Review

Hereditary Hearing Loss and Deafness Genes in Japan

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Hearing loss (HL) is the most common sensory impairment occurring at birth in developed countries. Epidemiological data show that more than one child in 1000 is born with HL, while more than 50% of prelingual HL cases are found to be hereditary. Approximately 70% of hereditary HL is nonsyndromic and subdivided to autosomal dominant (20%), autosomal recessive (75%), X-linked HL (1%), and maternally-inherited HL associated with the mitochondrial DNA mutation. More than 10 deafness genes have been reported to be responsible for nonsyndromic hereditary HL in Japan. Among them, the most prevalent causative genes, GJB2 and the mitochondrial DNA 12SrRNA are introduced. In addition, this study also refers to the specific genes responsible for the unique audiogram, mainly WFS1. Finally, the genes related to the enlargement of vestibular aqueduct of inner ear abnormality, SLC26A4, EYA1 and SIX1 are discussed. The clinical and genetic findings associated with these disorders including the results of a recent study are reviewed.

Key words: GJB2, SLC26A4, 12SrRNA, EYA1, SIX1

1. Introduction

Hearing loss (HL) is a full or partial decrease in the ability to detect or understand sounds. The types of HL include conductive HL (CHL), sensorineural HL (SNHL), and mixed HL (MHL). CHL is caused by problems in the outer and/or middle ear such as external meatal atresia and otosclerosis; SNHL is caused by problems in the inner ear, cochlear nerve, and/or central auditory pathway; and MHL is caused by a combination of both conductive and sensorineural components. The onset of HL is categorized as congenital, acquired or late-onset. Prelingual HL is either present at birth or begins before the age of five years, when language has normally been acquired. Postlingual HL occurs after the development of normal speech. The cause of HL is due to genetic (hereditary) and/or non-genetic (environmental) factors¹. Most hereditary HL is inherited as a simple Mendelian trait and is classified into nonsyndromic or syndromic according to the presence of other disorders, such as kidney, heart, or vision abnormalities¹. Owing to recent advances in molecular genetics, more than 130 loci and more than 40 causative genes for HL have now been identified (Van Camp G, Smith RJH. [homepage on the Internet]. Hereditary Hearing Loss Homepage, [updated 2008 May 28; cited 2009 Sep 14]. Available from: http://webh01.ua.ac.be/hhh/). This article reviews the hereditary HL and deafness genes seen particularly in the Japanese population.

2. Epidemiology of hearing loss

Epidemiological data show that HL is the most common defect at birth and the most prevalent sensory impairment in developed countries². In the United
More than 10 deafness genes have been reported to be associated with nonsyndromic hereditary HL in Japan. Mutations in GJB2, SLC26A4, and the mitochondrial DNA 1555A>G mutation are the major causes of the HL.

(1) GJB2

Gap junction protein, beta-2 (GJB2) is the most prevalent causative gene for nonsyndromic hereditary HL in various ethnic groups. Gap junction channels connect the cytoplasm of adjacent cells, allowing the diffusion of ions and small metabolites. They are transmembrane proteins which are formed at the appositional plasma membranes by related proteins families named connexines. GJB2 is found to constitute gap junctions between epithelial and connective tissue cells in the cochlea and serves as the structural basis for recycling endolymphatic potassium ions that pass through the sensory and supporting cells during the sound transduction process.

Most of HL related with GJB2 mutations are nonsyndromic recessive manner (DFNB1), while some mutations can cause nonsyndromic dominant hereditary HL (DFNA3). To date, more than 90 GJB2 mutations have been reported, most of which are found in patients with moderate to profound HL. Morton et al. reports that the rate of GJB2 mutations in the United States accounts for about 21% of all HL at birth and 15% at 4 years. The carrier rate for recessive deafness-causing GJB2 mutations in the general population is approximately one in 33 in the Midwestern United States. In Asian nations, GJB2 mutations account for approximately 20% of bilateral severe-to-profound nonsyndromic HL. In Japan, the GJB2 mutation has been recognized in 26.5 of autosomal recessive HL patients. The GJB2 allele variants are detected with a carrier rate of more than 2% among Japanese normal hearing populations. Our screening (refer to the supplemental data) of nonsyndromic HL patients including 70 prelingual HL patients, 25 congenital HL patients, and 46 autosomal recessive HL patients, shows that 13 (18.6%) patients with prelingual HL, 7 (28.0%) with congenital HL and 12 (26.1%) with autosomal recessive HL patients have either homozygous or compound heterozygous mutations in GJB2. This finding confirms that GJB2 is the most major causative gene for nonsyndromic hereditary HL in Japan.

The prevalent genotype of GJB2 mutations has a high ethnic predilection. By far the most commonly found mutations are deletions in two regions of GJB2: 35delG and 235delC. 35delG and 235delC are the GJB2 mutations.
Japanese hereditary hearing loss mutations detected high frequently in Caucasoid and East Asian populations, respectively. These mutations result in a frameshift and produce premature truncating protein. The large cross-sectional analyses of GJB2 genotype-phenotype correlation data suggest that the severity of HL associated with biallelic truncating mutations is significantly more severe than that associated with biallelic non-truncating mutations. Specifically, the HL with 35delG is more severe than that with non-truncating mutations including M34T, V37I, and L90P. V37I is the second most prevalent allele detected in the Japanese, followed by R143W, G45E and Y136X. Homozygous 235delC has exhibited a significantly more severe phenotype than homozygous V37I and the HL with V37I has a later age onset than those with the 235delC. Our study detects 235delC at the highest frequency with the rate of 20/38 (52.6%), followed by G45E, Y136X, V37I, and R143W. Homozygous 235delC causes severe-to-profound HL, while two patients associated with biallelic non-truncating mutations present moderate HL. The configuration of the audiogram is not related to either a genotype or a combination of mutations (Table I).

(2) 1555A>G mutation in the mitochondrial 12SrRNA

12SrRNA is encoded by nucleotides 648-1601 in mitochondrial DNA, and is also called MTRNR1. 12SrRNA molecules help assemble amino acids into the functioning proteins that carry out oxidative phosphorylation. The approximate prevalence of the 1555A>G mutation in the 12SrRNA is 0.5–2.4% in European HL patients and 0.2% in European general population. The 1555A>G mutation has also been frequently reported in various Asian countries including China and Balinese, and in Japan, we reports that 5.1% of nonsyndromic HL and 8.0% of familial HL carry the 1555A>G mutation. A sequential genetic analysis shows the prevalence of this mutation to be 1.2% among the sporadic cases and 5.7% among the familial cases. Among maternally inherited HL, the prevalence of this mutation is 17.2% (refer to the supplemental data).

The 1555A>G mutation is of particular interest as a key cause of antibiotic-induced HL. The pathogenic effect of the mutation is related to an alteration in the binding site for aminoglycosides and enlarges sensitivity to aminoglycoside ototoxicity. The antibiotic-induced HL appears dose-independently within a few days to weeks after the administration of aminoglycoside. However, the 1555A>G mutation has also been detected in HL patients not exposed to this antibiotic, and other genetic and environmental factors can also influence the presence of HL caused by the 1555A>G mutation.

This mutation is found to cause a diminished ability to repair cochlear damage from a variety of causes including noise. The HL in patients...
who are not exposed to aminoglycosides starts around the age of 20 years. The penetrance of HL in patients without aminoglycoside exposure is approximately 40% by the age of 30 years, and 80% by the age of 65 years. Therefore, the 1555A>G mutation does not necessarily show a maternal inheritance pattern and the patients with this mutation can show various phenotypes ranging from completely normal hearing to profound HL. The high prevalence and low penetrance suggest that genetic analysis before the administration of aminoglycoside antibiotics would be required for subjects consanguineous with HL patients, regardless of the presence of HL. In our study, the onset of HL varies from the prelingual period to 40 years of age and the rate of patients who show a maternal familial history is lower than half. The progression of HL is recognized in 8 of 12 (66.7%) patients. Antibiotic-induced HL is observed in only two cases. The severity of HL ranges from mild to profound (Table II). The 1555A>G mutation has been found to transmit in a homoplasmic state, however, a recent report describes patients with the mutation in a heteroplasmic state. Patients carrying less than 20% mutant copies are either asymptomatic or have a mild HL, whereas patients with more than 52% mutant copies have moderate to severe HL. The SNHL associated with the 1555A>G mutation is found to derive from cochlear dysfunction in examinations using speech audiometry, distortion-product otoacoustic emission testing, electrocochleography and auditory brainstem responses. In addition, the most severe damage to hair cells occurs toward the basal turn of the cochlea and the audiometric configuration in most cases results in high-frequency SNHL. Though the vestibular dysfunction has been thought to be less common in patients with the 1555A>G mutation, our previous study shows that this mutation can cause a dysfunction of either the saccule or the inferior vestibular nerve with a preserved function of the lateral semicircular canal based on the results of caloric response testing and vestibular evoked myogenic potentials.

### Table II. Summary of Patients With A1555G mutation in the mitochondrial t25S rRNA

<table>
<thead>
<tr>
<th>Age/Gender</th>
<th>Onset of HL</th>
<th>Familial History</th>
<th>Audiogram Severity</th>
<th>Configuration</th>
<th>Administration of Antibiotics</th>
<th>Progression of HL</th>
<th>Vertigo/Dizziness</th>
</tr>
</thead>
<tbody>
<tr>
<td>37/F</td>
<td>6</td>
<td>Maternally</td>
<td>Severe</td>
<td>Gently sloping</td>
<td>None</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>44/M</td>
<td>Congenital</td>
<td>Maternally</td>
<td>Severe</td>
<td>Steeply sloping</td>
<td>None</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>39/M</td>
<td>10</td>
<td>Maternally</td>
<td>Mild</td>
<td>Gently sloping</td>
<td>None</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>37/F</td>
<td>20</td>
<td>Maternally</td>
<td>Moderate</td>
<td>Steeply sloping</td>
<td>None</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>31/F</td>
<td>Congenital</td>
<td>Maternally</td>
<td>Profound</td>
<td>Flat</td>
<td>None</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>47/F</td>
<td>5</td>
<td>AR</td>
<td>Mild</td>
<td>Steeply sloping</td>
<td>Streptomycin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>35/F</td>
<td>34</td>
<td>AR</td>
<td>Moderate</td>
<td>Steeply sloping</td>
<td>None</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>36/F</td>
<td>36</td>
<td>AR</td>
<td>Severe</td>
<td>Steeply sloping</td>
<td>None</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>39/F</td>
<td>4</td>
<td>AR</td>
<td>Severe</td>
<td>Steeply sloping</td>
<td>None</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>75/F</td>
<td>37</td>
<td>Sporadic</td>
<td>Severe</td>
<td>Flat</td>
<td>Streptomycin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>56/M</td>
<td>40</td>
<td>Sporadic</td>
<td>Severe</td>
<td>Steeply sloping</td>
<td>None</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>40/F</td>
<td>5</td>
<td>Sporadic</td>
<td>Severe</td>
<td>Steeply sloping</td>
<td>None</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

AR: autosomal recessive

5. The deafness genes associated with low- and mid-frequency hearing loss

Although most hereditary HL causes high-frequency SNHL, some deafness genes are associated with low-frequency SNHL or mid-frequency SNHL. **DIAPH1** and **WFS1** are the representative deafness genes for low-frequency SNHL. TECTA and COL11A2 mutations can cause mid-frequency HL due to the abnormalities of the tectorial membrane in the inner ear. **WFS1** is a gene encoding an 890 amino-acid glycoprotein; wolframin, predominantly localized in the endoplasmic reticulum. Heterozygous mutations in the WFS1 can be responsible for nonsyndromic autosomal domi-
nent low-frequency SNHL (DFNA6/14/38). The majority of the mutations exist in exon 8 of the gene which encode the C-terminal domain of wolframin. Either homozygous or compound heterozygous mutations can cause Wolfram syndrome. The function within the inner ear is suggested to maintain calcium ion homeostasis, but the mechanism of hearing impairment mainly seen in low-frequencies in DFNA6/14/38 patients remains unclear.

Studies show that 75% of families affected with nonsyndromic autosomal dominant low-frequency SNHL carry the WFS1 mutations in Europe and the United States. However, the prevalence of detected mutations in Japan is much less than in Europe and the US. Most patients with the mutation show HL in their first or second decade, and it slowly progresses with age. However, the HL does not deteriorate to become profound. Our recent studies present two novel missense mutations in Japanese, A844T and K836T (refer to the supplemental data). The K836T in WFS1 is identified in affected family members who show mid-frequency SNHL in childhood but gradual progression of the audiometric thresholds at lower frequencies with age.

6. Deafness genes associated with enlargement of the vestibular aqueduct (EVA) syndrome

(1) Enlargement of the vestibular aqueduct syndrome

The vestibular aqueduct is a narrow bony canal that opens onto the medial surface of the temporal bone and passes to the vestibule of the inner ear. A membranous tube called the endolymphatic duct runs through the vestibular aqueduct to the endolymphatic sac on the posterior surface of the petrous portion of the temporal bone, where it comes into contact with the dura mater. First described by Valvassori, an enlargement of the vestibular aqueduct (EVA) syndrome is characterized by the presence of a much larger endolymphatic duct and sac than normal and is defined on CT as a diameter greater to or equal to 1.5 mm measured midway between the operculum and the common crus.

EVA is the most common malformation of the inner ear associated with HL. In the United States, 12% of deaf children at 4 years old are associated with EVA syndrome. EVA is observed in DFNB4, Pendred syndrome, BOR/BO syndrome, distal renal tubular acidosis with SNHL, and Waardenburg syndrome.

(2) DFNB4/Pendred syndrome

Pendred syndrome is an autosomal recessive disorder usually characterized by progressive severe-to-profound bilateral SNHL, vestibular dysfunction, and development of euthyroid goiter. DFNB4 was initially described in a Middle-Eastern Druze family with recessive nonsyndromic deafness and has been now recognized as nonsyndromic SNHL associated with EVA. DFNB4 and Pendred syndrome comprise a phenotypic spectrum of HL either with or without thyroid defects. Patients with DFNB4/Pendred syndrome may be unusually vulnerable to inner ear disease associated with head injury. Presumably this vulnerability occurs because there is an increased compliance of pressure waves in the brain to the inner ear.

The gene responsible for DFNB4/Pendred syndrome is known to be SLC26A4. SLC26A4 is mapped on 7q31, belonging to the sulfate ion transporter, and encodes a 780-amino acid (86-kD) protein known as pendrin. Pendrin seems to be responsible for the efflux of iodide in thyrocytes, and for mediating Cl/HCO3 exchange in inner ear. Pendrin regulates the pH of endolymphatic fluid by HCO3 secretion, and modifies inner ear acid-base homeostasis.

A sequence analysis of HL associated with EVA, except patients inherited as an autosomal dominant, has identified disease-causing mutations in approximately 80% of familial cases and in 20% of sporadic cases in the United States. A study of 274 East Asians and 318 South Asians with SNHL demonstrates that mutations in SLC26A4 are recognized in approximately 5% of both groups. Furthermore, an analysis in Japan shows that SLC26A4 mutations are responsible for 90% of families with Pendred syndrome, and for 78% of nonsyndromic familial HL with EVA. Three recurrent mutations in SLC26A4, being L236P, T416P, and IVS8+1G>A, account for approximately 50% of the variant alleles detected in DFNB4/Pendred syndrome in Caucasians of northern European descent. In East Asia, the spectrum of SLC26A4 mutations in the Chinese population reveals that IVS7-2A>G is the most frequent mutation which accounts for about half, while the most prevalent mutation in Korea is H723R with a rate of 40%. All of these mutations are accounted for approximately 50% of deafness-causing SLC26A4 mutations. The reason for the high prevalence of H723R is considered to be due to the founder effect rather than due to a mutational hot spot.

In our recent study, 10 patients with SNHL and EVA are screened for SLC26A4 mutation, excluding patients with dominant types of HL (refer to the supplemental data). Their phenotype shows Pendred syndrome in...
four patients and nonsyndromic HL in six patients. Mutations are identified in all four Pendred patients (100%) and one of 6 patients showing nonsyndromic HL (16.7%). The detected mutations include three missense and two splice site variants. H723R is the most common with a prevalence of 60% and a novel mutation, IVS5+1G>T is detected in that study. All patients carrying a biallelic SLC26A4 mutation show prelingual severe-to-profound HL and disequilibrium symptoms and 4/5 (80%) show progressive HL (Table III). The previous other report about the SLC26A4 mutation in Japan, similarly, shows that approximately 90% show progressive HL and 70% complain of vertigo. The goiterous phenotype in Pendred syndrome is not recognized in childhood and is found to develop with age. Therefore, an 11-year-old nonsyndromic female patient with homozygous H723R may be categorized as Pendred syndrome (Table III).

(3) BOR/BO syndrome

BOR syndrome is an autosomal dominant developmental disorder characterized by HL, anomalies of branchial arch system and renal malformations. It has an estimated prevalence of 1:40000 and 2% of profoundly deaf children are affected with BOR syndrome. H723R is the most common with a prevalence of 60% and a novel mutation, IVS5+1G>T is detected in that study. All patients carrying a biallelic SLC26A4 mutation show prelingual severe-to-profound HL and disequilibrium symptoms and 4/5 (80%) show progressive HL (Table III). The previous other report about the SLC26A4 mutation in Japan, similarly, shows that approximately 90% show progressive HL and 70% complain of vertigo. The goiterous phenotype in Pendred syndrome is not recognized in childhood and is found to develop with age. Therefore, an 11-year-old nonsyndromic female patient with homozygous H723R may be categorized as Pendred syndrome (Table III).

### Table III. Clinical features and analysis of SLC26A4 for the 10 subjects with EVA

<table>
<thead>
<tr>
<th>Age/Gender</th>
<th>Onset of HL</th>
<th>Severity of HL</th>
<th>Progression of HL</th>
<th>Vertigo/ Dizziness</th>
<th>Familial History</th>
<th>Phenotype</th>
<th>Genotype [allele1] + [allele2]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/F</td>
<td>7</td>
<td>Moderate</td>
<td></td>
<td></td>
<td>Sporadic</td>
<td>Nonsyndromic</td>
<td>H723R + [-]</td>
</tr>
<tr>
<td>3/M</td>
<td>1</td>
<td>Severe</td>
<td></td>
<td></td>
<td>AR</td>
<td>Nonsyndromic</td>
<td>[H723R] + [H723R]</td>
</tr>
<tr>
<td>11/F</td>
<td>3</td>
<td>Severe</td>
<td>+</td>
<td>+</td>
<td>Sporadic</td>
<td>Nonsyndromic</td>
<td>[H723R] + [H723R]</td>
</tr>
<tr>
<td>24/F</td>
<td>22</td>
<td>Mild</td>
<td>-</td>
<td>+</td>
<td>Sporadic</td>
<td>Nonsyndromic</td>
<td>[H723R] + [T410M]</td>
</tr>
<tr>
<td>24/F</td>
<td>Congenital</td>
<td>Profound</td>
<td>-</td>
<td>+</td>
<td>Sporadic</td>
<td>Pendred</td>
<td>[H723R] + [IVS15+5G&gt;A]</td>
</tr>
<tr>
<td>25/F</td>
<td>6</td>
<td>Severe</td>
<td>+</td>
<td>-</td>
<td>Sporadic</td>
<td>Nonsyndromic</td>
<td>[H723R] + [H723R]</td>
</tr>
<tr>
<td>28/F</td>
<td>1</td>
<td>Profound</td>
<td>+</td>
<td>+</td>
<td>AR</td>
<td>Nonsyndromic</td>
<td>[IVS5+1G&gt;T] + [G439R]</td>
</tr>
<tr>
<td>29/F</td>
<td>2</td>
<td>Profound</td>
<td>+</td>
<td>+</td>
<td>AR</td>
<td>Pendred</td>
<td>[H723R] + [H723R]</td>
</tr>
<tr>
<td>24/F</td>
<td>1</td>
<td>Profound</td>
<td>+</td>
<td>+</td>
<td>AR</td>
<td>Pendred</td>
<td>[IVS5+1G&gt;T] + [G439R]</td>
</tr>
</tbody>
</table>

AR: autosomal recessive

### Variation

Renal malformations are not infrequent and they range from mild hypoplasia to a complete absence. This syndrome can be called BO syndrome, when occurring without any renal malformation.

Three genes, EYA1, SIX5 and SIX1, are known to be associated with BOR/BO syndrome. The embryonic development of the ear depends on the EYA-SIX hierarchy of regulatory genes. EYA1 consists of 16 coding exons that extend over 156 kb, including a highly conserved particular structure in the Eya gene family known as the eya-homologous region (eyaHR). The eyaHR mediates interactions with the gene products of so in Drosophila. The vertebrate orthologues of so are members of the Six gene family. The SIX1 has two exons coding for a transcript of 1376 bp. SIX1 has a conserved SIX domain interacting of EYA1, and a homeodomain requiring for DNA binding. The normal expression of both EYA1 and SIX1 are necessary for appropriate development of the middle and inner ear. SIX5 demonstrates a high degree of homology to SIX1 and it is also known to interact directly with EYA1.

The EYA1 mutations are detected in approximately 40% of patients with BOR/BO syndrome. The SIX5 mutations are detected in 5.2% of patients with BOR/BO syndrome without EYA1 mutation. SIX1 mutations are thought to account for only a small proportion of patients with BOR/BO syndrome. In our study, five patients with BOR/BO syndrome are screened for the EYA1 and SIX1 mutation (refer to the supplemental data). The SIX1 and EYA1 mutations are detected in two and three patients, respectively (Table IV). These findings suggest that BOR/BO syndrome caused by SIX1 mutation is not so rare in Japanese and tends to lack apparent branchial anomalies, and thus may be...
Japanese hereditary hearing loss

Table IV. Clinical features and genetic analysis of the 5 subjects with BOR/BO

<table>
<thead>
<tr>
<th>Age/Gender</th>
<th>Onset of HL</th>
<th>Type of HL (Right / Left)</th>
<th>Severity of HL (Right / Left)</th>
<th>Other findings</th>
<th>Gene / Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/F</td>
<td>5</td>
<td>SNHL / SNHL</td>
<td>Mild / Moderate</td>
<td>Preauricular pit, EVA</td>
<td>SIX1/ [Y129C]</td>
</tr>
<tr>
<td>21/F</td>
<td>Childhood</td>
<td>SNHL / SNHL</td>
<td>Moderate / Moderate</td>
<td>Preauricular pit, EVA</td>
<td>SIX1/ [Y129C]</td>
</tr>
<tr>
<td>29/F</td>
<td>Childhood</td>
<td>SMHL / MHL</td>
<td>Mild / Moderate</td>
<td>Preauricular pit</td>
<td>EYA1/ [S189G]</td>
</tr>
<tr>
<td>9/M</td>
<td>5</td>
<td>SNHL / MHL</td>
<td>Mild / Moderate</td>
<td>Preauricular pit, Branchial fistula</td>
<td>EYA1/ [R407Q]</td>
</tr>
<tr>
<td>22/F</td>
<td>Childhood</td>
<td>MHL / SNHL</td>
<td>Moderate / Moderate</td>
<td>Preauricular pit, Branchial fistula, Renal anomaly, Pinnae deformity</td>
<td>EYA1/ [IVS11-1G&gt;A]</td>
</tr>
</tbody>
</table>

7. Conclusions

Based on epidemiological data, at least one child in 1000 is born with HL in developed countries and more than 50% of prelingual deafness cases are found to have hereditary HL. Therefore, a good understanding of the deafness genes is important to select the optimal treatment of patients with HL. This article reviewed the clinical and genetic characteristics of the most prevalent deafness genes such as GJB2, SLC26A4, and the mitochondrial DNA 1555A>G in Japanese. Furthermore, this study also elucidated the deafness gene, WFS1, whose mutation is closely related to low-frequency HL and SLC26A4, EYA1 and SIX1 which are regarded to cause EVA.

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Supplemental Data
In the present review paper, we used our genetic analysis data from 382 unrelated Japanese patients (235 females and 147 males; age, 0–84 y; mean age ± SD, 40.22 ± 18.83 years). The patients visited the Department of Otolaryngology, Tokyo Medical and Dental University between October 1999 and July 2008 and were suspected to have hereditary HL. DNA was extracted from peripheral blood lymphocytes using standard methods after obtaining written informed consent from each donor and/or guardians of the HL children. We first screened for GJB2 mutation and the mitochondrial DNA 1555A>G for all the 382 patients. The presence of WFS1 mutations were analyzed for four patients with autosomal dominant low-frequency SNHL. The coding exons including exon-intron boundaries of GJB2, WFS1, SLC26A4, EYA1, and SIX1; and mitochondrial DNA around the 1555 were amplified by polymerase chain reaction (PCR) in a thermal cycler (model 9700, PE Applied Biosystems, Foster City, CA) as described previously. The mitochondrial DNA 1555A<G was first screened on PCR restriction fragment length polymorphism using BsmA I (New England Biolabs, Beverly, MA) whenever the 1555A>G mutation was detected, the PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN), and were directly sequenced using the Applied Biosystems Prism BigDye Terminator Cycle Sequencing Ready Kit and an ABI Prism model 310 Genetic Analyzer. The other genetic analysis for GJB2, WFS1, SLC26A4, EYA1, and SIX1 was carried out by direct sequencing. All procedures were approved by the institutional review board at Tokyo Medical and Dental University.