understood. Although genotype-phenotype correlations have been obscure, the accumulation of patient data is gradually revealing the clinical characteristics of *OTOF*-related hearing loss. We believe that the clinical characteristics and genotype-phenotype correlation found in this study will support appropriate intervention and future treatment for *OTOF*-related hearing loss patients.

**Declarations**

**Funding**

This research was funded by a Health and Labor Sciences Research Grant for Research on Rare and Intractable diseases and Comprehensive Research on Disability Health and Welfare from the Ministry of Health, Labor and Welfare of Japan (S.U. 20FC1048), a Grant-in-Aid from Japan Agency for Medical Research and Development (AMED) (S.U.: 17kk0205010h0002, 18ek0109363h0001)

**Acknowledgements**

We thank all participant in the present study and collaborators for providing samples and clinical information.

**References**

1. Starr A, Picton TW, Sininger Y, Hood LJ CIB (1996) Auditory neuropathy. Brain 119:741–753
2. Akil O, Dyka F, Calvet C et al (2019) Dual AAV-mediated gene therapy restores hearing in a DFNB9 mouse model. Proc Natl Acad Sci 116:4496–4501. https://doi.org/10.1073/pnas.1817537116
3. Al-Moyed H, Cepeda AP, Jung S et al (2018) A dual-AAV approach restores fast exocytosis and partially rescues auditory function in deaf otoferlin knock-out mice. EMBO Mol Med e9396. https://doi.org/10.15252/emmm.201809396
4. Brandt T, Sack LM, Arjona D et al (2019) Adapting ACMG/AMP sequence variant classification guidelines for single-gene copy number variants. Genet Med 0: https://doi.org/10.1038/s41436-019-0655-2
5. Chandran JS, Sharp PS, Karyka E et al (2017) Site Specific Modification of Adeno-Associated Virus Enables Both Fluorescent Imaging of Viral Particles and Characterization of the Capsid Interactome. Sci Rep 7:1–17. https://doi.org/10.1038/s41598-017-15255-2
6. Chiu YH, Wu CC, Lu YC et al (2010) Mutations in the OTOF gene in Taiwanese patients with auditory neuropathy. Audiol Neurotol 15:364–374. https://doi.org/10.1159/000293992
7. Choi BY, Ahmed ZM, Riazuddin S et al (2009) Identities and frequencies of mutations of the otoferlin gene (OTOF) causing DFNB9 deafness in Pakistan. Clin Genet 75:237–243. https://doi.org/10.1111/j.1399-0004.2008.01128.x
8. Iwasa Y, ichiro, Nishio S ya, Sugaya A et al (2019) OTOF mutation analysis with massively parallel DNA sequencing in 2,265 Japanese sensorineural hearing loss patients. PLoS One 14:1–10. https://doi.org/10.1371/journal.pone.0215932

9. Kim BJ, Jang JH, Han JH et al (2018a) Mutational and phenotypic spectrum of OTOF-related auditory neuropathy in Koreans: Eliciting reciprocal interaction between bench and clinics. J Transl Med 16:1–13. https://doi.org/10.1186/s12967-018-1708-z

10. Kim BJ, Jang JH, Han JH et al (2018b) Mutational and phenotypic spectrum of OTOF-related auditory neuropathy in Koreans: Eliciting reciprocal interaction between bench and clinics. J Transl Med 16:1–13. https://doi.org/10.1186/s12967-018-1708-z

11. Kitao K, Mutai H, Namba K et al (2019) Deterioration in Distortion Product Otoacoustic Emissions in Auditory Neuropathy Patients with Distinct Clinical and Genetic Backgrounds. Ear Hear 40:184–191. https://doi.org/10.1097/AUD.0000000000000586

12. Mahdieh N, Shirkavand A, Rabbani B et al (2012) Screening of OTOF mutations in Iran: A novel mutation and review. Int J Pediatr Otorhinolaryngol 76:1610–1615. https://doi.org/10.1016/j.ijporl.2012.07.030

13. Marlin S, Feldmann D, Nguyen Y et al (2010) Temperature-sensitive auditory neuropathy associated with an otoferlin mutation: Deafening fever! Biochem Biophys Res Commun 394:737–742. https://doi.org/10.1016/j.bbrc.2010.03.062

14. Matsunaga T, Mutai H, Kunishima S et al (2012) A prevalent founder mutation and genotype-phenotype correlations of OTOF in Japanese patients with auditory neuropathy. Clin Genet 82:425–432. https://doi.org/10.1111/j.1399-0004.2012.01897.x

15. Miyagawa M, Nishio SY, Usami SI (2016) A comprehensive study on the etiology of patients receiving cochlear implantation with special emphasis on genetic epidemiology. Otol Neurotol 37:e126–e134. https://doi.org/10.1097/MAO.0000000000000936

16. Moser T, Starr A (2016) Auditory neuropathy-neural and synaptic mechanisms. Nat Rev Neurol 12:135–149. https://doi.org/10.1038/nrneurol.2016.10

17. Oza AM, DiStefano MT, Hemphill SE et al (2018) Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat 39:1593–1613. https://doi.org/10.1002/humu.23630

18. Rodríguez-Ballesteros M, Reynoso R, Olarte M et al (2008) 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 29:823–831. https://doi.org/10.1002/humu.20708

19. Rouillon I, Marcolla A, Roux I et al (2006) Results of cochlear implantation in two children with mutations in the OTOF gene. Int J Pediatr Otorhinolaryngol 70:689–696. https://doi.org/10.1016/j.ijporl.2005.09.006

20. Roux I, Safieddine S, Nouvian R et al (2006) Otoferlin, Defective in a Human Deafness Form, Is Essential for Exocytosis at the Auditory Ribbon Synapse. Cell 127:277–289. https://doi.org/10.1016/j.cell.2006.08.040
21. Santarelli R, Del Castillo I, Rodríguez-Ballesteros M et al (2009) Abnormal cochlear potentials from deaf patients with mutations in the otoferlin gene. JARO - J Assoc Res Otolaryngol 10:545–556. https://doi.org/10.1007/s10162-009-0181-z

22. Shearer AE (2019) Auditory Synaptopathy, Auditory Neuropathy, and Cochlear Implantation. 429–440. https://doi.org/10.1002/lio2.288

23. Smith RJH, Bale JF, White KR (2005) Sensorineural hearing loss in children. Lancet 365:879–890. https://doi.org/10.1016/S0140-6736(05)71047-3

24. Tekin M, Akcayoz D, Incesulu A (2005) A novel missense mutation in a C2 domain of OTOF results in autosomal recessive auditory neuropathy. Am J Med Genet 138 A:6–10. https://doi.org/10.1002/ajmg.a.30907

25. Varga R, Avenarius MR, Kelley PM et al (2006) OTOF mutations revealed by genetic analysis of hearing loss families including a potential temperature sensitive auditory neuropathy allele. J Med Genet 43:576–581. https://doi.org/10.1136/jmg.2005.038612

26. Vona B, Rad A, Reisinger E (2020) The many faces of dfnb9: Relating otof variants to hearing impairment. Genes (Basel) 11:1–23. https://doi.org/10.3390/genes1121411

27. Wang DY, Wang YC, Weil D et al (2010a) Screening mutations of OTOF gene in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy. BMC Med Genet 11:3–5. https://doi.org/10.1186/1471-2350-11-79

28. Wang K, Li M, Hakonarson H (2010b) ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:1–7. https://doi.org/10.1093/nar/gkq603

29. Wang Y, Lu Y, Cheng J et al (2018) Novel OTOF gene mutations identified using a massively parallel DNA sequencing technique in DFNB9 deafness. Acta Otolaryngol 6489:1–20. https://doi.org/10.1080/00016489.2018.1476777

30. Wroblewska-Seniuk KE, Dabrowski P, Szyfter W, Mazela J (2017) Universal newborn hearing screening: Methods and results, obstacles, and benefits. Pediatr Res 81:415–422. https://doi.org/10.1038/pr.2016.250

31. Wu CC, Tsai CY, Lin YH et al (2019) Genetic epidemiology and clinical features of hereditary hearing impairment in the Taiwanese population. Genes (Basel) 10:1–20. https://doi.org/10.3390/genes10100772

32. Yildirim-Baylan M, Bademci G, Duman D et al (2014a) Evidence for genotype-phenotype correlation for OTOF mutations. Int J Pediatr Otorhinolaryngol 78:950–953. https://doi.org/10.1016/j.ijporl.2014.03.022

33. Yildirim-Baylan M, Bademci G, Duman D et al (2014b) Evidence for genotype-phenotype correlation for OTOF mutations. Int J Pediatr Otorhinolaryngol 78:950–953. https://doi.org/10.1016/j.ijporl.2014.03.022

34. Zadro C, Ciorba A, Fabris A et al (2010) Five new OTOF gene mutations and auditory neuropathy. Int J Pediatr Otorhinolaryngol 74:494–498. https://doi.org/10.1016/j.ijporl.2010.02.004
35. Zhang QJ, Han B, Lan L et al (2016) High frequency of OTOF mutations in Chinese infants with congenital auditory neuropathy spectrum disorder. Clin Genet 90:238–246. https://doi.org/10.1111/cge.12744

36. Zheng D, Liu X (2020) Cochlear Implantation Outcomes in Patients With OTOF Mutations. Front Neurosci 14:1–7. https://doi.org/10.3389/fnins.2020.00447

**Tables**

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

**Figures**

**Figure 1**

Clinical characteristics of the OTOF-related hearing loss patients in this study. a) Hearing level in PTA or COR in very young children. b) Detection of hearing loss. c) Intervention for hearing loss. NA, not applicable; n, number of patients.
Figure 2

Detailed information of newborn hearing screening (NBHS). a) Results of NBHS for all patients in this study. b, c) Result of NBHS by each screening method (AABR and OAE).

A  Hearing threshold with CI (n=36)

B  CAP scale (n=33)

Figure 3

Performance of cochlear implantation for OTOF-related hearing loss. a) Hearing threshold with cochlear implants was available for 36 patients who had used CIs for more than 2 years. b) The CAP scale was available for 33 patients.

A  Status of OAE response (n=65)

B  Result at the last OAE testing

Figure 4
Detailed information on the disappearance of OAE response. a) Timing of the disappearance of OAE response in the ear in which OAE response remained longer or the current status of OAE response. b) Results at the last OAE testing.

Figure 5

Summary of genotype-phenotype correlations in this and previous studies. a) Mutation type and hearing severity in this and previous studies. b) Hearing severity of patients with two truncating mutations. c)
Hearing severity of patients with truncating and non-truncating mutations (excluding p.R1939Q). d) Hearing severity of patients with two non-truncating mutations (excluding p.R1939Q).

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Table1v5.xlsx
- Suppl.Table1.xlsx