Variants in *CDH23 C*ause Broad Spectrum of Hearing Loss: From Non-Syndromic to Syndromic Hearing Loss as Well as From Congenital to Age-Related Hearing Loss

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Abstract

Variants in the *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 *CDH23* variants cause broad phenotypes of non-syndromic hearing loss (DFNB12); from congenital profound hearing loss to late-onset high frequency-involved progressive hearing loss. In this study, using 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 *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, and the frequent *CDH23* variants that might be due to some founder effects. The present study demonstrated *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 combination) and phenotype (association of retinal pigmentosa, onset age) are shown to be well correlated, and are thought to be related to the residual function defined by the *CDH23* variants.

Introduction

The gene *CDH23*, 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 comprise the “Tip Link” structure of the stereocilia important for hair-cell function (Kazmierczak et al. 2007). Variants in the *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 *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 loss (ARHI) and noise-induced hearing loss may also be associated with *CDH23* variants (Miyagawa et al. 2012; Usami et al. 2012a, Kolwalski et al. 2014). Taken together, it has become clear that *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). *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; Kim et al. 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 (Zardani 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 using 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, in press). 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 HL patients (autosomal dominant sensorineural hearing loss; ADSNHL, 2,462; autosomal recessive sensorineural hearing loss; ARSNHL or sporadic, 6,912; and inheritance unknown, 2,220) 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 (shown in Supplementary Supplemental Table S1), 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 already 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 Xpres 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 (TMAP). 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 as <1% of several control database including the 1,000 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 1,208 Japanese exome variants) (http://www.genome.med.kyoti-u.ac.jp/SnpDB/index.html), the 8,300 Japanese genome variation database (https://jmorp.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 exon-specific 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

Detected Variants

A total of 123 possibly disease-causing *CDH23* variants were identified, 39 of which were previously reported and 86 were novel (Table 1). The variants consisted of 86 missense variants, 9 nonsense variants, 11 splicing variants and 17 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 are found, and 5) there is no contradiction with the family analysis. Based on the ACMG guidelines, the variants were categorized into 23 pathogenic, 39 likely pathogenic and 61 VUS. Among the mutations, p.P240L was the most common (33%; 191/578 allele), followed by p.R1588W (14%; 83/578 allele), p.R2029W (10%; 57/578 allele), and p.E956K (5%; 28/578 allele) (Fig. 1). The MAFs of these four highly prevalent variants in the Japanese and other ethnic groups are shown in Table 1. The MAFs in the Japanese population are significantly higher than those in the other ethnic groups. Several variants (4 out of 25 pathogenic variants, 3 out of 39 likely pathogenic variants, and 5 out of 62 VUS) were found in the DRE, DXNDN, or DXD motif (Table 1).

Various combinations of biallelic variants were detected and a total of 290 subjects were diagnosed as suffering hearing loss caused by *CDH23* variants (Table 2). Among them, 5 patients had associated visual impairment (Table 3).

As shown in Fig. 2A, an overall histogram of onset age was accumulated for the congenital/early-onset hearing loss population. It should be note that a small but significant number of patients showed late-onset (after the teens and up to the 60s) hearing loss. The age of onset seemed to be associated with specific variant combinations; i.e., the patients with p.[P240L];[P240L] and p.[P240L];[E956K] showed congenital onset, whereas those with p.[R1588W];[R1588W], p.[P240L]; [R1588W], and p.[P240L];[R2029W] showed late-onset hearing loss (Fig. 2A, B).

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

Progression was analyzed in the patients with four combinations of biallelic variants; p.[R1588W];[R1588W], p.[P240L];[R1588W], p.[P240L];[R2029W], and p.[R2029W];[R2029W] (Fig. 4). The patients with these four combinations of variants showed progressive hearing loss (Fig. 4).

Of the 103 cases for which clinical data were available, 56 used HAs and 44 used CI/EAS (Table 2).

Discussion

Frequency

The present large-cohort study revealed that the prevalence of *CDH23*-gene associated hearing loss was 2.38% (289/12,139) among SNHL probands and 3.73% (258/6,912) among ARSNHL/sporadic probands in this Japanese population. Some patients (n=32) were found in ADSNHL families, which is probably due to a pseudo-dominant 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 100,000 samples collected in a more unbiased manner, the present study indicated the prevalence of *CDH23*-gene associated hearing loss among non-syndromic SNHL patients. The *CDH23*-gene associated hearing loss, along with *SLC26A4*, is the second or third most frequent type of hearing loss after *GJB2*-gene associated hearing loss. (Nishio and Usami 2015; Usami and Nishio, submitted), and that *CDH23* variants are an important cause of non-syndromic SNHL.

Mutational spectrum

The present study demonstrated a total of 131 possible disease-causing *CDH23* variants, including 39 previously reported and 92 novel variants (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 few exceptions found in the cytoplasmic domain. Thirteen out of the 123 possible causative variants were found in the DRE, DXNDN, 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 *CDH23* (Supplemental Figure 1).

This study revealed that there are several common variants in the Japanese hearing loss population; p.P240L accounts for 33% of all *CDH23* variants in the Japanese population, followed by p.R1588W (14%), p.R2029W (10%), and p.E956K (5%). These 4 common variants account for 62% 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 was 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 control is exceptionally high compared to those in other ethnic groups (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

The majority of the causative variants (either pathogenic, likely pathogenic, or VUS) identified in this study (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; Ohshima et al. 2008; Miyagawa et al 2012). It has been reported that cases in which an Usher allele and DFNB12 allele are present in *trans* configuration, the DFNB12 allele is phenotypically dominant (Schultz et al. 2011).

In this study, the majority of patients with biallelic missense mutation or those with compound heterozygous of missense and truncating mutations showed the DFNB12 phenotype, which is generally in line with this rule. In our cohort, a limited number of patients were found to show the Usher phenotype (Table 3). Three out of five were associated with truncating mutations 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 the 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, they are supposed to be functionally null alleles.

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

Using 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*-gene associated hearing loss are congenital/early-onset, but nonetheless demonstrated that there is also a certain number of cases of late-onset (up to the 60s) progressive hearing loss. As shown in Fig. 2A, B, a wide range of onset ages (awareness of hearing loss) was found from congenital to 60+ years old, although the majority of cases were congenital or early-onset. Genotype (variant combination) 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 (Fig. 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 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 age-related hearing loss (ARHI) due to *CDH23* variants, both of which had hearing loss due to a homozygous mutation in p.R2029W (Usami et al. 2012). Such a late-onset phenotype is not surprising because 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, associated disease (circulatory disease and diabetes mellitus, etc.) play important causative roles in ARHI. Therefore, in addition to the monogenically inherited particular type of ARHI (late-onset hereditary hearing loss) 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 (Kolwalski et al., 2014). Taken together, these findings suggest that the residual function defined by the *CDH23* variants can cause various types of hearing loss. (Fig. 5).

Intervention perspective

In this study, at least 100 out of the 103 patients for which clinical data were available, used HAs or CI/EAS, indicating that these are common therapeutic interventions used in Japan. In terms of CI/EAS, our series of papers demonstrated the 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;

Miyagawa et al. 2013b; Moteki et al. 2017, 2018; Yoshimura et al. 2020; Usami 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). Genetic testing is also useful for estimating the presence of residual hearing for very young children whose residual hearing is difficult to measure by auditory brainstem response (ABR).

Declarations

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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.

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Tables

Due to technical limitations, table 1-3 is only available as a download in the Supplemental Files section.

Supplemental Table

Supplemental Table S1 is not available with this version.

Figures

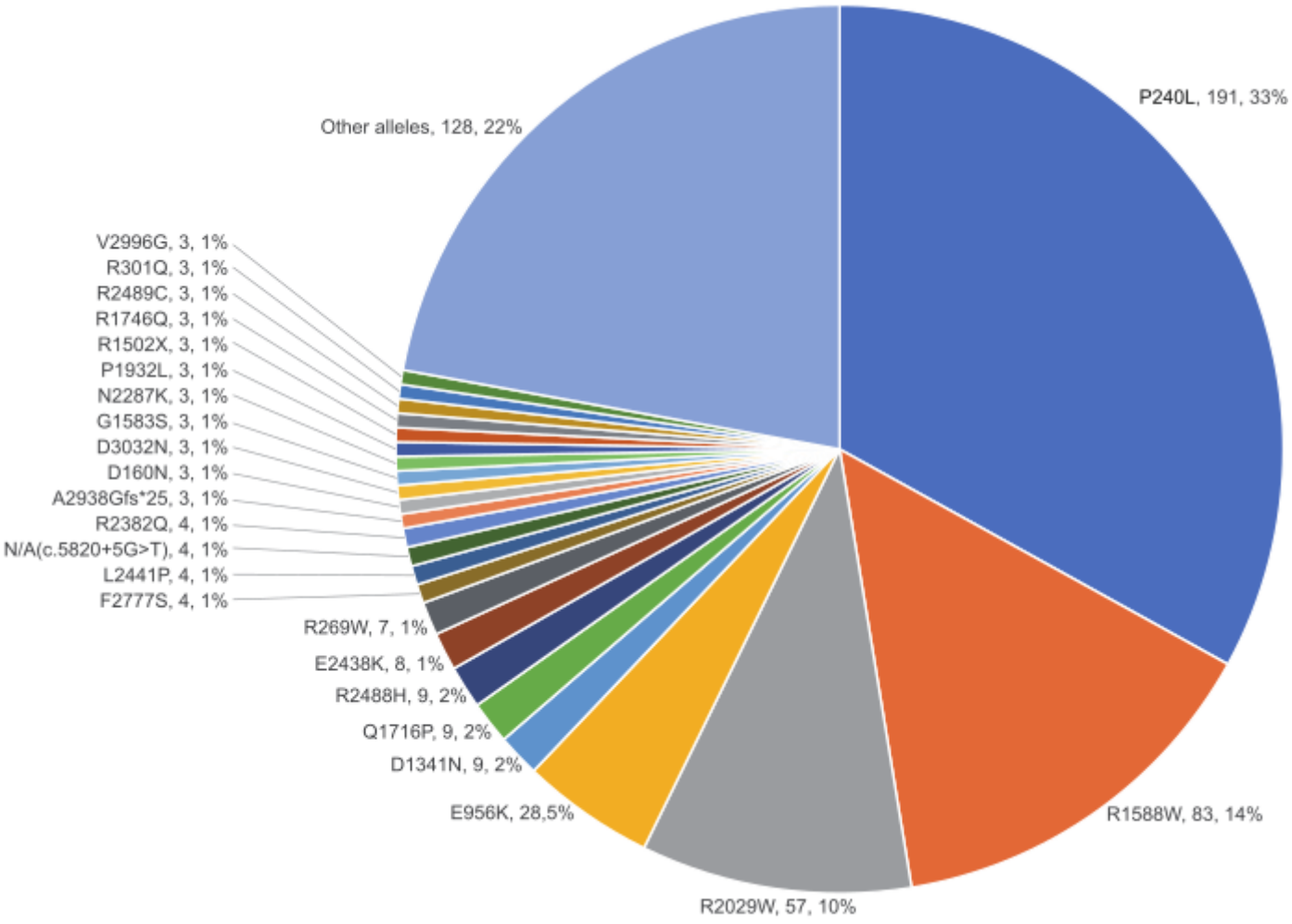

Figure 1

CDH23 variants identified in this study. A total of 123 possible disease-causing CDH23 variants were identified. The frequently found variants (p.P240L (33%), p.R1588W (14%), p.R2029W (10%), and p.E956K (5%) accounted for 62% of the mutations

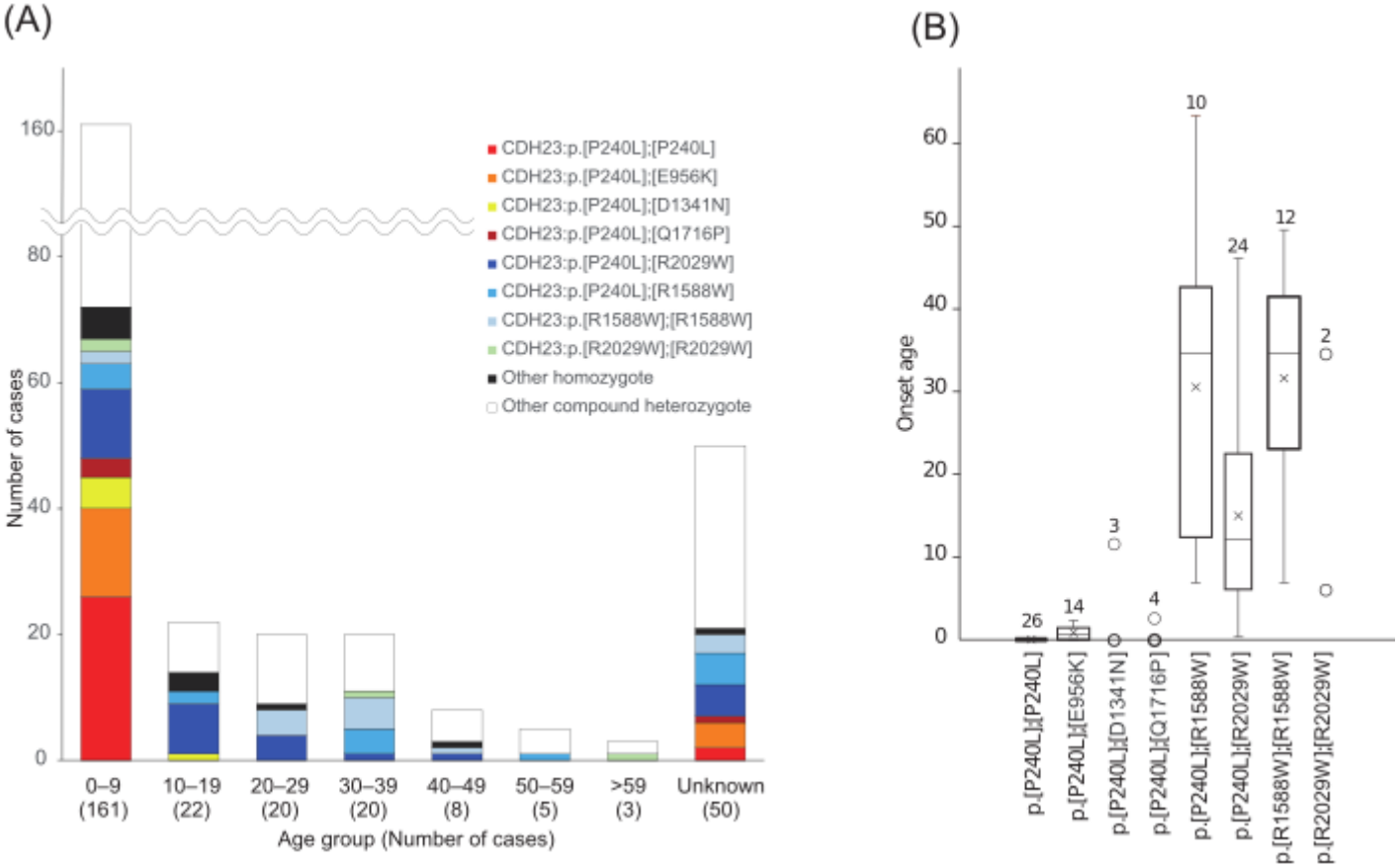

Figure 2

Age of onset and combination of CDH23 variants A: The age of onset and the combination of variants were closely related. The patients with p. [P240L] or p. [E956K] showed congenital/early-onset, while the patients involving p.[R1588W] or p. [R2029W] showed late-onset hearing loss. B: Frequently found combinations of CDH23 variants and onset age. The combinations p.[P240L]; p.[P240L] and p.[P240L]; p. [E956K] presented as congenital onset, whereas p.[R1588W]; p.[R1588W], p.[P240L]; p.[R1588W], and p. [P240L]; p.[R2029W] presented as late-onset hearing loss.

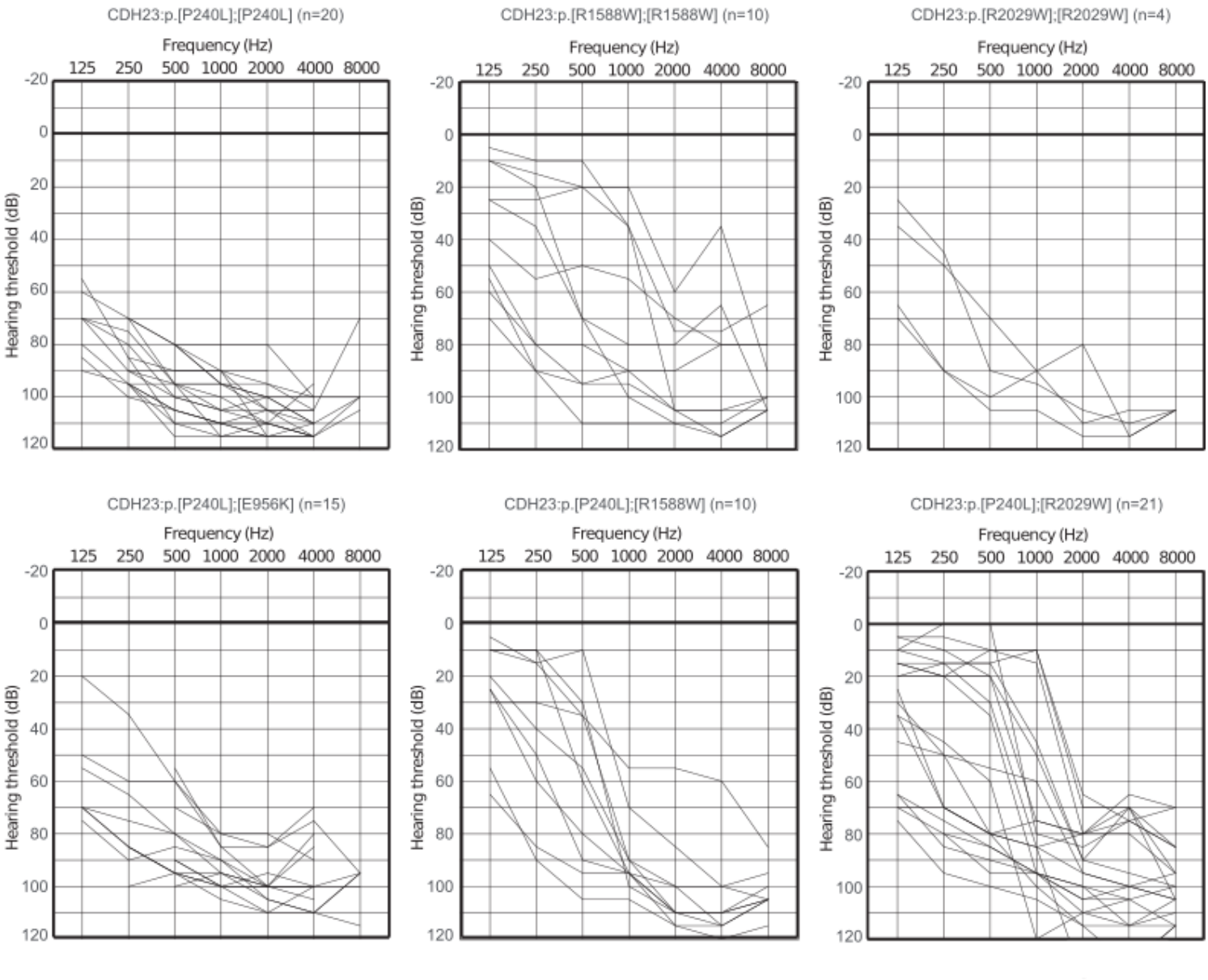

Figure 3

Genotype and audiograms The majority of the patients having p.[R1588W]; p.[R1588W], p.[P240L]; p.[R1588W], p.[P240L]; p.[R2029W], and p.[R2029W]; p.[R2029W] showed high frequency-involved hearing loss, in contrast to the patients with p.[P240L]; p.[P240L] and p.[P240L]; p.[E956K] who exhibit more severe hearing loss with limited residual hearing in the lower frequencies.

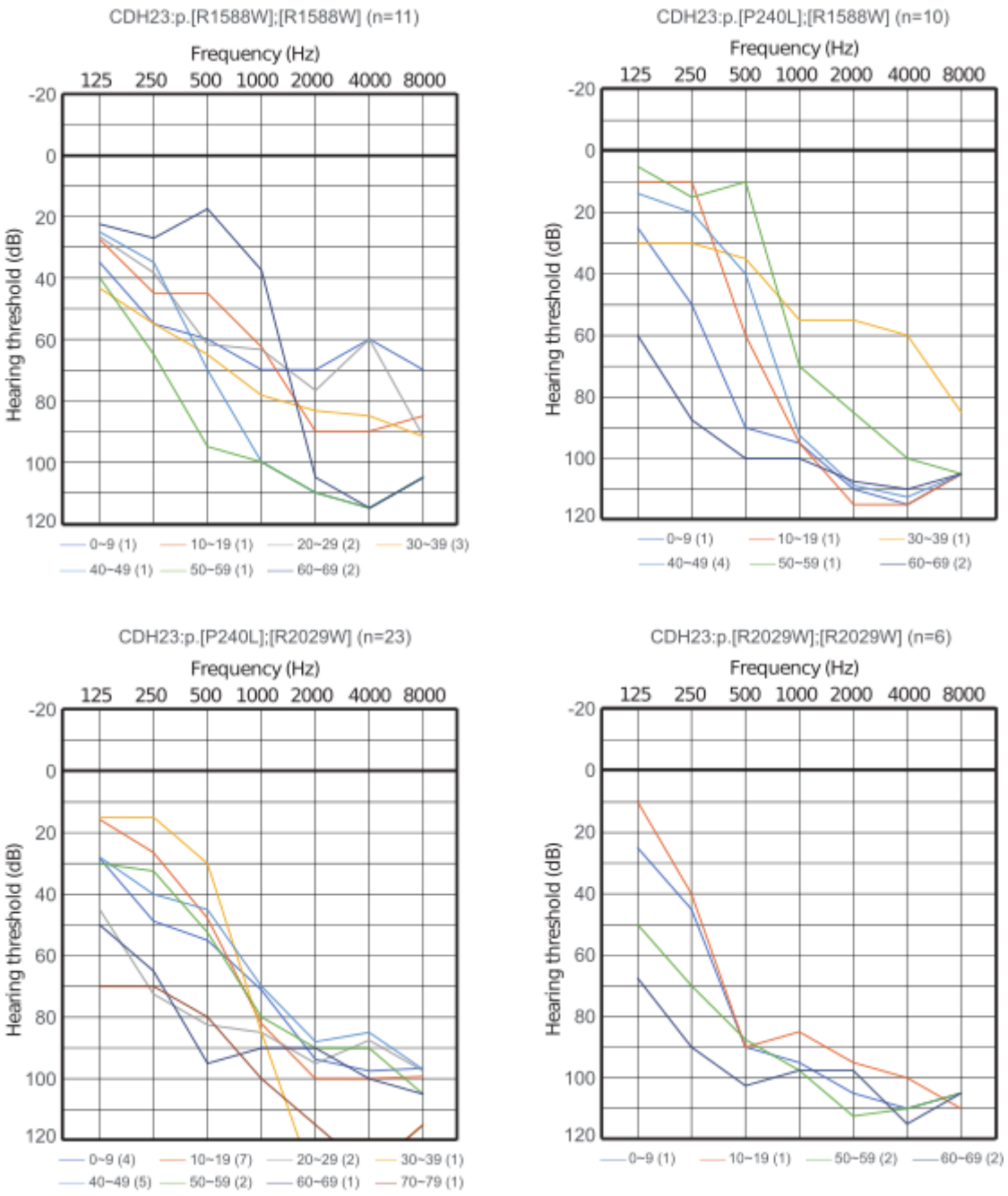

Figure 4

Progression of hearing loss in patients with CDH23 variants The patients with the four variant combinations involving p.[R1588W] or p. [R2029W] showed progressive hearing loss.

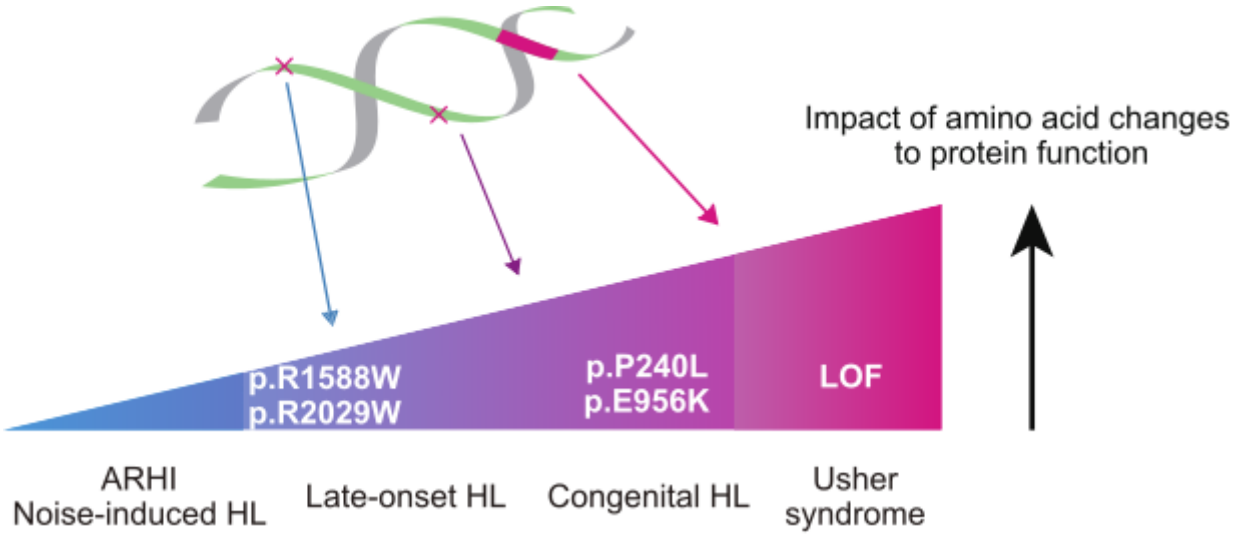

Figure 5

Image of genotype/phenotype correlations: It is presumed that various phenotypes are caused by the residual function of the CDH23 protein.

Supplementary Files

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

- SuppleFigure1.eps
- CDH23Table1SU.xlsx
- CDH23Table2SU.xlsx
- CDH23Table3SU.xlsx