Table 1 Mitochondrial DNA variants identified in this study

Gene	Mutation	Homo/ heteroplasmy	prelingual HL (N = 54)	Late-onset HL (N = 80)	Controls (N = 137)	freq in controls (%)	Japanese (N = 672)[a]	freq in Japanese (%)	conservation index[b]	Previous report[c]	mtDB[c] (N = 2704)	freq in mtDB (%)
12S rRNA	663A > G	homoplasmy	3	5	2	**1.5**	48	7.1	**29/51**	yes	86	3.2
	709G > A	homoplasmy	7	7	12	8.8	125	18.6	19/51	yes	444	16.4
	750A > G	homoplasmy	54	80	137	100.0	no data	no data	**49/51**	yes	2682	96.7
	752C > T	homoplasmy	0	0	9	6.6	17	2.5	44/51	yes	20	0.7
	827A > G	homoplasmy	4	3	3	**2.2**	25	3.7	**48/51**	yes	54	**2.0**
	904C > T	homoplasmy	1	0	0	**0.0**	0	**0.0**	**48/51**	none	0	**0.0**
	961insC	homoplasmy	1	0	3	**2.2**	1	**0.1**	9/51	yes	37	**2.0**
	961delT+ Cn	both	0	1	4(2)[d]	**2.9**	no data	no data	9/51	yes	no data	no data
	1005T > C	both	1	1(1)	1	**0.7**	1	**0.1**	**33/51**	yes	7	**0.3**
	1009C > T	homoplasmy	0	0	1	0.7	1	0.1	9/51	yes	2	0.1
	1041A > G	homoplasmy	0	4	5	3.6	11	**1.6**	**26/51**	yes	14	**0.5**
	1107T > C	homoplasmy	0	0	6	4.4	29	4.3	30/51	yes	34	1.26
	1119T > C	homoplasmy	1	2	7	5.1	20	3.0	20/51	yes	26	1.0
	1382A > C	homoplasmy	0	1	11	8.0	62	9.2	**38/51**	yes	65	**2.4**
	1438A > G	homoplasmy	54	80	137	100.0	662	98.5	**46/51**	yes	2620	96.9
$tRNA^{Ser}$ (UCN)	7501T > A	homoplasmy	0	3	0	**0.0**	1	**0.1**	15/51	yes	1	**0.0**

Mitochondrial gene variants that met the criterion for association with hearing loss (HL) are underlined and in bold type. [a]Data from the mtSNP database [48]. [b]Based on the results of the multiple alignment by ClustalW. See Additional File 1: Table S1 for information on the species used to calculate the sequence conservation. [c]Uppsala mtDB database [50]. [d]Each number in parentheses indicates the number of individuals with a heteroplasmic variant.

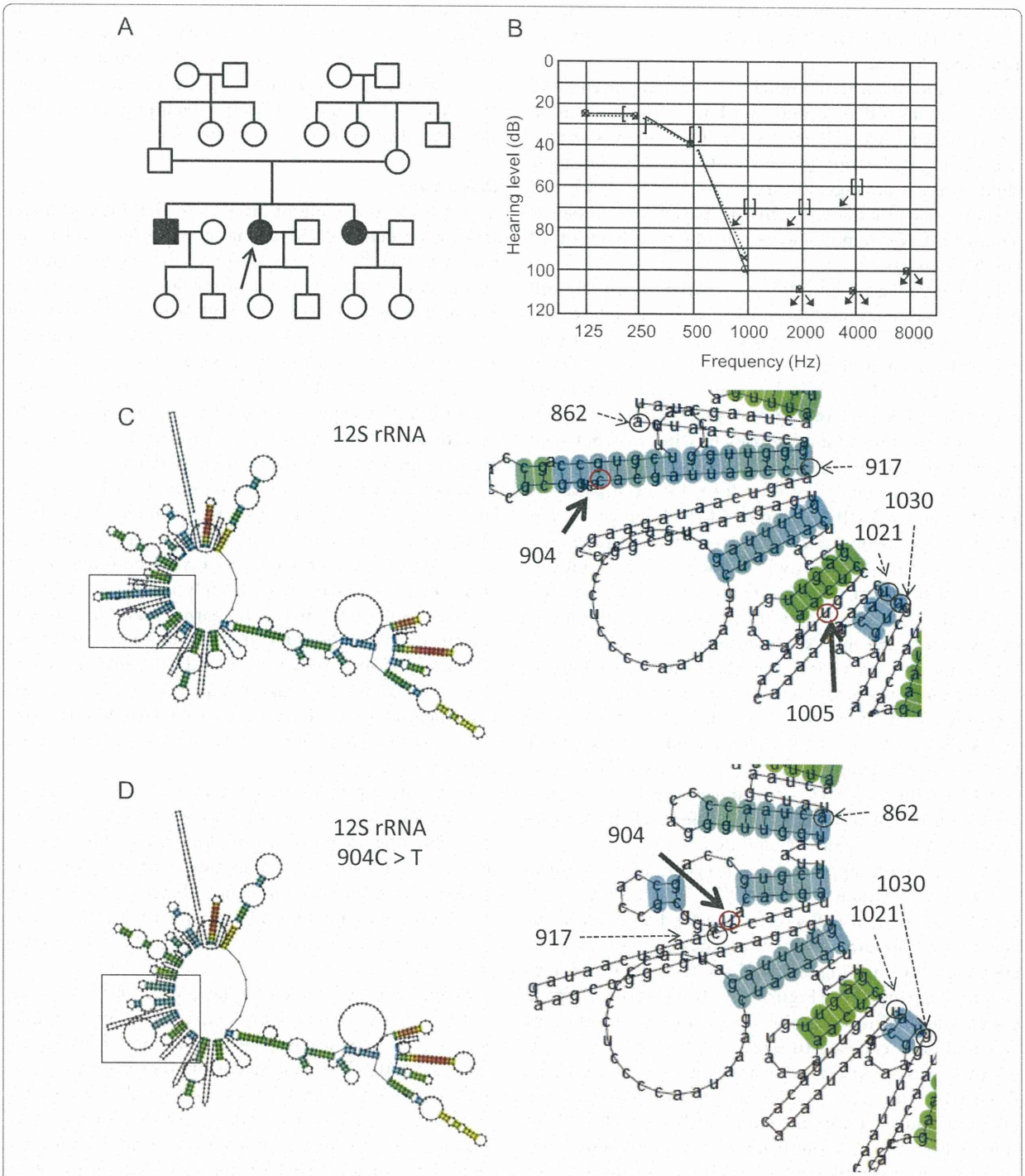

Figure 1 Pedigree of a family carrying the m.904C > T variant. (A) Pedigree of a family carrying the homoplasmic m.904C > T variant. Individuals with hearing loss are indicated by filled symbols. The arrow indicates the proband. (B) Audiogram of the proband of m.904C > T. Open circles with the line indicate the air conduction thresholds of the right ear; the X's with dotted line indicate the air conduction thresholds of the left ear; [, bone conduction thresholds of the right ear;], bone conduction thresholds of the left ear. Arrows indicate the scale-out level of hearing loss. (C, D) Secondary structures of wild-type 12S rRNA (C) and 12S rRNA with the m.904C > T (D) predicted by Centroid Fold. To the right is shown an enlargement of the region of predicted secondary structures surrounding nucleotide positions including 904 and 1005 (bold arrows with red circles). Positions 862, 917, 1021, and 1030 are marked by dashed arrows with black circles for easy comparison of the structural changes. Each predicted base pair is indicated by a gradation of color (red to blue) corresponding to the base-pairing probability from 1 (red) to 0 (blue) according to Centroid Fold.

stem-like structure from positions 1021 to 1030 (Figure 1C and 1D), implicating a significant role for 904C in 12S rRNA folding.

The homoplasmic m.1005T > C variant in the *12S rRNA* was found in a male patient with prelingual, severe hearing loss (Figure 2A, B). The patient's spouse had prelingual hearing loss owing to measles, and their child also had prelingual hearing loss. The m.1005T > C variant was not detected in the patient's spouse or daughter. DNA samples were not obtained from other family members.

The heteroplasmic m.1005T > C variant together with the homoplasmic mutation m.709G > A was detected in a male patient from a consanguineous marriage of parents with normal hearing (Figure 2C). In the proband (III:3), onset of hearing loss and diabetes mellitus occurred in his 40s. Among his five siblings, four (III:1, 2, 4, 6) also showed adult-onset hearing loss between age 20 and 50 years, but they did not have diabetes mellitus. The fifth sibling suffered from infantile paralysis and died at age 6 (III:5). Cloning of the fragment of *12S rRNA*, which demonstrated apparent heteroduplex formation (Figure 2D, arrow), yielded 12 of 54 clones (22%) with the m.1005T > C variant. However, the m.1005T > C variant was not detected in 24 clones derived from the mtDNA from each of these siblings, indicating that the variant was absent in the siblings or the frequency was less than 4%. The audiograms showed severe to profound hearing loss in the siblings III:1, 2, 3, and 4 (Figure 2E, F, 3A, B). The secondary structure of the *12S rRNA* variant predicted by Centroid Fold indicated that the m.1005T > C induces a gross structural alteration in the transcript, including nucleotide positions 862 to 917 (Figure 1C and 3C).

Three patients appeared to carry the homoplasmic m.7501T > A variant in $tRNA^{Ser(UCN)}$ (Figure 4A, C, E). One female patient suffered from episodic vertigo from age 27 years followed by tinnitus and fluctuant, moderate progressive hearing loss, and she had no familial history of hearing loss (Figure 4A, B). Another female patient suffered from tinnitus beginning at age 24 years and had been exposed to streptomycin from age 36 to 37 for treatment of tuberculosis (Figure 4C, D). She suffered from fluctuant, moderate hearing loss from her 50s and had no familial history of hearing loss. The third patient was a male from a consanguineous marriage of parents with normal hearing and showed non-progressive, severe hearing loss from childhood without tinnitus or vertigo (Figure 4E, F). Later, he was also found to have X-linked spinal and bulbar muscular atrophy (SBMA/Kennedy-Alter-Sung disease/Kennedy's disease). In this family, six of seven siblings showed hearing loss. Family members other than the proband did not participate in this study. According to the secondary structure prediction by Centroid Fold, the m.7501T > A in $tRNA^{Ser(UCN)}$ (which is transcribed as U in the reverse direction) causes an elongation of the D-arm in the transcript by reducing the size of the D-loop of $tRNA^{Ser(UCN)}$ (Figure 4G, H), which might affect biosynthesis of mitochondrial proteins [55].

Discussion

In our study, screening of mtDNA by dHPLC and direct sequencing detected 15 variants in *12S rRNA* and 1 variant in $tRNA^{Ser(UCN)}$. Comparison of the variant frequencies in controls, assessment of nucleotide conservation among mammalian species, and structural analysis of the transcript was used to select candidate mutations associated with hearing loss. No variants in $tRNA^{Leu(UUR)}$, $tRNA^{Lys}$, $tRNA^{His}$, $tRNA^{Ser(AGY)}$, or $tRNA^{Glu}$ were detected in the subjects studied here, suggesting that the mutations in these genes associated with hearing loss are not common in the Japanese population.

To our knowledge, the homoplasmic m.904C > T variant in *12S rRNA* has not been reported elsewhere. Lack of symptoms in the maternal relatives does not exclude mitochondrial transmission, because penetrance of *12S rRNA* mutations can be extremely low, as seen in the m.1555A > G associated with hearing loss [56]. Conservation of the nucleotides among mammals and gross alteration of the predicted secondary structure of the *12S rRNA* transcript suggest that the m.904C > T variant might affect auditory function by changing the efficiency with which mRNAs are transcribed to yield mitochondrial proteins.

A patient with the homoplasmic m.1005T > C variant in the *12S rRNA* had a child with prelingual hearing loss. The inheritance of hearing loss in the child is likely due to the transmission of an autosomal mutation, not mtDNA, from the male proband. Therefore, the data for this family may not provide unequivocal information about the pathogenicity of the m.1005T > C variant [4,22,27,30].

Identification of the heteroplasmic m.1005T > C variant in a patient with hearing loss is a novel finding, because this variant has been known only as homoplasmic [22,27,30,34]. We did not verify that the heteroplasmic m.1005T > C variant was correlated with hearing loss because four of five siblings of the proband had hearing loss without carrying the variant, whereas it might be associated with diabetes mellitus. However, it is difficult to exclude the possibility of association of the heteroplasmic variant detected in blood samples with mitochondrial diseases such as deafness. Frequencies of heteroplasmy of mtDNA vary considerably among tissues in the same individual (for instance, [37,57,58]). Therefore, it is possible that the frequency of the m.1005T > C variant in the inner ear cells of the

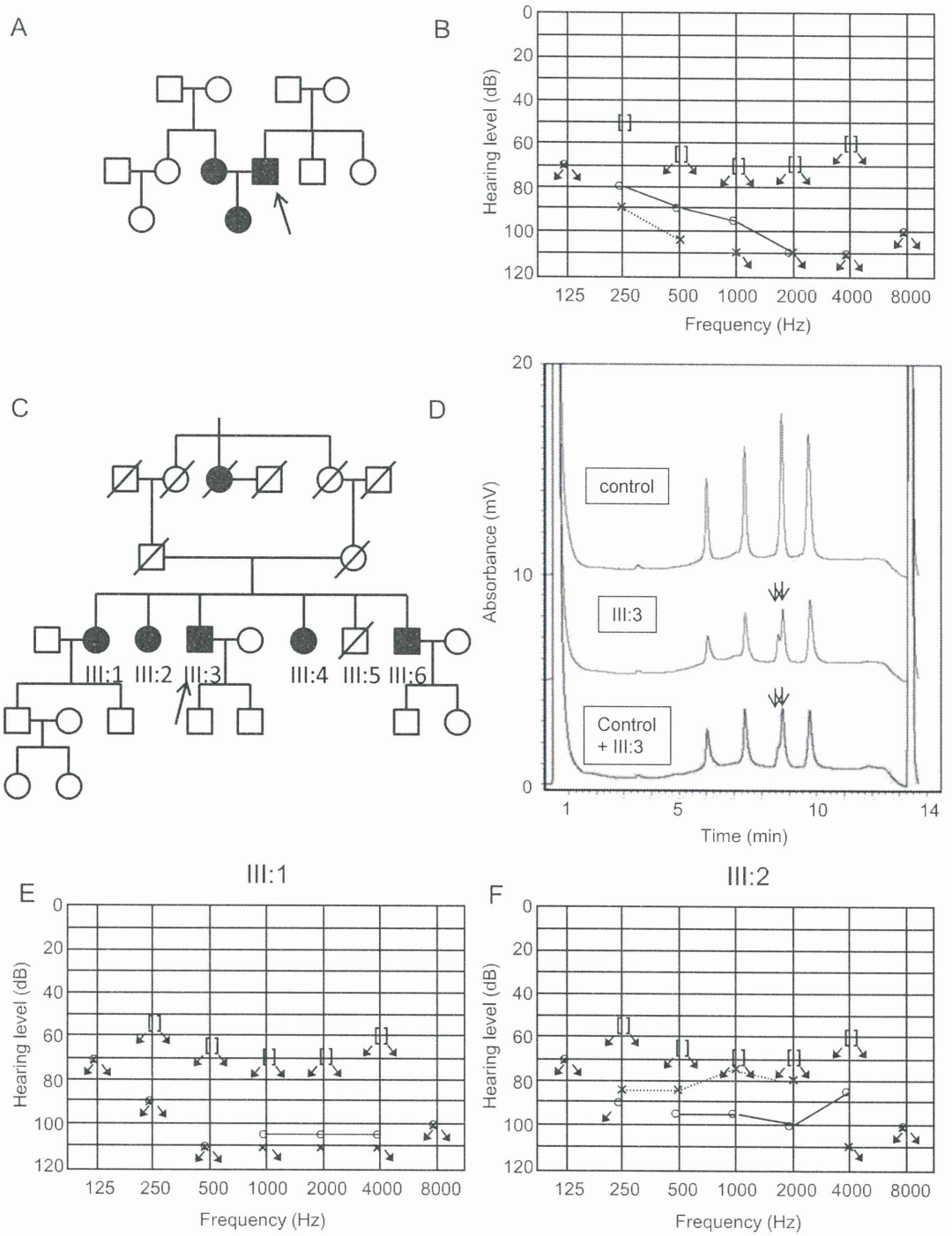

Figure 2 Pedigrees of families carrying the m.1005T > C variant. (A,B) Pedigree of a family carrying the homoplasmic m.1005T > C (A), and the audiogram of the proband (B). (C-F) Pedigree of a family carrying heteroplasmic m.1005T > C (C), and the chromatogram of dHPLC of the MT4 fragment of the proband (D). The arrows indicate split peaks of the fragment owing to the heteroplasmic m.1005T > C. Audiograms of the siblings (III:1, 2) are shown in (E-F).

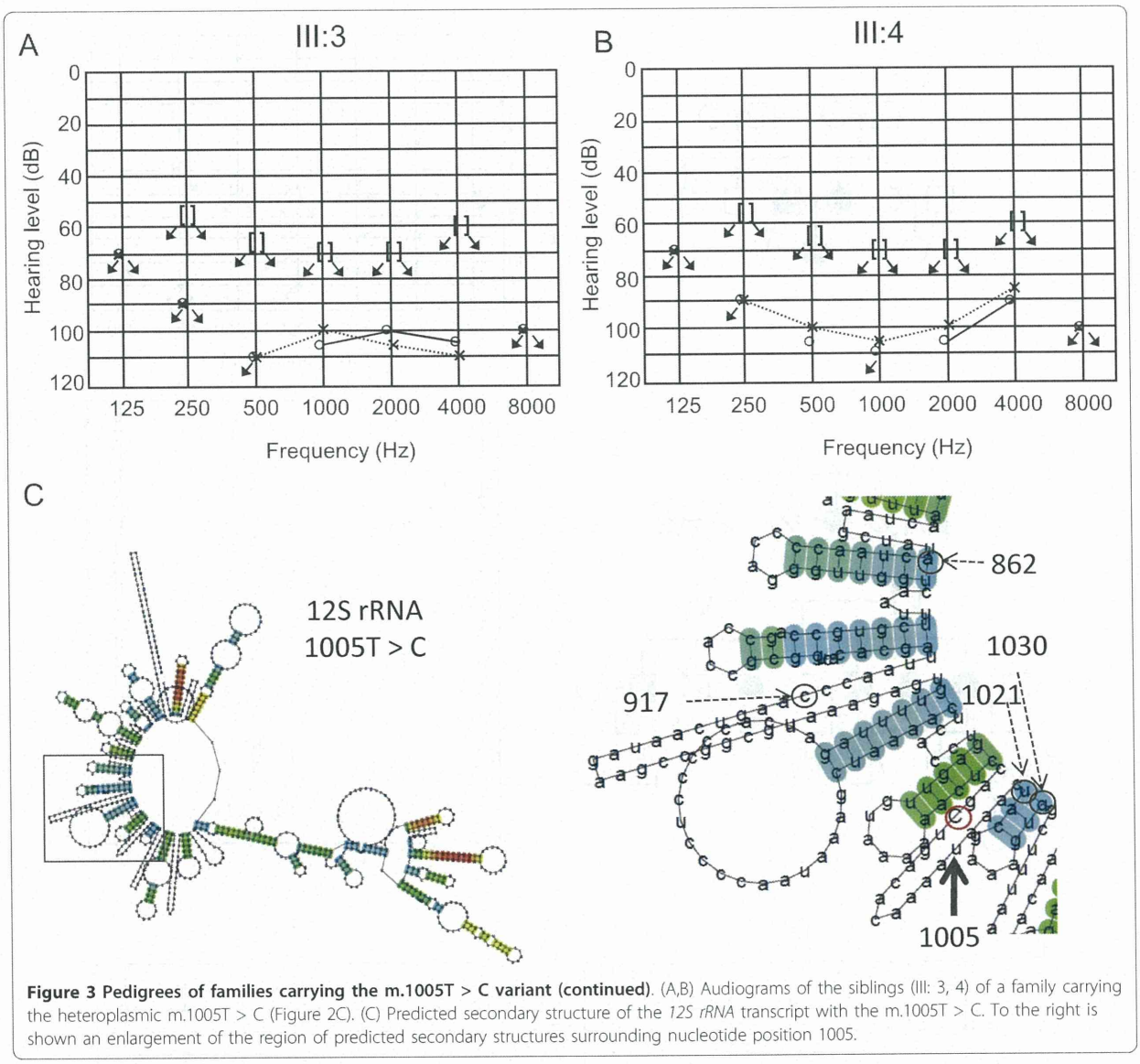

Figure 3 Pedigrees of families carrying the m.1005T > C variant (continued). (A,B) Audiograms of the siblings (III: 3, 4) of a family carrying the heteroplasmic m.1005T > C (Figure 2C). (C) Predicted secondary structure of the *12S rRNA* transcript with the m.1005T > C. To the right is shown an enlargement of the region of predicted secondary structures surrounding nucleotide position 1005.

siblings is much higher than in the blood cells and thus may underlie the hearing loss.

Another finding in this study is that three patients with postlingual hearing loss had the homoplasmic m.7501T > A variant in $tRNA^{Ser\ (UCN)}$. Various mutations in $tRNA^{Ser(UCN)}$, such as m.7445A > G [15,16], 7472insC [17,59], 7505T > C [60], 7510T > C [18], and 7511T > C [51,59,61], are associated with various types of hearing loss (syndromic or nonsyndromic, prelingual or late-onset), raising the possibility that the m.7501T > A variant, reported elsewhere without detailed investigation [33], is also associated with hearing loss. The low conservation of the variation at this position (29% among mammals) does not support the pathogenicity of the variant, in contrast to the much higher conservation at m.7472A (61%), 7505A (98%), 7510T (78%), and 7511T (98%). On the other hand, the m.7501T > A variant is predicted to modify the secondary structure of the D-arm in the $tRNA^{Ser(UCN)}$ transcript; the D-arm is important for the stability of the transcript and the general rate of mitochondrial protein synthesis [55]. Further investigation, such as haplogroup analysis or generating lymphoblastoid cell lines to measure endogenous respiration rates, may help to define the pathogenicity of the m.7501T > A variant.

All other variants found in this study, such as m.827A > G, 961insC, and 961delT + Cn, which have been discussed elsewhere with respect to their pathogenicity [21,22,27,30,62], were considered to be non-pathologic polymorphisms because they were found frequently in

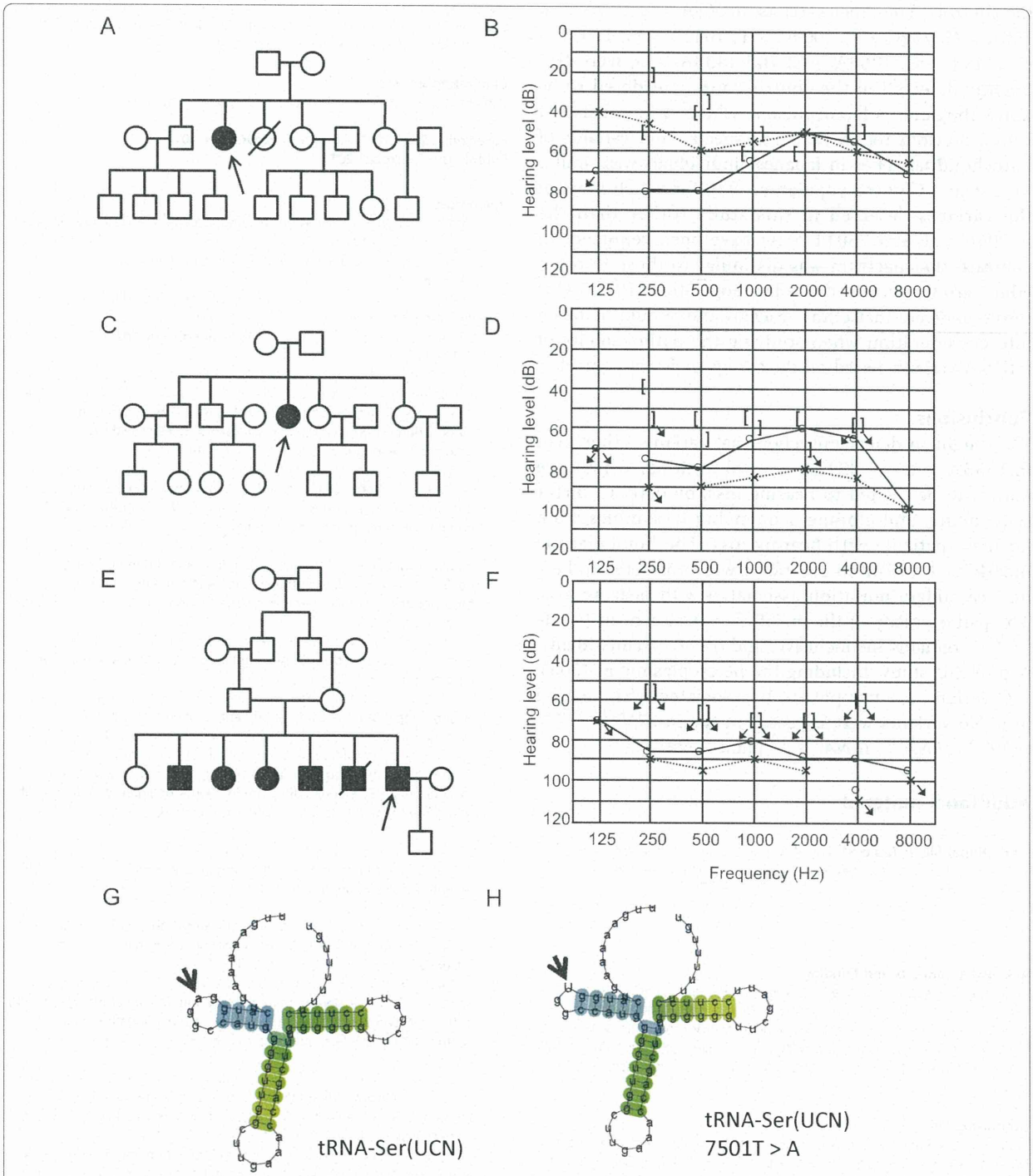

Figure 4 Pedigrees of families carrying the m.7501T > A variant. (A-F) Pedigrees of three families carrying the homoplasmic m.7501T > A, and audiograms of the probands (A and B, C and D, E and F). (G,H) Predicted secondary structure of the *tRNA*$^{Ser(UCN)}$ transcript (G) and the tRNA$^{Ser(UCN)}$ with m.7501T > A (H). Because the gene is transcribed in the reverse direction, thymine at 7501 (G) and adenine (H) are indicated as a and u, respectively (bold arrows).

the controls. The other variants, m.663A > G, 709G > A, 750A > G, 752C > T, 1009C > T, 1041A > G, 1107T > C, 1119T > C, 1382A > C, and 1438A > G, were frequently detected in the controls and considered to be nonpathogenic polymorphisms, which is in consistent with a previous report [27]. The spectrum of variants of mitochondrial genes in Japanese individuals was similar to that in a Chinese population [27], for which most of the variants detected in this study (other than the m.904C > T and 7501T > A) have been reported. In contrast, the spectrum was dissimilar to those in other ethnic groups such as the Polish population [19,63]. Our results indicate that ethnic background should be taken into consideration when studying the pathogenicity of mtDNA variants based on their frequencies in controls.

Conclusions

We sought to detect mitochondrial variants other than m.1555A > G or 3243A > G mutations, which are known to be related to hearing loss, by dHPLC, direct sequencing, and cloning-sequencing in samples from Japanese patients with hearing loss. The homoplasmic m.904C > T variant in *12S rRNA* was considered to be a new candidate mutation associated with hearing loss. The pathogenicity of the m.7501T > A variant in $tRNA^{Ser(UCN)}$ remains inconclusive, and other variants identified in this study, including the heteroplasmic m.1005T > C variant, are not positively associated with hearing loss. No variants were detected in the in $tRNA^{Leu(UUR)}$, $tRNA^{Lys}$, $tRNA^{His}$, $tRNA^{Ser(AGY)}$, and $tRNA^{Glu}$.

Additional material

Additional file 1: Table S1. List of animal species and the accession numbers of the mtDNA (GenBank) used to calculate nucleotide conservation.

Acknowledgements and Funding

This study was supported by a Health Science Research Grant (H16-kankakuki-006 to TM) from the Ministry of Health, Labor, and Welfare of Japan, a Grant-in-Aid for Clinical Research to TM from the National Hospital Organization, and by a Grant-in-Aid for Scientific Research (19592001 to TM) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Authors' contributions

HM participated in cloning and sequencing, data analysis, and drafted the manuscript. HKo, ET, ITK, and HKa established and conducted the dHPLC analysis, sequencing, and data analysis. HT, SU, and TO coordinated the study and helped with gene analysis. TM planned and organized the study, examined patients, analyzed data, and helped draft the manuscript. All the authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 6 June 2011 Accepted: 12 October 2011
Published: 12 October 2011

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Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2350/12/135/prepub

doi:10.1186/1471-2350-12-135
Cite this article as: Mutai *et al.*: Systematic analysis of mitochondrial genes associated with hearing loss in the Japanese population: dHPLC reveals a new candidate mutation. *BMC Medical Genetics* 2011 12:135.

Acta Oto-Laryngologica, 2011; 131: 1232–1236

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SHORT COMMUNICATION

Different cortical metabolic activation by visual stimuli possibly due to different time courses of hearing loss in patients with *GJB2* and *SLC26A4* mutations

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Abstract

Conclusion. We have demonstrated differences in cortical activation with language-related visual stimuli in patients who were profoundly deafened due to genetic mutations in *GJB2* and *SLC26A4*. The differences in cortical processing patterns between these two cases may have been influenced by the differing clinical courses and pathogenesis of hearing loss due to genetic mutations. Our results suggest the importance of hearing during early childhood for the development of a normal cortical language network. *Objectives.* To investigate the cortical activation with language-related visual stimuli in patients who were profoundly deafened due to genetic mutations in *GJB2* and *SLC26A4*. *Methods:* The cortical activity of two adult patients with known genetic mutations (*GJB2, SLC26A4*) was evaluated with fluorodeoxyglucose-positron emission tomography (FDG-PET) with a visual language task and compared with that of normal-hearing controls. *Results:* A patient with a *GJB2* mutation showed activation in the right auditory association area [BA21, BA22], and the left auditory association area [BA42] even with visual language task; in contrast, a patient with an *SLC26A4* mutation showed no significant activation in the corresponding area.

Keywords: *FDG-PET, visual language task, functional brain imaging*

Introduction

Functional brain imaging is an effective method for investigating the cortical processing of language, which has provided much evidence for the plasticity of the central auditory pathway following a profound loss of hearing [1–4]. Many previous studies showed that there is a capacity of the auditory cortex for cross-modal plasticity after auditory deprivation of the brain. Cerebral glucose metabolism in the primary auditory and related cortices in individuals with prelingual deafness was shown to decrease in younger patients, but to increase as they aged and, in fact, recover fully or even exceed the normal level of activation [5–7]. Children with prelingual deafness can acquire spoken language by cochlear implantation, but its efficacy decreases with age. The development of the auditory cortex is believed to depend on the patient's auditory experience within 'critical periods' in the early lifetime. Adults who had severe congenital hearing loss in their childhood may take advantage of hearing with cochlear implants if they had exploited residual hearing with hearing aids. It has been shown that low glucose metabolism in the temporal auditory cortex predicts a good cochlear implant outcome in prelingually deafened children, which suggests that low metabolism in the auditory cortex may indicate its potential of plasticity for spoken language acquisition [7].

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(Received 9 March 2011; accepted 29 May 2011)

ISSN 0001-6489 print/ISSN 1651-2251 online
DOI: 10.3109/00016489.2011.593719

Meanwhile, several etiological studies suggest that at least 60% of congenital hearing loss has genetic causes. Recent advances in molecular genetics have made genetic diagnosis possible [8]. The identification of the mutation responsible for hearing loss may provide some information as to cochlear damage, and help predict the time course and manifestations of hearing loss. Genetic testing can therefore be useful in decision-making regarding cochlear implantation and other necessary treatment.

Evaluation of brain function and diagnosing accurate etiology of hearing loss may be the keys to personalizing post-cochlear implantation habilitation programs and predicting the outcomes thereof.

In this study, we used 18 F-fluorodeoxyglucose (FDG) positron emission tomography (PET) to measure cortical glucose metabolism with a visual language task before cochlear implantation in profoundly deaf patients whose etiologies were identified by genetic testing.

Material and methods

Genetic diagnosis

Genetic screening was performed in two cases using an Invader assay to screen for 41 known hearing loss-related mutations [9] and direct sequencing for *GJB2* and *SLC26A4* mutations [10,11].

FDG-PET scanning and image analysis

FDG-PET scanning and image analysis were performed using the method described by Fujiwara et al. [12]. During the time period between the intravenous injection of 370 MBq 18 F-FDG (the dose was adjusted according to the body weight of each subject) and the PET scanning of the brain, the patients were instructed to watch a video of the face of a speaking person reading a children's book. The video lasted for 30 min, and several still illustrations taken from the book were inserted (for a few seconds each) to help the subjects to follow the story. The subjects were video-recorded to confirm that they were watching the task video. PET images were acquired with a GE ADVANCE NXi system (General Electric Medical Systems, Milwaukee, WI, USA). Spatial preprocessing and statistical analysis were performed with SPM2 (Institute of Neurology, University College of London, UK) implemented in Matlab (Mathworks, MA, USA). The cortical radioactivity of each deaf patient was compared with that of a control group of normal-hearing adults by a t test in the basic model of SPM2. The statistical significance level was set at $p < 0.001$ (uncorrected).

This study was approved by the Ethics Committee of Shinshu University School of Medicine and written consent was obtained from each participant.

Control group

The control group consisted of six normal-hearing right-handed adult subjects. The average (mean ± standard deviation) age of the normal-hearing subjects was 27.5 ± 3.8 years. The pure-tone average hearing levels were within 20 dB HL for all.

Case 1

A right-handed 22-year-old female with a *GJB2* mutation (235 delC homozygous) had hearing impairment that was noticed by her parents when she was 2 years old. She had used hearing aids ever since, but with insufficient hearing amplification. She used lip-reading and some sign language, and her speech was not intelligible to hearing people. Computed tomography (CT) findings of the middle and inner ear were normal. Her average pure-tone hearing levels were 102.5 dB for the right ear and 95 dB for the left ear (Figure 1A).

Case 2

A right-handed 26-year-old male with an *SLC26A4* mutation (H723R homozygous) had hearing impairment that was noticed by his parents when he was 2 years old, from which time he had used hearing aids bilaterally. He did not use lip-reading or sign language during the acquisition age for language. He obtained spoken language with hearing aids but had progressive hearing loss, and sometimes suffered vertigo attacks. His pronunciation was clear, and his speech was almost completely intelligible. CT findings exhibited an enlarged vestibular aqueduct on each side. His average pure-tone hearing levels were 106.2 dB for the right ear and 100 dB for left ear (Figure 1B).

Results

Figure 2 shows transaxial PET images of each participant's brain. The visual stimuli resulted in bilateral activation of the superior temporal gyrus, including Heschl's gyrus in case 1 with *GJB2* mutation (Figure 2A, white arrowhead). In contrast, in case 2 with *SLC26A4* mutation, the activation of the superior temporal gyrus was much lower than in case 1 (Figure 2B, white arrowhead).

Figure 3 shows supra-threshold clusters in each case. In case 1, activation higher than normal controls

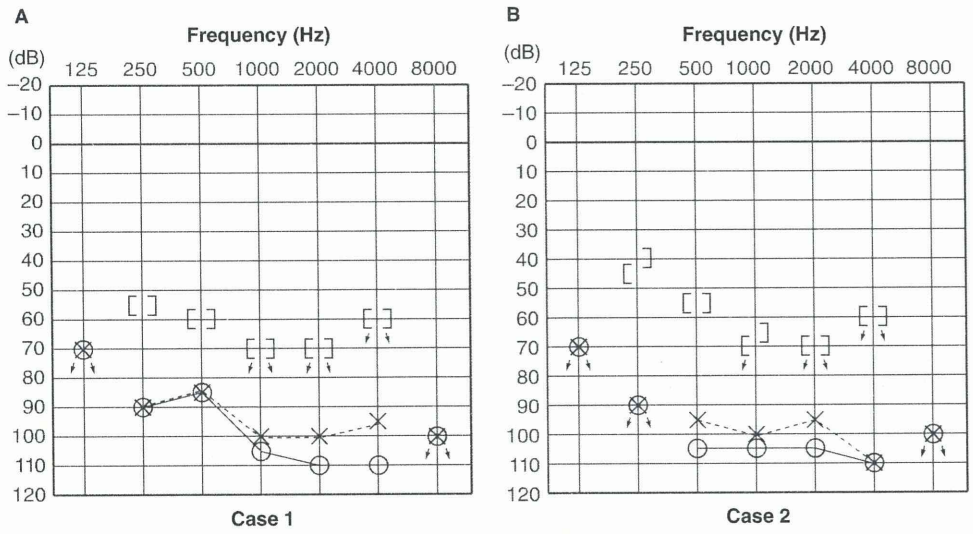

Figure 1. Pure-tone audiograms: (A) a 22-year-old female with a *GJB2* mutation; (B) a 26-year-old male with an *SLC26A4* mutation. There were no clear differences in hearing thresholds in these two cases.

was observed in the right auditory association area [BA21, BA22], and the left auditory association area [BA42] ($p < 0.001$). In case 2, the right superior frontal gyrus [BA9], and the middle temporal gyrus [BA20], showed higher activation than normal controls ($p < 0.001$).

Discussion

More than half of congenital hearing loss has been estimated to be from genetic causes, and phenotypes are affected by genetic mutations. There have been no reports of the influence of phenotype on brain function associated with hearing. This is the first report on evaluation of cortical processing of language in patients with genetic mutations as a main etiology of hearing loss. The auditory association area was activated bilaterally in case 1 (*GJB2* mutation), but not activated in case 2 (*SLC26A4* mutation). A previous study indicated that the temporal lobe is activated during speech-reading in normal subjects [13] and another study found that the temporal lobe is not activated when reading fluent speech from a talking face [14]. For the present study we used a

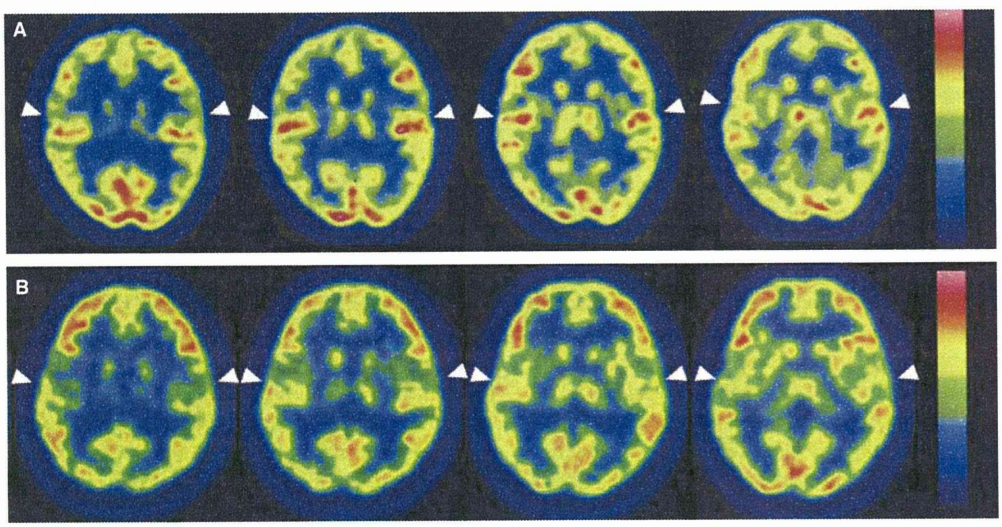

Figure 2. Transaxial PET images of each participant's brain: activation (arrowheads) of the superior temporal gyrus with visual language stimuli in each case. (A) Case 1 (*GJB2* mutation). The superior temporal gyri were strongly activated bilaterally. (B) Case 2 (*SLC26A4* mutation). The superior temporal gyri exhibited less activation than in case 1.

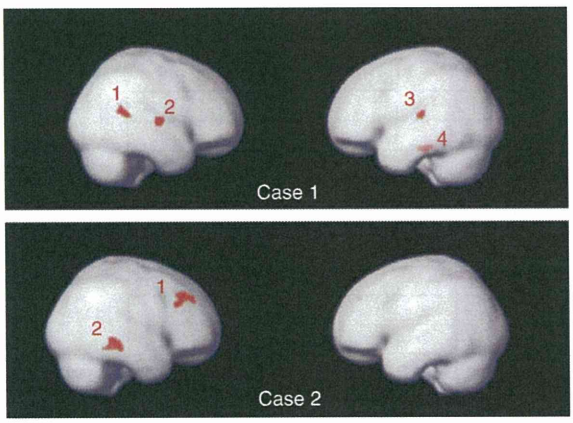

Figure 3. Cortical activation by language-related visual stimuli in the two profoundly deafened cases. Case 1 (*GJB2* mutation) showed significant activation in the right middle temporal gyrus [BA21] (1), superior temporal gyrus [BA22] (2), and left superior temporal gyrus [BA42] (3), and left cerebellum (4), while case 2 (*SLC26A4* mutation) exhibited significant activation in the right superior frontal gyrus [BA9] (1), and middle temporal gyrus [BA20] (2) (SPM2, $p < 0.001$, uncorrected).

fluent speech-reading task, similar to that described by Hall et al. [14]. Fujiwara et al. in a FDG-PET study using the same methods and task as the present study, showed that subjects with better spoken language skills had less temporal lobe activation [12].

To summarize these reports, the patients with hearing aids with better spoken language skills have less temporal lobe activation with a visual language task. Otherwise, Nishimura et al. [15] reported a sign language activation of the bilateral auditory association areas in a congenitally deafened subject. However, detailed clinical data for the subject – including his hearing levels, time course of hearing loss, and the cause of deafness – were not described. The different visual language activation patterns in the auditory cortices revealed in the current two profoundly deafened subjects with different genetic etiologies and hearing loss progressions may, thus, add further knowledge of the cross-modal plasticity brought about in the superior temporal association areas by lack of hearing.

The differences in cortical processing patterns between cases 1 and 2 – who both had hearing loss of cochlear origin – may have been influenced by the differing clinical courses of hearing loss. *GJB2* is currently known to be the most prevalent gene responsible for congenital hearing loss worldwide. Patients with severe phenotypes who have *GJB2* mutations are good candidates for implantation, because their hearing loss is of cochlear origin and non-progressive [16,17]. *SLC26A4* is known as a commonly found gene and is associated with enlarged vestibular aquaduct [11]. This phenotype includes congenital and progressive hearing loss, usually associated with vertigo [18]. In most cases hearing remains in low frequencies, enabling the understanding of spoken language with hearing aids. Cochlear implantation has resulted in remarkable improvements in auditory skills and speech perception for patients with profound hearing loss associated with *SLC26A4* mutations as well as *GJB2*.

Comparing case 1 (*GJB2* mutation) with case 2 (*SLC26A4* mutation), the crucial importance of the use of hearing aids during childhood up to age 6 years for acquisition of better hearing is evident. In case 1, even though she was able to hear sound with the use of hearing aids, she was unable to recognize enough speech language due to insufficient hearing amplification during the critical periods in her childhood. She therefore used lip-reading and some sign language in addition to hearing aids. Increased metabolism was observed by FDG-PET in the auditory association area, where no significant activation was found in the normal-hearing controls. In contrast, in case 2, a 26-year-old patient with an *SLC26A4* mutation, there was no significant activation in the corresponding area. He obtained rather hearing ability and spoken language by hearing aids with residual hearing at lower frequencies during his childhood. His hearing was supposed to be better than case 1, because 1) he did not use lip-reading or sign language during the acquisition age for language from anamnestic evaluation; 2) his pronunciation was clear, indicating better hearing (at least 40–50 dB) during the acquisition age for language; 3) from an etiological point of view, patients with *SLC26A4* mutation usually have mild to moderate hearing loss during childhood and this shows a progressive nature [18]. He had progressive hearing loss in the natural history as a phenotype of *SLC26A4* mutation. The difference in activation patterns in the cases with *GJB2* and *SLC26A4* mutations was clearly demonstrated by statistical processing with SPM, as well as in the PET scans. These results suggest the importance of hearing during early childhood for the development of a normal cortical language network, and that reorganization had occurred in the auditory cortex of the patient with a *GJB2* mutation; i.e. processing visual aspects of language in the superior temporal gyri. This implies that cross-modal plasticity as a consequence of the lack of hearing during the critical period for spoken language acquisition in early childhood was influenced by the time course of hearing loss characterized by genetic mutations.

Previous studies have suggested that auditory areas presented high accumulation of FDG with deafness of early onset, and plastic changes in auditory cortices

were strongly affected by the duration of auditory deprivation [1,5,6,19,20]. Since low activation of the auditory cortices with visual stimuli suggests the subject's lesser dependence on visual communication methods and substantial residual plasticity in his auditory cortices, case 2 with an *SLC26A4* mutation may be determined to be an appropriate candidate for cochlear implantation.

Accurate diagnosis of hearing loss and early cochlear implantation are important for successful spoken language development. The approach using PET could help those involved in the habilitation and education of prelingually deafened children to decide upon the suitable mode of communication for each individual.

Both of the patients received cochlear implantation after PET examination. Further follow-up of these cases may indicate that efficacy of the combination of genetic diagnosis and functional brain imaging helps to predict long-term outcomes of cochlear implantation. Examination of more cases is necessary to define the relationship of the varying cortical activation patterns with each genetic mutation.

Acknowledgments

We thank A.C. Apple-Mathews for help in preparing the manuscript. We also thank Masanori Sakaguchi MD and radiologic technologists, Kouichi Anraku and Hiroyuki Fujimoto, for excellent technical assistance.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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日本臨牀　第69巻・第2号（平成23年2月号）別刷

難聴の遺伝子診断

宇佐美真一

難聴の遺伝子診断

Molecular diagnosis of deafness

宇佐美真一

Abstract

Despite advances in discovery of deafness genes, clinical application still entails difficulties because of the genetic heterogeneity of deafness. In order to establish strategy for clinical application, we reviewed the genes responsible for hearing loss patients in Japan (Usami S et al; Acta Otolaryngol 128: 446–454, 2008), and discussed diagnostic strategy for mutation screening based on a mutation/gene database (Abe S et al; Genet Test 11: 333–340, 2007).

Our series of mutation screenings has revealed that mutations in *GJB2*, *SLC26A4*, and *CDH23*, and the 1555A>G mutation in the mitochondrial 12S rRNA, were the major causes of hearing loss in Japanese patients. Interestingly, spectrums of *GJB2*, *SLC26A4*, and *CDH23* mutations found in the Japanese population were quite different from those reported in populations with European ancestry. Our simultaneous screening of the multiple deafness mutations was based on the mutation spectrum of a corresponding population. The multicenter trial for this assay using an Invader panel revealed that approximately 40 % of congenital hearing loss subjects could be diagnosed. This assay will enable us to detect deafness mutations in an efficient and practical manner in the clinical platform.

Key words: deafness, vertigo, genetic testing

はじめに
—難聴を取り巻く環境の大きな変化—

この10年あまり難聴(特に先天性難聴)を取り巻く環境が，新生児聴覚スクリーニング，遺伝子診断，人工内耳の登場により大きく変化した．本稿ではこのうち難聴の遺伝子診断を中心に，難聴医療がどのように変わってきたかを概説する．

1. 難聴医療の進歩

a. 早期発見

先天性難聴は出生1,000人に1人生まれ，先天性疾患の中では最も高頻度に認められる疾患の一つである．難聴児の多くは難聴以外には何ら異常を示さない児であり，従来は言葉が出ない，音に対する反応がないなどをきっかけに2–3歳で発見されることが多かった．また原因も不明で，有効な治療法もなく，難聴児は補聴器を使用し，ろう学校に通学するという選択肢

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しかない時代が続いた．通常，子どもは両親の発音をまねて言語が次第に発達してくる．したがって正確な発音が聞き取れなければ正確な発音をすることは不可能である．言語発達には臨界期(2-4歳)がありその時期に十分な音の情報が入らない場合，発音や言語発達の獲得に不利になることが明らかになり，近年，新生児聴覚スクリーニングにより難聴を早期に発見し，早期に介入や療育を行い言語発達を促そうとする流れが定着している．現在多くの自治体で新生児聴覚スクリーニングが始まり難聴児が出生直後に診断されるようになってきている．

b. 原因の特定

難聴は長い間原因不明の疾患であったが，従来の疫学的な研究から先天性難聴の少なくとも50％は遺伝子の関与によるものと推測されていた．ヒトゲノムの解明に伴い，多くの疾患の原因遺伝子が特定されてきたが，難聴でもこの10年余りの間に多くの原因遺伝子が同定されてきている．原因遺伝子によって発症時期，進行性，前庭症状，随伴症状が異なることから，遺伝子診断は難聴の正確な診断，治療法の選択，予後の推測，合併症の予測，更には予防や遺伝カウンセリングといったものに関して重要な情報を提供してくれるようになってきた．今後数年のうちに難聴の分類は原因遺伝子ごとに再分類されていき，難聴患者に対する個別化医療が進んでいくと思われる．原因遺伝子の特定により，難聴のメカニズム，病態がピンポイントに理解可能になった．それに伴い近い将来難聴の医療にとって遺伝子診断は欠かせないものになることが予想される．

c. 人工内耳の発達

難聴に対する根本的な治療法の開発はこれからの課題になるが，重度の難聴患者では人工内耳が非常に効果を上げている．成人例(後天性難聴)ではいうまでもなく，現在重度の先天性難聴児に対する介入法としても人工内耳が普及し効果を上げている．従来重度難聴児に対しては補聴器では十分な補聴効果が得られない場合が多く，発音や言語発達に限界があったが，この10年余り先天性難聴児に対する人工内耳装用者が世界的に増え，その有効性が実証されている．

2. 原因の特定はなぜ必要か

改めていうまでもなく‘難聴’は症状名であり診断名ではない．難聴は原因不明の時代が長く続いたが，近年のヒトゲノム解析研究の発展により，多くの原因遺伝子が同定され報告されるようになり，もはや難聴は原因不明の疾患ではなくなってきている．例えば，内科医が‘腹痛’という診断名ではなく腹痛の原因を検索し患者にとって最適な治療法を考えるのと同じように，難聴の原因診断が可能になった現在，正確な診断は医療従事者側，療育関係者側，患者側からみても疾患に対するアプローチの王道であることはいうまでもなく，治療や療育を考えるうえでの出発点である．原因が異なる難聴児に対し同じ考え，同じプログラムで療育を進めていこうということ自体無理があるのは明らかで，今後は原因が異なる個々の難聴児に最適なオーダーメイドの療育プログラムが組まれていくことが望ましい．難聴児の両親にとっても，難聴の受容とともに原因を知り難聴の特徴を理解することは難聴と向き合う際の出発点であると考えられる．

3. なぜ難聴の遺伝子診断か

疫学的な研究により従来から先天性難聴の60-70％は遺伝子の関与によるものと推測されているが(**図1**)[1]，難聴の原因を知るためには遺伝学的検査が必要不可欠になってきている．近年，新生児聴覚スクリーニングによって難聴児が早期に発見され，人工内耳の発達によって高度難聴児でも聴覚を活用し言語発達を促すことが可能になってきた．小児難聴では早期に難聴の有無について診断がなされた後，難聴の原因診断を検索するための遺伝子診断のニーズが高まってきている．また患者サイドでも，なぜ難聴になったかということを知りたいというニーズが高まってきている．

図1は欧米のデータをまとめたものであるが，難聴原因遺伝子の中で特に高頻度で見いだされ

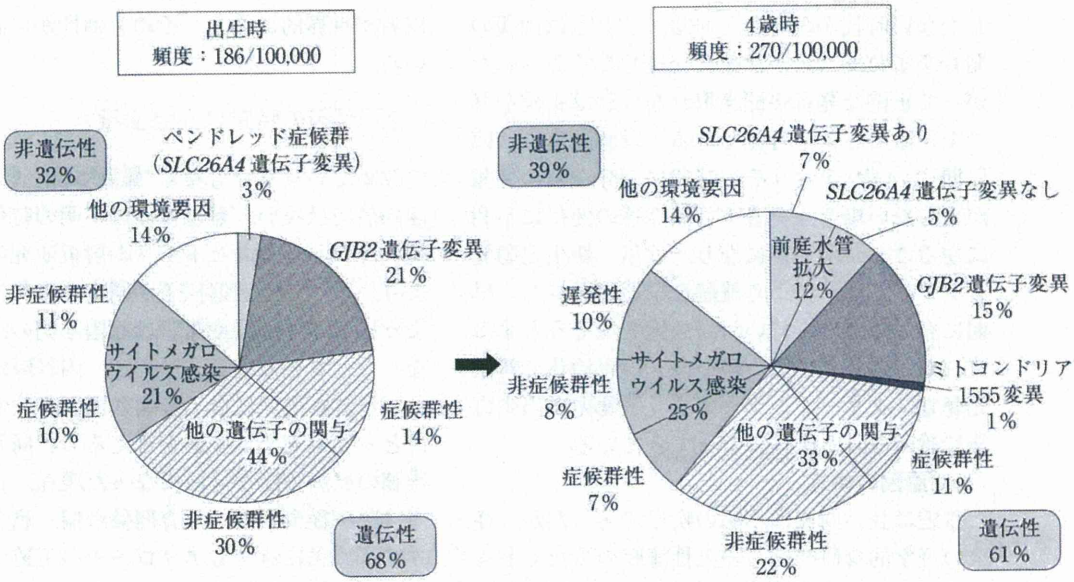

図1　小児期発症の難聴の原因(文献[1]より引用)

ているのが*GJB2*遺伝子変異による難聴で先天性難聴の約20％を占めることが知られている．次いで頻度が多いのが前庭水管拡大を伴う難聴(*SLC26A4*遺伝子変異が原因で引き起こされる)である．難聴は変動を繰り返し進行するのが特徴的であり，**図1**でも示されるように4歳時では前庭水管拡大を伴う難聴の割合が増加してくる．この2つの遺伝子で約30％を占め，その他の遺伝子が約30％を占める．このうち約1/3は'症候群性難聴'と呼ばれ，難聴のほか筋肉骨格系，腎尿路系，神経系，眼の異常，色素異常，代謝異常など種々の奇形や他の疾患を伴っている．症候群性難聴に関しては随伴する症候である程度診断が可能なものが多いが，遺伝子検索は確定診断や遺伝カウンセリングに有用となる．

4. 難聴の遺伝子診断の有用性

近年の分子遺伝学の進歩により難聴の病態が分子レベルで明らかになってきた．根本的な治療法はまだ開発されていないが，現時点でも，難聴の遺伝子診断が耳鼻咽喉科の日常臨床に応用され，次第にその有用性が認識されるようになっている[2,3]．有用性を**表1**にまとめたが，正確な診断は医師側，患者側からみても疾患に対するアプローチの王道であることはいうまでもなく，治療や療育を考えるうえでまず第一歩であり，難聴児の両親にとっても難聴の受容とともに原因を知り，難聴の特徴を理解することは難聴と向き合う際に重要であると考えられる．また，それぞれの遺伝子により臨床像が異なるので難聴の進行性の有無，変動の有無，随伴症状の有無を予測するのに有用である．また*GJB2*遺伝子などの場合，変異の種類によって難聴の程度が異なることが知られているので，介入法の選択(補聴器か人工内耳か)に有用な情報を提供してくれる．ミトコンドリア遺伝子1555変異などの場合，予防が可能であるなど，未発症の家族に対する予防が可能になっている．

表1　遺伝子診断の有用性

(1) 正確な診断
(2) 予後の推測 (難聴の進行，変動，随伴症状の予測)
(3) 治療法の選択
(4) 難聴の予防
(5) 遺伝カウンセリング
(6) 無駄な検査が省ける

(文献[2]より引用)

また遺伝形式が様々であるため遺伝カウンセリングの際の正確な情報提供に際しても原因となる遺伝子の同定が不可欠になってきた．'原因遺伝子を突き止めても治らないのであれば検査する必要はない'ということを言う患者(場合によっては医療従事者)がまだ多いのも事実である．しかしながら遺伝子治療，再生医療といった治療が近い将来可能になったときに，正確な診断ができていなければ，そのような治療が適応になるか否かもわからないこともまた事実である．

5. 難聴の原因遺伝子検索の特殊性：効率的な難聴の原因遺伝子スクリーニング

原因遺伝子の数に関しては従来から数十から100ほどの原因遺伝子が推測されているが，難聴は多種類の遺伝子が'難聴'という同じ表現型をとる(遺伝子異質性：locus heterogeneity)ために，実際に難聴を主訴に外来を受診した患者がどの原因遺伝子が関与しているかを推測することは困難である．現在までに日本人難聴患者からは合計10数種類の原因遺伝子が報告されているが(宇佐美真一'日本人難聴遺伝子データベースホームページ' http://ent.md.shinshu-u.ac.jp/deafgene.html)[4]，興味あることに，日本人で見いだされる変異は欧米人に見いだされる変異部位と大きく異なっていることが明らかになっている．これは創始者効果によるものであることが証明されており[4,5]，これらの日本人に特徴的な，あるいは頻度の高い遺伝子変異を網羅的，効果的にスクリーニングしていくことが原因を特定するために効率的であると考えられる．インベーダー法は複数の遺伝子において多数の変異を同時に検出可能なスクリーニング法として注目されているが，1回のアッセイでミトコンドリア遺伝子変異がホモプラスミーかヘテロプラスミーかも判定可能であり，従来のミトコンドリア遺伝子変異検出法と比較しても非常に優れた検査法である．日本人先天性・小児期発症難聴患者300余人における9遺伝子42変異の出現頻度の検討を行ったところ，約30％の患者で遺伝子変異の検出が可能であった[6]．多施設共同研究としてインベーダー法を用い10遺伝子47変異の有無について一次スクリーニングを実施，更に必要に応じ直接シークエンス法を用いた二次スクリーニングを行い変異確認，新規変異検索を行った結果，難聴患者の約35％(発症年齢が6歳以下の先天性難聴患者に限ると44.3％)の検出率が得られ，インベーダー法によるスクリーニングが臨床検査として有用であることが確認された(Usami et al.，投稿中)．

6. 先進医療としての難聴の遺伝子診断

多くの疾患でも同様に遺伝子解析研究が終了すると，多額の研究費を必要とする遺伝子解析自体が行われなくなるという現象が起き，費用負担の面から臨床に応用するという本来の最終目的の達成が困難になるという問題が生じている．

難聴に関しては，そのような問題点を踏まえ臨床応用の第一歩として2008年7月に'先天性難聴の遺伝子診断'が先進医療として承認され臨床診療として実施が開始されている．現在までに信州大学で実施した52症例での集計結果では約45％の症例で原因遺伝子が見いだされている(**図2**)．従来の先進医療ではすべてを自施設で行わなければならないという縛りがあり，実施可能な施設が限られていたが，2010年4月からは先進医療の共同実施(検査の受託側と委託側で共同し先進医療を実施)が可能になり，全国規模で難聴の遺伝子診断が臨床の現場で実施できるような体制作りが進んでいる．先進医療で承認された'先天性難聴の遺伝子診断'では遺伝学的検査を行い，結果を遺伝カウンセリングとともに返すまでを医療として位置づけている．後述のように各遺伝子に関して丁寧な説明と情報提供が行われている．

7. 日本人に多く見いだされる原因遺伝子

インベーダー法による網羅的な難聴遺伝子解析により，日本人難聴患者において高頻度で見いだされる遺伝子/遺伝子変異が次第に明らかになってきた．先天性難聴では*GJB2*遺伝子変

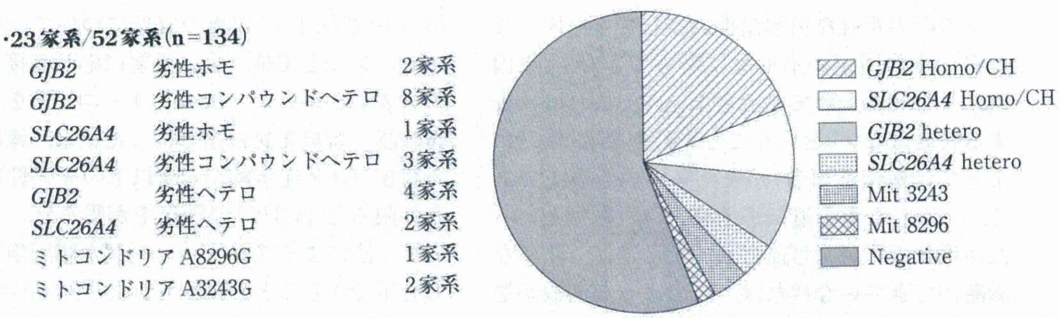

図2　信州大学における先進医療(先天性難聴の遺伝子診断)の現況

異，*SLC26A4*遺伝子変異が多く(**図2**)，後天性難聴にはミトコンドリア遺伝子変異が多く見いだされる．それぞれの遺伝子変異による難聴について解説するとともに，実際にどのように臨床で応用されているかを紹介する．

a. *GJB2*遺伝子変異による難聴：先天性難聴に最も多く見いだされる原因遺伝子

*GJB2*遺伝子は細胞間の結合様式の一つであるギャップ結合タンパク(コネキシン26)をコードする遺伝子で，内耳のカリウムイオンのリサイクルに重要な働きを担っていると考えられている．現時点で最も高頻度で見いだされる先天性難聴の原因遺伝子として全世界で研究が進められている．日本人難聴患者1,343例について*GJB2*遺伝子変異頻度を検討した結果では，191例(14.2％)に遺伝子変異が認められている[7]．難聴の発症年齢別(0–3歳，4–5歳，6歳–)では，0–3歳(先天性または言語獲得前難聴)の難聴患者の約25％に*GJB2*遺伝子変異が認められ，日本人先天性難聴患者の重要な原因の一つであることが明らかとなっている[7]．

現在全世界で，100種類以上の*GJB2*遺伝子変異が報告されており，変異の頻度および種類の分布は人種によって大きく異なっていることが報告されている[4]．日本人難聴患者には26種類の遺伝子変異が報告され，その中でも235delC変異の頻度が最も多く，次いでV37I，G45E/Y136X，R143W，176–191 del16 bp変異の順に多く認められている[7](**図3**)．*GJB2*遺伝子変異による難聴の場合，遺伝子型と難聴の程度には相関関係があることが明らかになっている[7](**図4**)．すなわち235delCをはじめ欠失，挿入変異，ストップ変異が含まれる場合，より高度の難聴になる傾向が報告されている．一方，ミスセンス変異の場合は軽度から中等度難聴の場合が多い傾向があることが報告されている．

発見年齢別に遺伝子型を検討した場合，0–3歳では235delCが58.5％と高く，発見年齢が高くなるほどその頻度は少なくなることが報告されている．一方，V37Iは発見年齢が高くなるほど頻度は高くなり，V37Iをもつ難聴患者は発見年齢が遅れることが明らかになっている[7](**図3**)．これは235delCを含む難聴患者は高度難聴を呈するのに比し，V37I変異は難聴が軽度であるため難聴の発見が遅れることが原因であると考えられている．また，V37I変異症例は日本人における*GJB2*遺伝子変異の中では2番目に多い変異であるが，対照(正常聴力)群では最も頻度が高い変異であることが明らかになっている．これはV37I変異症例の難聴が軽度であるために患者が病院を受診しない，もしくは診療医が患者の難聴が軽度であるため遺伝学的検査を勧めない可能性があるためと考えられている．

*GJB2*遺伝子変異では変異のタイプと聴力像に相関関係があることから，調整定常反応などの聴覚検査と組み合わせることにより重症度を予測し，治療法を選択する際に有用な情報となる．現在までに*GJB2*遺伝子変異による先天性難聴患者に対する人工内耳，あるいはミトコンドリア1555変異などによる後天性難聴患者に