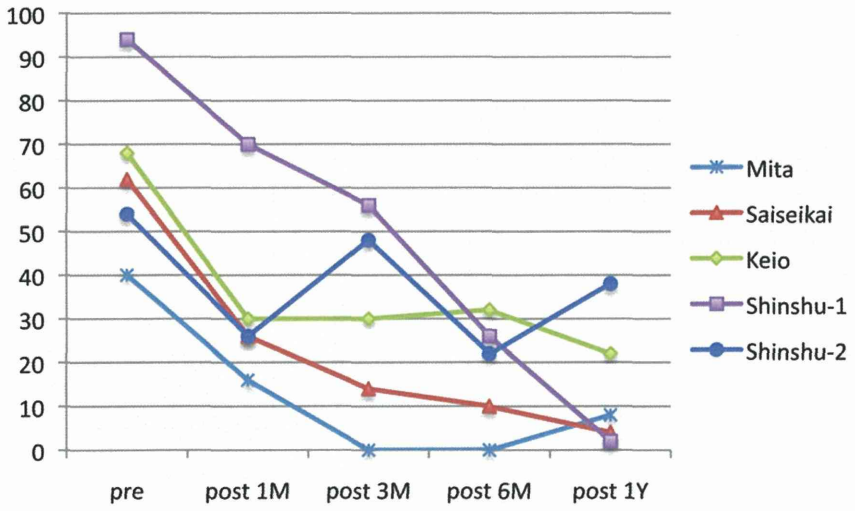

Figure 3. The time course of the THI scores for the five cases: All patients reported a marked reduction in tinnitus after cochlear implantation.

Conclusion

Cochlear implantation for Japanese-speaking, single-sided deafness patients resulted in improved speech perception, increased sound localization accuracy, and reduced tinnitus handicap. In the cases reported herein, the hearing assessment results gradually improved over time, particularly during the period from 6–12 months after implantation. It seems, however, that the speech perception ability might be unstable in the initial 1–6 months after implantation. These results suggest that long-term follow-up and auditory training are necessary after implantation and it is possible that CI fitting strategies could be optimized for use in patients with SSD.

Disclosure statement

Because CI for SSD patients had not yet been reimbursed in Japan, the devices were supplied by MedEL. Shinshu University Conflict of Interest Committee as well as the respective Conflict of Interest Committee of the other participating institutions approve the present clinical study. The authors alone are responsible for the content and writing of the paper.

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RESEARCH ARTICLE

SOD1 gene polymorphisms in sudden sensorineural hearing loss

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ABSTRACT

Conclusion The results suggest that SOD1 rs4998557 could be associated with susceptibility to SSNHL in the Japanese population. **Objectives** To assess the gene association with sudden sensorineural hearing loss (SSNHL). **Methods** A two-stage case control study was conducted to explore the relationship of the candidate genes to SSNHL. The 192 gene samples from SSNHL patients registered in the intractable inner ear disease gene bank were enrolled. As the candidate genes, 39 SNPs from 31 genes were selected for the first stage study. The second stage study examined whether the SOD1 gene polymorphisms, defined by significant differences between cases and controls in the first stage study, are associated with SSNHL. **Results** Significant differences were observed in four SNPs from three genes, Glutathione-S-transferase pai 1 (GSTP1), proteine kinase C heta (PRKCH), and superoxide dismutase 1 (SOD1), in terms of allele frequency between SSNHL patients and HapMap controls. In the SOD1 gene, a significant difference was observed in the dominant model study of the SNP rs4998557 in the second stage study. Furthermore, as a result of dividing SSNHL patients based on the clinical data, the difference was more apparent in the case of the over 60 dB group and the tinnitus–positive group.

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Introduction

Sudden sensorineural hearing loss (SSNHL) is among the diseases specified by the Japanese government as an 'intractable disease' resulting from an unidentifiable cause and without a clearly established treatment, and entailing a considerably high risk of disability.

Many hypotheses have been advocated to explain the etiology of SSNHL, including viral inflammation, ischemic events, and autoimmune responses. However, the cause of the pathology remains mostly unclear.

Recently, disease susceptibility genes have been identified in common diseases, such as diabetes mellitus, bronchial asthma, and cerebral infarction. The relevance of some genes to SSNHL, such as *MTHFR* [1,2], *PRKCH* [3], *CFH* [4], and *LTA* [5], has also been reported.

We are constructing a gene bank of intractable inner ear diseases including sudden deafness in collaboration with other institutions associated with the Sudden Deafness Research Committee of the Ministry of Health and Welfare, Japan.

In the present study, we examined gene associations with SSNHL using the samples accumulated for the above-mentioned bank. We conducted a two-stage case control study to explore the relationship of the candidate genes with SSNHL. In the first stage study, candidate genes reported in the past to have relevance to sudden deafness and other diseases considered to have related pathology were analyzed. In the second stage study, we used another group of control samples to examine whether the *SOD1* gene polymorphisms, defined as those showing a significant difference between cases and controls in the first stage study, are associated with SSNHL.

Materials and methods

Cases

A total of 192 gene samples from SSNHL patients registered in the intractable inner ear disease gene bank were included.

In the first stage study, 96 of the 192 gene samples were extracted at random and analyzed. All 192 samples were used for analysis in the second stage study. SSNHL was defined according to criteria established by the Sudden Deafness Research Committee of the Ministry of Health and Welfare, Japan (1973). Details of the criteria are shown in Table 1.

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Table 1. Criteria for the diagnosis of sudden deafness.

Main symptoms
(1) Sudden onset of hearing loss; patient can say clearly when it appeared
(2) Sensorineural hearing loss, usually severe
(3) Unknown cause
Accessory symptoms
(1) May be accompanied by tinnitus
(2) May be accompanied by vertigo, nausea, and/or vomiting, without recurrent episodes
(3) No cranial nerve symptoms other than from the eighth nerve
Definite: all of the above criteria
Probable: main symptoms 1 and 2

We performed gene association analysis using the patients' clinical data accumulated for the gene bank. Evaluation of hearing level was performed at 250, 500, 1000, 2000, and 4000 Hz. Data on the presence of vertigo and tinnitus was collected by interviews.

The clinical data for the 192 cases is shown in Table 2.

Controls

As controls in the first stage study, we used the allele frequency of the SNPs of Japanese in Tokyo from the HapMap database [6]. Most of the data were derived from 113 individuals, and some was from 45 individuals.

For the second stage study, we used the samples from 285 healthy adults with normal hearing from the Shinshu University control gene samples.

Ethics statement

The study protocol for DNA sampling from the patients and controls was reviewed and approved by the Ethics Committee of each collaborative institution, and written informed consent was obtained from all subjects.

SNP selection and genotyping

As the candidate genes, 39 SNPs from 31 genes were selected for the first stage study (Table 3).

(1) 14 SNPs from 12 genes reported to be related to SSNHL in the past were included.
(2) Since an ischemic event was conventionally considered to be related to the etiology of SSNHL, three SNPs from three genes reported to be associated with ischemic diseases were also selected.
(3) Because oxidative stress is considered to be a mechanism of inner ear injury, 13 SNPs from 13 genes related to the oxidative stress cascade and the protection against oxidative stress were analyzed.
(4) Since adrenal cortical steroid was used for medical treatment in general, seven SNPs from five of the steroid hormone receptor and inflammatory disorder-related genes were included.
(5) Two SNPs from two ion and water channel genes playing an important role in the inner ear were added to the analysis.

The SNPs were selected mainly by referring to previous reports. Some of the SNPs were selected from the list of SNPs/genes available in the NCBI database and using the search program of the LD block of the SNPs, SNP browser Software (Applied Biosystems, Foster City, CA).

Table 2. Clinical profiles of patients from the gene bank of intractable inner ear disorders.

Patient number	192
Age (years)	56.4 ± 14.4
Gender (M:F)	94:98
Affected ear (R:L)	95:97
Tinnitus (%)	86.4
Vertigo (%)	38.6
Initial PTA (dB)	73.9 ± 22.3

Real-time PCR using the Taqman probe (Applied Biosystems) was used for the typing of gene polymorphisms, with the reactions performed in 96-well microplates in StepOnePlus Real-Time PCR Systems (Applied Biosystems). Fluorescence was measured, and analyzed with System SDS software that uses an advanced multicomponent algorithm to calculate the distinct signal contribution of each allele of a marker.

Statistical analysis

In the first stage study, a Chi-square test was used to compare allele frequencies between SSNHL patients and the HapMap database as a control. Odds ratios were calculated with 95% confidence intervals.

A p value of less than 0.05 was considered statistically significant.

In the second stage study, simultaneous with the analysis of the allele frequency, we examined the effect of the minor allele of each SNP in two genetic models, dominant and recessive. Furthermore, the cases were divided into two groups based on hearing level and the existence of vertigo and tinnitus, and the same type of analysis was carried out.

Results

First stage study for the candidate genes

The call rate in SNP typing by this method was 99.4%. Hardy-Weinberg equilibrium (HWE) was tested using the Chi-square test and no SNPs showed significant deviation from HWE ($p < 0.05$). Significant differences in the allele frequencies were observed between SSHNL patients and HapMap controls in four SNPs from three genes among the 39 SNPs from 31 genes tested (Table 4).

Second stage study for the SOD1 gene

Among the genes with significant differences in the first stage study, we selected SNPs of the *SOD1* gene for the second stage study, because *SOD1* was the only gene with two significantly different SNPs between the cases and controls. The genotype distribution of the two polymorphisms is described in Table 5. No significant differences were found between the 192 SSNHL patients and 285 in-house controls in terms of the allele frequencies of the SNP rs4998557 and rs1041740. When a gene model was assumed, a significant difference was observed in

Table 3. Selected candidate SNPs and profiles for the first stage association study.

Gene	SNPs	Minor allele	MAF of control (%)	Function of the gene
(1) Genes reported to be associated with sudden sensorineural hearing loss				
MTHFR	rs1801133	T	39.0	remethylation of homocysteine to methionin
	rs1801131	G	18.6	
F2	rs2070850	C	47.7	coagulation factor 2
F5	rs2227244	C	18.8	coagulation factor 5
ITGB3	rs3851806	C	35.3	association for platelet activation
LTA	rs1041981	A	40.9	cytokine in the inflammation process (TNF-β)
NOS3	rs1799983	T	6.8	modulating flow-mediated vasodilation
PRKCH	rs2230500	A	1.3	one of the protein kinase C family
IL1A	rs1800587	A	11.1	inflammatory cytokine
CFH	rs1329423	A	48.8	regulatory protein during complement activation
	rs800292	A	41.6	
IL4R	rs1801275	G	12.4	regulate IgE antibody production in B cells
UCP2	rs660339	C	44.2	control of mitochondria-derived ROS
EDN1	rs5370	T	26.5	one of isoforms of human endothelin
(2) Ischemic disease–related genes				
APLNR	rs948847	G	32.3	regulation of blood pressure
PDE4D	rs702531	A	41.9	member of the phosphodiesterase
SURPINE1	rs2227631	A	42.3	plasminogen activator inhibitor type 1
(3) Oxidative stress–related genes				
GSTP-1	rs1695	G	8.8	conjugation of glutathione with xenobiotics
SOD1	rs4998557	A	45.6	convert superoxide anion into H_2O_2
	rs1041740	T	39.4	
GPX	rs1800668	A	10.2	catalyzes breakdown of H_2O_2 to H_2O and O_2
CAT	rs769217	T	49.1	catalyzes breakdown of H_2O_2 to H_2O and O_2
	rs475043	G	2.7	
GSR	rs3779647	T	37.2	reduces oxidized glutathione
	rs2253409	G	14.8	
	rs2251780	T	18.2	
GCLM	rs7549683	T	21.2	glutamate-cysteine ligase modifier sub-unit
SOD2	rs4880	G	9.7	convert superoxide anion into H_2O_2
MPO	rs7208693	A	14.3	produces strong oxidant, hypochlorous acid
PON1	rs662	T	28.4	hydrolyze specific oxidized lipids
(4) Steroid hormone receptors and imflammation–related genes				
IL7R	rs6897932	T	18.3	T-cell development
ESR1	rs2234693	C	42.0	estrogen receptor
	rs9340799	G	16.7	
NR3C1	rs4912910	A	40.7	glucocorticoid receptor
NR3C2	rs2070951	G	30.5	mineralocorticoid receptor
	rs5522	C	21.7	
FKBP5	rs9470080	T	33.5	glucocorticoid upregulates FKBP5 in cochlear
(5) Inner ear receptor genes				
AQP4	rs3763043	T	37.3	maintaining intracellular/extracellular water balance
KCNE1	rs2070358	T	38.5	K^+ ion channel

Table 4. Summary of the SNPs with significant differences in allele frequency.

		MAF (%)			
Gene	SNPs	Case ($n = 96$)	Control ($n = 113$)	p value	OR (95% CI)
SOD1	rs4998557	57.3	45.6	0.017	1.60 (1.09–2.36)
	rs1041740	29.7	39.4	0.038	0.65 (0.43–0.98)
GSTP-1	rs1695	15.1	8.8	0.047	1.83 (1.01–3.34)
PRKCH	rs2230500	15.4	1.3	1.159×10^{-7}	13.39 (5.13–34.97)

Table 5. Genotype distribution of the *SOD1* SNPs and summary of the statistical analysis.

	Case	Control	p value		
SNP/Genotypes	($n = 192$)	($n = 285$)	Allele frequency	Dominant	Recessive
rs4998557					
GG	40	62			
GA	89	154	0.148	0.039	0.809
AA	63	69			
rs1041740					
CC	102	134			
CT	66	119	0.336	0.801	0.156
TT	23	32			

the dominant model study of the SNP rs4998557 ($p = 0.039$, OR = 1.53, 95% CI = 1.02–2.29).

Genotype distribution and clinical data analysis

The cases were divided into two groups based on hearing level (over or under 60 dB) and the existence of vertigo and tinnitus. The distribution of genotype and allele frequencies in each subgroup is shown in Table 6. The difference in the dominant model study of the SNP rs4998557 was more apparent in the over 60 dB group ($p = 0.008$, OR = 1.86, 95% CI = 1.18–2.94) and tinnitus–positive group ($p = 0.026$, OR = 1.64, 95% CI = 1.07–2.52). No significant differences for the SNP rs1041740 were found.

Discussion

Although the etiology of SSNHL is unclear, it is considered to be a multifactorial disease, possibly caused by interactions between genetic and environmental factors. Several recent

Table 6. Genotype distribution and clinical data.

		Genotype			*p* value		
rs4998557		GG	GA	AA	Allele frequency	Dominant	Recessive
Initial PTA	60 dB>	9	24	10	0.991	0.891	0.903
	60 dB<	25	49	44	0.077	0.008	0.897
Tinnitus	positive	32	69	53	0.113	0.023	0.812
	negative	4	14	3	0.652	0.301	0.771
Vertigo	positive	14	34	20	0.504	0.375	0.833
	negative	20	48	33	0.203	0.097	0.68
rs1041740		CC	CT	TT			
Initial PTA	60 dB>	17	20	6	0.347	0.603	0.359
	60 dB<	67	38	13	0.162	0.951	0.074
Tinnitus	positive	83	52	18	0.307	0.866	0.149
	negative	10	8	3	0.869	0.671	0.957
Vertigo	positive	28	24	7	0.983	0.888	0.951
	negative	54	36	12	0.477	0.884	0.304

studies have reported associations between polymorphisms in the genes of some patients with SSNHL. One of these includes polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*) C677T [1,2]. Genetic linkage data for SSNHL are limited in Japan because the number of samples is limited in each research institution. For this study, we used samples from the gene bank collected from many institutions in Japan, the largest number of SSNHL case samples available to date.

First, we investigated the association between SSNHL and the 31 candidate genes. As a result of the first stage study, significant differences in allele frequency were observed in four SNPs from three genes, Glutathione-S-transferase pai 1 (*GSTP1*), proteine kinase C heta (*PRKCH*), and superoxide dismutase 1 (*SOD1*), between SSHNL patients and HapMap controls.

Glutathione-S-transferase (GST) enzymes catalyze the conjugation of glutathione to xenobiotic substrates and other compounds for the purpose of detoxification. This detoxification reaction involving glutathione is considered to play an important role in oxidative stress response, preventing damage to the cochlea caused by reactive oxygen species. *GSTP1* is one of the gene classes of GST and is most prominent in the inner ear of the rat [7]. The polymorphisms of other classes of GST, *GSTM1* and *GSTT1*, have been reported to be associated with the risk of SSNHL, but the frequencies in *GSTM1* and *GSTT1* null genotypes did not differ from those of control subjects [8].

Protein kinase C (PKC) is a serine-threonine kinase that regulates a wide variety of important cellular functions including proliferation, differentiation and apoptosis. PKCs are classified into various isoforms, and *PRKCH* is one of the novel PKC isoforms [9]. The *PRKCH* gene has been recently reported as a susceptible risk locus for enzyme cerebral infarction in Asians [10]. Also, the same SNP of *PRKCH* has been reported to have an association with SSNHL [3]. The results of those studies may indicate an underlying vascular pathogenesis of SSNHL.

SOD is an antioxidant enzyme that changes superoxide anions into oxygen and hydrogen peroxide. In the inner ear, it is known to have high activity comparable to that in the central nervous systems, such as the brain stem and cerebellum [11]. Moreover, SOD localizes widely in the cochlea, including the spiral ligament, stria vascularis, spiral ganglion cell, and Organ of corti [12]. The absence of *SOD1* resulted in hearing loss at an earlier age than in wild-type mice in a uniform B6 strain background [13]. A series of previous studies have evaluated the association between SNPs of SOD genes and susceptibility to noise-induced hearing loss [14–16]. There were statistically significant associations between some SOD1 SNPs and noise-induced hearing loss in Chinese workers [14]; however, no significant association was found in a Swedish population [15]. Concerning allele frequencies of SOD1 gene polymorphisms, there are large race-specific differences. For example, for the SNP rs4998557, minor allele frequency is ~10% in Europeans, while it is ~45% in Japanese. So SOD1 gene polymorphisms may contribute to inner ear pathology of such noise-induced hearing loss in Asian populations. Fortunato et al. [16] reported the association between SOD2 gene polymorphisms, other type of SOD, and noise-induced hearing loss. The SOD2 enzyme may also be involved in inner ear protection from noise-induced damage.

There have been a limited number of reports on the association between SSNHL and genetic polymorphisms of antioxidant enzymes including SOD. Teranishi et al. [17] previously reported the effects of polymorphisms in genes involved in oxidative stress response, SOD2, PON1, PON2, and GPX1, on the risk of susceptibility to SSNHL and Ménière's disease, but SOD1 gene polymorphisms were not involved in the study. Also in our study, no significant associations were observed between the risk of SSNHL and gene polymorphisms of SOD2, PON, or GPX.

In the *SOD1* gene, a significant difference was observed in the dominant model study of the SNP rs4998557 in the second stage study. Furthermore, as a result of dividing SSNHL patients based on their clinical data, the difference in the dominant model study of the SNP rs4998557 was more apparent in the over 60 dB group and the tinnitus–positive group. The association of Matrix Metalloproteinase-1 gene polymorphism with SSNHL has previously been shown in tinnitus–positive patients [18]. Also, regarding the relation of gene polymorphism of complement factor H with SSNHL, a higher relevance was observed in the SSNHL patients with diabetes mellitus as a complication [4]. Furthermore, a higher frequency of the minor allele of the PON1 polymorphism was observed in SSNHL cases with good recovery compared to those with poor recovery [17]. Potential etiologies of SSNHL may include various factors, so dividing patients based on their clinical data can lead to results the better reflects the pathogenesis for each group of SSNHL patients.

Although some of the candidate genes such as *MTHFR* [1,2], *CFH* [4], and *LTA* [5] had been reported to have a relation with SSHNL in the past, we failed to find any significant association between the SNPs of those reported genes and SSNHL.

MTHFR is an enzyme involved in the re-methylation of homocysteine to methionine.

The SNP (rs1801133) of *MTHFR* is the most common genetic cause of hyperhomocysteinemia [19], which is believed to promote atherosclerosis and atherothrombosis as risk factors for macroangiopathies and microvessel disease [20]. The association between SSNHL and the SNP of *MTHFR* has been reported among various populations [1,2]. In a report from Italy, the frequency of the T allele in controls was as low

as 30.5% [1]. There is a statistically significant difference in the allele frequencies between SSNHL patients in the present study and the controls in the Italian study. In a previous report from Japan, although there was a large control of 2000 or more subjects, the prevalence of SSNHL was based on self-reporting and the number in the SSNHL group was only ~30 [2]. With this small number of samples, there is a problem in power of statistical analysis, but with the addition of our samples it can be considered to be suitable for further analysis.

Some limitations to the present study should be considered when interpreting its findings. Although a two stage study was carried out, the SSNHL cases in the second stage study were not independent of the cases in the first stage, so this study did not serve as a form of perfect replication study, but was a combined study in design. We need to register more SSNHL patients for this gene bank, and further studies are needed to investigate the association with genetic factors in SSNHL.

Conclusion

The present study has demonstrated a significant association between *GSTP1*, *PRKCH*, and *SOD1* gene polymorphisms, and SSNHL in the first stage, and one *SOD1* gene was observed to have a significant difference in the dominant model of the SNP rs4998557 in the second stage. Furthermore, as a result of dividing SSHNL patients based on their clinical data, the difference in the dominant model study of the SNP rs4998557 was more apparent in the over 60 dB group and the tinnitus-positive group. Although potential etiologies of SSNHL may include various factors, in the majority of cases the cause is unclear. Therefore, a gene association study approach together with dividing patients based on their clinical data led to a result that better reflects the pathogenesis of SSNHL patients.

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Disclosure statement

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

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◆特集・突発性難聴 update

突発性難聴の遺伝的背景

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Abstract　突発性難聴は患者の QOL を著しく低下させるため疾患の克服が期待されている重要な疾患のひとつである．現在までに，局所循環障害，ウイルス感染，内リンパ水腫，自己免疫疾患などの複数の機序が推測されている．突発性難聴は，遺伝的要因と環境要因の双方が関与する多因子疾患であることが想定されており，候補遺伝子を対象にした遺伝相関解析が行われている．現在までに突発性難聴との関連が示唆されている遺伝的要因としては，プロトロンビン遺伝子，第Ⅴ因子，*MTHFR*，*NOS3*，などの遺伝子多型が関与することが明らかとなってきた．これらの遺伝子多型は，局所循環障害を引き起こすリスクファクターであり，突発性難聴発症のメカニズムに局所循環障害が関与することを支持するものである．また，筆者らが最近行った検討により酸化ストレスの関与も明らかとなってきた．今後さらなる大規模解析を行うことにより発症のメカニズム解明が期待される．

Key words　突発性難聴(idiopathic sudden sensorineural hearing loss)，遺伝子(gene)，多因子疾患(multifactorial disease)，遺伝相関解析(gene association study)

はじめに

突発性難聴は，1973 年に診断基準が作成され疾患概念が確立するとともに，1982 年には特定疾患(難病)として認められた疾患である．突発性難聴の診断は 1973 年に厚生労働省突発性難聴調査研究班により作成された「突発性難聴診断の手引き」が用いられている．突発性難聴は「原因不明」，「突然の発症」，「高度難聴」が診断の主要項目となっており，患者の QOL を著しく低下させる[1]ため疾患の克服が期待されている．従来から種々のアプローチで研究が行われているが，疾患概念自体が「原因不明」という疾患であるため，未だ確定的な発症メカニズムは不明である．

現在までに，局所循環障害，ウイルス感染，内リンパ水腫，自己免疫疾患などの複数の機序が推測されており，副腎皮質ステロイド薬，血管拡張薬，代謝改善薬，ビタミン製剤，高気圧酸素療法など，内耳循環障害やウイルス感染等による炎症抑制を想定した治療が行われているが，エビデンスの高い効果的な治療方法は確立していない状況である．この原因の一つとして，突発性難聴という疾患の中に，発症原因の異なる複数の疾患が混在しているため，単一の治療法では有効性のエビデンスが出ない可能性が高い．我が国で行われた単剤の効果を比較した研究でも有意差が出ていない．また，突発性難聴の診断基準確立後，ムンプスウイルスの不顕性感染による難聴，外リンパ瘻による難聴に関する診断基準が作成され，鑑別を要する疾患として認識されるようになってきた．

多因子疾患としての突発性難聴

近年の分子遺伝学的検査手法の発達により，糖尿病や高血圧，ガンといった様々な疾患に遺伝子

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が関与することが明らかとなってきた．これらの疾患は，単一遺伝子によるメンデル疾患と異なり，疾患に複数の遺伝子が関与することに加えて環境要因も関与する多因子疾患であることが報告されている．このような多因子疾患の遺伝子解析には，遺伝相関解析が用いられることが多い．

遺伝相関解析は，ある疾患の原因となる真の変異があると仮定し，その周辺に存在するマーカーは連鎖して遺伝するという法則を利用し，患者集団とコントロール集団のヒトゲノム中に認められる一塩基多型マーカー(SNPsマーカー)の遺伝子型を決め，頻度の比較を行うことにより疾患に関連する遺伝子を見出す解析手法である．

糖尿病や高血圧など罹患者の多い疾患に関しては，全ゲノム中に存在する多型を網羅的に解析する全ゲノム相関解析(Genome Wide Association Study；GWAS)が広く用いられているが，突発性難聴のように罹患者頻度が低い疾患の場合には十分な検出力を有した検討を行うことが困難であるため，推定される病態から関与する遺伝子候補を選別して遺伝相関解析を行う候補遺伝子解析が用いられている．

2015年4月時点で，突発性難聴を対象に遺伝相関解析を行った検討は37件行われている(表1)[2)~40)]．また，2012年にLinらが報告したメタアナリシスの文献が非常に良くまとまっている[41)]

突発性難聴の医学的リスクファクター

突発性難聴のリスクファクターとしては，推定病態である局所循環障害，ウイルス感染，内リンパ水腫，自己免疫疾患などに関連が深い因子を対象にした様々なバイオマーカーに関する検討が行われている．

Linらが行ったメタアナリシスの結果では，高血圧は突発性難聴患者群では13.6%に認めるのに対してコントロール群では0.5%に認めるに留まっていた．また，糖尿病は重要なリスクファクターになっており，突発性難聴患者群の有病率が6.5%であるのに対して，コントロール群ではわずか0.15%に認めるのみであった．また，過去(あるいは現在)の喫煙歴は突発性難聴患者群では36%であるのに対してコントロール群では19.1%であった．また，飲酒に関しては，突発性難聴患者群では11.8%で1日にワイン2杯以上であるのに比較し，コントロール群では15.1%であった．これらの因子は全て局所循環障害のリスクファクターであり，突発性難聴の病態に局所循環障害が関与することを強く示唆する．一方，メタアナリシスの結果からは，血中コレステロール値(Total－，HDL－，LDL－，TG)，フィブリノーゲン値はコントロールとの間で差が認められず，古典的な循環障害ではうまく説明の付かない部分もみられる．しかしながら，突発性難聴症例では，血中の葉酸濃度が有意に低いことが報告されている．また，ホモシステインの濃度に関しては報告により異なるが，突発性難聴群で有意に高いとする報告もあり，動脈硬化による局所循環障害がリスクファクターとなっていることが示唆されている．

また，酸化ストレスの関与を支持するデータも報告されている．突発性難聴患者群ではコントロール群と比較してコエンザイムQ10の血中濃度が有意に低いことが報告されている．また，ω9不飽和脂肪酸およびネルボン酸(一価不飽和脂肪酸の一種)の血中濃度が有意に低いことも報告されている．いずれも，血液中の抗酸化力の低下を示す報告であり，酸化ストレスが突発性難聴に関与する可能性が支持されている．

突発性難聴の遺伝学的リスクファクター(局所循環障害関連因子)

医学的リスクファクターと同様に，遺伝学的リスクファクターに関する検討も，推定病態である局所循環障害，ウイルス感染，内リンパ水腫，自己免疫疾患などに関連が深い因子を対象に前述の候補遺伝子解析による検討が行われている(表1)．

局所循環障害に関連する因子としては，血小板

表 1. 突発性難聴を対象に行われた候補遺伝子解析

著者名	雑誌名・出版年	解析人数	コントロール人数
Yeo SW et al.	Acta Otolaryngol 2000	35	206
Yeo SW et al.	Arch Otolaryngol Head Neck Surg 2001	41	206
Rudack C et al.	Hear Res 2004	85	85
Capaccio P et al.	Genet Med 2005	45	135
Görür K et al.	Otol Neurotol 2005	46	95
Fatini C et al.	Clin Appl Thromb Hemost 2005	80	80
Nam SI et al.	Life Sci 2006	97	614
Capaccio P et al.	Am J Otolaryngol 2005	67	134
Amor-Dorado JC et al.	Acta Otolaryngol 2005	33	145
Rudack C et al.	Thromb Haemost 2006	142	84
Gross M et al.	Audiol Neurootol 2006	81	264
Cadoni G et al.	Otol Neurootol 2006	80	80
Capaccio P et al.	Laryngoscope 2007	100	200
Ballesteros F	Audiol Neurootol 2009	99	
Um JY et al.	Clin Appl Thromb Hemost 2010	97	587
Uchida Y et al.	Laryngoscope 2010	33	2141
Lee EJ et al.	J Laryngol Otol. 2010	33	68
Mosnier I et al.	Audiol Neurootol 2011	96	179
Fusconi M et al.	Audiol Neurootol 2011	40	120
Nam SI et al.	Laryngoscope 2011	99	530
Furuta T et al.	Int J Immunogenet 2011	68	2202
Lan MY et al.	Eur Arch Otorhinolaryngol 2011	24	36
Um JY et al.	Otol Neurootol 2011		
Hiramatsu M et al.	J Neurogenet 2012	72	2010
Uchida Y et al.	J Neurogenet 2011	33	2188
Nishio N et al.	Gene 2012	72	2161

候補遺伝子	解析多型	有意差	
HLA-DRB1	＊14	○	
HLA-DRB1	＊04	×	
HLA-DRB1	＊14	×	
GP Ⅰa	c.807C>T	○	
MTHFR	c.677C>T	×	
MTHFR	c.677C>T	○	
MTHFR	c.1298A>C	○	
F V	Leiden	○	Odds 比：2.08
NOS3	c.894G>T	○	Odds 比：2.81
NOS3	c.-786T>C	×	
IL4	p.Q576R	○	Odds 比：2.58(CI 95%，1.84-3.60)
MTHFR	c.677C>T	○	
	c.1298A>C	○	
HLA-DRB1	＊0403	○	Odds 比：11.97(CI 95%，1.99-91.60)
HLA-DRB1	＊04		
GP Ⅰa	c.807C>T	○	喫煙がリスクファクター
MTHFR	c.677C>T	×	
MTHFR	c.1298A>C	×	
MTR	c.2756A>G	○	
GSTM1		×	
GSTT1		×	
MTHFR	c.677C>T	○	
F Ⅱ	c.20210G>A	○	ホモシステイン・コレステロール・フィブリノーゲン高値／葉酸低値
Gly Ⅲa	A1/A2	○	
F V	Leiden	○	
F V	Leiden	×	
TNF-α	c.-308G>A	×	
TNF-β	c.252A>G	○	Odds 比：1.534(CI 95%，1.12-2.10)
MTHFR	c.677C>T	○	Odds 比：1.687(CI 95%，1.023-2.780)
MTHFR	c.677C>T	×	
F V	Leiden	×	高血圧，循環器疾患の家族歴有意差あり，個人の循環器疾患のリスクファクターは関連無し
F Ⅱ	c.20210G>A	×	
MTHFR	c.677C>T	○	
F V	Leiden	×	
F Ⅱ	c.20210G>A	×	
MMP1	c.-1607insG	○	
IL1A	rs1800587	○	Odds 比：25.89(CI 95%，12.19-54.98)
IL1B	rs16944	×	
F V	Leiden	×	
F Ⅱ	c.20210G>A	×	
GSTM1		×	
GSTT1		×	
P450		×	
IL6	c.-572C>G	○	Odds 比：1.734(CI 95%，1.080-2.783)
IL4R	c.1902G>A	×	
IL10	c.-592A>C	×	
TNF-α	c.-863C>A	×	
TNFRSF1B	c.593G>A	×	
VEGF	c.936C>T	×	
VEGF	c.-2578C>A	×	
VEGF	c.-1154G>A	×	
PRKCH	c.1424G>A	○	Odds 比：1.770(CI 95%，1.024-3.060)
CHF	p.Y402H	○	Odds 比：1.820(CI 95%，1.025-3.232)

Teranishi M et al.	DNA Cell Biol 2012	84	2107
Chien CY et al.	Audiol Neurootol 2012	160	178
Fusconi M et al.	Int J Audiol 2012	49	210
Ballesteros F et al.	Audiol Neurootol 2012	118	161
Um JY et al.	Immunophalmacol Immunotoxicol 2013	102	595
Teranishi M et al.	Free Radic Res 2013	83	2048
Nishio N et al.	Life Sci 2013	85	2136
Uchida Y et al.	Laryngoscope 2013	72	2159
Liu B et al.	Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2013	735	1230
Cadoni G et al.	Laryngoscope 2015	87	107
Castiglione A et al.	BioMed Res Int 2015	200	400

因子である第Ⅴ因子(プロアクセレリン)のLeiden変異(p. R509Q)に関する検討が多く行われている．第Ⅴ因子のLeiden変異は，静脈血栓塞栓症のリスクファクターであることが報告されており，コーカソイドに比較的多く認められる変異である．突発性難聴と第Ⅴ因子Leiden変異に関してはメタアナリシスの結果では有意差を認め，突発性難聴のリスクファクターであることが示唆されている．しかしながら，有色人種においては非常に稀な変異であるため，Leiden変異のみで突発性難聴の原因を説明することは困難である．また，プロトロンビン(第Ⅱ因子)のc. 2021G>A多型に関しての検討が行われているが，突発性難聴患者群とコントロール群の間の有意差はメタアナリシスの結果においても報告により異なるため，現時点では関与は明確ではない．血液凝固補助因子として血小板のコラーゲン粘着反応に関与するグリコプロテインであるGP Ⅰa/Ⅱa，*ITGB3* (Gly Ⅲa)遺伝子多型に関する検討も行われており，突発性難聴との関連が報告されている．

また，主に血管拡張作用に関与する一酸化窒素合成酵素であるeNOS(NOS3)の多型c. 894G>Tが突発性難聴群において有意に高頻度であることが報告されていることもからも，局所循環障害の関与が示唆されている．

さらにまた，メチレンテトラヒドロ葉酸還元酵素である*MTHFR*遺伝子のc. 677C>T多型，c. 1298A>C多型と突発性難聴の関連に関して多く検討されている．c. 677C>T多型はメチレンテトラヒドロ葉酸還元酵素の酵素活性を野生型(CC

	GPX	rs1050450	×	
	PON1	rs662	×	
	PON1	rs854560	×	
	PON2	rs7493	×	
	SOD2	rs4880	×	
	HSP70	rs2075800	○	Odds 比：0.59(P＝0.019)
	MTHFR	c.C677T	○	ホモシステイン高値
	ITGB3	rs5918	×	
	ITGA2	rs1126643	○	
	IL1B	c.-511C>T	○	Odds 比：9.111(CI 95%，1.441-57.618；P＝0.022)
	IL1B	c.3953C>T	○	
	MTR	rs1805087	×	
	MTRR	rs1801394	×	
	NOS3	rs1799983	○	Odds 比：2.108(CI，1.343-3.309)
	Cav1	rs3840634	×	メニエール病では有意
	MTNR1B	rs1387153	×	
	NADH/NADPHp22phox	rs4673	×	
	MT5178	rs28357984	×	
	AQP4	rs2075575	×	
	AQP5	rs3736309	×	メニエール病では有意
	ERα1	rs2234693	×	
	ERα2	rs9340799	×	
	EDN1	p.K198N	○	Odds 比：2.209(CI 95%，1.140-4.281)
	F II	c.20210G>A	○	9論文のメタアナリシス／Odds 比：1.79(CI 95% 1.06-3.01；P＝0.03)
	MCP1	c.2518A>G	×	
	E-secretin	p.S128R	×	
	IL6	c.174C>G	○	
	FPN1	c.-8C>G	○	Odds 比：4.27(CI 95%，2.65-6.89；P＝0.001)
	TF	p.P570S	×	
	HFE	p.H63D	×	
	HFE	p.C282Y	×	
	HEPC	c.-582A>G	×	

型）と比較し，CT のヘテロ型で 65%に，TT のホモ型では 30%にそれぞれ低下させることが報告されている．MTHFR タンパク質の活性低下は，メチオニン代謝を低下させ，ホモシステインがメチオニンおよびシステインに変わる過程が阻害されるため，結果的にホモシステインが増加につながる．ホモシステインは LDL コレステロールと結合した後に，LDL コレステロールの酸化を引き起こす．酸化 LDL はマクロファージにより処理されるが，酸化 LDL 量が多い場合にはマクロファージとともに血管壁に付着することで動脈硬化を促進すると考えられており，局所循環障害の原因として重要な因子であると考えられている．メタアナリシスの結果では，どちらの多型も有意に突発性難聴症例に多く認められており，*MTHFR* 遺伝子の活性低下により，ホモシステインの増加を介した突発性難聴発症のリスクファクターになっていることが示唆されている．実際に，医学的リスクファクターとして，突発性難聴患者群において，葉酸低値，ホモシステイン高値が報告されていることからも，*MTHFR* 遺伝子の関与が推測されている．

突発性難聴の遺伝学的リスクファクター（免疫応答関連因子）

また，ウイルス感染や自己免疫疾患との関連としては種々のサイトカインの遺伝子多型および HLA-DRB の多型に関して検討が行われている．IL-1α の c. −899C>T 多型，IL-1β の c. −511C>T，c. 3953C>T，IL-4 の p. Q576R，IL-6 の c.

−572C>G, c. 174G>C, TNFβのc. 252A>G多型と突発性難聴との間に相関が認められることが報告されている．これらの因子の多型はサイトカイン活性の変化を誘起し，結果的に炎症反応が増強されることが発症メカニズムとして想定されている．

突発性難聴の遺伝学的リスクファクター（酸化ストレス関連因子）

活性酸素種は動脈硬化をはじめとした循環障害，リンパ球の成熟を抑制することによる免疫機能の低下等に関与することが報告されており，直接的な内耳障害のみならず，内耳での循環障害や感染などを介して突発性難聴を引き起こす原因となっている可能性が考えられる．

特に，近年多くの疾患や老化と活性酸素との関連が多数報告されており，内耳障害に関しても，動物実験において活性酸素による有毛細胞の障害や，騒音・耳毒性薬物による内耳障害への関与が報告されている．また，加齢による内耳活性酸素の増加などが報告されている．さらには抗酸化剤の投与がこうした内耳障害に対する保護作用を示すという報告もあり，活性酸素種が内耳障害に関連していることを支持するものと考えられる．また，前述ホモシステインの増加により引き起こされる動脈硬化においてもLDLコレステロールの酸化を介した経路が支持されており，局所循環障害にも関与する重要な因子となっていると考えられる．

酸化ストレス関連遺伝子としては，*GSTM1*, *GSTT1*, *GPX*, *PON1*, *PON2*, *SOD2*の遺伝子多型に関して検討が行われているが，現在までに有意差を認めた遺伝子は報告されていない．筆者らは，厚生労働科学研究難治性疾患克服研究事業「急性高度難聴に関する調査研究」班の研究の一環として，突発性難聴と活性酸素種の関連を明らかにする目的で，呈色クロモゲン法を用いて血中の過酸化物を測定した．その結果，突発性難聴症例では治療前の測定にて酸化ストレス度は有意に高く，全身的な高酸化ストレス状態が発症に何らかの影響を及ぼしている可能性が推測された．治療前の酸化ストレス度と治療効果との間には関連性が認められ，治療効果を予測する因子となる可能性が考えられた．治療後の酸化ストレス度については治療前と比較して低下する傾向を認めた．しかし治療効果と治療後の酸化ストレス度の間には有意な関連性は認めなかったことを見出し報告した[42]．

さらに，原因不明な病態に対するアプローチとして突発性難聴患者を対象に，難治性内耳疾患の遺伝子バンクプロジェクトとして，急性高度難聴に関する調査研究班・前庭機能異常に関する調査研究班との共同研究という形で突発性難聴症例の登録および遺伝子サンプルの収集を行った．本遺伝子バンクに集積されたサンプル数は250に達し，突発性難聴患者の遺伝子バンクとしては，世界でも最大規模のバンクと考えられる．バンクに集積されたサンプルの一部（突発性難聴患者96名）を利用し候補遺伝子相関解析を行った．解析を行った遺伝子としては，過去に突発性難聴に関連すると報告されている遺伝子（*MTHFR*や*PRKCH*など），虚血性疾患に関連することが報告されている遺伝子（*AGTRL1*：日本人の脳梗塞関連遺伝子など），酸化ストレス関連の遺伝子（*GSTP1*や*SOD1/2*など），ステロイドホルモン受容体や炎症性疾患に関連した遺伝子（*NR3C1*（グルココルチコイド受容体）や*ESR*など）に関して検討を行った（Kitoh et al. 投稿中）．

その結果*SOD1*, *PRKCH*, *GSTP1*遺伝子にてアレル頻度に有意差を認めた．一方，過去に突発性難聴との関連が報告されていた*MTHFR*, *NOS3*, *LTA*遺伝子などでは差を認めなかった．また，有意差の認められた遺伝子を対象に，解析対象症例数を192例に増やすとともに，信州大学で収集した聴力正常で突発性難聴等の耳疾患の既往のない成人コントロール（285例）との間で比較を行ったところ，*SOD1*遺伝子のrs4998557多型について優性遺伝モデルで有意差を認めた．この

ように予備的な解析(候補遺伝子関連解析)を実施したところ，酸化ストレス処理に関連する遺伝子である*SOD1*遺伝子の多型で患者群とコントロール群の間で有意差を認める結果となり，突発性難聴の発症と酸化ストレスの関連を強く示唆する結果となった．

考　察

突発性難聴は疾患に複数の遺伝子が関与することに加えて環境要因も関与する多因子疾患であることが示唆されているが，家族歴に関する報告は非常に少ない．Gäcklerらの報告によると[43]，突発性難聴の患者186名(ただし，騒音性難聴などを含む)およびコントロール75名を対象に，家系内における突発性難聴罹患者の有無に関して検討を行った所，突発性難聴患者群では36名の家系内罹患者がいたのに対し，コントロールでは11名の家系内罹患者が認められた．興味深いことに，突発性難聴患者群では，家系内に2名以上の突発性難聴患者を認める家系では，突発性難聴の発症年齢が有意に若年であり，また聴力の改善も有意に低いことが明らかとなった．

また，突発性難聴患者における循環器障害の既往に関しては有意な差は認められていないものの，Leeらは，突発性難聴患者12例中4例がその後2ヶ月以内に脳梗塞イベントを有したこと，また，Linらは突発性難聴患者群では，コントロール群と比較して5年以内の脳梗塞のイベントのリスクが1.6倍となることを報告している．

以上を総合的に考えると，脳梗塞などの循環障害イベントでは問題にならないような微小な局所の循環障害が突発性難聴を引き起こす重要な要因であること，また，血栓の生成を高めるような遺伝的背景を有する突発性難聴患者においては，その後の脳梗塞などの発症リスクが高いことが示唆される．特に家系内に突発性難聴の罹患者がいる場合には，遺伝的リスクファクターを有している可能性が考えられる．突発性難聴に関与する遺伝的因子としては，プロトロンビン遺伝子，第Ⅴ因子，*MTHFR*, *NOS3*, などの遺伝子多型が関与することが明らかとなってきた．また，酸化ストレス応答遺伝子の関与も明らかとなってきた．

しかしながら，現在までに行われた突発性難聴を対象にした遺伝相関解析は，いずれも症例数に乏しく，遺伝統計学的に計算を行うと十分な検出力を有した検討が行われていない場合も多い．また，症例数が少ないことよりGWAS解析は行われておらず，新規の候補遺伝子を明らかにするためには，国際的共同研究も視野に入れた検討が必要であろう．今後，さらに症例数を増加させた大規模解析を行うことにより，突発性難聴の発症メカニズムを解明するとともに，個々の原因に応じた医学的介入法が確立することが期待される．

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Ethnic-Specific Spectrum of *GJB2* and *SLC26A4* Mutations: Their Origin and a Literature Review

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Abstract

Objective: The mutation spectrum of the *GJB2* and *SLC26A4* genes, the 2 most common genes causing deafness, are known to be ethnic specific. In this study, the spectrum of the reported *GJB2* and *SLC26A4* mutations in different populations are reviewed and considered from a human migration perspective.

Methods: Fifty-two and 17 articles on *GJB2* and *SLC26A4* mutations, respectively, were reviewed through the PubMed database from April 1996 to September 2014. The 4 most prevalent mutations were selected and compared. A cluster analysis was subsequently performed for these selected mutations.

Results: The present review of frequent mutations shows the ethnic-specific *GJB2* and *SLC26A4* gene mutation spectrum. A cluster analysis of the *GJB2* and *SLC26A4* genes revealed similarities between ethnic populations.

Conclusion: The mutation spectrum reviewed in this study clearly indicated that the frequent mutations in the *GJB2* and *SLC26A4* genes are consistent with the founder mutation hypothesis. A comparison with the Y-chromosome phylogenetic tree indicated that these mutations may have occurred during human migration.

Keywords

GJB2, *SLC26A4*, mutation spectrum, c.35*del*G, c.235*del*C, p.H723R, Y-chromosome

Introduction

Hereditary hearing loss affects approximately 1 in 1000 infants in developed countries, and genetic causes account for at least 50% of all childhood hearing loss.[1] Mutations in the *GJB2* gene are the most common genetic cause of both congenital and hereditary hearing loss worldwide. A series of studies has demonstrated that 15% to 25% of patients with congenital hearing loss have a *GJB2* mutation.[2-5] To date, more than 100 *GJB2* variations have been reported (see the Connexin-deafness homepage: davinci.crg.es/deafness/), and hearing loss ranges from mild to profound according to genotype differences; therefore, genotype-phenotype correlations are well documented, and this type of hearing loss is thought to be nonprogressive. The detailed audiologic features, including genotype-phenotype correlations and progression in patients with these mutations, have been well studied.[4-6]

Mutations in the *SLC26A4* gene are thought to be the second most common cause of inherited hearing loss. Based on our genetic screening, *SLC26A4* is the second most common responsible gene in Japanese patients with deafness. Mutations in the *SLC26A4* gene are responsible for a broad phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss (NSHL) with an enlarged vestibular aqueduct (EVA). The prevalent association of *SLC26A4* mutations with EVA in these patients (93% in Pendred syndrome and 77% in NSHL with EVA) indicates the importance of this gene in the pathophysiology of this category of hearing impairment,[7] and more than 160 mutations have been found in *SLC26A4* (Pendred/BOR homepage: http://www.healthcare.uiowa.edu/labs/pendredandbor/).

The *GJB2* and *SLC26A4* gene mutation spectrum is ethnic specific. Ethnic background should be considered when performing genetic testing. Thus, knowledge of ethnic and regional differences in the *GJB2* and *SLC26A4* mutation spectrum could help guide genetic testing and assist in clinical decision making. In this study, we reviewed the spectrum of *GJB2* and *SLC26A4* mutated alleles worldwide.

The frequencies of the *GJB2* and *SLC26A4* gene mutations can be considered through the footprints of human migration. A cluster analysis of the 4 most frequent mutations

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was performed to speculate about the origins of the prevalent *GJB2* and *SLC26A4* mutations and to compare them to a phylogenetic tree of the Y-chromosomal haplogroups.

Methods

Literature Search

The PubMed database was searched for the period from April 1996 to September 2014. The following keywords were used to identify all studies on hearing loss: "*GJB2*" or "connexin26" and "*SLC26A4*" or "Pendred syndrome."

A total of 1634 and 695 articles were identified that discussed the *GB2* and *SLC26A4* mutations, respectively.

Inclusion Criteria

Titles and abstracts were screened, and 240 articles about *GJB2* and 125 about *SLC26A4* were chosen for a full-length review according to the following criteria.

1. The article was published in a peer-reviewed journal in the English language.
2. The prevalence of the *GJB2* and *SLC26A4* mutation alleles among all individuals with sensorineural hearing loss was used.
3. All exons and frank regions of all hearing loss probands were sequenced.

When several prevalence studies were from the same country, those articles thought to have the largest number of mutated alleles were chosen; thus, 52 *GJB2* and 17 *SLC26A4* reports were included in this study.

4. The 4 most frequent variants were selected and are summarized in Table 1 and Figure 1 (for *GJB2*), and Table 2 and Figure 2 (for *SLC26A4*).

Cluster Analysis

A cluster analysis was performed to identify the similarities between ethnic populations. Allele frequencies for each selected variant in the ethnic populations were standardized to a *z*-score prior to the cluster analysis. After standardization, the cluster analysis was performed by calculating the Euclidean distance and using Ward's clustering method. All cluster analyses were performed with R version 3.1.2 and the heatmap.3 program including the plug-in package GMD version 0.3.3 (http://CRAN.R-project.org/package=GMD).

Results and Discussion

Frequent GJB2 Mutations

The prevalence of the *GJB2* mutation is summarized in Table 1 and Figure 1.

The c.35*del*G mutation was the most prevalent among the *GJB2* variants. c.35*del*G was predominant throughout Europe, the Middle East, North Africa, North and South America, and Australia. The frequencies of the c.35*del*C mutation had ranges of 71.1% to 100%, 68.6% to 93.8%, 50% to 84.2%, 51.3% to 100%, and 38% in Europe, North Africa, the Middle East, North and South America, and Australia, respectively. c.35*del*G frequency in Europe was higher than that in other regions around the world with a predominance of the c.35*del*C mutation.

The c.235*del*C and p.V37I mutations were most prevalent in East and Southeast Asia, c.235*del*C was predominant in East Asia (range, 51.9%-66%), and p.V37I was predominant in Southeast Asia including Taiwan (range, 52.9%-88%).

The p.W24X mutation was the most prevalent allele in South Asia (range, 40%-67.2%), and other specific populations were characterized by other mutations, that is, c.167*del*T in Israel, p.R143W in Ghana, c.-23+1G>A in Mongolia, and p.S199F in Colombia. The second most prevalent alleles in each country showed a pattern. The c.235*del*C mutation was common in Mongolia and Thailand. The p.V37I mutation was the second most prevalent in Japan (16.6%), China (20.4%), and some regions of Africa (2%-8.6%). The p.M34T, p.L90P, and c.313_326*del*14bp mutations were prevalent in the Caucasian population, and the c.167*del*T and c.257_259*del*CGC mutations were the second most prevalent in the Middle East, whereas c.-23+1G>A and p.W77X mutations were the second most prevalent in South Asia.

GJB2 Mutation Cluster Analysis

We performed a cluster analysis of the *GJB2* mutated allele frequencies in each ethnic population shown in Table 1 by calculating the Euclidean distance and using Ward's clustering method (Figure 3).

The Japanese, Korean, and Chinese populations, which were characterized by the c.235*del*C and c.299_300*del*AT mutations, were grouped into 1 cluster, and the Mongolian population, characterized by the c.-23+1G>A mutation, was located outside the Japanese–Chinese cluster. This result clearly indicates the similarities in the genetic backgrounds of the Japanese, Chinese, and Korean populations. The Mongolian population carried a slightly different genetic background; however, it resembled those of the Japanese–Chinese cluster to some extent. The Thai, Taiwanese, Malaysian, and Indonesian populations were characterized by the p.V37I and p.R32H mutations and were grouped into 1 cluster. The Bangladeshi, Indian, and Pakistani populations, which were characterized by the p.W24X and p.W77X mutations, were also grouped into 1 cluster. Just outside of the above clusters, there were the Ghanan and Israeli populations, with the p.R143W and c.167*del*T mutations. All of these populations were organized into 1 large cluster.

Table 1. The Spectrum of *GJB2* Mutant Alleles.

Country	No. of Screened Patients	No. of Mutant *GJB2* Alleles	Variant Alleles, No. (%)																																			References
			c.35*delG*	p.V37I	c.235*delC*	p.W24X	c.167*delT*	c.-23+1G>A	p.R143W	p.S199F	p.M34T	c.313_326 *del*14bp	p.L90P	c.257_259 delCGC	c.299_300 *del*AT	p.W77X	c.171_191 *del*16bp	p.G45E Y136X	p.E47X	p.R184P	p.R32H	p.W77R	c.333_334 *del*AA	c.290 *ins*A	c.269 *ins*T	c.51*del* 12*ins*A	p.V84L	p.M1V	p.V95M	p.I203K	c.257_259 delCGC	p.M34L	c.294_295 *del*GA	Others	
East Asia																																						
Japan	1343	283	0 (0)	47 (16.6)	147 (51.9)	0 (0)	0 (0)	0 (0)	18 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	11 (3.9)	0 (0)	15 (5.3)	34 (12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	18 (6.3)	Tsukada et al, 2010[4]
China	2063	899	10 (1.1)	183 (20.4)	509 (56.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	82 (9.1)	0 (0)	31 (3.4)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	51 (5.6)	Dai et al, 2009[3]
Korea	174	47	0 (0)	1 (2.1)	31 (66)	0 (0)	0 (0)	0 (0)	4 (8.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (12.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (10.6)	Shin et al, 2012[8]
Taiwan	1017	209	0 (0)	184 (88)	25 (22)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Wu et al, 2011[9]
Central Asia																		0 (0)																			
Mongolia	534	77	2 (2.5)	6 (7.7)	16 (20.5)	0 (0)	0 (0)	42 (53.8)	1 (1.3)	0 (0)	0 (0)	1 (1.3)	0 (0)	0 (0)	4 (5.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (5.1)	Tekin et al, 2010[10]
Southeast Asia																		0 (0)																			
Indonesia	120	6	0 (0)	4 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Snoeckx et al, 2005[11]
Malaysia	91	17	0 (0)	9 (52.9)	0 (0)	4 (23.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (11.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.9)	0 (0)	1 (5.9)	0 (0)	Zainal et al, 2012[12]
Thailand	166	52	0 (0)	37 (71.1)	10 (19.2)	3 (5.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	1 (1.8)	0 (0)	0 (0)	Wattanasirichaigoon et al, 2004[13]
South Asia																		0 (0)																			
India	303	60	0 (0)	0 (0)	2 (3.3)	52 (86.7)	1 (1.7)	3 (5.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Padma et al, 2009[14]
Pakistan	196	25	0 (0)	0 (0)	0 (0)	10 (40.0)	2 (8.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.0)	2 (8.0)	0 (0)	10 (40.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Santos et al, 2005[15]
Bangladesh	53	28	0 (0)	0 (0)	0 (0)	14 (50.0)	0 (0)	5 (19.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (10.7)	3 (10.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)	Bajaj et al, 2008[16]
Oceania																		0 (0)																			
Australia	364	142	54 (38.03)	21 (14.8)	2 (1.4)	4 (2.8)	3 (2.1)	5 (3.5)	1 (0.7)	0 (0)	23 (16.3)	0 (0)	2 (1.4)	0 (0)	2 (1.4)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	3 (2.1)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	20 (14.1)	Dahl et al, 2013[17]
Middle East																		0 (0)																			
Iran	2322	802	547 (68.9)	2 (0.2)	1 (0.1)	31 (3.9)	16 (2)	38 (4.8)	7 (0.9)	0 (0)	0 (0)	30 (3.8)	1 (0.1)	39 (4.9)	8 (1.0)	3 (0.4)	0 (0)	0 (0)	2 (0.2)	18 (2.2)	20 (2.5)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	45 (10.9)	Bazazzadegan et al, 2012[18]
Israel	230	98	38 (38.8)	1 (1.0)	0 (0)	1 (1.0)	45 (45.9)	1 (1.0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (4.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (7.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Brownstein et al, 2009[19]
Turkey	406	184	155 (84.2)	0 (0)	0 (0)	2 (1.1)	0 (0)	9 (4.9)	1 (0.5)	0 (0)	0 (0)	1 (0.5)	0 (0)	11 (6.0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.5)	Tekin et al, 2007[20]
Saudi Arabia	130	8	4 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Imtiaz et al, 2011[21]
Kuwait	100	21	18 (86.0)	0 (0)	0 (0)	0 (0)	3 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Al-Sebeih et al, 2014[22]
Syria	50	43	36 (83.7)	0 (0)	0 (0)	0 (0)	5 (11.6)	0 (0)	0 (0)	0 (0)	2 (4.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Al-Achkar et al, 2011[23]
Europe																		0 (0)																			
Russia	16	15	11 (73.3)	0 (0)	1 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.3)	Posukh et al, 2005[24]
Slovakia	273	171	122 (71.3)	6 (3.5)	0 (0)	15 (8.8)	3 (1.8)	3 (1.8)	0 (0)	0 (0)	6 (3.5)	0 (0)	4 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10	Minarik et al, 2012[25]
Belarus	241	437	397 (93.0)	0 (0)	5 (1.2)	0 (0)	6 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)	27 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.5)	Danilenko et al, 2012[26]
Estonia	391	210	173 (82.4)	0 (0)	0 (0)	0 (0)	1 (0.5)	0 (0)	5 (2.4)	0 (0)	27 (12.9)	2 (1.0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Teek et al, 2010[27]
Poland	102	82	73 (87.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.2)	6 (7.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.4)	Wiszniewski et al, 2001[28]
Croatia	58	51	41 (80.4)	2 (3.9)	0 (0)	1 (2.0)	0 (0)	1 (2.0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Sansović et al, 2009[29]
Czech	156	134	111 (82.8)	0 (0)	0 (0)	13 (9.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (3.7)	2 (0.7)	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.1)	Seeman et al, 2004[30]
Bulgaria	51	47	45 (95.6)	0 (0)	0 (0)	1 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Popova et al, 2012[31]
Romania	45	40	32 (80.0)	0 (0)	0 (0)	2 (5.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (5.0)	0 (0)	1 (2.5)	1 (2.5)	0 (0)	0 (0)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Radulescu et al, 2012[32]
Austria	204	66	47 (71.2)	0 (0)	0 (0)	3 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.5)	2 (3)	7 (10.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (9)	Janecke et al, 2002[33]
Netherlands	222	47	35 (74.5)	1 (2.1)	0 (0)	0 (0)	0 (0)	3 (6.3)	1 (2.1)	0 (0)	1 (2.1)	2 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (8.4)	Santos et al, 2005[34]
France	207	395	287 (72.7)	0 (0)	1 (0.3)	4 (1.0)	6 (1.5)	6 (1.5)	0 (0)	0 (0)	0 (0)	12 (3)	11 (2.8)	6 (1.5)	0 (0)	0 (0)	0 (0)	0 (0)	12 (3)	3 (0.9)	2 (0.6)	4 (1.2)	1 (0.3)	0 (0)	1 (0.3)	0 (0)	0 (0)	3 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	37 (9.4)	*Marlin* et al, 2005[35]
Germany	228	76	54 (71.1)	0 (0)	0 (0)	2 (2.6)	2 (2.6)	0 (0)	0 (0)	0 (0)	9 (11.8)	0 (0)	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (6.6)	Zoll et al, 2003[36]
Italy	NA	534	478 (89.7)	0 (0)	0 (0)	1 (0.2)	8 (1.5)	7 (1.3)	0 (0)	0 (0)	1 (0.2)	0 (0)	7 (1.3)	4 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.6)	7 (1.3)	0 (0)	0 (0)	7 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	24 (4.5)	Andrea et al, 2002[37]
Spain	576	396	351 (82)	2 (0.5)	0 (0)	1 (0.3)	7 (1.6)	0 (0)	4 (1.2)	0 (0)	1 (0.3)	2 (0.6)	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.9)	7 (1.6)	0 (0)	1 (0.3)	3 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16 (4.0)	Rabionet et al, 2000[38]
Greece	210	145	139 (95.9)	0 (0)	0 (0)	2 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.4)	0 (0)	NA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	Pampanos et al, 2002[39]
Portugal	53	25	16 (64)	0 (0)	0 (0)	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (20)	Nogueira et al, 2011[40]
Sweden	79	49	43 (87.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.0)	0 (0)	0 (0)	0 (0)	1 (2.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.1)	Carlsson et al, 2012[41]
Finland	71	30	26 (86.7)	1 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Löppönen et al, 2003[42]
Norway	254	85	72 (84.7)	0 (0)	0 (0)	2 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.4)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (6.0)	Siem et al, 2010[43]
Greenland	45	3	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Homøe et al, 2012[44]
North America																																					
USA/Canada	7401	1952	1001 (51.3)	183 (9.4)	36 (1.8)	33 (1.7)	83 (4.3)	0 (0)	16 (0.8)	6 (0.3)	256 (13.1)	28 (1.4)	46 (2.4)	8 (0.4)	3 (0.2)	3 (0.2)	2 (0.1)	0 (0)	22 (1.1)	7 (0.4)	2 (0.1)	0 (0)	5 (0.3)	0 (0)	6 (0.4)	2 (0.1)	0 (0)	10 (5.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	193 (9.9)	Putcha et al, 2007[45]
Mexico	76	42	32 (76.2)	0 (0)	0 (0)	0 (0)	2 (4.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (12.0)	de la Luz Arenas-Sordo et al, 2012[46]
South America																																					
Brazil	300	75	56 (74.7)	1 (1.3)	1 (1.3)	1 (1.3)	1 (1.3)	0 (0)	0 (0)	0 (0)	4 (5.3)	0 (0)	1 (1.3)	0 (0)	0 (0)	0 (0)	1 (1.3)	0 (0)	0 (0)	2 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (5.3)	Batissoco et al, 2009[47]
Colombia	112	80	37 (46.3)	0 (0)	0 (0)	0 (0)	1 (1.3)	0 (0)	0 (0)	39 (48.8)	3 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Tamayo et al, 2009[48]
Argentina	94	60	40 (66.7)	2 (3.3)	0 (0)	0 (0)	2 (3.3)	1 (1.6)	5 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.3)	1 (1.6)	0 (0)	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (8.3)	Gravina et al, 2010[49]
Chile	113	28	28 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Cifuentes et al, 2013[50]
Africa																																					
Egypt	111	35	24 (68.6)	2 (8.6)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (12.9)	Snoeckx et al, 2005[51]
Algeria	59	45	36 (80.0)	3 (6.7)	0 (0)	0 (0)	2 (4.4)	2 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Ammar-Khodja et al, 2009[52]
Morocco	81	65	61 (93.8)	3 (4.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Abidi et al, 2007[53]
Kenya/Sudan	589	13	10 (76.9)	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15.4)	Gasmelseed et al, 2004[54]
Tunisia	95	52	45 (86.5)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (6.0)	1 (2)	0 (0)	0 (0)	2 (4.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Trabelsi et al, 2013[55]
Uganda	126	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	Javidnia et al, 2014[56]
Ghana	365	121	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	110 (90.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.3)	0 (0)	0 (0)	0 (0)	2 (1.7)	0 (0)	0 (0)	0 (0)	5 (4.1)	Hamelmann et al, 2001[57]

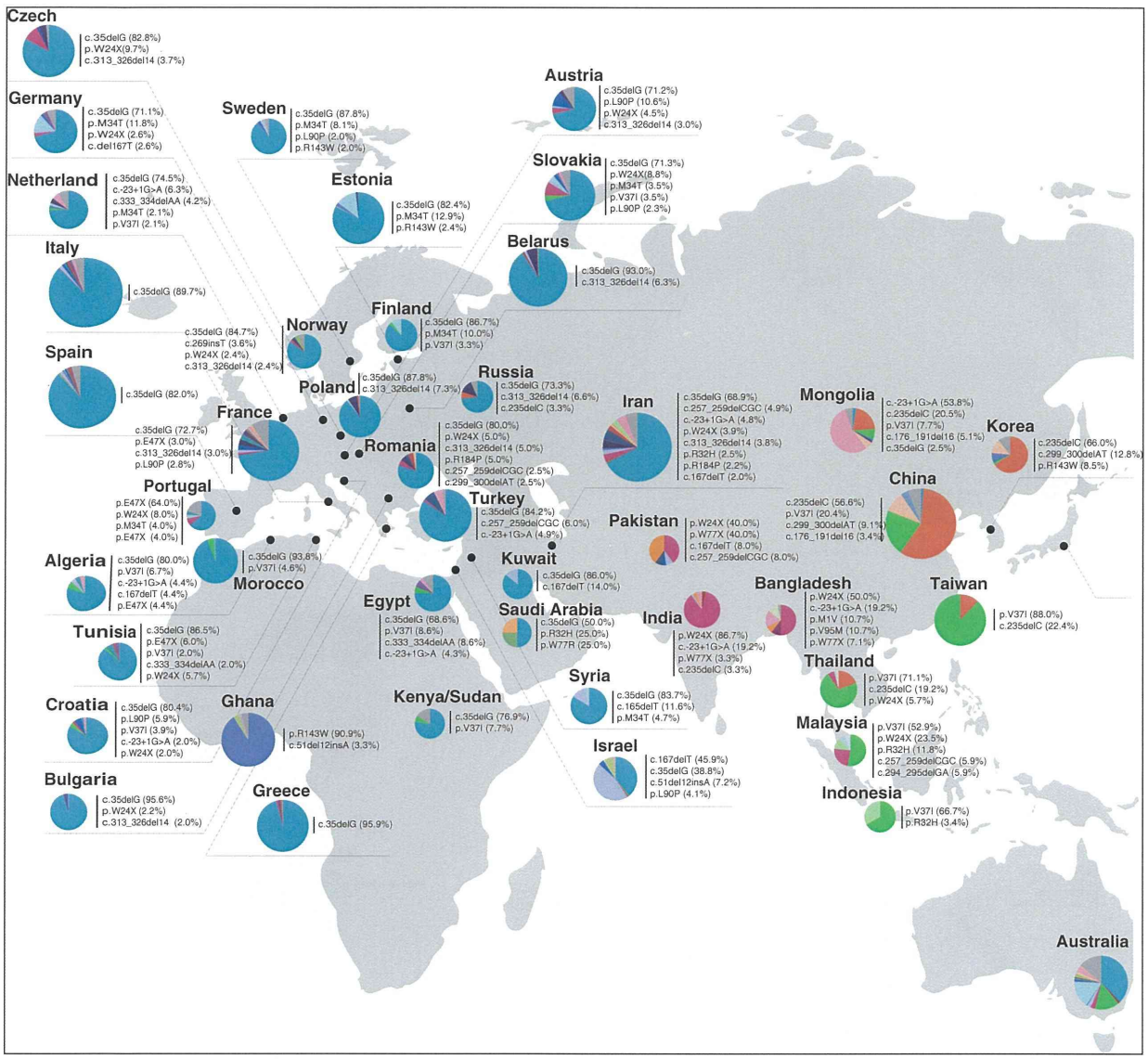

Figure 1. The spectrum of *GJB2* mutations.
A larger circle indicates a larger number of mutated alleles.

(continued)

In contrast, the other populations listed above, which were characterized by the c.35*del*G, c.313_326*del*14, and p.M34T mutations, were grouped into 1 large cluster. These results reveal that the European, Middle Eastern, North African, American, and Australian genetic backgrounds were similar but distinguishable from the East Asian, Southeast Asian, South Asian, and Sub-Saharan African populations.

Ethnic-Specific GJB2 *Mutations and Their Suspected Origin*

We conducted a cluster analysis to speculate on the origin of these frequent mutations, and we compared the results with the human Y-chromosomal haplogroup tree, which is applicable to human migration and expansion investigations.[72-74] Based on these data, the footprints of the human migration route began to emerge.[72-74] There is no doubt that all of these gene mutations migrated according to this route. Therefore, we compared the *GJB2* mutation clustering results of the ethnic populations and the distribution of the *GJB2* mutations to the proposed human migration routes deduced from the Y-chromosome haplotype tree[74] (Figure 4).

*The c.35*delG *Mutation*

The *GJB2* c.35*del*G mutation is the most common cause of hearing loss worldwide and is identified frequently in