Variants in \textit{CDH23} cause a broad spectrum of hearing loss: from non-syndromic to syndromic hearing loss as well as from congenital to age-related hearing loss

Shin-ichi Usami$^1$ · Yuichi Isaka$^1$ · Maiko Miyagawa$^1$ · Shin-ya Nishio$^1$

Received: 16 June 2021 / Accepted: 6 January 2022 / Published online: 12 January 2022
© The Author(s) 2022

Abstract

Variants in the \textit{CDH23} gene are known to be responsible for both syndromic hearing loss (Usher syndrome type ID: USH1D) and non-syndromic hearing loss (DFNB12). Our series of studies demonstrated that \textit{CDH23} variants cause a broad range of phenotypes of non-syndromic hearing loss (DFNB12); from congenital profound hearing loss to late-onset high-frequency-involved progressive hearing loss. In this study, based on the genetic and clinical data from more than 10,000 patients, the mutational spectrum, clinical characteristics and genotype/phenotype correlations were evaluated. The present results reconfirmed that the variants in \textit{CDH23} are an important cause of non-syndromic sensorineural hearing loss. In addition, we showed that the mutational spectrum in the Japanese population, which is probably representative of the East Asian population in general, as well as frequent \textit{CDH23} variants that might be due to some founder effects. The present study demonstrated \textit{CDH23} variants cause a broad range of phenotypes, from non-syndromic to syndromic hearing loss as well as from congenital to age-related hearing loss. Genotype (variant combinations) and phenotype (association with retinal pigmentosa, onset age) are shown to be well correlated and are thought to be related to the residual function defined by the \textit{CDH23} variants.

Introduction

The \textit{CDH23} gene, a member of the cadherin superfamily, encodes calcium-dependent cell–cell adhesion glycoproteins and is known to be expressed in both the inner and outer hair cells in the cochlea. Encoded protein cadherin 23 comprises the “Tip Link” structure of the stereocilia important for hair cell function (Kazmierczak et al. 2007). Variants in the \textit{CDH23} gene are responsible for both syndromic hearing loss (Usher syndrome type ID: USH1D) and non-syndromic hearing loss (DFNB12) (Bolz et al. 2001; Bork et al. 2001). A series of studies indicated that \textit{CDH23} variants cause a broad range of phenotypes of non-syndromic hearing loss (DFNB12), from congenital profound hearing loss to high-frequency-involved progressive hearing loss (Wagatsuma et al. 2007; Miyagawa et al. 2012). Meanwhile, several recent studies have suggested that certain types of age-related hearing impairment (ARHI) and noise-induced hearing loss may also be associated with \textit{CDH23} variants (Miyagawa et al. 2012; Usami et al. 2012a; Kowalski et al. 2014). Taken together, it has become clear that \textit{CDH23} variants are likely to cause a broad range of hearing loss phenotypes.

To date, more than 190 pathologic variants have been reported for the Usher phenotype and 200 pathologic variants for the non-syndromic hearing loss phenotype (DFNB12) (Stenson et al. 2003). \textit{CDH23}-related hearing loss has been reported in many countries with diverse ethnic backgrounds, including Cuba (Bolz et al. 2001), Germany (Bolz et al. 2001), Japan (Wagatsuma et al. 2007; Miyagawa et al. 2012; Mizutari et al. 2015), Korea (Kim et al. 2015, 2016), China (Lu et al. 2014), India (Bork et al. 2001; Ganapathy et al. 2014; Vanniya et al. 2018), Pakistan (Bork et al. 2001; Park et al. 2020), Saudi Arabia (Ramzan et al. 2020), Iran (Zardadi et al. 2020), Qatar (Alkowari et al. 2017), Turkey (Atik et al. 2015), Israel (Ashkenazi, Mizurahi, Sephardi) (Brownstein et al. 2011), Palestine (Abu Rayyan et al. 2020) and the Netherlands (Seco et al. 2017).
We have previously reported the mutational spectrum and clinical features of CDH23-associated hearing loss, as well as certain genotype/phenotype correlations. Meanwhile, in Japan, as genetic testing for deafness has been reimbursed by the National Health Insurance system since 2012, it has become a standard diagnostic tool for deafness. ENT clinicians can order genetic testing by application to the National Health Insurance system, allowing samples to be collected in a more unbiased manner. Currently, DNA samples as well as clinical data from more than 10,000 patients have been collected from 102 collaborative centers participating in the deafness consortium (Usami and Nishio, 2021). As the accuracy of the diagnostic strategy using massive parallel sequencing (MPS) has improved over the last few years, this study was conducted using large-cohort data to revise the mutational spectrum of CDH23 as well as to verify if there are certain genotypes correlated with such a wide range of phenotypes.

Subjects and methods

Subjects

For this study, a total of 12,139 Japanese hearing loss patients and controls (autosomal dominant sensorineural hearing loss; ADSNHL, 2462; autosomal recessive sensorineural hearing loss; ARSNHL or sporadic, 6912; inheritance unknown, 2220; unilateral hearing loss, 212; and normal hearing control subjects, 333) were recruited from 102 otolaryngology departments nationwide. Among these subjects, we selected patients with CDH23 variants based on MPS for 63 target genes. Prior to participation in this study, which was approved by the Shinshu University Ethical Committee and the ethical committee within each participating institution, written informed consent was obtained from all patients (or from their next of kin, caretaker or guardian in case of minors or children). Clinical information and peripheral blood samples were obtained from each subject and from all their consenting relatives. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Shinshu University School of Medicine (No. 387-4 September 2012 and No. 576-2 May 2017).

MPS analysis

Sixty-three genes (Nishio et al. 2015) reported to be causative of non-syndromic hearing loss (Hereditary Hearing loss Homepage; http://hereditaryhearingloss.org/) were analyzed in this study. The detailed protocols for targeted enrichment and DNA sequencing have been described elsewhere (Nishio et al. 2015). In brief, amplicon libraries were prepared using the Ion AmpliSeq Custom Panel, with the Ion AmpliSeq Library Kit 2.0 and the Ion Xpress Barcode Adapter 1–96 Kit (Life Technologies) according to the manufacturer’s instructions. After the amplicon libraries were prepared, equal amounts of the libraries for six patients were pooled for one Ion PGM sequence reaction and those for 45 patients were pooled for one Ion Proton system sequence reaction with an Ion P1 chip or an Ion S5 system sequence reaction with an Ion 540 chip according to the manufacturer’s instructions. The sequence data were mapped against the human genome sequence (build GRCh37/hg19) with the Torrent Mapping Alignment Program. Subsequently, DNA variants were piled up with the Torrent Variant Caller plug-in software included in the Torrent Suit (Life Technologies). After variant detection, the effects of the variants were analyzed using ANNOVAR software (Wang et al. 2010). The missense, nonsense, insertion/deletion and splicing variants were selected among the identified variants. Variants were further selected from < 1% of several control databases including the 1000 genome database (http://www.1000genomes.org/), the 6,500 exome variants (http://evs.gs.washington.edu/EVS/), The Genome Aggregation Database (https://gnomad.broadinstitute.org), the human genetic variation database (dataset for 1208 Japanese exome variants) (http://www.genome.med.kyotio-u.ac.jp/SnpDB/index.html), the 8300 Japanese genome variation database (https://jmrp.megabank.tohoku.ac.jp/202102/) and the 333 in-house Japanese normal hearing controls. The filtering procedures were performed using our original database software as described previously (Nishio and Usami 2017). The pathogenicity of the identified variants was evaluated in accordance with the American College of Medical Genetics (ACMG) standards and guidelines (Richards et al. 2015) with ClinGen hearing loss clinical domain working group expert specification (Oza et al. 2018). To validate the identified variant, Sanger sequencing analysis was performed using PCR and exonspecific custom primers according to the manufacturer’s instructions. All primers were designed using the web version of Primer 3 plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi).

Results

As a result of the large-cohort MPS analysis, we identified 307 probands with CDH23-associated hearing loss. Among the 307 probands, 29 were identified from ADSNHL or maternally inherited cases, whereas 273 were identified from ARSNHL or sporadic cases, with no familial information available for five cases. A total of 126 possibly disease-causing CDH23 variants were identified, 37 of which were previously reported and 89 were novel (Supplementary Table 1). The variants consisted of 90 missense
variants, 9 nonsense variants, 11 splicing variants and 16 frameshift deletion variants. Variants were defined as likely causative variants if the following criteria were fulfilled: (1) pathogenic or likely pathogenic based on the ACMG criteria, or (2) in the case of variants of uncertain significance (VUS) based on the ACMG criteria, where significant CADD scores (>20) were observed, (3) biallelic variants found in recessive inheritance cases, (4) no other candidate variants were found and (5) there was no contradiction with the family analysis (if samples from family member were available). Based on the ACMG guidelines, the variants were categorized into 24 pathogenic, 40 likely pathogenic and 62 VUS. Among the mutations, p.P240L was the most common (32.9%; 202/614 allele), followed by p.R1588W (14.5%; 89/614 allele), p.R2029W (10.3%; 63/614 allele) and p.E956K (4.7%; 29/614 allele) (Fig. 1). The minor allele frequencies (MAFs) of these four highly prevalent variants in the Japanese and other ethnic groups are shown in Supplementary Table 1. The MAFs in the Japanese population are significantly higher than those in the other ethnic groups. Fourteen variants (4 out of 24 pathogenic variants, 4 out of 40 likely pathogenic variants and 6 out of 62 VUS) were found in the DRE, DXNDN, or DXD motif (Supplementary Table 1).

Various combinations of biallelic variants were detected and a total of 307 subjects were diagnosed as suffering hearing loss caused by $\text{CDH23}$ variants (Supplementary Table 2). Among them, six patients had associated visual impairment (Supplementary Table 3).

As shown in Fig. 2A, an overall histogram of onset age was compiled for the congenital/early-onset hearing loss population. It should be noted that a small but significant number of patients showed late-onset (from the 2nd to the 7th decade) hearing loss. The age of onset seemed to be associated with specific variant combinations; i.e., the patients with p.[P240L];[P240L], p.[P240L];[E956K], p.[P240L];[D1347N] and p.[P240L];[Q1716P] showed congenital onset, whereas those with p.[P240L];[R1588W], p.[P240L];[R2029W], p.[P240L];[R2488H], p.[R1588W];[R1588W] and p.[R2029W];[R2029W] showed late-onset hearing loss (Fig. 2A, B).

With regard to genotype/phenotype correlations, in contrast to the patients with p.[P240L];[P240L], p.[P240L];[E956K] and p.[P240L];[D1347N] and...
p.[P240L];[Q1716P] who exhibited more severe hearing loss with limited residual hearing in the lower frequencies, the majority of patients with p.[P240L];[R1588W], p.[P240L];[R2029W], p.[R1588W];[R1588W] and p.[R2029W];[R2029W] showed high-frequency-involved hearing loss (Fig. 3).

Progression was analyzed in patients with residual hearing with four combinations of biallelic variants: p.[P240L];[R1588W], p.[P240L];[R2029W], p.[R1588W];[R1588W] and p.[R2029W];[R2029W] showed high-frequency-involved hearing loss (Fig. 3).

Of the 198 cases for which clinical data were available, 59 used hearing aids (HAs) and 120 used cochlear implants or electric acoustic stimulation (CI/EAS), with 13 cases not wearing any aids and 6 unknown cases (Supplementary Table 2).

With regard to the effects of hearing aids and cochlear implantation, hearing threshold (dB) and monosyllable perception score (%) were found to be significantly improved after these interventions (Fig. 5).

Discussion

Frequency

As described in “Results”, through the screening for 63 deafness genes in this study, we focused on patients with CDH23 variants. No other candidate variants were found in these patients. The present large-cohort study revealed that the prevalence of CDH23-associated hearing loss was 2.64% (307/11,594) among bilateral SNHL probands and 3.95% (273/6912) among ARSNHL/sporadic probands in this Japanese population. Some patients (n = 29) were found in ADSNHL families, which is probably due to a pseudodominant inheritance pattern. These frequencies are slightly higher than those in our previous report (1.6% in total, 2.5% in ARSNHL) (Miyagawa et al. 2012), but this is due to differences in the methodology; this study used sequencing for the entire exons of the CDH23 gene in contrast with the previous screening based on TaqMan for a limited number of common variants. Based on the use of over 10,000 samples collected in a more unbiased manner, the present study indicated the prevalence of CDH23-associated hearing loss among non-syndromic SNHL patients. CDH23-associated hearing loss, along with SLC26A4, is the second or third
Fig. 3 Genotype and audiogram configurations. With regard to genotype/phenotype correlations, in contrast to the patients with p.[P240L];[P240L], p.[P240L];[E956K] p.[P240L];[D1347N] and p.[P240L];[Q1716P] who exhibited more severe hearing loss with limited residual hearing in the lower frequencies, the majority of patients with p.[P240L];[R1588W], p.[P240L];[R2029W], p.[R1588W];[R1588W] and p.[R2029W];[R2029W] showed high-frequency-involved hearing loss. The bold lines indicate the average hearing threshold for each genotype.
most frequent type of hearing loss after \(GJB2\)-associated hearing loss. (Nishio and Usami 2015; Usami and Nishio, 2021), and \(CDH23\) variants are an important cause of non-syndromic SNHL. However, hearing loss due to \(CDH23\) variants is a frequent cause of hearing loss, not only in the Japanese population, but also in many ethnicities, such as Netherlanders, Palestinians, Egyptians, and Jews (see Usami and Nishio 2021 for review).

Fig. 4 Progression of hearing loss in patients with \(CDH23\) variants. The figure shows the hearing level for each age group. The patients with the four variant combinations involving \(p.[R1588W]\) or \(p.[R2029W]\) showed progressive hearing loss
Fig. 5 Benefits of HAs and/or CI/EAS. HAs and/or CI/EAS show good outcomes for patients with CDH23 variants. A Hearing threshold of the patients with/without HA or CI/EAS. B Monosyllable perception score of the patients with/without HA or CI/EAS. The corresponding two groups were tested by t-test. The number on each group indicates the number of patients. *p < 0.05, **p < 0.01
Mutational spectrum

The present study demonstrated a total of 126 possible disease-causing CDH23 variants, including 37 previously reported and 89 novel variants (Supplementary Table 1). As in our previous reports on DFNB12 (Miyagawa et al. 2012), a majority of variants was found in the EC domain, with only a few exceptions found in the cytoplasmic domain. Fourteen out of the 126 possible causative variants were found in the DRE, DXDN and DXD motifs, which are thought to be important for calcium binding. These highly conserved EC calcium-binding motifs are thought to be essential for linearization, rigidification, and dimerization of the cadherin molecules (Nagar et al. 1996; Angst et al. 2001). It should be noted that p.E956K is located in the DRE motif, which is in agreement with the comparatively severe DFNB12 phenotype. According to recent computer analysis for the prediction of the impact of amino acid changes to protein structures, some possible pathologic variants are predicted to cause severe damage to the protein function of cadherin 23 (Supplementary Fig. 1).

This study revealed that there are several common variants in the Japanese hearing loss population, with p.P240L accounting for 32.9% of all CDH23 variants in the Japanese population, followed by p.R1588W (14.5%), p.R2029W (10.3%) and p.E956K (4.7%). These four common variants account for 62.4% of all variants. For such recurrent variants, founder effects have been demonstrated in many deafness genes: for example, with regard to GJB2, it is reported that c.35delG, which is predominant throughout Europe, the Middle East, North Africa, North and South America and Australia; and c.235delC, which is commonly found in East Asians, are due to founder effects (see review; Tsukada et al. 2015). The p.P240L variant in the CDH23 gene, the most frequent variant in the Japanese as well as the Korean population, has been proven to be due to a founder effect using the STR marker (Kim et al. 2015). In fact, the MAF in Japanese controls is exceptionally high compared to those in other ethnic groups (Supplementary Table 1), which is consistent with the fact that there are many patients with CDH23-related hearing loss due to the founder effect.

DFNB12 phenotype vs. Usher phenotype

In this study, we were able to collect current clinical data for approximately two-thirds (198/307) of the patients. According to the age at which clinical information was collected (see Supplementary Table 2) and the age range of patients with each combination of biallelic variants, visual symptoms were not observed, despite the inclusion of a large number of patients aged 10 years and older. Phenotype classification (DFNB12 or Usher phenotype) is shown in Supplementary Table 2. It is necessary to take into consideration that patients with “non-syndromic (NS)” hearing loss up to the second decade may experience visual symptoms in the future. However, some cases with the same variant do not present with visual symptoms even in the third decade. For example, in terms of p.[P240L];[P240L], 22 patients were within the second decade, so the term NVS (no visual symptom to date) is used; however, as no visual symptoms were observed in two patients over the third decade, it was speculated that this variant shows a non-syndromic phenotype. The same results can be observed for many variants. The majority of patients (192/198) were found to not have any associated visual symptoms; therefore, most patients with the CDH23 variants are likely to present with a non-syndromic phenotype.

This finding seems to be related to the fact that the majority of the causative variants (either pathogenic, likely pathogenic or VUS) identified in this study (Supplementary Table 1) were missense mutations, which are supposed to have a residual function. With regard to genotype/phenotype correlations, the DFNB12 phenotype is reported to be associated with biallelic missense mutations, whereas the USH1D phenotype is associated with presumably functional null alleles, including nonsense, splice-site, frameshift or some missense mutations (Bork et al. 2001; Wagatsuma et al. 2007; Oshima et al. 2008; Miyagawa et al. 2012). It has been reported that cases in which an Usher allele and DFNB12 allele are present in the trans configuration show a non-syndromic phenotype (Schultz et al. 2011). In this study, No. 3330 with a biallelic homozygous missense mutation (p.[R2489C];[R2489C]) showed a USH phenotype, whereas p.[R1588W];[R2489C] and p.[Y2301H];[R2489C], which had a compound heterozygous HL, showed a non-syndromic phenotype (Supplementary Table 2). In this study, the majority of patients with biallelic missense mutations or those with compound heterozygous missense and truncating mutations showed the DFNB12 phenotype, which is generally in line with this rule. In our cohort, a limited number of patients (6/307) were found to show the Usher phenotype (Supplementary Table 3). Five out of six cases were associated with at least one truncating mutation and had visual impairment (Usher phenotype), which also supports this rule. However, patient No. 3330 with a biallelic homozygous missense mutation (p.[R2489C];[R2489C]) and patient No. 4177 with a nonsense mutation and missense mutation (p.[Y288X];[G2017S]) also showed the Usher phenotype. Although the functional significance of these missense mutations is unknown, we presume that they are functionally null alleles based on the phenotypes identified herein. Therefore, future functional analysis will be necessary to resolve this inconsistency. When discussing genotype–phenotype correlations, we must take into account that there are always exceptions.
Of course, it is important to pay attention to the visual symptoms during the follow-up period, but other clinical symptoms predicting the Usher phenotype include a delay in starting to walk. USH1D is known to be associated with vestibular dysfunction. Among six Usher phenotype patients in this study, only one patient (No. 3687 in Supplementary Table 3) had undergone vestibular function testing. The patient had no bilateral response on the caloric test. In this study, instead, we collected clinical information, thinking that “the month at which the head is supported when sitting” and “the month at which walking started” could be indirect evidence of a developmental delay and vestibular dysfunction. As a result, it was clarified that five out of six patients with Usher syndrome had a delayed start to walking, which could be indirect proof of vestibular dysfunction (Supplementary Table 2). This was also supported by the fact that most of the patients had no delay in starting to walk; that is, there were not many cases of CDH23-related hearing loss with the Usher phenotype, as inferred from the clinical data. Although it is difficult to evaluate the vestibular function in young children, it may be possible to identify the USH phenotype more accurately by performing pediatric vestibular assessments (Dhondt et al. 2018).

A wide range of hearing loss: clinical characteristics and genotype/phenotype correlations

Based on the data for more than 10,000 hearing loss patients, the present updated study clearly demonstrated that CDH23 variants cause a wide range of hearing loss from non-syndromic hearing loss (DFNB12) to syndromic hearing loss and Usher syndrome type ID (USH1D). Also, the present results showed that most cases of CDH23-associated hearing loss are congenital/early onset. Nonetheless, a certain number of cases of late-onset (up to the 60 s) progressive hearing loss were also identified. As shown in Fig. 2A, B, a wide range of onset ages (awareness of hearing loss) was found, ranging from congenital to 60+ years old, although the majority of cases were congenital or early onset. Genotype (variant combinations) and phenotype (onset age) were shown to be well correlated. The patients with p.[P240L];[P240L] and p.[P240L];[E956K] showed congenital and severe hearing loss, whereas the patients with the p.R2029W or p.R1588W variant showed late-onset high-frequency-involved hearing loss (Figs. 2A, B, 3). We have previously reported the clinical characteristics of CDH23-related hearing loss to be high-frequency-involved progressive hearing loss (Miyagawa et al. 2012). With regard to audiogram configurations, the majority of patients had some residual hearing in the lower frequencies, as reported previously (Wagatsuma et al. 2007; Miyagawa et al. 2012). Further, the progressive nature of the hearing loss was demonstrated by serial audiograms (Miyagawa et al. 2012), and reconfirmed using audiograms with the average for each age plotted (Fig. 4). To date, several replication studies stating the same clinical features have been reported (Mizutari et al. 2015; Kim et al. 2016; Ramzan et al. 2020). Combined with these reports, the present large-cohort data confirmed that this clinical feature tends to be constant regardless of the type of variant.

In addition, we have previously reported some types of ARHI due to CDH23 variants, both of which had hearing loss due to a homozygous mutation in p.R2029W (Usami et al. 2012a). Such a late-onset phenotype is not surprising as a series of animal studies have shown that CDH23 variants are involved in the C57BL/6 mouse strain, which is the most common mouse model for ARHI (Noben-Trauth et al. 2003). ARHI is believed to be a typical complex disorder associated with both genetic factors and environmental factors. Degeneration and age-related changes in the cochlea might be accelerated by accumulated external and internal factors. These environmental factors, including exposure to noise, ear disease, ototoxic drugs and associated disease (circulatory disease, diabetes mellitus, etc.) play important causative roles in ARHI. Therefore, in addition to the monogenically inherited particular type of ARHI shown in this study, various SNPs may be involved in susceptibility to ARHI. It should be noted...
that variants of CDH23 are reported to be associated with noise-induced hearing loss (Kowalski et al. 2014) (Fig. 5). Taken together, these findings suggest that the residual function defined by the CDH23 variants can cause various types of hearing loss, from non-syndromic to syndromic hearing loss as well as from congenital to age-related hearing loss (Fig. 6).

**Intervention perspective**

In this study, 198 patients for whom clinical data concerning interventions were available used HAs or CI/EAS, indicating that these are common therapeutic interventions used in Japan. Figure 5 clearly shows the benefits of HAs and/or CI/EAS. In terms of CI/EAS, our series of papers demonstrated that CI is a good therapeutic option for patients with hearing loss over all frequencies, and EAS is a good option for patients with residual hearing (Usami et al. 2012b, 2020; Miyagawa et al. 2013; Moteki et al. 2017, 2018; Yoshimura et al. 2020). As a significant portion of patients with CDH23 variants have residual hearing, it is extremely important to perform atraumatic CI surgery to preserve residual hearing for this particular category of patients. With regard to post-operative residual hearing after EAS, we have demonstrated that the hearing preservation rate among patients with mutations in stereocilia-related genes, such as CDH23, MYO7A, or MYO15A, was statistically better compared to the patients with other etiologies (Yoshimura et al. 2020). With regard to the progression of hearing loss in patients with CDH23 mutations, it is better to use long electrodes for CI/EAS that can cover low-frequency region (Usami et al. 2020). Genetic testing is also useful for estimating the presence of residual hearing for very young children for whom residual hearing is difficult to measure by auditory brainstem response (ABR) (Usami et al. 2012b).

**Conclusion**

This paper clearly shows the mutational spectrum of CDH23 in Japanese hearing loss patients as well as genotype–phenotype correlations revealed through genetic analysis using more than 10,000 patients. In the present large-cohort study, variants in CDH23 were shown to cause a broad spectrum of hearing loss: from non-syndromic to syndromic hearing loss as well as from congenital to age-related hearing loss. Although these results are based solely on the analysis of Japanese hearing loss patients, the fundamental rule is believed to be the same regardless of racial differences.

**Acknowledgements** We would like to thank the following 102 collaborative hospitals for participating in the Deafness Gene Study Consortium: Asahikawa Medical University, Shinon ENT Clinic, Hokkaido University, Sapporo Medical University, Hakodate Central General Hospital, Hiroaki University, Iwate Medical University, Tohoku Rosai Hospital, Tohoku University, Akita University, Omagari Kousei Medical Center, Yamagata University, Fukushima Medical University, Fukushima Medical University Aizu Medical Center, Hoshi General Hospital, Hitachinaka General Hospital, University of Tsukuba, Dokkyo Medical University, Jichi Medical University, Gunma University, TAKASAKI Ear Nose & Throat Clinic, Kawagoe Otology Institute, Jichi Medical University Saitama Medical Center, National Rehabilitation Center for Persons with Disabilities, National Defense Medical College, Showa General Hospital, Nippon Medical School, Juntendo University, Tokyo Medical and Dental University, Kamio Memorial Hospital, Keio University, Tokyo Medical University, Tokyo Metropolitan Children’s Medical Center, Akasaka Toranomon Clinic, Toranomon Hospital, The Jikei University School of Medicine, International University of Health and Welfare, Abe ENT Clinic, Kitasato University, Yokohama City University Medical Center, Kanagawa Children’s Medical Center, Tokai University, Yokohama City University, Niigata University, Kanazawa University, University of Fukui, University of Yamanashi, Miyagawa ENT Clinic, Shinshu University, Gifu University, Nagara Medical Center, Kizawa Memorial Hospital, Kyoai Clinic, Japanese Red Cross Shizuoka Hospital, Hamamatsu University School of Medicine, Nagoya University, Chubu Rosai Hospital, Aichi Children’s Health and Medical Center, National Mie Hospital, Mie University, Shiga Medical Center for Children, Shiga University School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto University, Kyoto Teishin Hospital, Osaka Medical College, Osaka University, Kansai Medical University, Kitanohospital, National Hospital Organization Osaka National Hospital, Japanese Red Cross Osaka Hospital, Osaka Women's and Children's Hospital, Izumi Clinic, Hyogo Prefectural Amagasaki General Medical Center, Hyogo Medical University, Kobe University, Kobe City Medical Center General Hospital, Nara Medical University, Wakayama Medical University, Tottori University, Okayama University, Hiroshima City Hiroshima Citizens Hospital, Hiroshima University, Hiroshima Prefectural Hospital, Yamaguchi University, Tokushima University, Ehime University, Kochi University, Fukukoua Children's hospital, Kyushu University, Fukukoua University, Kurume University, Saga University, Kanda ENT Clinic, Nagasaki University, Oita University, University of Miyazaki, Kagoshima City Hospital, Kagoshima University, University of the Ryukyus. We also thank Fumiko Tomioka and Sachiko Matsuda for their technical assistance with this research.

**Funding** This research was funded by the 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.: H29-Nanchitou(Nan)-Ippan-031, 20FC1048). Grants-in-aid from the Japan Agency for Medical Research and Development (AMED) (S.U.: 16kk0205010h0001, 17kk0205010h0002, 18kk0205010h0003, 17ek0109114h0003, 18ek0109363h0001, 19ek0109363h0002, 20ek0109363h0003) and a Grant-in-Aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (S.U.: 15H02565).

**Declarations**

**Conflicts of interest** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00439-022-02431-2.
Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abu Rayyan A, Kamal L, Casadei S et al (2020) Genomic analysis of inherited hearing loss in the Palestinian population. Proc Natl Acad Sci USA 117:20070–20076

Alkwari MK, Vozi D, Bhagat S et al (2017) Targeted sequencing identifies novel variants involved in autosomal recessive hereditary hearing loss in Qatari families. Mutat Res Fund Mol Mech Mutagen 800:29–36

Angst BD, Marozzuci C, Magee AI (2001) The cadherin superfamily: diversity in form and function. J Cell Sci 114:629–641

Atik T, Onay H, Aykut A et al (2015) Comprehensive analysis of deafness genes in families with autosomal recessive nonsyndromic hearing loss. PLoS ONE 10:e0142154

Bolz H, von Brederlow B, Ramirez A et al (2001) Mutation of $CDH23$, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. Nat Genet 27:108–112

Bork JM, Peters LM, Riazuddin S et al (2001) Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene $CDH23$. Am J Hum Genet 68:26–37

Brownstein Z, Friedman LM, Shahin H et al (2011) Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families. Genome Biol 12:R89

Dhoff C, Dhogo J, Maes L (2018) Vestibular assessment in the pediatric population. Laryngoscope 129:490–493

Ganapathy A, Pandey N, Srivastapathy CS et al (2014) Non-syndromic hearing impairment in India: high allelic heterogeneity among mutations in $TMPS3$, $TMC1$, $USHIC$, $CDH23$ and $TMIE$. PLoS ONE 9:e84773

Kazmierczak P, Sakaguchi H, Tokita J et al (2007) Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. Nature 449:87–91

Kim SY, Kim AR, Kim NK et al (2015) Strong founder effect of $P240L$ in $CDH23$ in Koreans and its significant contribution to severe-to-profound nonsyndromic hearing loss in a Korean pediatric population. J Transl Med 13:263

Kim BJ, Kim AR, Lee C et al (2016) Discovery of $CDH23$ as a significant contributor to progressive postlingual sensorineural hearing loss in Koreans. PLoS ONE 11:e0165680

Kowalski TJ, Pawelczyk M, Rajkowska E et al (2014) Genetic variants of $CDH23$ associated with noise-induced hearing loss. Otol Neurotol 35:358–365

Lu Y, Zhou X, Jin Z et al (2014) Resolving the genetic heterogeneity of prelingual hearing loss within one family: Performance comparison and application of two targeted next generation sequencing approaches. J Hum Genet 59:599–607

Miyagawa M, Nishio SY, Usami SI (2012) Prevalence and clinical features of hearing loss patients with $CDH23$ mutations: a large cohort study. PLoS ONE 7:e40366

Miyagawa M, Nishio SY, Ikeda T et al (2013) Massively parallel DNA sequencing successfully identifies new causative mutations in deafness genes in patients with cochlear implantation and EAS. PLoS ONE 8:e75793

Mizutari K, Mutai H, Namba K et al (2015) High prevalence of $CDH23$ mutations in patients with congenital high-frequency sporadic or recessively inherited hearing loss. Orphanet J Rare Dis 10:60

Moteki H, Nishio SY, Miyagawa M et al (2017) Long-term results of hearing preservation cochlear implant surgery in patients with residual low frequency hearing. Acta Otolaryngol 137:516–521

Moteki H, Nishio SY, Miyagawa M et al (2018) Feasibility of hearing preservation for residual hearing with longer cochlear implant electrodes. Acta Otolaryngol 138:1080–1085

Nagar B, Overduin M, Ikura M et al (1996) Structural basis of calcium-induced E-cadherin rigidification and dimerization. Nature 380:360–364

Nishio SY, Usami SI (2015) Deafness gene variations in a 1120 nonsyndromic hearing loss cohort: molecular epidemiology and deafness mutation spectrum of patients in Japan. Ann Otol Rhinol Laryngol 124:495–508

Nishio SY, Usami SI (2017) The clinical next-generation sequencing database: A tool for the unified management of clinical information and genetic variants to accelerate variant pathogenicity classification. Hum Mutat 38:252–259

Nishio SY, Hayashi Y, Watanabe M, Usami SI (2015) Clinical application of a custom AmpliSeq library and ion torrent PGM sequencing to comprehensive mutation screening for deafness genes. Genet Test Mol Biomarkers 19:209–217

Noben-Trauth K, Zheng QY, Johnson KR (2003) Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss. Nat Genet 35:21–23

Oshima A, Jaijo T, Aller E et al (2008) Mutation profile of the $CDH23$ gene in 56 probands with Usher syndrome type I. Hum Mutat 29:E37–E46

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

Park HR, Kanwal S, Lim SO et al (2020) Homozygous mutations in Pakistani consanguineous families with prelingual nonsyndromic hearing loss. Mol Biol Rep 47:9979–9985

Ramzan K, Al-Numair NS, Al-Ageel S et al (2020) Identification of novel $CDH23$ variants causing moderate to profound progressive nonsyndromic hearing loss. Genes 11:1474

Richards S, Aziz N, Bale S et al (2015) 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 17:405–424

Schultz JM, Bhatti R, Madeo AC et al (2011) Allelic hierarchy of $CDH23$ mutations causing non-syndromic deafness DFNB12 or Usher syndrome USH1D in compound heterozygotes. J Med Genet 48:767–775

Seco CZ, Wesdorp M, Feenstra I et al (2017) The diagnostic yield of whole-exome sequencing targeting a gene panel for hearing impairment in The Netherlands. Eur J Hum Genet 25:308–314

Stenson PD, Ball EV, Mort M et al (2003) Human gene mutation database (HGMD®): 2003 update. Hum Mutat 21:577–581

Tsuakada K, Nishio SY, Hattori M et al (2015) Ethnic-specific spectrum of $GJB2$ and $SLC26A4$ mutations: their origin and a literature review. Ann Otol Rhinol Laryngol 124:615–628

Usami SI, Nishio SY (2021) The genetic etiology of hearing loss in Japan revealed by 10K social health insurance-based genetic testing. Hum Genet. https://doi.org/10.1007/s00439-021-02371-3

Usami SI, Miyagawa M, Suzuki N et al (2012a) Genetics and presbycusis-monogenic form of age related hearing impairment caused by $CDH23$ mutations. J Hear Sci 2:52–55

 Springer
Usami SI, Miyagawa M, Nishio SY et al (2012b) Patients with CDH23 mutations and the 1555A>G mitochondrial mutation are good candidates for electric acoustic stimulation (EAS). Acta Otolaryngol 132:377–384

Usami SI, Nishio SY, Moteki H et al (2020) Cochlear implantation from the perspective of genetic background. Anat Rec 303:563–593

Vanniya SP, Chandru J, Pavithra A et al (2018) Recurrence of reported CDH23 mutations causing DFNB12 in a special cohort of South Indian hearing impaired assortative mating families—an evaluation. Ann Hum Genet 82:119–126

Wagatsuma M, Kitoh R, Suzuki H et al (2007) Distribution and frequencies of CDH23 mutations in Japanese patients with non-syndromic hearing loss. Clin Genet 72:339–344

Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:e164

Yoshimura H, Moteki H, Nishio SY et al (2020) Genetic testing has the potential to impact hearing preservation following cochlear implantation. Acta Otolaryngol 140:438–444

Zardadi S, Razmara E, Asgaritarghi G et al (2020) Novel homozygous variants in the TMCI and CDH23 genes cause autosomal recessive nonsyndromic hearing loss. Mol Genet Genomic Med 8:e1550

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.