European Journal of Human Genetics (2003) 11, 744–748

www.nature.com/ejhg

ARTICLE

Mutations in the *COCH* gene are a frequent cause of autosomal dominant progressive cochleo-vestibular dysfunction, but not of Meniere's disease

Shin-ichi Usami*[,1], Kentaro Takahashi[1], Isamu Yuge[1], Akihiro Ohtsuka[1], Atsushi Namba[2], Satoko Abe[3], Erik Fransen[6], Laszlo Patthy[4], Gottfried Otting[5] and Guy Van Camp[6]

[1]*Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan;* [2]*Department of Otorhinolaryngology, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan;* [3]*Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan;* [4]*Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary;* [5]*Australian National University, Research School of Chemistry, Canberra ACT 0200, Australia;* [6]*Department of Medical Genetics, University of Antwerp, Antwerp, Belgium*

The *COCH* gene is the only gene identified in man that causes autosomal dominantly inherited hearing loss associated with vestibular dysfunction. The condition is rare and only five mutations have been reported worldwide. All affected families showed a similar progressive hearing loss and vestibular dysfunction. Since Meniere's disease-like symptoms have also been described in some families, it was suggested that *COCH* mutations might be present in some patients diagnosed with Meniere's disease. In this study, using a Japanese population, we performed a *COCH* mutation analysis in 23 patients from independent families with autosomal dominant hearing impairment, four of whom reported vestibular symptoms, and also in 20 Meniere's patients. While a new point mutation, A119T, was found in a patient with autosomal dominant hearing loss and vestibular symptoms, no mutations were found in the Meniere's patients. Like all other previously identified *COCH* mutations, the mutation identified here is a missense mutation located in the FCH domain of the protein. The current mutation is located in close spatial proximity to W117, in which a mutation (W117R) had previously been associated with autosomal dominant hearing loss. Model building suggests that, like the W117R mutation, the A119T mutation does not affect the structural integrity of the FCH domain, but may interfere with the interaction with a yet unknown binding partner. We conclude that mutations in the *COCH* gene are responsible for a significant fraction of patients with autosomal dominantly inherited hearing loss accompanied by vestibular symptoms, but not for dominant hearing loss without vestibular dysfunction, or sporadic Meniere's disease.
European Journal of Human Genetics (2003) **11**, 744–748. doi:10.1038/sj.ejhg.5201043

Keywords: *COCH*; FCH domain; deafness; vertigo; Meniere's disease

Introduction

The *COCH* gene is known to be responsible for non-syndromic autosomal dominant hearing loss parallelled by vestibular symptoms (DFNA9).[1] To date, five *COCH* mutations have been reported in families of European ancestry.[1–5] (Table 1) These include three private mutations in families from the US, a single founder mutation in many

*Correspondence: Dr Shin-ichi Usami, Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. Tel: 81 263 37 2666; Fax: 81 263 36 9164;
E-mail: usami@hsp.md.shinshu-u.ac.jp
Received 6 December 2002; revised 31 March 2003; accepted 14 April 2003

Table 1 Characteristics of currently known *COCH* mutations

Mutation (amino acid)	*Protein domain*	*Mutation (nucleotide)*	*Exon*	*Families*	*References*
P51S	FCH	151C>T	4	Several Belgian and Dutch	Fransen *et al*[3,4] and De Kok *et al*[2]
V66G	FCH	197T>G	4	one American	Robertson *et al*[1]
G88E	FCH	263G>A	5	one American	Robertson *et al*[1]
I109N	FCH	326T>A	5	one Australian	Kamarinos *et al*[5]
W117R	FCH	349T>C	5	one American	Robertson *et al*[1]
A119T	FCH	355G>A	5	one Japanese	This study

The numbering is based on the *COCH* sequence with GenBank accession number AF006740. The numbering of nucleotides starts with the A of the start codon AUG as nucleotide 1, as recommended by the HUGO nomenclature committee. In some publications, the numbering is different, including 56 base pairs upstream of AUG. The amino acids were numbered with the starting methionine as residue 1.

families in Belgium and The Netherlands, and a private Australian mutation. For some mutations, the penetrance of vestibular symptoms is complete (the Belgian–Dutch P51S mutation and the Australian I109N mutation). For the US mutations (V66G, G88E, W117R), it was unclear whether the vestibular phenotype was present in all carriers of the *COCH* mutation. It is therefore not excluded that the penetrance of the vestibular symptoms varies across families, according to the mutation.

Fransen *et al*[3] and Verstreken *et al*[6] reported a large DFNA9 family where, apart from hearing loss and vestibular dysfunction, 25% of the carriers of the *COCH* mutation suffered from recurrent episodes of dizziness, associated with tinnitus, aural fullness, nausea, and vomiting. Such episodes are very similar to the vertigo attacks encountered in Meniere's disease. Meniere's disease is a term generally used to describe patients with disabling episodes of combined vestibular and auditory disturbance. Both familial and sporadic forms occur. The etiology of this frequent pathology is not known, but it is probably complex, involving multiple environmental causes and predisposing genes.

Due to the similarity between DFNA9 and Meniere's disease, Fransen *et al*[3] suggested that the possibility of a *COCH* mutation should be considered in patients diagnosed with Meniere's disease. However, there are also clear clinical differences between these two conditions. In Meniere's disease, the hearing loss that accompanies the vertigo attack is usually unilateral and fluctuating, affecting mainly the low frequencies at an early stage of the disease, whereas DFNA9 is characterized by bilateral and progressive hearing loss, starting in the high frequencies and gradually affecting all frequencies.

The biological function of *COCH* is unclear. *COCH* mRNA is known to be predominantly expressed in the inner ear[7,8] and recent proteomic analysis indicated that the *COCH* protein, cochlin, constitutes 70% of all inner ear proteins,[9] suggesting that this protein plays an important role in the inner ear. The human *COCH* gene contains two different types of domains, a region homologous to a domain in factor C of Limulus (FCH, factor C homologous domain; sometimes also referred to as LCCL domain)[10] and two von Willebrand factor A-like domains (vWFA1 and 2). Interestingly, all mutations reported so far were detected in the FCH domain.

Families with autosomal dominant cochleo-vestibular dysfunction are rare compared to those with autosomal dominant hearing impairment without vestibular symptoms. In addition, both these conditions are very rare in comparison with Meniere's disease. To analyze the contribution of *COCH* mutations to various forms of hearing and vestibular dysfunction in the Japanese population, we performed *COCH* mutation analysis in patients with autosomal dominant hearing loss with and without vestibular symptoms, as well as in patients with sporadic Meniere's disease.

Subjects and methods

Subjects

In the present study, we used individuals with Japanese ancestry. All patients and family members gave informed consent to be included in this study. A blood sample was obtained from each individual and DNA was isolated using standard procedures.

Autosomal dominant families

We analyzed 23 subjects from independent families with probable autosomal dominant sensorineural hearing loss (with two or more generations affected). The number of patients in each family ranged from 3 to 65, with a median of 29.2. The severity of hearing loss (a three-frequency average of 500, 1000, 2000 Hz) varied from mild to profound. The hearing loss was mild (30–49 dB) in six patients (15%), moderate (50–69 dB) in nine (40%), severe (70–89 dB) in two (5%), and profound (>90 dB) in six (15%). The age of onset varied from congenital to 40 years. In seven patients (30.4%) the hearing loss was progressive and four patients (17.4%) complained of vertigo.

Meniere's disease patients

Diagnostic criteria for 20 Meniere's patients were according to the guidelines of the American Academy of Otolaryngology-Head and Neck Surgery.[11] In brief, the patients were selected by the following clinical features: (1) two or more definitive spontaneous episodes of vertigo lasting 20 min

or longer; (2) audiometrically documented hearing loss on at least one occasion; (3) tinnitus or aural fullness. The age of the Meniere's disease patients ranged from 33 to 63 years (mean 50.15). None of the Meniere's disease patients had a family history.

Clinical examination

Pure-tone audiometry was performed in all the patients. The criteria used to consider an individual affected was bilateral sensorineural hearing loss of more than 30 dB in at least one frequency with pure-tone audiometry. For patients with a history of vertigo, a caloric test was performed. CT examination was performed for all patients, but no malformations of the inner ear were identified.

Mutation analysis of the *COCH* gene

All 12 *COCH* exons were PCR amplified using previously described intronic primers.[1] Amplification products were purified using QIAquick (Qiagen) or gel purified by electroelution, and sequenced using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Fragments were analyzed using an ABI 377 automated sequencer.

Structure analysis

The mutation found in the present study was modelled onto the nuclear magnetic resonance (NMR) structure of the FCH domain.[12] The program MOLMOL[13] was used for manual, interactive modelling and to plot Figure 1.

Results

A mutation analysis was performed by genomic DNA sequencing of all 12 *COCH* exons in 23 patients with autosomal dominant hearing loss and in 20 patients with Meniere's disease. No mutations were found in the Meniere's disease patients. A novel *COCH* mutation was found in one affected member of a family that had affected subjects in two generations (Figure 1). The proband visited our ENT clinic at the age of 47 years because of recurrent dizziness/vertigo and gradually progressive hearing loss associated with tinnitus that had started at the age of 35 years. Pure-tone audiograms showed profound deafness for all frequencies. Caloric testing showed bilateral decreased response. No other obvious clinical abnormalities were noted. Anamnestically, the deceased mother of the proband also had suffered from hearing loss and recurrent episodes of vertigo, starting in her 30s.

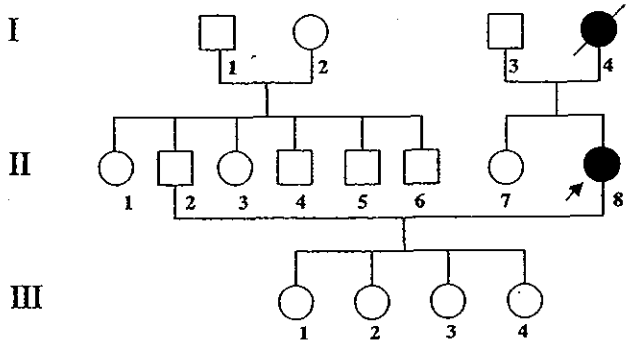

Figure 1 Pedigree of the family bearing a *COCH* mutation. Filled symbols represent affected members.

The novel *COCH* mutation detected in this Japanese family is a heterozygous 355A>G mutation in exon 5, resulting in an alanine to threonine substitution at residue 119 of the *COCH* protein (A119T). This alanine residue is evolutionarily conserved in the mouse *Coch* gene, but not in chicken. This mutation was not found in unaffected members in the family (Figure 1; III–1,2,3,4). To exclude that the A119T variation is a common polymorphism, 96 unrelated random individuals of Japanese origin were analyzed. None of them showed this mutation. No other DNA variations that would lead to amino-acid changes or could affect splice sites were found.

Inspection of the three-dimensional structure of the FCH domain showed that the side chain of A119 is quite solvent-exposed. Using a χ^1 angle of $-60°$, a Thr side chain could be modelled at the site of this residue without introduction of steric clashes in the existing structure (Figure 2).

Discussion

In this study, we found a novel *COCH* mutation, A119T, in a Japanese pedigree with hereditary sensorineural hearing loss associated with vestibular impairment. Although only one affected family member was genotyped, the identified mutation is most likely disease-causing. A119T is not a common variation in the *COCH* gene since it was not found in unaffected family members or controls, nor has it ever been reported in any other mutation analysis of the *COCH* gene. The clinical picture of the Japanese patient is highly similar to previously reported patients with a *COCH* mutation,[6] and the disease was likely familial with a segregation pattern compatible with autosomal dominant inheritance.

Previously, five *COCH* mutations have been reported (P51S, V66G, G88E, I109N, W117R); all in families with European ancestry.[1–3] (Table 1) A119T- is the first *COCH* mutation identified in a patient of nonEuropean ancestry. In Japanese patients, *MYO7*[14], *KCNQ4*[15], and *TECTA*[16] already have been reported as responsible for nonsyndromic autosomal dominant sensorineural hearing loss. This study adds *COCH* as a fourth gene.

Including the present mutation, all six mutations in the *COCH* gene were missense mutations located in the conserved cysteine-rich factor C homologous (FCH) do-

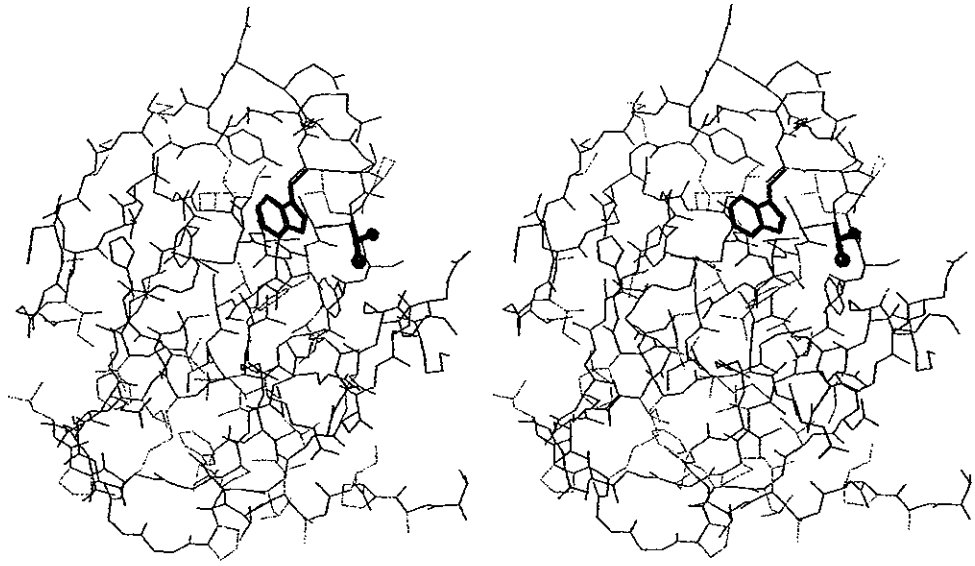

Figure 2 Stereo representation of a model of the FCH domain with alanine at position 119 replaced by threonine, based on the conformer closest to the mean conformer of the NMR structure. All bonds connecting heavy atoms are shown. The side chain of threonine at position 119 is drawn in a ball-and-stick representation, with red and gray spheres identifying the location of the side-chain oxygen and methyl carbon, respectively. The side chain of tryptophan at position 117 is highlighted with bold lines. Backbone bonds are drawn in magenta. Side chains are drawn in yellow for hydrophobic residues (Ala, Cys, Ile, Leu, Met, Phe, Pro, Trp, Val), gray for uncharged hydrophilic residues (Asn, Gln, Ser, Thr, Tyr), red for negatively charged residues (Asp, Glu), and blue for positively charged residues (Arg, His, Lys). As residue 119 is located on the surface of the domain and its side chain is solvent exposed, the A119T mutation is unlikely to interfere with the structural integrity of the FCH domain.

main (Table 1). This suggests an important specific role for this domain in the functioning of *COCH* in the inner ear.

The three-dimensional solution structure of this domain has recently been reported.[12] It assumes a unique fold that is affected by the majority of the previously reported point mutations. Among the reported mutations, P51S, V66G, G88E, and I109N result in misfolding of the FCH domain. The W117R mutation, however, is correctly folded and is stable as the wild-type protein. This residue is consequently thought to participate in the interaction with a binding partner.[12] Also, the A119T mutation is unlikely to interfere with the structural integrity of the FCH domain. Interestingly, the side chains of A119 and W117 are spatially close (Figure 1), and it is therefore possible that these two mutations interfere with the same process.

Histological analysis of temporal bones of patients with a *COCH* mutation revealed acidophilic deposits, described as a mucopolysaccharide-like substance, in the cochlea and vestibular end organs.[17–19] *In situ* hybridization and immunohistochemical analysis showed expression of *COCH* in the fibrocytes of the spiral ligament, the spiral limbus, osseous spiral lamina, and the stroma of the vestibular end organs. The deposits were found at exactly the same places in temporal bone sections.[8] It was therefore suggested that mutant protein may have a direct or indirect effect on these fibrocytes and lead to degeneration. This may have a negative impact on the inner ear, possibly by interfering with K^+ recycling, which is thought to play an important role in ion homeostasis.

The clinical features found in the Japanese patient with the *COCH* mutation, who showed progressive sensorineural hearing loss starting in the fourth decade of life in combination with recurrent vertigo attacks, are in line with the previous reports of DFNA9 families. The sensorineural hearing loss caused by *COCH* mutations initially affects only the high frequencies, but progresses to include the lower frequencies, leading to severe to profound loss across all frequencies. The onset is in adulthood, ranging from the third to the fifth decade, and penetrance of the hearing loss is complete. Meniere's disease-like symptoms such as vertigo attacks have only been reported in patients from the Belgian–Dutch families, the present patient, and in a few patients from the Australian family.

No mutations were detected in our 20 sporadic patients diagnosed with Meniere's disease, suggesting that *COCH* mutations may not be a major cause for typical Meniere's disease, at least not for the sporadic form. Among the 23 patients originating from autosomal dominant families analyzed in this study, four had vertigo complaints and the A119T mutation was found in one of these four. No mutations were found in any of the autosomal dominant families without vestibular complaints. The absence of mutations in the hearing loss families without vestibular

symptoms and in Meniere's disease patients indicates that *COCH* mutation screening has little chance of success in these patients, and is best limited to autosomal dominant families with adult onset combined hearing loss and vestibular dysfunction.

Acknowledgements

We thank the families who participated in the present project. We also thank Ms C Kawashima for technical assistance and Ms AC Apple-Mathews for help in preparing the manuscript. This work is supported by the Ministry of Health and Welfare, Japan (SU), a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (SU), the University of Antwerp (GVC), and the Flemish fund for Scientific Research (EF) (GVC).

References

1 Robertson NG, Lu L, Heller S *et al*: Mutations in a novel cochlear gene cause DFNA9, a human nonsyndromic deafness with vestibular dysfunction. *Nat Genet* 1998; 20: 299–303.
2 de Kok YJ, Bom SJ, Brunt TM *et al*: A Pro51Ser mutation in the COCH gene is associated with late onset autosomal dominant progressive sensorineural hearing loss with vestibular defects. *Hum Mol Genet* 1999; 8: 361–366.
3 Fransen E, Verstreken M, Verhagen WI *et al*: High prevalence of symptoms of Meniere's disease in three families with a mutation in the COCH gene. *Hum Mol Genet* 1999; 8: 1425–1429.
4 Fransen E, Verstreken M, Bom SJ *et al*: A common ancestor for COCH related cochleovestibular (DFNA9) patients in Belgium and The Netherlands bearing the P51S mutation. *J Med Genet* 2001; 38: 61–65.
5 Kamarinos M, McGill J, Lynch M, Dahl H: Identification of a novel COCH mutation, I109N, highlights the similar clinical features observed in DFNA9 families. *Hum Mutat* 2001; 17: 351.
6 Verstreken M, Declau F, Wuyts FL *et al*: Hereditary otovestibular dysfunction and Meniere's disease in a large Belgian family is caused by a missense mutation in the COCH gene. *Otol Neurotol* 2001; 22: 874–881.
7 Robertson NG, Skvorak AB, Yin Y *et al*: Mapping and characterization of a novel cochlear gene in human and in mouse: a positional candidate gene for a deafness disorder, DFNA9. *Genomics* 1997; 46: 345–354.
8 Robertson NG, Resendes BL, Lin JS *et al*: Inner ear localization of mRNA and protein products of COCH, mutated in the sensorineural deafness and vestibular disorder, DFNA9. *Hum Mol Genet* 2001; 10: 2493–2500.
9 Ikezono T, Omori A, Ichinose S, Pawankar R, Watanabe A, Yagi T: Identification of the protein product of the Coch gene (hereditary deafness gene) as the major component of bovine inner ear protein. *Biochim Biophys Acta* 2001; 1535: 258–265.
10 Trexler M, Banyai L: Patthy L: The LCCL module. *Eur J Biochem* 2000; 267: 5751–5757.
11 Committee on Hearing and Equilibrium guidelines for the diagnosis and evaluation of therapy in Meniere's disease. American Academy of Otolaryngology-Head and Neck Foundation, Inc. *Otolaryngol Head Neck Surg* 1995; 113: 181–185.
12 Liepinsh E, Trexler M, Kaikkonen A *et al*: NMR structure of the LCCL domain and implications for DFNA9 deafness disorder. *Embo J* 2001; 20: 5347–5353.
13 Koradi R, Billeter M, Wuthrich K: MOLMOL: a program for display and analysis of macromolecular structures. *J Mol Graph* 1996; 14: 51–55, 29–32.
14 Liu XZ, Walsh J, Tamagawa Y *et al*: Autosomal dominant non-syndromoic deafness (DFNA11) caused by a mutation in the myosin VIIA gene. *Nat Genet* 1997b; 17: 268.
15 Akita J, Abe S, Shinkawa H, Kimberling WJ, Usami S: Clinical and genetic features of non-syndromic autosomal dominant sensorineural hearing loss: KCNQ4 is a responsible gene in Japanese. *J Hum Genet* 2001; 46: 355–361.
16 Iwasaki S, Harada D, Usami S, Nagura M, Takeshita T, Hoshino T: Association of clinical features with mutation of TECTA in a family with autosomal dominant hearing loss. *Arch Otolaryngol Head Neck Surg* 2002; 128: 913–917.
17 Khetarpal U, Schuknecht HF, Gacek RR, Holmes LB: Autosomal dominant sensorineural hearing loss: pedigrees, audiologic findings, and temporal bone findings in two kindreds. *Arch Otolaryngol Head Neck Surg* 1991; 117: 1032–1042.
18 Khetarpal U: Autosomal dominant sensorineural hearing loss: further temporal bone findings. *Arch Otolaryngol Head Neck Surg* 1993; 119: 106–108.
19 Merchant SN, Linthicum FH, Nadol Jr JB: Histopathology of the inner ear in DFNA9. *Adv Otorhinolaryngol* 2000; 56: 212–217.

J Hum Genet (2003) 48:564–570
DOI 10.1007/s10038-003-0079-2

ORIGINAL ARTICLE

Satoko Abe · Shin-ichi Usami · Yusuke Nakamura

Mutations in the gene encoding KIAA1199 protein, an inner-ear protein expressed in Deiters' cells and the fibrocytes, as the cause of nonsyndromic hearing loss

Received: 7 July 2003 / Accepted: 31 August 2003 / Published online: 24 October 2003

Abstract We report three possibly disease-causing point mutations in one of the inner-ear-specific genes, *KIAA1199*. We identified an R187C mutation in one family, an R187H mutation in two unrelated families, and an H783Y mutation in one sporadic case of nonsyndromic hearing loss. In situ hybridization indicated that the murine homolog of *KIAA1199* mRNA is expressed specifically in Deiters' cells in the organ of Corti at postnatal day zero (P*n*) P0 before the onset of hearing, but expression in those cells disappears by day P7. The signal of *KIAA1199* was also observed in fibrocytes of the spiral ligament and the spiral limbus through to P21, when the murine cochlea matures. Thus, the gene product may be involved in uptake of potassium ions or trophic factors with a particular role in auditory development. Although the R187C and R187H mutations did not appear to affect subcellular localization of the gene product in vitro, the H783Y mutation did present an unusual cytoplasmic distribution pattern that could underlie the molecular mechanism of hearing impairment. Our data bring attention to a novel candidate for hearing loss and indicate that screening of mutations in inner-ear-specific genes is likely to be an efficient approach to finding genetic elements responsible for deafness.

Keywords cDNA microarray · *KIAA1199* mutations · Nonsyndromic deafness · Deiters' cells · Fibrocytes

Nucleotide sequence data reported herein are available in the DDBJ/EMBL/GenBank databases; for details, see the electronic database section of this article.

S. Abe · Y. Nakamura (✉)
Laboratory of Molecular Medicine,
Human Genome Center,
Institute of Medical Science,
The University of Tokyo,
4-6-1 Shirokanedai,
Minato-ku, Tokyo 108-8639, Japan
E-mail: yusuke@ims.u-tokyo.ac.jp
Tel.: +81-3-54495372
Fax: +81-3-54495433

S. Usami
Department of Otorhinolaryngology,
Shinshu University School of Medicine,
3-1-1 Asahi, Matsumoto 390-8621, Japan

Introduction

Hereditary hearing loss is a highly heterogeneous sensory disorder in genetic terms. So far, more than 70 deafness loci have been mapped on human chromosomes, and 29 genes controlling hearing have been identified (Hereditary Hearing Loss home page). The cochlea is composed of structurally and functionally diverse cell types; many forms of deafness involve defects in the hair cells that work as mechanosensors either directly or indirectly. The deafness genes already identified encode a wide variety of proteins, including gap junctions, ion channels, extracellular matrix proteins, transcription factors, and cell/cell adhesion molecules. Obviously, identification of genes that confer proper function of a cochlear-cell type or site is central to an understanding of the molecular mechanism of normal development and maintenance of hearing.

To supplement the candidate-gene approach based on linkage analyses, alternative strategies for reaching that goal rely on recent developments, such as inner-ear cDNA libraries, RT-PCR tests for candidate genes, and expression profiling of inner-ear genes by microarray technologies to identify genes specifically or preferentially expressed in the ear. These advances can also reduce the number of candidates to be tested within a mapped deafness locus. Using a cDNA microarray for analysis, we recently reported *CRYM* (MIM 123740) as one candidate for nonsyndromic deafness from a set of 52 genes specifically or exclusively expressed in the human inner ear (Abe et al. 2003). More information about genes that are specific to or preferentially expressed in the inner ear will revolutionize our knowledge of the molecular processes involved in auditory function.

In this study, we screened these 52 genes one by one for mutations in patients with hearing loss and identified the disease-associated mutations of KIAA1199 protein

gene (*KIAA1199*), as it shows no significant homology to any known function domain. We also show the unique localization and development of *KIAA1199* in the mouse cochlea.

Materials and methods

Candidate gene selection and cloning of the full-length *KIAA1199*

Among a set of 52 genes specifically or preferentially expressed in the in cochlea and vestibule through cDNA microarray analysis (Abe et al. 2003), we screened each gene using direct sequencing of the coding exons PCR products. We focused on the KIAA1199 protein gene (*KIAA1199*), one of a collection of genes that showed a high level of expression in the inner ear, and it was selected for our further analysis. We established the full-length *KIAA1199* cDNA sequence derived from the original entry XM_051860 by using RT-PCR experiment and direct sequencing. Entry XM_051860 of *KIAA1199* mRNA sequence has been replaced in the GenBank. Sequence of major transcript of *KIAA1199* has been submitted to GenBank (accession number AB103330). To confirm our cDNA microarray data, we performed semiquantitative RT-PCR experiments using cDNAs derived from the vestibule and from the 29 normal tissues using single-round amplified RNAs, as reported previously (Abe et al. 2003). Primers used to amplify specific *KIAA119* products were F: CTAATGCAAGGGTCTCACAC and R: ACCAGCTATTTCGGCAACAG; the PCR proceeded for 30 cycles of 95°C for denaturing, 55°C for annealing, and 72°C for extension. The PCR products of 348 bp lengths were electrophoresed on a 2% agarose gel and visualized by UV light.

Northern-blot analysis

A gel-purified 505 nt cDNA fragment of *KIAA1199* mRNA (GenBank accession number AB103330, nucleotides 6495–6999), the same product as that spotted onto the cDNA microarray slides, was used to probe a northern blot. A human multitissue blot (Clontech, Palo Alto, CA, USA) hybridized with the DNA probe that labeled with random-primed [32P]dCTP using Rediprime DNA labeling system (Amersham Life Science, Cleveland, OH, USA). The condition of hybridization and washing was carried out as previously described (Koyama et al. 1995).

Patients

We described earlier the ascertainment of 192 Japanese patients with or without families in which probands sustained congenital or childhood-onset bilateral nonsyndromic sensorineural hearing loss (Abe et al. 2003). With the exception of hearing impairment, no patient showed untoward neurological signs. Written informed consent was obtained from all family members who participated in this study. Pure-tone audiometry was systematically performed for each individual; in younger children, auditory function was assessed by the auditory brainstem response (ABR) and conditioned orientation reflex audiometry. The control group consisted of 96 unrelated Japanese volunteers whose normal hearing was confirmed by auditory testing.

Mutation screening of *KIAA1199*

We screened DNA of one affected person from each family. The exon/intron structure of *KIAA1199* was generated using a combination of nuclei acid sequence database homology search from NCBI and BAC sequence (GenBank accession number AC027808). Twenty-seven DNA fragments encompassing the 28 coding exons of *KIAA1199* and their flanking sequences were PCR amplified from genomic DNA (10–20 ng) using the 27 primer pairs listed in Table 1. PCR products were sequenced by using 27 additional internal primers (Table 1). Details of the methods for PCR amplification and sequencing analysis were as described elsewhere (Abe et al.2003). PCR products were sequenced using 27 additional internal primers (Table 1).

Table 1 *Primers used in this study. F forward, R reverse, S sequence*

Exon	F	R	S
2	CCCATTCTGTCACTGCCTGTC	CCAACCCCTTCATTTTGTGG	GGCTCTGTTTATCTCCATGTC
3	TCTCTCAGCTCTCTAAGGTC	ACCAAATGCCAACATGAACC	GCCTTAAAGATGCATGGGAG
4	CCTTCAGATACTGAGGGATG	TTACAGGCAAGCTTGCCCTC	ATGGGGAGTGCTTAGGGAGT
5	GTGTGACTGGCTCTATGGTG	AAGGCAGGGATTCCCATCTG	AGCTGTGACTCCTAGGTTC
6	CATGTTAGCTGTCGCAGCAC	CATTTAGGATGGTACGGCTG	GAGAGGAGATGGGAAAGGTA
7	GAATTTCCCCCAAACAGCAG	CTTCCATGTAAGGTGGCCAG	GCCATGCTCTGAGAAAAGTG
8	GATGATCATCTCTTCCCAAG	AGTGGTCCTACTGGTATAAGAG	TGATCATCTCTTCCCAAGTG
9	TGCTTGGAGACAACACTGAG	GCTGGACCCAGGAGAGAATC	CACAGCACCCAAGTGACAAG
10	ATAGTGTTGCAGCAGGGACA	CTGCTCCCGTGTCACAAAAG	AGCACTGTCTCACTGAGAG
11	CTTTACCAAGAGTGGGATGG	GCCATAGCTGACTGATACAG	AGCCAGTGATCTGGGTTTTG
12	GTAACATGAGACCACTGTGC	CTTCTCAAACTTCCAGAAGG	AGGCATGGCGATGAGTAAG
13	ACTGAGTACTAAGTGGAGAC	GGATGGTTCAATCCCCAAAG	CTTTGGAATATGGGCACCAG
14	CCTGGTGTACGGCTGAGACT	GAAGCTGGAGCCGTAGGTGG	TGGGCTGAGCTGAGTGAAC
15	CAGAGTGCCCAACCTGCAC	ACCACCCCCAGTCTGTCGT	GTAAGCCTCGGCTGTCTC
16	TCCTGTGGGTGACGGCAGTAG	GTGCAGGAACCACCACAGGAG	ACCACCACAGGAGATTTGTG
17	TCAGAAGTATGCAGTGAGGC	GTACCCCCAGCAACTAGCTC	TGACTGTGAGACGATGCCTG
18	AGGCAATGCGAATGGGTTTC	CCTGGCACTGTAGGCTGAAG	CCTGGGCACTGTGAGTAGAG
19 and 20	CAGTTTGACTCCAAGGGCAG	ATTCCTCTGGCTGGTAGAAG	GGAAGTGTCCTTCTCTCCAC
21	CCCAGGAAGTCTCTGCTACC	CCCAGCCTAGTTGGAGGAG	GGCAAACATGGCGTTTAGAC
22	CGTCAGTGGAAATCGGAAAC	CTTTCTGGCCTGAAACAGAG	CAGGCATAGAGTAGACACTC
23	GTGCGGTCTATAGTCAGATG	CTGGGTGATGTCAACCCCTC	CTAGCCTGTGGTTTCGTTAG
24	ACTCAGTCACCACGACAGCA	TTGGCATTAACTACATGGCTG	AAAGGCCAAGGGGACAAGAT
25	CGCTGGGTCTACCTGCTATG	GGGTAGTCATTCCCCTCCTC	CTGAGTGAGGGACCTAATTG
26	CAATTAGGTCCCTCACTCAG	AGAAGAGGCATGGAACAGGT	TTTCCTGTGACTGGGGATAC
27	GCCACTATTTGACCCACTAC	GACTGCCTGAGCTTTTCCAG	TAGGCAACTTCTGCCTTCAG
28	CCTCTGGGCACTGCTAACTC	GCAGTGCAGGGGTGGTAAAC	CCCACGTTTAGTACAAAGC
29	CACATGTCAGGCACCATCAG	CACTGGTCTGCTGCCAAGA	AGCTGAGCACAGAGCTCTTC

Cloning of mouse homolog of *KIAA1199*

To determinate mouse *KIAA1199* cDNA sequence, human *KIAA1199* cDNA sequence was aligned to publicly available mouse cDNA sequences through the BLAST searches of the NCBI database. Four ESTs, BB62981, BB620830, AY007815, and NM_030728, have shown high identity with more than 85% of nucleotides. The complete mouse *KIAA1199* homolog cDNA sequence was determined and confirmed by ESTs connection PCR and direct sequencing, and have been submitted to GenBank (GenBank accession number AB103331).

In situ hybridization

A 388 nt fragment spanning part of the murine counterpart of *KIAA1199* (GenBank accession number AB103331, nucleotides 3721–4108), was cloned into pBlueScript SK(-) vector (Stratagene, Cedar Creek, TX, USA). Probes were prepared by in vitro transcription of linealized plasmid using T7 polymerase for the generation of sense and antisense RNA probes. Mice at postnatal days (P*n*) P0, P7, and P21 were dissected prior to fixation in10% neutral formalin. Tissues were embedded in paraffin, and then tissue sectioning was carried out through the midmodiolar plane to provide for the well-defined morphological structure of the auditory apparatus. Serial tissue sections (6 μm) were hybridized with digoxigenin-labeled RNA probes as described before.

Generation of a HA-tagged *KIAA1199* protein construct and mutagenesis

Wild-type *KIAA1199* cDNA fragment by adding a 3'-tag encoding the HA epitope, excluding the predicted signal peptide (codon, 1–30), was generated by PCR using the following primers: sense 5'-CGTGGTACCCAGACCGTGCATCATGG GAGCTGCTGG-GAGGCAGGACTTC -3', and antisense 5'-AAGCTCGAGT-CAAGCGTAGTCTGGGACGTCGTATGGGTACAACTTCTT CTTCTTCACCACAGG –3', containing KpnI and XhoI sites. This amplicon was purified and digested with KpnI and XhoI, and subcloned into pCDNA 3.1(+) mammalian expression vector (Invitrogen, San Diego, CA, USA). Three R187C, R187H, and H783Y mutants were created by an inverse PCR reaction using 5'phosphorelated primers with their 3'ends facing away each other, containing the desired mutations. PCR was performed using KOD(+) DNA polymerase (TOYOBO, Tokyo, Japan) on wild-type KIAA1199 plasmid template. To circularize, the DNA fragments were ligated. R187C primers: F 5'- GTGGAGTTATT GTTCATGTCATCGACC-3', R 5'- AGTGGCCCCAGCT CCTTTCA-3'; R187H primers: F 5'-TGGAGTTATTGTT CATGTCATCGAC-3', R 5'-TGGTGGCCCCAGCTCCTTT-CAA-3'; H783Y primers: F 5'-TACTTCATTGCCTA CAA-GAACCAGGACCA-3', R 5'-TCTGATGAT GGCCGGCTCC CGGGGCT-3'. All constructs were then verified by direct sequencing.

Immunofluorescence

Transfections were performed on COS-7 cells using FuGENE6 (Roche Molecular Biochemicals, Indianapolis, IN, USA) diluted in OPTIMEM medium (Gibco BRL, Grand Island, NY, USA) according to the manufacturer instructions. 5×10^4 cells cultured on in 2-well glass chamber slides were transfected with 1 μg of each construct for immunofluorescence experiments. At 24 h, 48 h, 72 h, and 96 h after transfection, cells were fixed and treated as described elsewhere (Tsujikawa et al. 1999). They were first incubated overnight at 4°C with mouse anti-HA tag antibody (1:1000) (Santa Cruz Biotechonology, USA), followed by the FITC-conjugated goat anti-mouse IgG (1:3000) (ICN/Cappel, Costa Mesa, CA, USA) for 60 min at 25°C. Slides were examined by means of fluorescence microscopy.

Results

Expression of *KIAA1199*

KIAA1199 (GenBank accession numbers XM_051860 and AB103330) showed a high level of expression in the inner ear (Cy3/Cy5 ratio: 56.54 in the cochlea, 22.55 in the vestibule) by cDNA microarray analysis (Abe et al. 2003). We confirmed predominant expression of *KIAA1199* in the inner ear by means of semiquantitative RT-PCR experiments (data not shown). Northern-blot analysis of multiple human tissues using a partial *KIAA1199* cDNA probe yielded a transcript approximately 7 kb long that was expressed only in prostate and testis (data not shown).

Genomic and predicted protein structure of *KIAA1199*

Comparison of cDNA sequences (GenBank accession number AB103330) with archived genomic sequences (GenBank accession number AC027808) revealed that the gene spans approximately 170 kb and consists of 29 exons (exons 2–29 corresponding to the coding elements). The cDNA sequence contains a predicted 4083-bp ORF encoding a 1361-aa protein, and the PSORT II program (http://psort.ims.u-tokyo.ac.jp/form2.html) predicted that its N-terminal 30 amino acid portion was a cleavable signal peptide. The molecular weight of the mature 1331 amino acid protein was calculated to be 150 kDa. A BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) revealed 38% identity (63% similarity) to transmembrane protein 2 (TMEM2; GenBank accession number NP_037522, MIM 605835), which has a wide tissue distribution that includes human fetal cochlea (Scott et al. 2000). Part of the gene product (aa 55–155) revealed 38% identity (57% similarity) to PKHD1 protein (fibrocystin; GenBank accession number NP_733842), whose mutant forms are responsible for polycystic kidney and hepatic disease 1 (MIM 606702). Analysis with the ProDom program (http://prodes.toulouse.inra.fr/prodom/2002.1/html/home.php) identified one region of homology (aa 187–278) with O-linked mannose beta1,2-N-acetylglucosaminyltransferase (UDP-GLCNAC; GenBank accession number NP_001373), and with FAM3-family proteins such as human FAM3A (GenBank accession number P98173) and 3B (GenBank accession number P58499), mouse FAM3B (GenBank accession number Q9D309), human FAM3C (GenBank accession number Q92520) and 3D (GenBank accession number AAH15359), and hypothetical protein M70.4 of *Caenorhabditis elegans* (GenBank accession number AAK71390). No apparent functional domain or motif was predicted by any of the multiple computer programs we invoked.

Table 2 Variations detected in the coding region of *KIAA1199*

Exon	Nucleotide change	Amino acid change	Allele frequency in patients	Allele frequency in control	Comparison with mouse homolog
5	559 C → T	R187C	1/ 384	0/192	Conserved
5	560 G → A	R187H	2/384	0/192	Conserved
13	1594C → T	L532L	1/384	1/192	
14	1857 G → A	P619Pr	1/384	ND	
18	2348A → G	H783R	28/384	10/192	
18	2347 C → T	H783Y	1/384	0/192	Conserved
18	2399 T → C	D800D	2/384	ND	
24	3327 A → G	V1109I	1/384	0/192	Not conserved
25	3507 C → G	P1169A	2/360	2/192	

Table 3 Variations detected in the intronic region of *KIAA1199*

Nucleotide change	Allele frequency in patients	Allele frequency in control
IVS1 + 185 A → G	194/372	ND
IVS3 + 112 G → A	178/372	ND
IVS6 −109 G → T	158/372	ND
IVS7 −61 A → G	42/344	ND
IVS12 + 57 A → G	94/368	0.3240 (JSNP data)
IVS16 + 133 A → C	77/366	ND
IVS25 ins 27 bp	9/360	5/192
IVS26 + 63 C → G	78/360	ND

KIAA1199 mutations analyzed in this study

We identified nine DNA variants, including six that would alter the predicted amino acid sequence of *KIAA1199*, as summarized in Table 2: six missense alterations (R187C, R187H, H783R, H783Y, V1109I, and P1169A) and three synonymous substitutions (L532L, P619P, and D800D). We observed no R187C, R187H, H783Y, or V1109I in any of the 192 chromosomes from the control group without hearing loss, but R187H was found in two unrelated families with hearing-impaired members, suggesting that four missense (R187C, R187H, H783R, or V1109I) mutations may be potentially disease causing. We also detected eight SNPs in intronic regions (Table 3). To clarify whether pathogenic changes might result from rare genetic variations, we investigated segregation in some members of family 69. In family 69 (Fig. 1A), an R187C allele was derived from the unaffected mother and an L532L synonymous substitution from the unaffected father. The DNA sample from other family members was not available.

Distribution of *KIAA1199* mRNA in the developing murine cochlea

At P0, the immature period of the organ of Corti in the mouse, *KIAA1199* mRNA was localized to the fibrocytes of the spiral ligaments and the spiral limbus in the expansive connective tissue region surrounding perilymph and endolymph (Fig. 2a). In addition, *KIAA1199* mRNA was expressed at the Deiters' cells, which are supporting structures of outer hair cells (OHCs) at their basal pole, as shown in Fig. 2b. *KIAA1199* signal in the Deiters' cells observed at P0 was not present by P7, before the onset of hearing (data not shown). The widespread signal of *KIAA1199* mRNA at the connective tissue elements has been restricted, but its expression in the lateral region of the spiral ligament and the fibrocytes of the spiral limbus was maintained at P7 and P21 (data not shown).

Subcellular localization study

To evaluate the effect of three nonsynonymous amino acid changes (R187C, R187H, and H783Y) on functional consequences of these three amino acid changes in the different areas of the protein structures, we performed transiently transfection experiments in the COS-7 cells. At all time points examined after transfection (24 h, 48 h, 72 h, and 96 h, respectively), wild-type KIAA1199 protein was localized predominantly in the cytoplasmic region with some scattered spaces (Fig. 3a). The localization of the R187C and R187H mutants were very similar to that of the wild-type protein (data not shown). The remaining H783Y mutant also showed the cytoplasmic distributed pattern, but the edge and surface showed an irregular and worm-eaten pattern in most transfected cells (Fig. 3b). In cellular extracts of transfected cells with normal and three mutated proteins, ~150 kDa bands representing full-length KIAA1199 protein were detected by western blots using an anti-HA antibody (data not shown).

Discussion

We screened these 52 genes in this series for mutations in patients with hearing loss. In those experiments, *KIAA1199* showed a high level of expression in the inner ear by cDNA microarray analysis (Abe et al. 2003). Although *KIAA1199* mRNA is also expressed in the fetal cochlea (Human Cochlear cDNA library and EST database), we did not initially focus on this gene, because its function was unknown and its physical location at chromosome 15q24 was not among the previously known deafness loci. However, since our microarray and RT-PCR data indicated preferential expression of this gene in the inner ear, we selected it for further analysis.

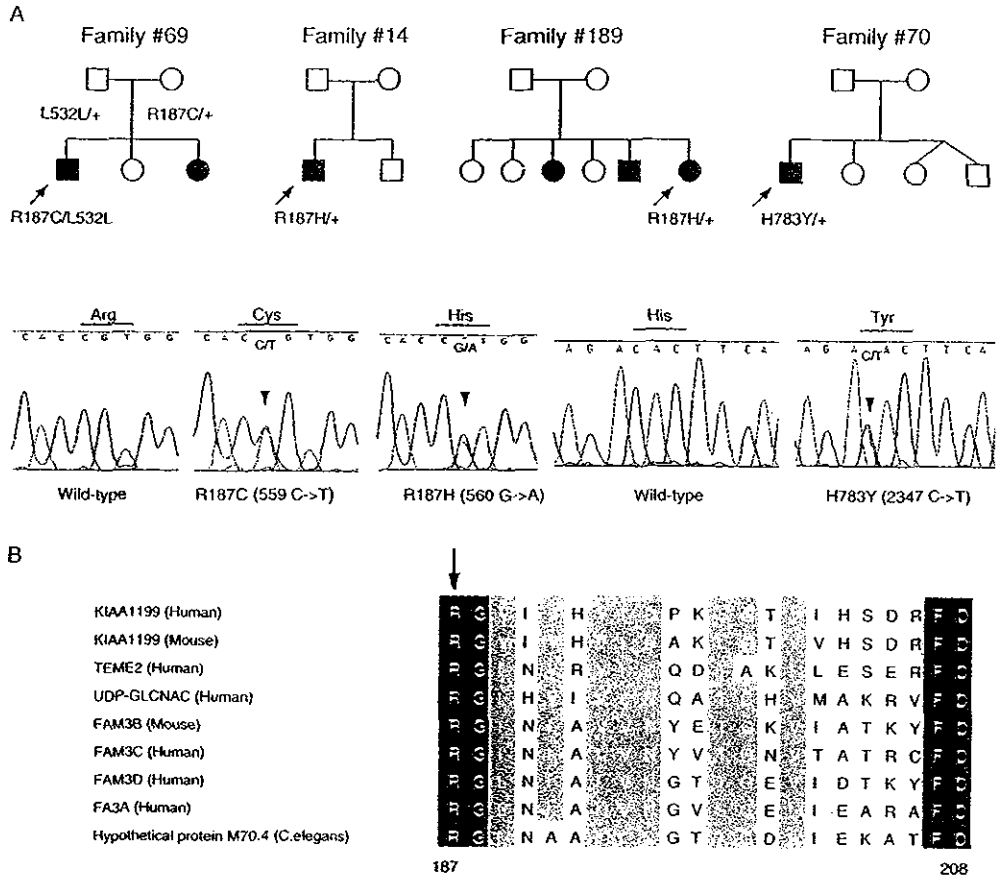

Fig. 1A, B Detection of *KIAA1199* mutations. **A** Three missense mutations in four probands (*arrows*), two with family history (families 69 and 189) and two sporadic cases with nonsyndromic hearing loss (families 14 and 70). Sequence chromatograms from wild-type protein are shown. The proband in family 69 and his mother carried a heterozygous 559 C->T transition in exon 5, which results in an arginine to cysteine change (R187C). Another variation, 1594C->T (L532L), was derived from his father. Probands in families 14 and 189 are heterozygous for 560 G->A in exon 5, which replaces arginine with histidine (R187H). The patient in family 70 is heterozygous for a 2347 C->T change in exon 18, which substitutes tyrosine for histidine (H783Y). Since DNA samples were not available from any other family members, we were unable to examine the segregation of mutations any further. **B** Alignment of KIAA1199 protein, TMEM2, FAM3-family proteins, UDP-GLCNAC, and hypothetical protein M70.4 of *Caenorhabditis elegans* by means of the ProDom program. Residues between codons 187 and 208 are indicated. An *arrow* indicates the position of the R187 codon

In the present study, we identified nine DNA variants in the KIAA1199 protein gene (Table 2). Segregation study in family number 69 showed that an R187C allele was derived from the unaffected mother and an L532L synonymous substitution from the unaffected father (Fig. 1A). Since DNA samples were not available from any other family members, we were unable to examine the segregation of mutations any further. Hence, to assess the significance of the missense substitutions found in our study population, we examined whether the substitutions had occurred at amino acid residues that were conserved in the murine homologue of *KIAA1199* (GenBank accession number AB103331). Among fo missense (R187C, R187H, H783Y, or V1109I) mut tions, V1109I mutation was not conserved between h man and mouse sequence (Table 2); therefore, it seen unlikely to be a disease-causing mutation.

Although the H783R mutation also occurred in t conserved H783 codon, it was observed in ten of the 1 alleles from individuals with normal hearing (Table 2 suggesting that this replacement represent a comm polymorphism. On the other hand, the R187 residue widely conserved among FAM3-family proteins, as w as in UDP-GLCNAC, TMEM2, and hypothetical pr tein (*Caenorhabditis elegans*). This conservation ov evolutionarily distant species (Fig. 1B) suggests th substitutions at R187 could significantly affect t function of KIAA1199 protein. Phenotypically, the wi variation in the onset of hearing loss and the shape a severity of the audiograms was seen in affected indivi uals harboring each of three mutations (Table 4). No of them showed abnormal vestibular symptoms.

To investigate the biological significance of this ge product in the auditory system, we performed in si hybridization experiments using murine mRNA. In t mouse, the counterpart of *KIAA1199* is widely expressi in the fibrocytes of the spiral ligament and the spi limbus at P0 (Fig. 2a). These cell types, rich in io transporting molecules, are involved in generating e docochlear potential. At P7, after maturation but befo the onset of hearing, and at P21, the expansive sig

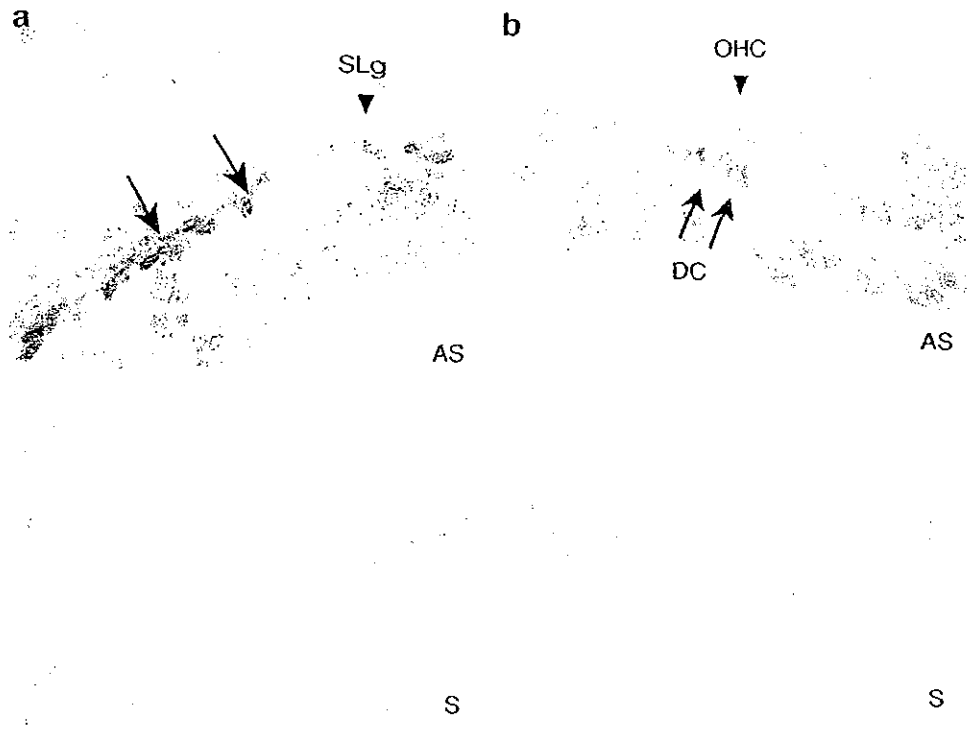

Fig. 2a, b In situ hybridization of *KIAA1199* mRNA in the mouse cochlea at postnatal day (P*n*) P0. Probes were prepared from a 388 nucleotide fragment spanning part of the murine counterpart of *KIAA1199* and were labeled with digoxigenin. *AS* antisense probe, *S* sense probe. Images were obtained at a magnification of 400×. **a** Localization of *KIAA1199* mRNA in the expansive connective tissue region surrounding perilymph and endolymph (*arrows*). *SLg* spiral ligaments (*arrowhead*). **b** Specific expression of mouse *KIAA1199* mRNA in Deiters' cells (*DC, arrow*), which are supporting structures for outer hair cells (*OHCs, arrowhead*) at their basal poles

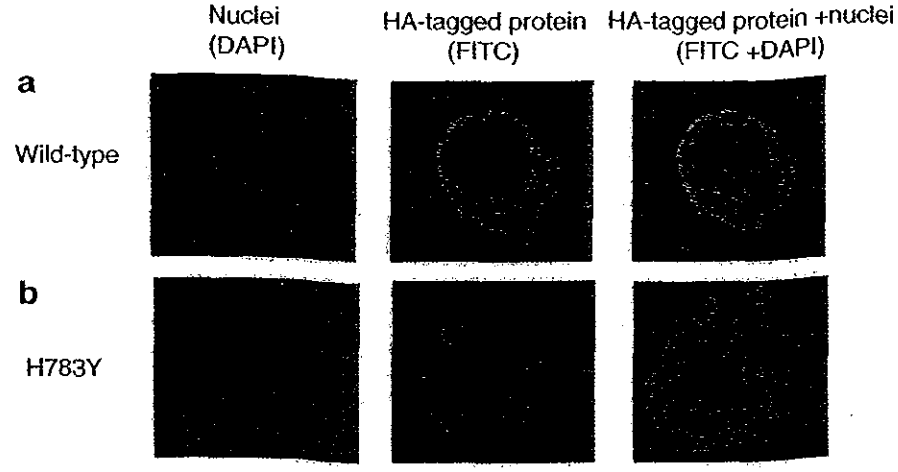

Fig. 3a, b Subcellular localization of KIAA1199 protein in COS-7 cells transiently transfected with wild-type (**a**) or H783Y (**b**) KIAA1199 proteins with HA-tags at their C-termini. Nuclei are counterstained with DAPI (*blue*). **a** Localization of wild-type protein (*green*) in cytoplasm. **b** Representative pattern of the irregular and worm-eaten appearance of the edge and surface observed in 80% of the cells transfected with the H783Y mutant

Table 4 Phenotype and genotype of patients harboring *KIAA1199* mutations

Family number	Mutations	Inherited form	Deafness onset	Severity	Progression of hearing loss	Audiogram pattern
69	R187C	Recessive	Congenital	Profound	–	Symmetric, all frequencies
14	R187H	Simplex	6 years	Moderate	+	Asymmetric, all frequencies
189	R187H	Recessive	Postlingual	Mild	+	Symmetric, mid-high frequency
70	H783Y	Simplex	Postlingual	Moderate	+	Symmetric, mid-high frequency

was confined, but its expression was maintained in the lateral region of the spiral ligament and in fibrocytes of the spiral limbus (data not shown). Maintenance of expression through to the cochlea-matured stage implies that KIAA1199 protein might play a key role in ion homeostasis. We detected a transient signal of *KIAA1199* mRNA in Deiters' cells at day P0 (Fig. 2b), but those signals were no longer observed at P7 (data not shown).

Unlike humans, mice are unable to hear at birth; the first two postnatal weeks are critical for inner-ear development in mice (Rybak et al. 1992). Deiters' cells, a type of supporting structure in the epithelium of the organ of Corti, are thought to maintain the optimal set

point of cochlear amplifiers, such as OHCs or stereocilia to account for the remarkable sensitivity of the cochlea to sound (Flock et al. 1999). Moreover, since Deiters' cells are closely apposed to the OHCs, extending the phalangeal process apically to contribute to the reticular lamina, these cells may also play an important role as a trophic intermediary for the OHCs (Mothe and Brown 2001). In addition to mechanical and trophic support, these cells contribute to uptake of potassium ions from sensory hair cells and to diffusion of K^+ through gap junctions to adjacent supporting cells and fibrocytes (Boettger et al. 2002). Therefore, specific expression in Deiters' cells within the organ of Corti suggests that KIAA1199 protein may mediate a cochlear epithelial network involving potassium, or expedite passage of other neurotrophic factors between OHCs and the supporting cells before the onset of hearing. Even though no KIAA1199 mRNA is expressed in sensory hair cells, *KIAA1199* mutations may induce perturbation of sensory epithelia and lead to auditory deficits; this hypothesis is consistent with the early appearance of Deiters' cells during development.

We then investigated functional consequences of three nonsynonymous amino acid changes (R187C, R187H, and H783Y) occurring at conserved residues, none of which were detected in any normal controls, by forcing the expression of normal or mutated proteins in COS-7 cells. At all time points examined after transfection (24 h, 48 h, 72 h, and 96 h, respectively), wild-type KIAA1199 protein was localized in the cytoplasmic region with some scattered spaces (Fig. 3a). R187C and R187H mutants indicated patterns very similar to that of the wild-type protein (data not shown), excluding the possibility that the primary detrimental effect was caused in these cases by aberrant subcellular distribution. Therefore, we speculate that both mutations may instead interfere with interaction and cooperation with unknown binding partners. In contrast, the H783Y mutant showed an irregular and worm-eaten pattern in most transfected cells (Fig. 3b), suggesting that incorrect cytoplasmic localization of the H783Y mutant may underlie a molecular process leading to hearing loss.

We have provided here the first report that KIAA1199 protein may be essential for auditory function and that its mutated forms may cause nonsyndromic hearing loss. The mode of inheritance in our test families indicated either autosomal-recessive transmission or simplex cases; we were unable to detect any pathogenic mutations in the other *KIAA1199* allele of any affected individual. Possible explanations for penetrance in cases harboring just one missense change include: (1) a second mutation outside the coding sequence, perhaps in an intron or promoter (Lerer et al. 2001), or (2) de novo occurrence in simplex cases. Alternatively, mutations in other genes may be impairing auditory function on their own (Abe et al. 2001; de Brouwer et al. 2003), although the *GJB2* gene (MIM 121011) and the mitochondrial gene encoding *12S rRNA* (MIM 561000) have already been screened and excluded from this premise (Abe et al. 2003). A full understanding of the consequences of each mutation reported here will require additional study, as will efforts to determine the precise mechanisms operating in the pathophysiology of hearing loss associated with *KIAA1199* mutations.

Acknowledgements We thank all of the families that participated in this study. We are also grateful to Yumi Kondoh for help with direct sequencing of DNA. This work was supported in part by Research for the Future Program Grant #00L01402 from the Japan Society for the Promotion of Science.

References

Abe S, Kelley PM, Kimberling WJ, Usami SI (2001) Connexin 26 gene (GJB2) mutation modulates the severity of hearing loss associated with the 1555A–>G mitochondrial mutation. Am J Med Genet 103:334–338

Abe S, Katagiri T, Saito-Hisaminato A, Usami S, Inoue Y, Tsunoda T, Nakamura Y (2003) Identification of CRYM as a candidate responsible for nonsyndromic deafness, through cDNA microarray analysis of human cochlear and vestibular tissues. Am J Hum Genet 72:73–82

Boettger T, Hubner CA, Maier H, Rust MB, Beck FX, Jentsch TJ (2002) Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. Nature 416:874–878

de Brouwer AP, Pennings RJ, Roeters M, Van Hauwe P, Astuto LM, Hoefsloot LH, Huygen PL, van den Helm B, Deutman AF, M Bork J, Kimberling WJ, Cremers FP, Cremers CW, Kremer H (2003) Mutations in the calcium-binding motifs of CDH23 and the 35delG mutation in GJB2 cause hearing loss in one family. Hum Genet 112:156–163

Flock Å, Flock B, Fridberger A, Scarfone E, Ulfendahl M (1999) Supporting cells contribute to control of hearing sensitivity. J Neurosci 19:4498–4507

Koyama K, Sudo K, Nakamura Y (1995) Isolation of 115 human chromosome 8-specific expressed-sequence tags by exon amplification. Genomics 26:245–253

Lerer I, Sagi, M, Ben-Neriah Z, Wang T, Levi H, Dvorah A (2001) A deletion mutation in GJB6 cooperating with a GJB2 mutation in *trans* in non-syndromic deafness: a novel founder mutation in Ashkenazi Jew. Hum Mut

Mothe AJ, Brown IR (2001) Expression of mRNA encoding extracellular matrix glycoproteins SPARC and SC1 is temporally and spatially regulated in the developing cochlea of the rat inner ear. Hear Res 155:161–174

Rybak LP, Whitworth C, Scott V (1992) Development of endocochlear potential and compound action potential in the rat. Hear Res 59:189–194

Scott DA, Drury S, Sundstrom RA, Bishop J, Swiderski RE, Carmi R, Ramesh A, Elbedour K, Srikumari Srisailapathy CR, Keats BJ, Sheffield VC, Smith RJ (2000) Refining the DFNB7-DFNB11 deafness locus using intragenic polymorphisms in a novel gene, TMEM2. Gene 246:265–274

Tsujikawa M, Kurahashi H, Tanaka T, Nishida K, Shimomura Y, Tano Y, Nakamura Y (1999) Identification of the gene responsible for gelatinous drop-like corneal dystrophy. Nat Genet 21:420–423

European Journal of Human Genetics (2003) 11, 916–922

www.nature.com/ejhg

ARTICLE

Distribution and frequencies of *PDS* (*SLC26A4*) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese

Koji Tsukamoto[1], Hiroaki Suzuki[1], Daisuke Harada[1], Atsushi Namba[2], Satoko Abe[3] and Shin-ichi Usami*[,1]

[1]*Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan;* [2]*Department of Otorhinolaryngology, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan;* [3]*Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan*

Molecular diagnosis makes a substantial contribution to precise diagnosis, subclassification, prognosis, and selection of therapy. Mutations in the *PDS (SLC26A4)* gene are known to be responsible for both Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct, and the molecular confirmation of the *PDS* gene has become important in the diagnosis of these conditions. In the present study, *PDS* mutation analysis confirmed that *PDS* mutations were present and significantly responsible in 90% of Pendred families, and in 78.1% of families with nonsyndromic hearing loss associated with enlarged vestibular aqueduct. Furthermore, variable phenotypic expression by the same combination of mutations indicated that these two conditions are part of a continuous category of disease. Interestingly, the *PDS* mutation spectrum in Japanese, including the seven novel mutations revealed by this study, is very different from that found in Caucasians. Of the novel mutations detected, 53% were the H723R mutation, suggesting a possible founder effect. Ethnic background is therefore presumably important and should be noted when genetic testing is being performed. The *PDS* gene mutation spectrum in Japanese may be representative of those in Eastern Asian populations and its elucidation is expected to facilitate the molecular diagnosis of a variety of diseases.

European Journal of Human Genetics (2003) **11**, 916–922. doi:10.1038/sj.ejhg.5201073
Published online 24 September 2003

Keywords: *PDS* (*SLC26A4*); Pendred syndrome; enlarged vestibular aqueduct; Japanese; founder effect

Introduction

The Pendred syndrome is defined as an autosomal recessive disorder characterized by congenital sensorineural hearing loss and goiter.[1] It is supposed that Pendred syndrome, the most common form of syndromic deafness, is as frequent as 7.5–10 per 100 000 persons and accounts for about 10% of hereditary hearing impairment.[2,3] In spite of such a high frequency, the precise clinical diagnosis has long been hampered by the variable phenotypes, the degree of involvement of goiter, appearance from congenital to complete absence, and the perchlorate discharge test

*Correspondence: Dr S Usami, Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. Tel: 81-263-37-2666; Fax: 81-263-36-9164; E-mail: usami@hsp.md.shinshu-u.ac.jp

which is nonspecific to Pendred syndrome.[3] However, the recent identification of the *PDS* gene[4] as being responsible for Pendred syndrome enables us to perform molecular evaluation of this syndrome. Simultaneously, the *PDS* gene has also been reported to cause 'nonsyndromic hearing loss with enlarged vestibular aqueduct (EVA)',[5] suggesting that the *PDS* gene may cover a wider range of diseases from typical Pendred syndrome to nonsyndromic hearing loss with EVA. Therefore, molecular genetic testing is becoming more important to diagnose this category of disease caused by the *PDS* gene.

The frequencies and nature of mutations are known to be influenced by ethnic differences and geographic isolation. Although much is known regarding the association of *PDS* mutations with deafness in populations of European descent, data about the ethnic groups that form the majority of the world's population is scarce. In the present study, screening of *PDS* mutations was carried out to confirm their contribution to the deafness in patients diagnosed with Pendred syndrome or nonsyndromic hearing loss with EVA, and to reveal the spectrum of the *PDS* gene in Japanese.

Materials and methods

A total of 10 Japanese families, in which some individuals had been diagnosed with Pendred syndrome, and 32 Japanese families, in which some individuals had bilateral sensorineural hearing loss associated with EVA but without goiter, participated in the current study. We defined Pendred syndrome patients as those having either a palpable goiter or abnormal perchlorate discharge. Computerized tomography scan was used to diagnose EVA patients (according to the criteria of EVA: a diameter greater than 1.5 mm at the midpoint between the common crus and the external aperture), and they were clinically well characterized by repeated auditory examinations and long follow-up periods. Briefly, the EVA patients had congenital, high frequency involved, fluctuating, sometimes progressive, sensorineural hearing loss.[6,7] There were no cases of Mondini malformation (cochlear hypoplasia) of the inner ear in the current subjects.

DNA samples from 96 unrelated Japanese, who had normal hearing, were used as controls. All participants gave informed consent for genetic analysis.

Mutation analysis

Intronic polymerase chain reaction (PCR) amplification primers flanking each exon[4] were used to detect mutations. Exons 1–21 of *PDS* were amplified from genomic DNA samples by PCR. A 5-min denaturation at 95°C was followed by 37 three-step cycles (95°C for 30 s, 55°C for 1 min, 72°C for 1 or 3 min), followed by 72°C for 10 min, and ending with a holding period at 4°C in a Perkin-Elmer thermal cycler. The PCR products were directly sequenced after removal of unincorporated dNTPs and primers by incubation at 37°C for 30 min with 50–100 ng PCR product with 0.1 μl exonuclease I (Amersham Life Science, Cleveland, USA) and 1 μl shrimp alkaline phosphatase (Amersham Life Science). The enzymes were heat-inactivated at 80°C for 15 min. An aliquot of 6 pmol of either the forward or the reverse primer was used in standard cycle sequencing reactions with ABI Big Dye terminators, and run on an ABI 377 sequencer.

Results

Table 1[5,8–23] summarizes all reported *PDS* mutations including the 18 mutations detected in the affected families in this study, which were 10 missense mutations, one stop mutation, four frameshift mutations, and three splice site mutations. The evolutionary conservation of the amino acids affected by the missense mutations, on the basis of the alignment of the closely related mouse pendrin protein and rat pendrin protein, are shown in Table 1 and Figure 1. All 10 missense mutations had occurred at the conservative residues among humans, mice, and rats (Figure 1). Mutations were identified in nine out of the 10 typical Pendred families (90%) and 25 out of the 32 families with sensorineural hearing loss associated with EVA (78.1%). Mutations were either homozygous, compound heterozygous, or heterozygous but with no other mutations detectable (Table 2).

Missense mutations

The 10 missense mutations detected were: P123S (367C>T in exon 4); M147V (439A>G in exon 5); K369E (1105A>G in exon 9); A372V (1115C>T in exon 9); N392Y (1174A>T in exon 10); C565Y (1694G>A in exon 15); S657N (1970G>A in exon 17); S666F (1997C>T in exon 17); T721M (2162C>T in exon 19), and H723R (2168A>G in exon 19).

Stop mutation

An 1829C>A substitution was found in exon 17, leading to S610X.

Frameshift mutations

Four frameshift mutations were detected: 322delC in exon 4 causing a frameshift at codon 108 and leading to a stop codon at position 139; 917delT in exon 7 causing a frameshift at codon 307 and leading to a stop codon at position 308; 1652insT in exon 15 causing a frameshift at codon 551 and leading to a stop codon at position 556; and a small 2111 insertion of GCTGC in exon 19 causing a frameshift leading to an amino-acid sequence change from codon 704, followed by a stop at codon 722.

Table 1 All reported PDS mutations (including the current ones)

Exon	*Nucleotide change*	*Amino-acid change*	*Japanese*	*Evolutionary conservation*	*Reference*
2	85G>C	E29Q		Yes	Campbell *et al*[8]
3	IVS2-2A>G	Splice acceptor		NA	Lopez-Bigas *et al*[9]
3	279delT	Stop at 96		NA	Kopp *et al*[10]
4	314A>G	Y105C		Yes	Campbell *et al*[8]
4	317C>A	A106D		Yes	Campbell *et al*[8]
4	322delC	Stop at 139	○	NA	This study
4	336insT	Stop at 180		NA	Coyle *et al*[11]
4	349C>T	L117F		Yes	Reardon *et al*[12]
4	367C>T	P123S	○	Yes	This study
4	395C>T	T132I		Yes	Lopez-Bigas *et al*[9]
4	406delTCTCA	Stop at 179		NA	Lopez-Bigas *et al*[9]
4	412G>T	z138z		Yes	Van Hauwe *et al*[13]
4	IVS4+7A>G	Splice donor		NA	Lopez-Bigas *et al*[9]
5	416G>C	G139A		Yes	Van Hauwe *et al*[13]
5	439A>G	M147V	○	Yes	This study
5	580C>T	T193I		Yes	Adato *et al*[15]
6	IVS5-1G>A	Splice acceptor	○	NA	This study
6	626G>T	G209V		Yes	Van Hauwe *et al*[13]
6	707T>C	L236P		Yes	Van Hauwe *et al*[13]
6	753delCTCT	Stop at 286		NA	Coyle *et al*[11]
6	754T>C	S252P		Yes	Park *et al*[16]
7	IVS6-2A>G	Splice acceptor		NA	Coucke *et al*[17]
7	783insT	Stop at 286		NA	Campbell *et al*[8]
7	811G>C	D271H		Yes	Van Hauwe *et al*[13]
7	917delT	Stop at 308	○	NA	Usami *et al*[5]
7	IVS7+1G>A	Splice donor		NA	Van Hauwe *et al*[13]
8	IVS7-2A>G	Splice acceptor	○	NA	Coucke *et al*[17]
8	IVS8+1G>A	Splice donor	○	NA	Coyle *et al*[11]
9	IVS8-2A>G	Splice acceptor		NA	Yong *et al*[18]
9	1003T>C	F335L		Yes	Campbell *et al*[8]
9	1105A>G	K369E	○	Yes	Usami *et al*[5]
9	1115C>T	A372V	○	Yes	Usami *et al*[5]
9	1146delC	Stop at 394		NA	Van Hauwe *et al*[13]
10	1151A>G	E384G		Yes	Coyle *et al*[11]
10	1174A>T	N392Y	○	Yes	Park *et al*[16]
10	1181delTCT	S394del		NA	Yong *et al*[18]
10	1197delT	Stop at 431		NA	Everett *et al*[4]
10	1226G>A	R409H		Yes	Van Hauwe *et al*[13]
10	1229C>T	T410M	○	Yes	Coyle *et al*[11]
10	1246A>C	T416P		Yes	Van Hauwe *et al*[13]
11	1284delTGC	A429del		NA	Coyle *et al*[11]
11	1334T>G	L445W		Yes	Van Hauwe *et al*[13]
11	1337A>G	Q446R		Yes	Reardon *et al*[12]
12	1334insAGTC	Stop at 467		NA	Coyle *et al*[11]
12	1341delG	Stop at 453		NA	Everett *et al*[4]
13	1439T>A	V480D		Yes	Scott *et al*[19]
13	1468A>C	I490L		Yes	Li *et al*[20]
13	1489G>A	G497S		Yes	Li *et al*[20]
13	1523C>A	T508N		Yes	Bogazzi *et al*[21]
13	1536delAG	Stop at 524		NA	Coyle *et al*[11]
13	IVS13+9C>G	Splice donor		NA	Yong *et al*[18]
14	1584insC	Stop at 526		NA	Park *et al*[16]
14	1588T>C	Y530H		Yes	Coyle *et al*[11]
15	IVS14-7A>G	Splice acceptor		NA	Park *et al*[16]
15	1652insT	Stop at 556	○	NA	Namba *et al*[22]
15	1666T>C	Y556H		Yes	Lopez-Bigas *et al*[9]
15	1667A>G	Y556C		Yes	Coyle *et al*[11]
15	1694G>A	C565Y	○	Yes	Van Hauwe *et al*[13]
16	1790T>C	L597S		Yes	Campbell *et al*[8]
17	1829C>A	S610X	○	NA	This study
17	1898delA	Stop at 634		NA	Van Hauwe *et al*[13]
17	1958T>C	V653A		Yes	Scott *et al*[19]
17	1970G>A	S657N	○	Yes	This study
17	1997C>T	S666F	○	Yes	This study
17	2000T>G	F667C		Yes	Everett *et al*[4]
17	2015G>A	G672E		Yes	Coyle *et al*[11]

Table 1 Continued

Exon	Nucleotide change	Amino-acid change	Japanese	Evolutionary conservation	Reference
17	2027T>A	L676Q		Yes	Park *et al*[16]
17	2111*ins*GCTGG	Stop at 722	O	NA	Usami *et al*[5]
19	2127delT	Stop at 719		NA	Coyle *et al*[11]
19	2162C>T	T721M	O	Yes	Usami *et al*[5]
19	2168A>G	H723R	O	Yes	Van Hauwe *et al*[13]
19	2182insG	Y728X		NA	Fugazzola *et al*[23]
21	2343A>G	X781W		NA	Lopez-Bigas *et al*[9]

O: mutations found in Japanese.

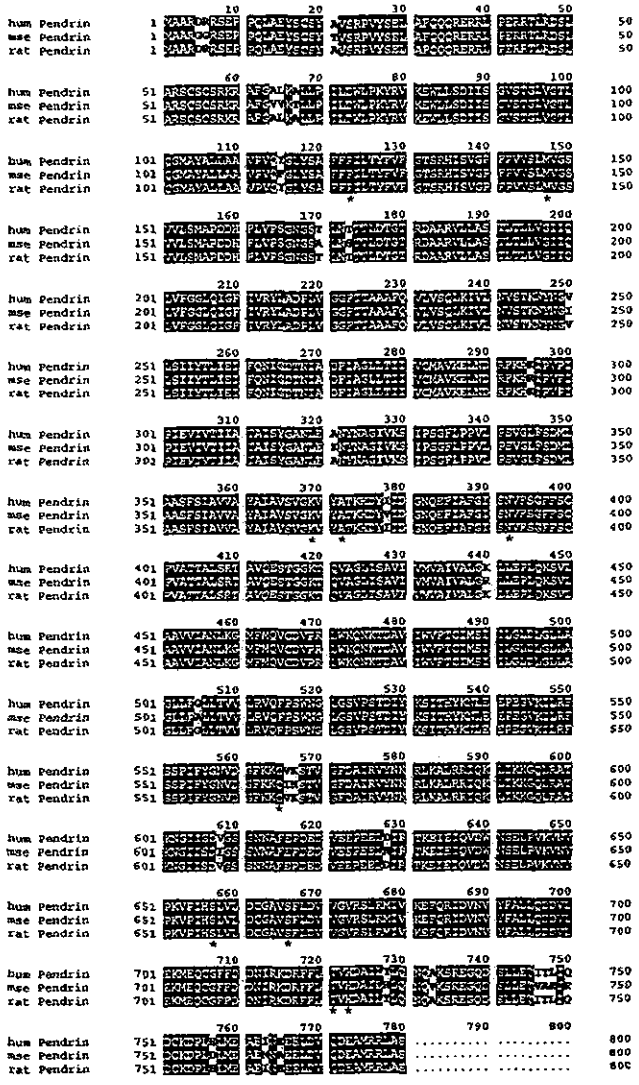

Figure 1 *Multiple-sequence alignment of selected proteins with significant sequence homology to human pendrin. The amino-acid sequence of human pendrin (hum-pendrin) is aligned relative to the sequences of the mouse pendrin (mse-pendrin) and rat pendrin (rat-pendrin). Positions exhibiting absolute identity among the three proteins are shown with a black background. The black background regions lead evolutionary conservation. The regions with asterisks (*) denote the missense mutations detected in our study.*

Splice site mutations

The three splice site mutations found were: IVS5-1G>A (exon 6 acceptor splice site); IVS7-2A>G (exon 8 acceptor splice site); and IVS8+1G>A (exon 8 donor splice site).

Mutations found in unaffected controls

Of the 18 mutations identified in the Pendred syndrome and EVA families, only the H723R mutation was found in a single control subject (one out of 192 alleles).

Differences between Japanese and those with *Caucasoid ancestry*

Figure 2 shows a schematic representation of the *PDS* gene and the approximate positions of mutations found in Japanese, and those found in families with European ancestry.

Genotype–phenotype correlation

Table 2 summarizes phenotype–genotype correlation. One mutation, IVS5-1G>A, was found in the Pendred syndrome family, whereas 11 mutations, P123S, M147V, K369E, C565Y, S657N, S666F, S610X, 322delC, 917delT, IVS7-2A>G, and IVS8+1 G>A were detected in families with nonsyndromic hearing loss associated with EVA. The other six mutations, A372V, N392Y, T721M, H723R, 1652insT, and 2111ins5bp, were found in both EVA and Pendred syndrome families.

Discussion

Recent advances in molecular genetics as well as radiology have provided reliable diagnostic criteria for Pendred syndrome. After identification of the gene responsible for Pendred syndrome, *PDS*,[4] it has become possible to diagnose this syndrome from the molecular genetic viewpoint. This is noteworthy because of the possible molecular diagnostic applications for affected individuals. Furthermore, mutations in *PDS* were found in nonsyndromic hearing loss associated with EVA, suggesting that this gene may give rise to different pathologic phenotypes.[5]

The appearance of EVA by CT/MRI is demonstrated to be a reliable radiological marker, and has become one of the reliable diagnostic criteria for Pendred syndrome.[24] According to Phelps *et al*,[24] EVA was found in a majority of Pendred syndrome patients, but Mondini deformity, which

Table 2 Genotype–phenotype correlations in the current study

Family-patient #	*Phenotype*	*Allele 1*	*Allele 2*
#1	Pendred	–	
#2	NSEVA	917delT	
#3	NSEVA	–	
#4-P	NSEVA	T721M	H723R
#4-B	NSEVA	T721M	H723R
#4-F	Normal	T721M	
#4-M	Normal	H723R	
#5-P	Pendred	A372V	H723R
#5-B	Pendred	A372V	H723R
#5-M	Normal	H723R	
#6-P	NSEVA	A372V	2111ins5bp
#6-F	Normal	A372V	
#6-M	Normal	2111ins5bp	
#7	NSEVA	H723R	
#8	NSEVA	H723R	
#9	NSEVA	–	
#10-P	NSEVA	K369E	H723R
#10-F	NA	K369E	
#10-M	NA	H723R	
#11	Pendred	A372V	1652insT
#12	NSEVA	H723R	
#13	NSEVA	2111ins5bp	
#14	NSEVA	H723R	
#15	NSEVA	N392Y	H723R
#16	Pendred	H723R (homo)	
#17-P	NSEVA	S610X	S657N
#17-M	NA	S610X	
#17-A	NSEVA	S610X (homo)	
#18	NSEVA	–	
#19	NSEVA	–	
#20	NSEVA	H723R	
#21	NSEVA	–	
#22-P	NSEVA	IVS7-2A>G	H723R
#22-S	NSEVA	IVS7-2A>G	H723R
#22-F	Normal	H723R	
#22-M	Normal	IVS7-2A>G	
#23	NSEVA	T721M	
#24-P	NSEVA	IVS8+1G>A	H723R
#24-F	Normal	H723R	
#24-M	Normal	IVS8+1G>A	
#25	NSEVA	P123S (hetero)	
#26	NSEVA	IVS7-2A>G	H723R
#27	NSEVA	–	
#28	NSEVA	H723R (homo)	
#29-P	NSEVA	S666F	H723R
#29-M	NSEVA	S666F (homo)	
#30	NSEVA	C565Y	H723R
#31-P	NSEVA	H723R (homo)	
#31-S	NSEVA	H723R (homo)	
#31-F	Normal	H723R	
#31-M	Normal	H723R	
#32-P	Pendred	IVS5-1G>A	H723R
#32-F	Normal	H723R	
#32-M	Normal	IVS5-1G>A	
#33-P	NSEVA	H723R (homo)	
#33-B	NSEVA	H723R (homo)	
#33-M	NA	H723R	
#34	Pendred	H723R (homo)	
#35	Pendred	H723R (homo)	
#36	Pendred	T721M	H723R
#37	Pendred	A372V	H723R
#38	Pendred	H723R (homo)	
#39	NSEVA	—	
#40	NSEVA	322delC	
#41	NSEVA	M147V	H723R
#42-P	NSEVA	H723R (homo)	
#42-F	Normal	H723R	
#42-M	Normal	H723R	

Pendred, Pendred syndrome; NSEVA, nonsyndromic hearing loss with EVA; NA, patient less than 1 year of age and cannot be accurately diagnosed; P, proband; B, brother; S, sister; F, father; M, mother; A, aunt.

had been thought to be a characteristic radiological feature of Pendred syndrome, was not found. Based on molecular genetics and radiological evaluation, Pendred syndrome and nonsyndromic hearing loss associated with EVA are currently thought to be a continuum of disease caused by *PDS* mutations; that is, hearing loss associated with EVA with/without goiter.

In the present study, causative mutations have been identified in 90% of typical Pendred families and 78.1% of those with sensorineural hearing loss associated with EVA. The results confirmed a new category of disease caused by mutations in *PDS*, encompassing a range from 'classic' Pendred syndrome to nonsyndromic hearing loss associated with EVA.

To date, 66 mutations causing Pendred syndrome and nonsyndromic hearing loss with EVA have been reported in the *PDS* gene (Table 1). We have previously reported seven mutations, K369E, A372V, T721M, H723R, 917delT, 1652insT, and 2111ins5bp, in Japanese families with nonsyndromic hearing loss with EVA or Pendred syndrome.[5,22] Kitamura *et al*[25] reported the T410M mutation in a family with nonsyndromic hearing loss with EVA. The present study added 12 mutations including seven novel *PDS* mutations: 322delC, P123S, M147V, IVS5-1G>A, S610X, S657N, and S666F. It is likely that these are pathologic mutations rather than rare or functionally neutral polymorphic changes because: (1) none of the novel mutations represented here were found in any of the controls, (2) these mutations were highly associated with affected subjects in which mutations were found to be homozygous or compound heterozygous, also indicating that they may be disease-causing mutations, and (3) all of the missense mutations found in our study had occurred at amino-acid residues that were conserved in the rat and the mouse (Figure 1). The present study revealed a unique spectrum of *PDS* mutations quite different from that found in the populations with European ancestry. Of the 19 mutations found in Japanese, only five mutations were also found in European populations (Figure 2), further suggesting a founder effect of these mutations as demonstrated in frequent mutations in *GJB2*.[26,27] In the Caucasoid popula-

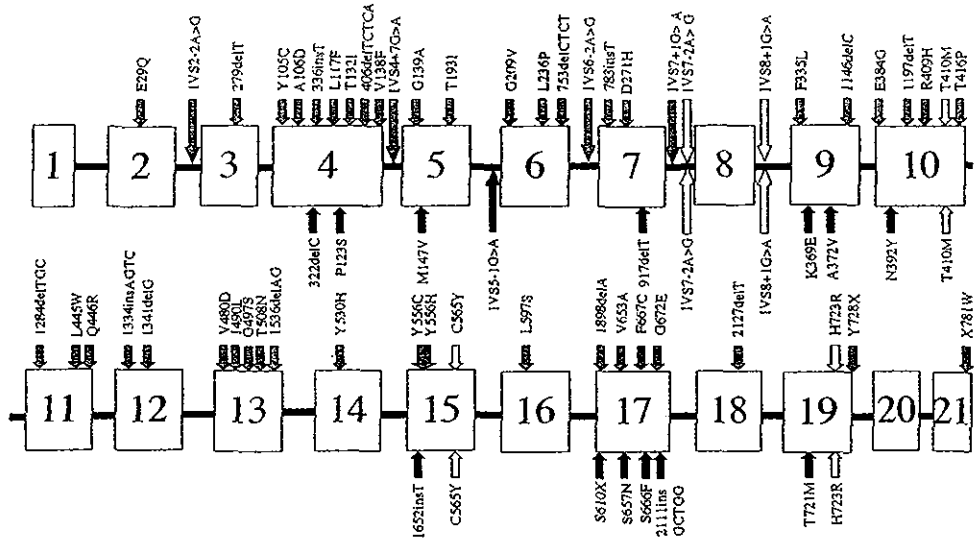

Figure 2 Differences in *PDS* mutation spectrums between Japanese and the populations with European ancestry. Shaded arrows indicate approximate localization of *PDS* mutations found in the Caucasoid populations, filled arrows are localization of mutations found in Japanese and white arrows are the mutations that have been reported in both Caucasoid and Japanese families.

tions, three frequent mutations, L236P (16%), T416P (15%), and IVS8 + 1G > A (14%), account for nearly half of all *PDS* mutant alleles.[8] In contrast, these mutations are rare in Japanese (L236P, 0%; T416P, 0%; and IVS8 + 1G > A, 2%), whereas H723R accounted for 53% of *PDS* mutations. A recent report suggested that the cause of the frequent mutation (H723R) was the existence of a common ancestor rather than a mutational hot spot.[16] The spectrum of *PDS* mutations revealed by the present study will facilitate the effective molecular diagnosis of a category of disease caused by the *PDS* gene.

Pendrin, the transmembrane protein encoded by the *PDS* gene, is expressed in the inner ear, thyroid gland, and kidney.[4,19,28] In Xenopus oocytes and Sf9 cells, it has been demonstrated that pendrin transports chloride and iodine, and mediates the exchange of chloride and formate.[19,29] The mutations associated with Pendred syndrome showed complete loss of pendrin-induced chloride and iodide transport, whereas alleles associated with nonsyndromic sensorineural hearing loss with EVA showed partial transport function, which may be sufficient to maintain thyroid function and eliminate goiter.[19] With regard to genotype–phenotype correlation, four mutations, P123S, S610X, S666F, and H723R, were found to be homozygous (Table 2). In these cases, the phenotype would reflect the function of the respective mutations. The former three mutations caused nonsyndromic sensorineural hearing loss with EVA, and therefore may have residual transport function. However, the homozygous H723R mutation caused either Pendred syndrome or nonsyndromic sensorineural hearing loss with EVA, making the story more complex. Actually, patient #38 showed a huge goiter and tracheostomy was necessary because of dyspnea,[30] whereas three out of eight patients did not show any goiter. Phenotypic variability has also been reported in two families carrying the same *PDS* missense mutation (L445W).[31] A modifier gene or environmental factor (iodine uptake, nutrition, etc.) may contribute to such variability.

In the present study, one out of 10 families with Pendred syndrome and seven out of 32 families with nonsyndromic hearing loss associated with EVA did not show any mutations. This suggests that mutations possibly exist in the activate cryptic splice sites in the introns or that they occur in the promoter region, which was not analyzed in this study. An alternative explanation may be that other genes were responsible for those families without *PDS* mutations. According to Hulander *et al*,[32] Foxi1 is an upstream regulator of pendrin, and defective pendrin-mediated chloride ion resorption in the endolymphatic duct/sac epithelium accounts for the phenotype seen in Foxi1-null mice. Therefore, it is possible that mutations in *FOXI1* cause Pendred syndrome in humans.

In conclusion, the present study confirmed that a range of disease from 'classical' Pendred syndrome to nonsyndromic hearing loss associated with EVA is dependent on the *PDS* mutations. Our findings also represented a unique spectrum of the *PDS* gene in Japanese, which is distinct from that found in the populations with European ancestry. The present study clearly indicates that the establishment of databases for different populations will facilitate the molecular diagnosis of a clinically complex disease.

Acknowledgements

We thank all the subjects who participated in the present project. We also thank Dr Kyoko Nagai (Gunma University School of Medicine), Dr Satoshi Iwasaki (Hamamatsu University School of Medicine), Dr Tatsuo Matsunaga (International University of Health and Welfare), Drs Noriko Minami and Yumiko Hosoya (Iwate Medical University), Drs Hajime Ishinaga and Atsushi Yuta (Mie University), Dr Tetsuya Tono (Miyazaki Medical College), Dr Yukihiko Kanda (Nagasaki University School of Medicine), Dr Hitoshi Sato (Niigata University School of Medicine), Drs Tatsuhiko Harada and Ryuichi Osanai (Saitama Medical School), Drs Hitome Kobayashi and Yasuya Nomura (Showa University), and Dr Tatsuya Yamasoba (Tokyo University) for sending the samples of their patients. This work was supported by the Ministry of Health and Welfare, Japan (SU), and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (SU).

References

1 Pendred V: Deaf mutism and goitre. *Lancet 2* 1896; 532.
2 Fraser GR: Association of congenital deafness with goitre (Pendred's syndrome): a study of 207 families. *Ann Hum Genet* 1965; 28: 411–422.
3 Reardon W, Coffey R, Phelps PD *et al*: Pendred syndrome: 100 years of underascertainment? *Q J Med* 1997; 90: 443–447.
4 Everett LA, Glaser B, Beck JC *et al*: Pendred syndrome is caused by mutations in a putative sulphate transporter gene (*PDS*). *Nat Genet* 1997; 17: 411–422.
5 Usami S, Abe S, Weston MD *et al*: Non-syndromic hearing loss associated with enlarged vestibular aqueduct is caused by *PDS* mutations. *Hum Genet* 1999; **104**: 188–192.
6 Valvassori GE, Clemis JD: The large vestibular aqueduct syndrome. *Laryngoscope* 1978; **88**: 723–728.
7 Abe S, Usami S, Shinkawa H: Three familial cases of hearing loss associated with enlargement of the vestibular aqueduct. *Ann Otol Rhinol Laryngol* 1997; **106**: 1063–1069.
8 Campbell C, Cucci RA, Prasad S *et al*: Pendred syndrome, DFNB4, and *PDS/SLC26A4*: identification of eight novel mutations and possible genotype–phenotype correlations. *Hum Mutat* 2001; **17**: 403–411.
9 Lopez-Bigas N, Melchionda S, de Cid R *et al*: Identification of five new mutations of *PDS/SLC26A4* in Mediterranean families with hearing impairment. *Hum Mutat* 2002; **18**: 548.
10 Kopp P, Arseven OK, Sabacan L *et al*: Phenocopies for deafness and goiter development in a large inbred Brazilian kindred with Pendred's syndrome associated with a novel mutation in the PDS gene. *J Clin Endocrinol Metab* 1999; **84**: 336–341.
11 Coyle B, Reardon W, Herbrick JA *et al*: Molecular analysis of the PDS gene in Pendred syndrome. *Hum Mol Genet* 1998; 7: 1105–1112.
12 Reardon W, O Mahoney CF, Trembath R *et al* : Enlarged vestibular aqueduct: a radiological marker of Pendred syndrome, and mutation of the PDS gene. *Q J Med* 2000; 93: 99–104.
13 Van Hauwe P, Everett LA, Coucke P *et al*: Two frequent missense mutations in Pendred syndrome. *Hum Mol Genet* 1998; 7: 1099–1104.
14 Lopez-Bigas N, Rabionet R, de Cid R *et al*: Splice-site mutation in the *PDS* gene may result in intrafamilial variability for deafness in Pendred syndrome. *Hum Mutat* 1999; **14**: 520–526.
15 Adato A, Raskin L, Petit C *et al*: Deafness heterogeneity in a Druze isolate from the Middle East: novel OTOF and PDS mutations, low prevalence of GJB2 35delG mutation and indication for a new DFNB locus. *Eur J Hum Genet* 2000; 8: 437–442.
16 Park HJ, Shaukat S, Liu XZ *et al*: Origins and frequencies of *SLC26A4 (PDS)* mutations in east and south Asians: global implications for the epidemiology of deafness. *J Med Genet* 2003; 40: 242–248.
17 Coucke PJ, Van Hauwe P, Everett LA *et al* : Identification of two different mutations in the PDS gene in an inbred family with Pendred syndrome. *J Med Genet* 1999; 36: 475–477.
18 Yong AM, Goh SS, Zhao Y *et al*: Two Chinese families with Pendred's syndrome: radiological imaging of the ear and molecular analysis of the pendrin gene. *J Clin Endocrinol Metab* 2001; 86: 3907–3911.
19 Scott DA, Wang R, Kreman TM: Functional differences of the *PDS* gene product are associated with phenotypic variation in patients with Pendred syndrome and non-syndromic hearing loss (DFNB4). *Hum Mol Genet* 2000; 9: 1709–1715.
20 Li XC, Everett LA, Lalwani AK *et al*: A mutation in *PDS* causes non-syndromic recessive deafness. *Nat Genet* 1998; 18: 215–217.
21 Bogazzi F, Raggi F, Ultimieri F *et al*: A novel mutation in the pendrin gene associated with Pendred's syndrome. *Clin Endocrinol (Oxf)* 2000; 52: 279–285.
22 Namba A, Abe S, Shinkawa H *et al*: Genetic features of hearing loss associated with ear anomalies: *PDS* and *EYA1* mutation analysis. *J Hum Genet* 2001; 46: 518–521.
23 Fugazzola L, Mannavola D, Cerutti N *et al*: Molecular analysis of the Pendred's syndrome gene and magnetic resonance imaging studies of the inner ear are essential for the diagnosis of true Pendred's syndrome. *J Clin Endocrinol Metab* 2000; 85: 2469–2475.
24 Phelps PD, Coffey RA, Trembath RC *et al*: Radiological malformations of the ear in Pendred syndrome. *Clin Radiol* 1998; 53: 268–2273.
25 Kitamura K, Takahashi K, Noguchi Y *et al*: Mutations of the Pendred syndrome gene (*PDS*) in patients with large vestibular aqueduct. *Acta Otolaryngol* 2000; 120: 137–141.
26 Van Laer L, Coucke P, Mueller RF *et al*: A common founder for the 35delG *GJB2* gene mutation in connexin 26 hearing impairment. *J Med Genet* 2001; 38: 515–518.
27 Ohtsuka A, Yuge I, Kimura S *et al*: GJB2 deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. *Hum Genet* 2003; **112**: 329–333.
28 Royaux IE, Wall SM, Karniski LP *et al* : Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. *Proc Natl Acad Sci USA* 2001; 98: 4221–4226.
29 Scott DA, Wang R, Kreman TM *et al*: The Pendred syndrome gene encodes a chloride–iodide transport protein. *Nat Genet* 1999; 2: 440–443.
30 Ishinaga H, Shimizu T, Yuta A *et al* : Pendred's syndrome with goiter and enlarged vestibular aqueducts diagnosed by *PDS* gene mutation. *Head Neck* 2002; **24**: 710–713.
31 Masmoudi S, Charfedine I, Hmani M *et al*: Pendred syndrome: phenotypic variability in two families carrying the same *PDS* missense mutation. *Am J Med Genet* 2000; 90: 38–44.
32 Hulander M, Kiernan AE, Blomqvist SR *et al*: Lack of pendrin expression leads to deafness and expansion of the endolymphatic compartment in inner ears of *Foxi1* null mutant mice. *Development* 2003; 130: 2013–2025.

耳喉頭頸 75(2)：101-105，2003

特集 薬物による聴覚障害

2. アミノ配糖体抗生物質による難聴 update —遺伝子学的アプローチ—

宇佐美真一*

I. アミノ配糖体抗生物質による難聴

ストレプトマイシンを初めとするアミノ配糖体抗生物質は広い抗菌スペクトルをもち，比較的安価であることから現在でも多くの国々で使用されているが，アミノ配糖体抗生物質には耳毒性，腎毒性といった副作用があることからわが国では使用頻度がかなり減ってきている。しかし，アミノ配糖体抗生物質のもつ抗結核菌作用，抗緑膿菌作用，抗 MRSA 作用などを期待して使用する場合も多く，また最近では副作用の少ない新世代のアミノ配糖体抗生物質も出始めている。表 1 に現在わが国で用いられているアミノ配糖体抗生物質の一覧を示した。

アミノ配糖体抗生物質による難聴は両側性，対称性の高音障害型の感音難聴を示す。オージオグラムでは，通常，まず 8,000 Hz が急墜し進行するに従い中低音域にも難聴がみられるようになる。難聴に先立ち耳鳴を自覚するといわれており，重篤な難聴の予防には耳鳴の発現に注意することが必要である。難聴は非可逆的で，ストレプトマイシン投与を中止した後も進行する場合がある。側頭骨病理および動物実験から，アミノ配糖体抗生物質による障害部位はコルチ器の有毛細胞，特に外有毛細胞が易受傷性が高く，次いで内有毛細胞が障害を受け，引き続いてラセン神経節が変性することが知られている。また，これらの障害は蝸牛の基底回転から始まり，次第に上方回転に及ぶことが知られている[1]。この形態的変化は，初期には高音障害型の聴力像を呈し，進行するに従い中低音域も障害されるという臨床像とよく一致する。硫酸ストレプトマイシンには，前述の聴覚障害に比較し平衡障害をきたすことが多いことが知られている。

表 1 わが国で現在用いられているアミノ配糖体抗生物質（商品名）

硫酸ストレプトマイシン（硫酸ストレプトマイシン）
硫酸カナマイシン（カナマイシン）
硫酸アミカシン（ビクリン，アミカマイシン）
硫酸ゲンタマイシン（ゲンタシン）
硫酸ベカナマイシン（カネンドマイシン）
硫酸リボスタマイシン（ビスタマイシン）
硫酸ジベカシン（パニマシン）
トブラマイシン（トブラシン）
硫酸シソマイシン（シセプチン）
硫酸ミクロノマイシン（サガミシン）
硫酸フォーチミシン（フォーチミシン）
硫酸アストロマイシン（ネチリン，ベクタシン）
硫酸イセパマイシン（イセパシン，エクサシン）
硫酸アルベカシン（ハベカシン）
硫酸フラジオマイシン（フラジオマイシン）

これらの副作用の出現には一般に 3 つの要素が関係しているといわれている。すなわち 1）投与量，2）局所の濃度，3）遺伝的要素（感受性の違い）である。

1. 投与量

一般的に投与総量が増すと副作用の頻度が増えるとされ，ストレプトマイシンでは 1 日 1 g 注射で 20 g 前後で副作用をみることが多いとされる。しかし，後述のようにアミノ配糖体抗生物質に遺伝的に易受傷性をもつ患者では，1 回の投与でも難聴をきたすことがあり注意が必要である。

* 信州大学医学部耳鼻咽喉科学教室
（〒390-8621 長野県松本市旭 3-1-1）