Table 2: DNA variants observed in 56 Usher I probands for the *CDH23* gene. All are presumed to be pathogenic. None of the variants have been observed in 192 control chromosomes.

Nucleotide[1,2] (codon change)	Exon	Domain	Citation	Proportion of Usher Chromosomes	Proportion control	National origin
Missense						
c.1096G>A, p.A366T	10	EC4	(c)	1/112	0/192	USA
c.2263C>T, p.H755Y	20	EC7		1/112	0/192	USA
c.3268G>A, p. V1090I	27	EC10		2/112	0/192	Spain
c.3293A>G, p.N1098S	27	EC10		1/112	0/192	USA
c.4488G>C, p.Q1496H	35	EC14	(e)	1/112	nd	Spain
c.5363C>T, p.P1788L	40	EC17		1/112	0/192	Spain
c.5734C>T, p.R1912W[3]	43	EC18		2/112	0/192	Spain
c.5788G>A, p.D1930N	43	EC18		1/112	0/192	Spain
c.6049G>A, p.G2017S[3]	45	EC19	(d)	2/112	0/192	Spain
c.7127A>T, p.D2376V	50	EC22		2/112	0/192	USA
c.7589C>T, p.T2530I	53	EC24		1/112	0/192	USA
c.8311G>A, p.G2771S	58	EC25		1/112	0/192	Spain
c.8903T>C, p.V2968A	60	EC27		1/112	0/192	Spain
Stop						
c.6337C>T, p.Q2113X	47	EC20		1/112	0/192	USA
Deletion						
c.6346_6347delTT	47	EC20		1/112	0/192	Spain
c.6392delC	47	EC20		1/112	0/192	Spain
c.6511delC	47	EC20		1/112	0/192	Spain
Splice						
c.336+1G>A	4		(a)	5/112	nd	Swedish
c.2289+1G>A	20		(a)	1/112	0/192	USA
c.6050-9G>A	46		(b)	5/112	0/192	Spain, USA
c.8722+1delG	59			1/112	0/192	Spain

[1] Nucleotide and codon numbering is based on the sequence NM_022124.3. [2] Novel mutations are shown in bold font. [3] These two variants occurred together in an apparent homozygous state. Which one is the pathologic substitution cannot be determined with certainty at this time. (a) Astuto et al., 2002; (b) von Brederlow et al., 2002; (c) Ouyang et al., 2005; (d) Roux et al., 2006; (e) Bolz et al., 2001

Missense mutations

All of the ten novel missense mutations reported in this study are in EC domains. According to the analysis using the ConSeq program, two are functional residues. Seven residues are exposed residues and three of them are buried. The c.3293A>G, p. N1098S was in the DXNDN calcium binding motif. No missense mutation that disrupts the LDRE and DXD calcium binding motif was observed. No mutation having the potential of disrupting an N-linked glycosylation was detected.

Deletion mutations

Three novel deletion mutations, c.6346_6347delTT, c.6392delC and c.6511delC, were observed. All of these mutations are in the EC20. They create the frame shift leading to a subsequent premature stop codon.

c.6346_6347delTT creates a stop codon at p.2128. c.6392delC creates a stop at p.2142 and c.6511delC create it at codon p.2181.

Splice-site mutations

One novel splice-site deletion variant, c.8722+1delG, was observed in intron 59. The previously reported c.336+1G>A mutation was observed in three probands from Sweden. An c.5924-9G>A mutation that creates novel acceptor-site (von Brederlow et al., 2002) was detected in three probands from both the United States and Spain. The c.336+1G>A and c.5924-9G>A alleles have been reported as the most frequent mutations in *CDH23*. In the present study, they are also the most frequent pathologic DNA variants.

Neutral DNA variants

Tables 3a and 3b and Table 4 list the DNA variants, many novel, observed in the present study that are presumed to be non-pathogenic. Table 3a lists missense variants that are believed to be neutral, while 3b lists the isocoding variants. Table 4 lists variants occurring in the introns that were reliably detected by our sequencing strategy. Many of the variants are polymorphic and, unless otherwise noted, all were in Hardy-Weinberg equilibrium.

Table 3a. Presumed neutral missense variants occurring within the coding region of the *CDH23* gene

Variant[1,2]	Exon	Usher[3]	Control[3]
c.1469G>C, p.G490A	14	20/112	
c.1487G>A, p.S496N	14	88/112	
c.2236G>A, p.V746I	20	0/112	1/192
c.2830G>A, p.S944G	24	0/112	2/112
c.2878G>A, p.E960K	24	1/112	2/192
c.3268G>A, p.V1090I	27	2/112	0/192
c.3664G>A, p.A1222T	30	6/112	8/192
c.3707G>A, p.R1236Q	30	0/112	1/112
c.3845A>G, p.N1282S	31	1/112	1/192
c.4045C>T, p.R1349C	31	0/112	2/192
c.4051A>G, p.D1351N	31	90/112	147/192
c.4310G>A, p.R1437Q	34	4/112	
c.4723G>A, p.A1574T	37	23/112	
c.4858G>A, p.V1520M	38	5/112	
c.5023G>A, p.V1675I	38	13/112	
c.5996C>G, p.T1999S	45	49/112	89/192
c.6130G>A, p.E2044C	46	36/112	52/192
c.6847G>A, p.V2283I	49	2/106	0/192
c.7073G>A, p.R2853Q	50	37/112	52/192
c.7139C>T, p.P2380L	50	35/112	52/192
c.7467C>T, p.R2489R	52	1/112	
c.7762G>C, p.E2588Q	54	2/112	
c.9373T>C, p.F3125L	65	5/112	

[1] Nucleotide and codon numbering is based on the sequence NM_022124.3. [2] Novel mutations are shown in bold font. [3] The numerator is the count of the allele to the right of the '>' mark.

Table 3b. Presumed neutral isocoding variants occurring within the coding region of the *CDH23* gene

Variant[1,2]	Exon	Usher	Control
c.366T>C, p.V122V	5	33/110	
c.510C>T, p.S170S	6	1/112	
c.1038G>A, p.P346P	10	6/112	
c.1053C>T, p.S351S	10	1/112	
c.1185C>T, p.S395S	12	1/112	
c.2316T>C, p.N772N	21	33/112	
c.2388T>C, p.D796D	21	33/112	
c.2424G>A, p.G808G	22	3/112	
c.2976G>A, p.T992T	25	1/112	
c.3009T>C, p.S1003S	25	8/112	
c.3231T>G, p.P1077P	27	2/112	0/192
c.3801C>T, p.T1267T	31	0/112	1/192
c.3999G>A, p.V1333V	31	0/112	1/192
c.4299T>A, p.P1433P	34	3/112	
c.4341T>C, p.D1447D	34	2/112	
c.4509C>T, p.G1503G	36	2/112	1/192
c.4773G>A, p.P1591P	37	1/112	
c.5100C>T, p.Y1700Y	39	20/112	
c.5523G>A, p.V1841V	42	1/112	
c.6648C>T, p.A2216A	47	0/112	1/192
c.6852G>C, p.L2284L	49	2/112	0/192
c.6918G>A, p.L2306L	49	1/106	0/192
c.7572G>A, p.A2524A[1]	53	23/112	16/170
c.7630T>C, p.L2544L	53	0/112	1/192
c.8574C>T, p.D2858D	59	0/112	2/192
c.8895C>T, p.P2965P	60	14/112	33/192
c.9873G>A, p.T3291T	69	12/112	
c.9903C>T, p.P3301P	69	1/112	

[1] Nucleotide and codon numbering is based on the sequence NM_022124.3. [2] Novel mutations are shown in bold font. [3] Significantly out of HW equilibrium in the Usher sample but in equilibrium in controls; the less common allele is more frequent in the Usher group.

DISCUSSION

Sixty-eight pathologic DNA variants have been described so far (Bolz et al.,2001, Bork et al.,2001, von Brederlow et al., 2002, Astuto et al., 2002, Brouwer et al., 2003, Ouyang et al., 2005, Schultz et al., 2005, Roux et al., 2006). In this study, 21 pathologic DNA variants were observed in 18 out of the 56 probands who have been previously screened for mutation of MYO7A. MYO7A gene defects account for Usher syndrome type I from in 39% in the United States and the United Kingdom (Ouyang et al., 2005) and in 47.9% in Spain (Jaijo et al., 2006). Adjusting for the results of these reports, it is estimated that 15 - 20 % of patients with Usher syndrome type I are due to *CDH23* pathology.

Fifteen novel pathologic variants were detected in this study. The missense mutations were all observed in EC domain. c.3293A>G, p.N1098S, seen here in an Usher syndrome proband, was within a DXNDN calcium binding motif. No missense mutation was observed in this series that disrupted the LDRE or DXD calcium binding motifs.

Table 4. Presumed neutral variants observed in the introns of the *CDH23* gene

Nucleotide Change[1,2]	Exon-Specific PCR product	Frequency in	
		Usher Chromosomes	Control Chromosomes
c.145+26C>T	2	1/112	
c.145+135C>T	2	28/112	
c.429+13G>A	5	33/110	
c.429+18T>C	5	1/110	
c.429+26A>G	5	33/110	
c.624+64C>T	6	43/112	
c.832+110G>A	8	46/112	
c.1753-78A>T	16	50/112	
c.1753-56T>C	16	1/112	
c.1753-43C>T	16	2/112	
c.1987-123G>A	18	22/112	
c.2060-38G>T	19	4/112	
c.2060-19C>G	19	3/112	
c.2290-19G>A	21	1/112	
c.2397+26T>C	21	33/112	
c.2588-86C>A	23	2/112	
c.3373+54C>T	27	1/112	0/192
c.3580-67G>A	30	0/112	2/192
c.4210-53C>T	34	1/112	
c.4360-71G>A	35	3/112	
c.4488+32C>G	35	49/112	
c.4489-85T>G	36	1/112	0/192
c.4617+63C>T	36	1/112	0/192
c.4618-77T>C	37	25/110	
c.4846-49T>C	38	51/112	
c.5187+44C>G	39	44/112	
c.5187+73C>T	39	12/112	
c.5187+99T>C[2]	39	32/112	
c.5503-111A>G	42	1/112	
c.5503-44T>C	42	24/112	
c.5503-10A>G	42	21/112	
c.5924-90G>A	45	4/112	2/192
c.6712+8G>A	47	1/112	0/112
c.6712+67C>T	47	1/112	3/112
c.6830-81G>A	49	26/102	46/182
c.7055-16A>G	50	41/112	53/192
c.7224+85C>T	50	1/112	0/192
c.7225-22C>T	51	9/112	
c.7362+59G>C	51	2/112	
c.7482+64C>T	52	2/112	
c.7631-26A>G	54	1/112	
c.8178+26G>T	56	1/112	
c.8980-14C>A	61	1/112	
c.9077+8G>A	61	24/112	

c.9078-50G>C	62	1/108
c.9198+14G>A	62,63	1/112
c.9319+11G>A	64	14/112
c.9319+72_73delTC	64,65	22/112
c.9320-93C>G	65	14/112
c.9320-34C>T	65	3/112
c.9380+109G>A	65	5/112
c.9380+111C>T	65	9/112

[1.] Nucleotide and codon numbering is based on the sequence NM_022124.3. [2.] Novel mutations are shown in bold font.

Interestingly, out of 47 *CDH23* variants observed associated with the Usher syndrome type I phenotype (Bolz et al., 2001, Bork et al., 2001, von Brederlow et al., 2002, Astuto et al., 2002, Ouyang et al., 2005, and Roux et al., 2006) only one has occurred within the highly conserved peptide sequences LDRE, DXNDN, and DXD which are responsible for calcium-binding. On the other hand, ten of 21 misssense mutations observed in patients with nonsyndromic hearing loss (Bork et al., 2001, Astuto et al., 2002, Brouwer et al., 2002, Schultz et al., 2005) occurred within the DXNDN, LDRE and DXD calcium binding motifs. These observations suggest that deficiency of the calcium-dependent cell adhesion is more important for cochlear cell integrity but not for the retina.

CDH23 has 31 potential N-linked glycosylation sites. So far, no missense mutations in the potential N-linked glycosylation sites of *CDH23* nor any mutations that create new potential glycosylation sites have been reported. N-Glycans modify proteins at asparagine residues within the consensus sequence NX(S/T), where X can be any amino acid with the exception of proline (Helenius and Aibe, 2001, Kukuruzinska and Lennon, 1998). The role of glycosylation of cadherin 23 is unclear.

Technical error, hidden mutations in gene regions, such as introns and regulatory regions, or large deletions involving one or more exons may account for the individuals who carry single *CDH23* heterozygous mutation. Digenic inheritance of Usher phenotype involving *CDH23* and *PCDH15* was reported (Qing, Y.Z. et al., 2005). So the possibility also exists that these individuals are *CDH23* carriers and that the second causative allele is at a different locus, presumably *PCDH15* (USH1F).

Better understanding of the biochemical and physiologic consequences of mutations within the different *CDH23* domains will help to shed light on which functions are important for normal functioning of the hair cells in the cochlea and rod photoreceptors in the retina. Such knowledge could ultimately lead to better therapies for Usher type I DFNB12 hearings loss, and possibly even age related hearing loss.

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REVIEW ARTICLE

The responsible genes in Japanese deafness patients and clinical application using Invader assay

SHIN-ICHI USAMI[1], MICHIO WAGATSUMA[1], HISAKUNI FUKUOKA[1], HIROAKI SUZUKI[1], KEITA TSUKADA[1], SHINYA NISHIO[1], YUTAKA TAKUMI[1] & SATOKO ABE[2]

[1]*Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto and* [2]*Division of Advanced Technology and Development, BML, Inc., Saitama, Japan*

Abstract

Discovery of deafness genes has progressed but clinical application lags because of the genetic heterogeneity. To establish clinical application strategy, we reviewed the frequency and spectrum of mutations found in Japanese hearing loss patients and compared them to those in populations of European ancestry. Screening revealed that in Japanese, mutations in *GJB2*, *SLC26A4*, and *CDH23*, and the mitochondrial 12S rRNA are the major causes of hearing loss. Also, mutations in *KCNQ4*, *TECTA*, *COCH*, *WFS1*, *CRYM*, *COL9A3*, and *KIAA1199* were found in independent autosomal dominant families. Interestingly, spectrums of *GJB2*, *SLC26A4*, and *CDH23* mutations in Japanese were quite different from those in Europeans. Simultaneous screening of multiple deafness mutations based on the mutation spectrum of a corresponding population using an Invader panel revealed that approximately 30% of subjects could be diagnosed. This assay will enable us to detect deafness mutations in an efficient and practical manner in the clinical platform. We conclude that specific racial populations may have unique deafness gene epidemiologies; therefore, ethnic background should be considered when genetic testing is performed. Simultaneous examination of multiple mutations based on a population's spectrum may be appropriate and effective for detecting deafness genes, facilitating precise clinical diagnosis, appropriate counseling, and proper management.

Keywords: *GJB2, SLC26A4, CDH23, mitochondria, founder effect, hot spot, hearing impairment, genetic testing*

Discovery of deafness genes and clinical application

From several etiological studies, at least 50% of childhood hearing loss has been estimated to be of genetic etiology, with the other 50% due to environmental causes, including newborn delivery problems, acoustic trauma, ototoxic drug use, and prenatal/postnatal infection [1]. Newborn hearing screening programs and subsequent early hearing loss detection have developed rapidly, leading to improved outcomes in cognitive and linguistic ability, and better educational management of deaf children [2]. Along with early discovery of hearing loss and subsequent intervention programs, much attention has been paid to the determination of the hearing loss etiology. Genetic testing, therefore, is now vital to enable a precise diagnosis, predict the severity of hearing loss, estimate associated abnormalities, select appropriate habilitation, prevent hearing loss, and provide improved genetic counseling. There has been considerable progress in the discovery of deafness genes, but clinical application still entails difficulties due to the genetic heterogeneity of deafness. Nearly 100 genes are thought to cause hereditary hearing loss, but a number of these may result in similar phenotypes that entail no abnormalities other than hearing loss. The costs and time required for screening genes one by one are prohibitive, but it is now thought that certain mutations

Correspondence: 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

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are recurrent. A screening strategy that focuses on those frequently reported recurrent mutations, expected to be commonly encountered in the clinical setting, might be a breakthrough for clinical application.

Our series of studies has revealed genes responsible for Japanese deafness patients, and the differences in mutation spectrum between Japanese (who are probably representative of other Asian populations) and populations with European ancestry. To construct a deafness gene database for Japanese subjects and to establish an efficient genetic testing method, the current study 1) summarized the frequency and the spectrum of the mutations found in the Japanese deafness population in comparison with those reported in the populations of European ancestry, and 2) considered whether a diagnostic strategy based on a mutation/gene database followed by Invader assay would be adequate for genetic screening for such heterogeneous genetic disorders.

Genes responsible for deafness

Genes that were responsible for non-syndromic hearing loss in Japanese patients and the frequencies of mutations mainly based on our screening series are summarized in Table I.

We chose references that describe mutations and frequency in each gene in Japanese.

The major cause of hearing loss in Japanese patients and clinical relevance

The present review summarized the 15 genes responsible for non-syndromic hearing loss in Japanese patients (including some who may have visited an ENT clinic as a non-syndromic hearing loss patient), a number that suggests there may be many genes involved in deafness in Japanese patients. As predicted, there are many frequent and recurrent mutations within the reported mutations. So far, mutations in *GJB2*, *SLC26A4*, *CDH23*, and the 1555A→G mitochondrial mutation were found to be the major causes of hearing loss in Japanese patients. These mutations are prevalent and therefore commonly encountered in the clinic, and the clinical application of this information will be important.

GJB2

In large-scale screening using more than 1000 samples (1227 sensorineural hearing loss patients with various onsets and etiology), 11.3% (259/2454) of the alleles had some mutations. A total of 13 mutations in *GJB2* have so far been reported in Japanese subjects [3]. When restricting the subjects to only those with congenital SNHL, 18.3% (62/338) had at least one *GJB2* mutation detected by Invader assay [4]. Of the mutations identified, 235delC was the most frequent, followed by V37I, G45E, and Y136X [3–5].

GJB2 is currently known to be the most prevalent gene responsible for congenital hearing loss worldwide [1], and therefore is closely related to universal infant hearing screening programs. In our screening, approximately 20% of congenital subjects had at least one mutation in *GJB2*, confirming that *GJB2* is the major causative gene in Japanese deafness patients [5].

It has been known that prevalent *GJB2* mutations have a high ethnic predilection (see The connexin-deafness homepage; http://davinci.crg.es/deafness/): 35delG is quite common in the Caucasoid population, 167delT was reported as prevalent in Ashkenazi Jews, R143W in a restricted village in Africa, and 235delC in Asian populations. The 235delC mutation was found to be the most prevalent mutation in our screening, accounting for 37.6% (38/101) of the hearing-impaired persons [5].

Investigation regarding a founder effect for the 235delC mutation by means of single nucleotide polymorphism (SNP) analysis indicated that this mutation was derived from a common ancestor [3]. In contrast, the 35delG mutation, the most prevalent mutation in Caucasoid populations, was not detected in our population, indicating that this mutation is seldom seen in Japanese subjects.

There have been general rules that inactivating mutations (deletion mutations and stop mutations) show more severe phenotypes compared with those caused by non-inactivating mutations (missense mutations) [6–8]. As well as a highly accurate diagnosis, these genotype-phenotype correlation data could provide prognostic information to help decide the strategy of intervention with hearing, i.e. whether a child should receive cochlear implantation or hearing aids. For the patients with severe phenotypes who have *GJB2* mutations, genetic information would aid decision-making regarding cochlear implantation, because their hearing loss is of cochlear origin and therefore they are good candidates for implantation. In fact, cochlear implantation has resulted in remarkable improvements in auditory skills and development of speech production for patients with profound hearing loss associated with *GJB2* mutations [9].

SLC26A4

SLC26A4 is also a common responsible gene in Japanese patients associated with enlarged vestibular

Table I. Responsible genes reported in Japanese patients (in order of reported year).

Genes	References	Frequency
Mitochondrial *3243A→G*	Goto et al., 1990	0.3% (1/319 Usami et al., 2000 [15]) – 3% (3/100 Oshima et al., 1999 [26]) of the SNHL patients who visited outpatient clinics
Mitochondrial *1555A→G*	Hutchin et al., 1993	3% (11/319 Usami et al., 2000 [15]) – 5% (7/138 Noguchi et al., 2004 [18]) of the SNHL patients who visited outpatient clinics 33% (7/21 Usami et al., 2000 [15], 2/6 Noguchi et al., 2004 [18]) of the SNHL patients due to aminoglycoside injection 10% (14/140) of patients who received cochlear implantation 57% (13/22) of patients who received cochlear implantation due to aminoglycoside-induced hearing loss (Usami et al., 2000 [15])
MYO7A	Liu et al., 1997	Not available (single DFNA11 family)
POU3F4	Hagiwara et al., 1998	Not available (single DFN3 family)
GJB2	Fuse et al., 1999	11.3% (259/2454) alleles $n=1227$ SNHL patients (Ohtsuka et al., 2003 [3]) 18.3% (62/338) congenital SNHL (Abe et al., 2007 [4])
SLC26A4	Usami et al., 1999 [11]	90% (9/10) Pendred syndrome families 78% (25/32) non-syndromic hearing loss Associated with EVA families (Tsukamoto et al., 2003 [12])
KCNQ4	Akita et al., 2001 [22]	1/16 ADSNHL (Akita et al., 2001 [22])
Mitochondrial *7511T→C*	Ishikawa et al., 2002	Not available (single maternally inherited family)
TECTA	Iwasaki et al., 2002 [23]	Not available (single mid-frequency involved family)
WFS1	Komatsu et al., 2002	3/182 ADSNHL 3/10 low-frequency ADSNHL 0/64 ARSNHL (Fukuoka et al., 2007 [24])
COCH	Usami et al., 2003 [21]	1/23 ADSNHL 0/20 Meniere's disease (Usami et al., 2003 [21])
CRYM	Abe et al., 2003 [25]	2/192 congenital SNHL (Abe et al., 2003 [25])
KIAA1199	Abe et al., 2003 [27]	4/192 congenital SNHL (Abe et al., 2003 [27])
COL9A3	Asamura et al., 2005 [28]	2/147 SNHL (Asamura et al., 2005 [28])
CDH23	Wagatsuma et al., 2007 [14]	5/64 AR congenital SNHL (Wagatsuma et al., 2007 [14]).

SNHL, sensorineural hearing loss; AD, autosomal dominant; AR, autosomal recessive.

aquaduct (EVA). Based on genetic evaluations, the historical concept of two distinct categories of disease, Pendred syndrome and non-syndromic hearing loss associated with EVA, has changed and they should be considered and treated as a continuum of diseases caused by the same gene [10,11]. The prevalent association of *SLC26A4* mutations in these patients (90% Pendred syndrome, 78% non-syndromic hearing loss associated with EVA) indicates the importance of this gene in the pathophysiology of this category of hearing impairment [12]. Our recent study confirmed clinical characteristics of 38 patients with EVA caused by biallelic *SLC26A4* mutations. These included congenital (100%), fluctuated (92.3%), and progressive (92.3%) hearing loss usually associated with vertigo (70.6%) and/or goiter (27.8%) during long-term follow-up [13]. Among the mutations identified, H723R was the most common. There was great variation regarding hearing loss severity even with the same mutations, suggesting that other factors may contribute to such variability. Unlike in the case of *GJB2*, phenotype cannot be predicted from the genotype [13]; however, the clarification of clinical

features will enable more appropriate genetic counseling and proper medical management for these patients.

CDH23

Mutations in the *CDH23* gene are known to be responsible for both Usher syndrome type ID (USH1D) and non-syndromic hearing loss (DFNB12), and the molecular confirmation of the *CDH23* gene, encoding a molecule that forms the lateral links between the stereocilia, has become important in the diagnosis of these conditions.

We have identified 6 patients (from 5 families) with mutations in *CDH23* out of 64 recessive hearing loss families, indicating that these mutations are one of the major known genetic causes of hereditary hearing loss in Japanese subjects 14]. A total of 17 variants (possible disease-causing mutations) were found in this screening. Among them, four mutations were confirmed to be responsible for deafness by segregation study. Although it is difficult to screen this gene because of the many exons as well as polymorphisms, much attention should be paid to it when performing genetic testing of hearing loss patients. Similar to *GJB2* and *SLC26A4*, the *CDH23* mutation spectrum in Japanese is very different from that found in Caucasians [14], indicating a possible founder effect.

1555A→G mutation in the mitochondrial 12S rRNA

The 1555A→G mitochondrial mutation has been identified as the most prevalent mitochondrial mutation [15]. This mutation has been reported to be associated with aminoglycoside injection, although there have been some patients with no history of injection [16,17]. In our series of screenings in the hearing impaired population, the 1555A→G mitochondrial mutation was found in approximately 3–5% of outpatients and 33% of subjects with a history of aminoglycoside injection [15,18]. In addition, it has also been found in congenital/early onset sensorineural hearing loss patients, suggesting that it is important to screen for this mutation in patients with that condition [4]. Although the mitochondrial 1555A→G mutation has been thought to transmit in a homoplasmic state, recent reports have shown heteroplasmy cases [4,19]. For the family members who do not have hearing loss, it is important to prevent severe hearing loss by avoiding use of aminoglycoside antibiotics; therefore we distribute a drug use warning card to them [20].

Less frequent genes

In contrast to the four genes discussed above, the occurrence of mutations of the *COCH* [21], *KCNQ4* [22], *TECTA* [23] *WFS1* [24], and *CRYM* [25] genes in our group of deaf subjects was low (Table I). The 3243A→G mutation in the mitochondrial tRNA (Leu(UUR)) was found in 0.3% of SNHL patients in our screening [15] but 3% in another report [26], the higher figure being due to sampling bias, indicating that further evaluation of this mutation is needed. A few patients with *COL9A3,* and *KIAA1199* have also been found, but due to the rather small families, replicable data are needed to confirm whether these mutations are really pathologic [27,28]. There are still only small numbers of autosomal dominant sensorineural hearing loss (ADSNHL) families whose responsible genes have been identified. Continuous collection and analysis of clinical data are necessary to clarify how these genes are involved in Japanese ADSNHL.

Comparison with the mutation spectrum

As shown in Figures 1–3, spectrums of *GJB2*, *SLC26A4*, and *CDH23* mutations found in the Japanese population were quite different from those reported in populations with European ancestry. This suggests that each racial population may have a unique epidemiology of deafness genes and there are specific common founder mutations, for example, 35delG, 235delC, R143W in the *GJB2* gene, and H723R in the *SLC26A4* gene. SNP analysis proved a founder event for the origin of the prevalent mutations in the Japanese [3,29]. These ethnic-specific mutation spectrums indicate that an individual's ethnic background should be borne in mind when genetic testing is performed.

Screening strategy based on recurrent mutations

The reason for the recurrence of some mutations has been explained either by the existence of common founders or a mutational hot spot. SNP analysis has proved that 235delC in the *GJB2* gene and H723R in the *SLC26A4* gene are common founder mutations, thus explaining their frequency [3,29]. Different combinations of *GJB2, SLC26A4* and *CDH23* mutations exist in the Japanese, indicating that ethnic background should be considered when performing genetic testing. On the other hand, there are certain hot spots for frequent genes, for example, in the *KCNQ4* gene, a mutation (W276S) has been found independently in three families originating from Europe and Japan. Haplotype analysis using

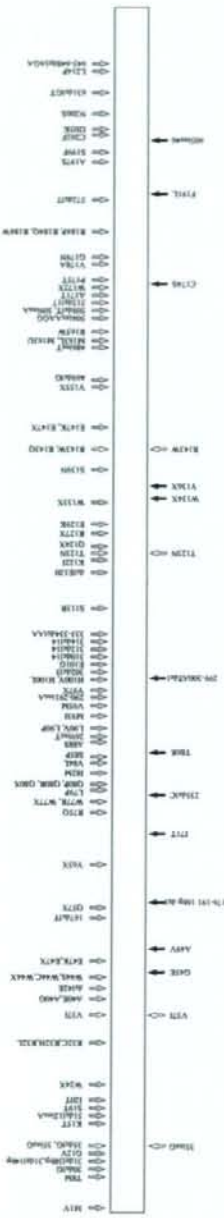

Figure 1. Differences in *GJB2* mutation spectra between Japanese populations and populations with European ancestry. Shaded arrows indicate mutations found in Caucasoid populations; filled arrows indicate mutations found in Japanese; white arrows indicate mutations reported in both Caucasoid and Japanese families.

microsatellite markers excluded a single founder mutation and therefore it is most likely a hot spot mutation [30]. In the case of the mitochondrial 1555A→G mutation, which has been reported in many ethnic populations, phylogenetic analysis of independent families with that mutation has suggested that it is sporadic and has multiplied through the evolution of the mtDNA [31]. Therefore, it is conceivable that this mutation does not have a common founder, but may be a mutational hot spot.

From the above facts, in the search for responsible genes in deafness patients, prior screening based on recurrent mutations and ethnic databases of deafness genes should be done. These facts indicate that each population may not have its own responsible genes, but may have its own mutational spectrum within the same catalog of deafness genes.

Invader assay as a simultaneous mutation screening

Simultaneous technology, including the microarray approach as well as Invader assay, has developed rapidly and will likely facilitate great improvements in medical management and genetic counseling. The use of a microarray approach for multi-gene deafness mutation detection has been recently reported [32,33]. We have recently succeeded in screening deafness genes effectively by means of an Invader assay panel (including 41 known mutations of 9 known deafness genes) [4], enabling diagnosis of approximately 30% of the congenital hearing loss patients who had at least one mutation in *GJB2*, *SLC26A4*, and/or the mitochondrial 12S rRNA, in accordance with results in our mutation screening series. The Invader technology has excellent sensitivity and accuracy and is advantageous for the following reasons: 1) reduced contamination due to being PCR-independent, 2) extremely simple operation that can be learned quickly and does not require PCR-purification or post-hybridization washing skills, 3) detection of low-level heteroplasmy, and 4) amenability to automation. Genetic screening based on the mutation spectrum of a corresponding population may be an appropriate and effective strategy for detecting mutations in causative deafness genes.

Although mutation spectra have been demonstrated to be dependent on each population, mutations within the prevalent four genes have been extensively reported across many populations and are currently recognized to be the most common deafness genes worldwide. Therefore, the present results are consistent with a series of reports and

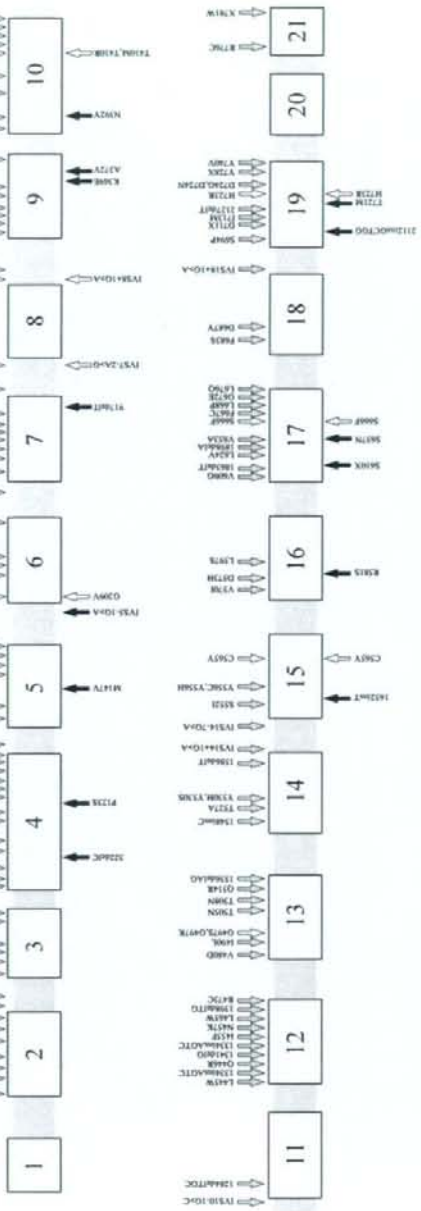

Figure 2. Differences in *SLC26A4* mutation spectra between Japanese populations and populations with European ancestry. Shaded arrows indicate mutations found in Caucasoid populations; filled arrows indicate mutations found in Japanese; white arrows indicate mutations reported in both Caucasoid and Japanese families.

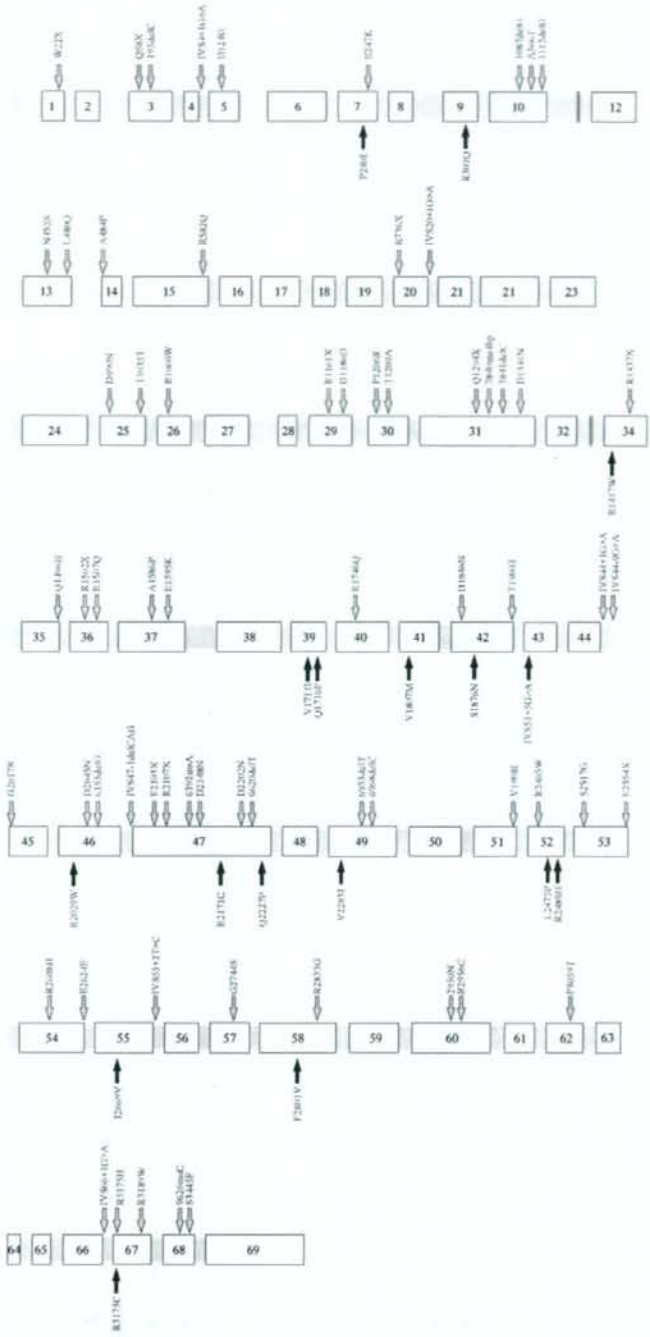

Figure 3. Differences in *CDH23* mutation spectra between Japanese populations and populations with European ancestry. Shaded arrows indicate mutations found in Caucasoid populations; filled arrows indicate mutations found in Japanese.

this strategy may also be applicable to other populations.

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