Audiologic Phenotype and Progression in GJB2 (Connexin 26) Hearing Loss

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Objectives: To document the audiologic phenotype of children with biallelic GJB2 (connexin 26) mutations, and to correlate it with the genotype.

Design: Prospective, observational study.

Setting: Tertiary care children’s hospital.

Patients: Infants and children with sensorineural hearing loss (SNHL).

Intervention: Sequencing of the GJB2 (connexin 26) gene.

Main Outcome Measures: Degree and progression of SNHL.

Results: From December 1, 1998, through November 30, 2006, 126 children with biallelic GJB2 mutations were identified. Of the 30 different mutations identified, 13 (43%) were truncating and 17 (57%) were nontruncating; 62 patients had 2 truncating, 30 had 1 truncating and 1 nontruncating, and 17 had 2 nontruncating mutations. Eighty-four patients (67%) initially had measurable hearing in the mild to severe range in at least 1 of 4 frequencies (500, 1000, 2000, or 4000 Hz). Of these 84 patients with residual hearing, 47 (56%) had some degree of progressive hearing loss. Patients with 2 truncating mutations had significantly worse hearing compared with all other groups. Patients who had 1 or 2 copies of either an M34T or a V37I allele had the mildest hearing loss.

Conclusions: Hearing loss owing to GJB2 mutations ranges from mild to profound and is usually congenital. More than 50% of patients will experience some hearing loss progression, generally gradually but occasionally precipitously. Hearing loss severity may be influenced by genetic factors, such as the degree of preserved protein function in nontruncating mutations, whereas hearing loss progression may be dependent on factors other than the connexin 26 protein. Genetic counseling for patients with GJB2 mutations should include the variable audiologic phenotype and the possibility of progression.


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SENSORINEURAL HEARING LOSS (SNHL) is the most common congenital sensory impairment, with an incidence of 1 to 2 per 1000 for bilateral severe to profound losses (>70 dB) and up to 4 per 1000 if mild to moderate and unilateral losses are included. The most frequently identified causes of pediatric SNHL are divided into 3 categories: infectious, anatomic, and genetic. The most common infectious cause is congenital cytomegalovirus; the most common anatomic findings are the presence of enlarged vestibular aqueducts and other inner-ear anomalies, some of which have a genetic basis; and the most common genetic causes are mutations in the gap junction β2 gene (GJB2) (OMIM 121011) encoding the connexin 26 (Cx26) protein. In developed countries, more than 50% of SNHL cases have a genetic origin; 30% are syndromic and the remainder are nonsyndromic. Of the nonsyndromic cases, approximately 80% are autosomal recessive, 15% to 17% are autosomal dominant, 2% to 3% are X-linked, and about 1% are mitochondrial. Of all nonsyndromic autosomal recessive cases of hearing loss, as many as half are caused by GJB2 mutations.1

In 1997, GJB2 was reported as the first autosomal recessive gene implicated in nonsyndromic SNHL.2,3 This gene encodes the Cx26 protein and segregates at the DFNB1 locus on chromosome 13q12. More than 100 mutations have been described for the GJB2 gene, with most associated with recessive hearing loss.4 One
mutation, 35delG, is the most common, particularly among white populations. This mutation results in a frameshift and premature termination of the protein. A second mutation, 167delT, has a high frequency in the Ashkenazi Jewish population, and a third mutation, 235delC, is frequent among Asian populations. In addition, there are many other GJB2 mutations, including missense and nonsense mutations, small deletions and insertions, and several mutations for which the clinical implications are unknown. At least 9 dominant GJB2 mutations are associated with nonsyndromic SNHL and 8 with dominant syndromic SNHL. GJB2 was first identified in patients with severe to profound bilateral SNHL, and, therefore, diagnostic clinical testing was initially offered to patients with that phenotype. However, it is now well recognized that GJB2 hearing loss ranges from mild to profound, with some cases demonstrating incomplete penetrance or delayed onset of hearing loss. In addition, some mutations, including M34T and V37I, seem to be associated with a milder audiologic phenotype than others. Although there have been some reports of hearing loss progression, there are no studies, to our knowledge, that have examined many patients over long periods to reliably identify the percentage of patients whose hearing loss progresses. Although recent multicenter studies, 1 international and 1 US-based, described marked variability in the presenting audiologic phenotype, progression rate was not assessed because multiple sequential audiograms were not available for most participants.

In a 2001 report about GJB2-related hearing loss, 4 of 19 participants with mild to severe hearing impairment had progression of their hearing loss. In this report, we describe the longitudinal audiologic features for a large cohort of pediatric patients with biallelic pathogenic GJB2 mutations, including documentation of progression of the hearing loss.

METHODS

PATIENTS

Starting December 1, 1998, children from birth to age 21 years with SNHL or mixed hearing loss who were seen in the outpatient clinics of the Department of Otolaryngology at Children's Hospital Boston were eligible for GJB2 testing. This study was approved by the hospital institutional review board.

Patients of both sexes and all races were offered testing. As of April 30, 2002, all patients were also tested for the 309-kb GJB6-D13S1830 (Cx30) deletion described by del Castillo and colleagues in 2002. Initially, only children who had a bilateral, audiometrically profoundly, clinically nonsyndromic phenotype were offered GJB2 testing. Subsequently, testing was expanded to include patients with a less severe audiologic phenotype and to patients who had either other nonaudiologic clinical findings or other potential causes of the hearing loss.

GENETIC TESTING

GJB2 (Cx26) gene testing was performed in the Clinical Laboratory Improvement Amendments--approved Genetics Diagnostic Laboratory at Children's Hospital Boston. For all tests, genomic DNA was extracted from each patient's blood specimen according to the Gentra PureGene protocol (Qiagen Inc, Valencia, California). DNA was amplified by polymerase chain reaction (PCR) with 2 primer sets that amplified the entire open-reading frame of the GJB2 gene. The PCR product was then sequenced bidirectionally in 2 parts using the external PCR primers plus 2 internal sequencing primers on an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, California). The 309-kb GJB6-D13S1830 deletion was detected by PCR, as previously published. This deletion has been found in some participants who were double heterozygous for both the Cx30 deletion and a GJB2 mutation or homozygous for the 309-kb Cx30 deletion. More recently, a 232-kb GJB6-D13S1834 deletion has been described; however, in the present study, we did not look for this deletion.

CLASSIFICATION OF GJB2 MUTATIONS

All but 1 mutation identified had been previously reported. The exception is the 453_460del8ins9 mutation, which is presumed to be pathogenic because of its predicted truncation of the Cx26 protein. Although most variants in this study are generally accepted to be pathogenic, M34T and V37I have been reported to be both pathogenic and benign. We present data that support the more recent reports that these variants are pathogenic but result in a milder audiologic phenotype. All mutations were classified as truncating or nontruncating mutations. Truncating mutations include nonsense mutations, deletions and insertions that introduce a shift in the reading frame, or mutations predicted to affect translation initiation (eg, M1V). Nontruncating mutations contain amino acid substitutions. These groupings were made to differentiate mutations based on predicted severity, with truncating mutations generally resulting in transcript or protein degradation or a protein product that has either no, or very abnormal, function and therefore would be expected to result in a more severe hearing loss compared with nontruncating mutations. However, it is possible that some missense mutations could also lead to a protein with complete loss of function.

GENETIC COUNSELING

Genetic counseling was provided through the Division of Genetics, Children's Hospital Boston, and, since February 2005, by a genetic counselor in the hospital's Department of Otolaryngology. Genetic counseling was offered to all patients before and after genetic testing.

AUDIOMETRIC EVALUATION

All audiometric testing was performed in the Department of Audiology using age-appropriate techniques (visual reinforcement audiometry, conditioned-play audiometry, and/or conventional “hand-raising” audiometry) by audiologists seasoned in pediatric audimetric testing. Testing also included auditory brainstem-evoked response (ABR) testing in newborns, infants, and young children, otoacoustic emission testing to further confirm and characterize the hearing loss, and behavioral and frequency-specific testing in children who were old enough to participate. A combination of behavioral and electroneurophysiologic audimetric tests was often used to confirm the diagnosis of hearing loss. Hearing loss was categorized as mild (21-40 dB hearing level [dBHL]), moderate (41-55 dBHL), moderately severe (56-70 dBHL), severe (71-90 dBHL), or profound (>90 dBHL). Hearing loss was also classified as conductive, sensorineural, or mixed. The severity of hearing was noted for each ear separately.
Determination of progression of hearing loss or fluctuation of hearing was based on pure-tone behavioral audiograms. The first ear-specific pure-tone audiogram that was judged by the audiologist as having “good” reliability was compared with the most recently available audiogram with good reliability for each patient. Audiograms for patients for whom reliability of responses were in question were not included in the analysis of progression or fluctuation. According to clinical protocols, children with SNHL were seen every 3 months following new identification of SNHL or mixed hearing loss, so serial audiograms and/or electrophysiologic results were obtained for all patients. A 4-frequency pure-tone average (PTA) (500, 1000, 2000, and 4000 Hz) was calculated for each ear for each audiogram. Progression of fluctuation was documented if thresholds had worsened (or improved in the case of fluctuation) by 10 dB or more at 1 or more frequencies in the same ear and confirmed on retest, or by 15 dB at 1 or more frequencies in 1 ear compared with the prior audiogram and confirmed on retest, and provided that middle-ear immittance measures or pneumatic otoscopic testing did not suggest middle-ear fluid or significant negative middle-ear pressure. Similarly, definitive worsening or fluctuation of hearing was noted if the 4-frequency PTA decreased or improved by 10 dB. In patients younger than 3 years, if hearing loss progression or fluctuation was suspected based on behavioral testing, but the results were in question, ABR and otoacoustic emission were used for confirmation of the change. These definitions for progression (or fluctuation) of hearing loss were based on known reliability of pure-tone audiometry using the different test techniques. Conventional audiometry using the modified Hughson-Westlake procedure (as is standard audiologic practice and is used at Children’s Hospital Boston) is known to have a test-retest reliability of 5 dB, whereas visual reinforcement audiometry is known to provide reliable responses in 90% of infants and toddlers. In addition, longitudinal studies of hearing thresholds in hearing-impaired infants with stable hearing loss showed no significant change in hearing threshold levels across behavioral audiometric techniques, and retest reliability in hearing-impaired infants younger than 12 months has been shown to be better than 10 dB. For a child to be identified as having a significant change in hearing, thresholds must have changed by more than the known test-retest reliability of behavioral audiometry and confirmed on retest. Furthermore, averaging across frequencies eliminated the possibility that a child would be labeled as having a change in hearing based on a subtle change at a single frequency. Finally, including only those audiograms labeled as having “good” reliability decreases the likelihood of a child being misdiagnosed with progressive hearing loss based on less than optimal audiometric results.

These guidelines for progression were applied to the frequencies from 500 through 8000 Hz. Data for the 250-Hz frequency was not used to calculate progression or PTA because it can be difficult to separate vibrotactile from auditory thresholds at this frequency. In addition, we did not use any behavioral data that were not center specific, which meant that no sound field data were used. However, if sound field behavioral data suggested a significant worsening of hearing, additional frequency-specific and ear-specific ABR testing was often performed in younger children to rule out the possibility that the suspected worsening of hearing was caused by poor reliability of behavioral testing. This ABR data could then be compared with prior ABR data, and to support behavioral thresholds going forward.

STATISTICAL METHODS

The degree of baseline hearing loss was compared among genotypes by 1-way analysis of variance, with the significance level for pairwise comparisons adjusted by the Tukey-Kramer method.

Between December 1, 1998, and November 30, 2006, a total of 126 patients with pathogenic biallelic Cx26 mutations were identified (Table 1). There were 50 boys (40%) and 76 girls (60%), with ages ranging from birth to 21 years; 104 were white, 17 Asian, 2 African American and white, 2 Asian and white, and 1 of unknown race. Ten participants were Hispanic (all white). The sample included 11 sets of 2 siblings each and 1 set of identical twins. A total of 30 mutations were identified and are listed, with their frequencies, in Table 1. Only 1 mutation has not been reported, 453_460del8ins9, which is presumed to be pathogenic owing to its predicted truncation of the Cx26 protein.

There were 62 patients with homozygous mutations, of which 43 were 35delG/35delG, and 64 who were compound heterozygous. The other homozygous mutations were 167delT/167delT (3 patients), 235delC/235delTC (2 patients), M34T/M34T (3 patients), V37L/V37T (9 patients), 313_326del14/313_326del14 (1 patient), and V84L/V84L (1 patient). Ninety-four of 126 patients (75%) had at least 1 33delG mutation. Of the 30 types of GJB2 mutations among 126 patients, 13 (43%) were truncating and 17 (57%) were nontruncating (Table 2).

Of the 126 patients, 42 (33%) could not be evaluated for progression because they presented with bilateral pro-

<table>
<thead>
<tr>
<th>Table 1. Characteristics of 126 Patients With SNHL and GJB2 Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Girls</td>
</tr>
<tr>
<td>Boys</td>
</tr>
<tr>
<td>Race/ethnicity</td>
</tr>
<tr>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Asian</td>
</tr>
<tr>
<td>African-American and white</td>
</tr>
<tr>
<td>Asian and white</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Progressive hearing loss</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Not evaluable</td>
</tr>
<tr>
<td>Baseline hearing loss, median (range), dB</td>
</tr>
<tr>
<td>Baseline age, median (range), y</td>
</tr>
</tbody>
</table>

Abbreviation: SNHL, sensorineural hearing loss.

*Data are given as number (percentage) of patients unless otherwise indicated.

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found SNHL at all frequencies (n=25) or had insufficient follow-up data to evaluate progression (n=17). Eighty-four (67%) had measurable hearing in at least 1 of 4 frequencies (500, 1000, 2000, or 4000 Hz) in the mild to severe range on their initial audiogram. One patient with 35delG/35delG had normal hearing documented by ABR at birth but progressed to a mild sloping to moderate SNHL by age 15 months.

Table 2. GJB2 Mutations Identified in 126 Patients With SNHL

<table>
<thead>
<tr>
<th>Mutation</th>
<th>No. (%) of 225 Alleles Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Truncating</td>
<td></td>
</tr>
<tr>
<td>35delG</td>
<td>137 (53.9)</td>
</tr>
<tr>
<td>167delT</td>
<td>22 (8.7)</td>
</tr>
<tr>
<td>235delC</td>
<td>8 (3.1)</td>
</tr>
<tr>
<td>313_326del14bp</td>
<td>5 (2.0)</td>
</tr>
<tr>
<td>E47X</td>
<td>4 (1.6)</td>
</tr>
<tr>
<td>M1V</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>W24X</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Q57X</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>176_191del16</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>299_300delAT</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>333_334delAA</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>453_460del8</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>631_632delST</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>188 (74.1)</td>
</tr>
<tr>
<td>Nontruncating</td>
<td></td>
</tr>
<tr>
<td>V37I</td>
<td>23 (9.4)</td>
</tr>
<tr>
<td>M34T</td>
<td>22 (9.1)</td>
</tr>
<tr>
<td>V84L</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>L90P</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>N206S</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>S199F</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>T8M</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>G12V</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>K15T</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>R32C</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>I35S</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>V95M</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>[E114G;V27I](in cis)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>S198N</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>R143W</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>V153I</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>R184P</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (26.1)</td>
</tr>
</tbody>
</table>

Abbreviation: SNHL, sensorineural hearing loss.  
*a Novel variants.  
*b Indicates 2 mutations that occur on the same allele.

Table 3. Severity of Hearing Loss at Baseline Visit in 118 Patients With 2 Mutations in the GJB2 Gene, as Related to Presence of Truncating (T), Nontruncating (NT), and M34T or V37I (MV) Alleles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All Patients (N=126)</th>
<th>No. (%) of Patients</th>
<th>Better Ear 4-Frequency PTA, db</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/T</td>
<td>79</td>
<td>73 (62)</td>
<td>90.8 (2.9)</td>
</tr>
<tr>
<td>T/NT</td>
<td>14</td>
<td>13 (11)</td>
<td>62.5 (6.9)</td>
</tr>
<tr>
<td>NT/NT</td>
<td>3</td>
<td>2 (2)</td>
<td>60.0 (17.5)</td>
</tr>
<tr>
<td>T/NT</td>
<td>16</td>
<td>16 (14)</td>
<td>32.0 (6.2)</td>
</tr>
<tr>
<td>NT/NT</td>
<td>14</td>
<td>14 (12)</td>
<td>25.5 (6.6)</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>118 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: PTA, pure-tone average.  
a For a pairwise comparison, see Table 2.  
b Eight patients were culturally deaf and did not provide audiograms.  
c Data are given as mean (SEM), from analysis of variance. Baseline audiometric measurements were not available for 8 patients.

Patients were considered to have progression of hearing loss if thresholds had worsened by 10 dB or more at 2 or more frequencies in the same ear or by 15 dB at 1 or more frequencies in 1 ear compared with the prior audiogram. The original 126 patients, 84 (67%) had enough residual hearing in at least 1 of 4 frequencies (500, 1000, 2000, or 4000 Hz) to allow for calculation of progression. To calculate progression, the patient had to have at least 2 audiograms from which an accurate PTA could be calculated; the median number of visits (beyond baseline) from which PTA could be calculated was 4 (range, 1-18). Of the 84 patients, 47 (56%) had some degree of progressive hearing loss. The median time to the onset of progressive hearing loss was 13 months after initial presentation, but there was great variability across the group (range, 1-110 months). Although in most cases the hearing loss progressed fairly gradually, there was 1 case of an 8-year-old patient with
2 truncating GJB2 mutations (35delG/299_300delAT) who had rapid progression of hearing loss from mild to profound over several months. Although audiograms that preceded clinical evidence of hearing loss were not available, the patient had completely normal speech and language and above-average academic achievement, suggesting that until shortly before we saw the child the hearing had been normal or only mildly impaired (Figure 2). None of the demographic or genetic classifications shown in Table 4 differed significantly between those with and without progressive loss.

Figure 3 uses Kaplan-Meier curves to show the development of hearing loss progression after the baseline audiologic evaluation. In contrast to the initial hearing loss, the incidence of progression did not differ significantly according to genotype (log-rank \( H^2 = 2.28; P = .51 \)). In addition, neither baseline age (\( P = .33 \)) nor initial level of hearing loss (\( P = .77 \)) was significantly associated with time to onset of progressive loss.

**COMMENT**

GJB2 is the most common recessive genetic cause of SNHL. GJB2 mutations were first identified in individuals with profound bilateral SNHL, and, therefore, diagnostic clinical testing was only offered to patients with that audiologic phenotype. Most initial reports described the audiologic phenotype as moderate to profound and/or were based on single audiograms so that progression was difficult to judge. In a 2001 report of GJB2-related hearing loss, 42 children aged 1 week to 16 years who had SNHL and biallelic GJB2 mutations were described. Of the 42, 23 (53%) were congenitally deaf and 19 (45%) were hearing impaired. Four of the 19 hearing-impaired children had progression of their hearing loss, with dramatic progression to bilateral profound hearing loss in 2 patients and a much milder progression in 2 others. Of the 23 congenitally deaf children, 18 (78%) were biallelic for 35delG and/or 167delT mutations. In con-

**Table 4. Progressive Hearing Loss in Patients With SNHL and 2 GJB2 Mutations**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Progressive Hearing Loss</th>
<th>No Progressive Hearing Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>47 (100)</td>
<td>37 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>29 (62)</td>
<td>24 (65)</td>
</tr>
<tr>
<td>Boys</td>
<td>18 (38)</td>
<td>13 (35)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>40 (85)</td>
<td>24 (65)</td>
</tr>
<tr>
<td>White, Hispanic</td>
<td>3 (6)</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (4)</td>
<td>7 (19)</td>
</tr>
<tr>
<td>African American and white</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Asian and white</td>
<td>1 (2)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Truncating alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28 (60)</td>
<td>19 (51)</td>
</tr>
<tr>
<td>1</td>
<td>13 (28)</td>
<td>10 (27)</td>
</tr>
<tr>
<td>0</td>
<td>6 (13)</td>
<td>8 (22)</td>
</tr>
<tr>
<td>M34T or V37I Alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, with T/T</td>
<td>28 (60)</td>
<td>19 (51)</td>
</tr>
<tr>
<td>0, with T/NT</td>
<td>4 (9)</td>
<td>6 (16)</td>
</tr>
<tr>
<td>0, with NT/NT</td>
<td>1 (2)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>1, with T/NT</td>
<td>9 (19)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>2, with NT/NT</td>
<td>5 (11)</td>
<td>7 (19)</td>
</tr>
<tr>
<td>Age at baseline, median (range), y</td>
<td>2.0 (0.5-13.9)</td>
<td>3.1 (0.4-15.1)</td>
</tr>
<tr>
<td>Baseline hearing loss, median (range), dB</td>
<td>69 (9-118)</td>
<td>46 (5-111)</td>
</tr>
<tr>
<td>Time observed, median (range), mo</td>
<td>13 (1-110)</td>
<td>34 (3-122)</td>
</tr>
</tbody>
</table>

Abbreviations: NT, nontruncating; T, truncating.

*Data are given as number (percentage) of patients unless otherwise indicated.

*Four-frequency pure-tone average in the better ear.

*Time to the first audiogram, showing progression as defined in the “Results” section.

*Time between the first and most recent audiogram, without progressive hearing loss, as defined.
of others. The 2005 report by Snoeckx et al repre-
senting loss than those patients with truncating or nontrun-
cating mutations (Figure 1). In addition, patients who
had at least 1 M34T or V37I mutation had less severe hear-
ing loss. Of these 47, 47 (56%) have had pro-
gression of their hearing loss. Three of the 47 patients
who had progression have other diagnoses that may
have contributed to their hearing loss. One had congen-
tital cytomegalovirus (235delC/235delC), and a second
(35delG/M34T) has cystic fibrosis and has received many
courses of systemic and inhaled aminoglycosides; this
second patient has been tested for 1553A→G and 1494C→T
mutations in the 12SrRNA mitochondrial gene, and re-
sults were negative. The third patient (35delG/35delG)
had congenital syphilis and was treated effectively in in-
fancy. Two of these 3 have progressed to bilateral pro-
found hearing loss and have received cochlear implants.
For all 3 of these patients, the medical diagnosis was
thought to be the cause of their hearing loss before GJB2
testing was performed. In all 3 cases, however, the de-
gree or configuration of the hearing loss was not be-
thought to be consistent with the medical diagnosis, so ge-
etic testing was pursued and the results are included in
the final analysis of this article. Although it is impos-
sible to say with 100% certainty that these 3 patients had
progression of SNHL owing to GJB2 mutations, it seems
probable; the patient with syphilis was definitively treated
in infancy, and the patient with cystic fibrosis did not have
the sharply downward sloping audiogram of most pa-
tients with aminoglycoside ototoxicity. The patient with
congenital cytomegalovirus had a very symmetrical hear-
ing loss, which is less characteristic of cytomegalovirus
and more so of GJB2. An additional important point for
these patients is that they have a definite genetic cause
of hearing loss, which previously would not have been
considered given their medical diagnoses.

The degree of severity of the hearing loss at initial pre-
sentation is statistically related to the type of mutation.
Patients who had 1 or 2 nontruncating mutations had a
milder audiologic phenotype than those who had 2 trun-
cating mutations (Figure 1). In addition, patients who
had at least 1 M34T or V37I mutation had less severe hear-
ing loss than those patients with truncating or nontrun-
cating mutations not involving M34T or V37I. These re-
sults support our initial findings, as well as those of
others. The 2005 report by Snoeckx et al represents a
1531-participant, multicenter, international study of GJB2 audiologic phenotype-genotype in which our re-
search group participated. In this study, 79% of patients
had mild to moderate hearing loss. The current data, as
well as the larger aggregate data in the Snoeckx et al study,
demonstrate that the degree of hearing loss was gener-
amely more severe in the patients with 2 truncating mu-
tations compared with those with 2 nontruncating mu-
tations or 1 of each. Furthermore, this larger multicenter
study also found milder hearing loss in those with at least
1 M34T or V371 mutation.

The mechanism of the varying phenotype (including
severity and presence or absence of progression) in pa-
tients with GJB2 is unclear. In 1995, Kikuchi and col-
leagues showed immunochemical localization of Cx26
to 2 groups of cells in the cochlea: nonsensory epithelial
cells and connective tissue cells. Gap junction channels,
which in the cochlea are comprised of Cx26 and other
connexin proteins, are thought to help maintain the en-
docochlear potential, which is essential for hair cell ex-
citation and function, by facilitating passage of potas-
sium ions between cells. A 2002 study using a targeted
knockout mouse model of Cx26 showed normal devel-
ment of the inner ear with cell death occurring soon
after the onset of hearing. These investigators suggest that
Cx26 deficiency may lead to an increase in extracellular
potassium, which in turn may cause death of supporting
cells through oxidative stress. However, it is now un-
derstood that different mutations have varying effects on
the function of gap junctions, with some mutations lead-
ing to protein absence and others leading to expressed
proteins with altered function. These mutation-specific
differences as well as differences in other genetic and en-
vironmental modifiers are likely to explain the variability
in the severity and progression of hearing loss.

In the present study and in the one by Snoeckx et al, trun-
cating mutations included nonsense mutations as well
as deletions, insertions, and duplications that intro-
duced a shift in the reading frame. The nontruncating
mutations included amino acid substitutions. For the trun-
cating mutations, the protein product is generally not
made, is quickly degraded, or is extremely abnormal. For
the nontruncating mutations, it is possible that a pro-
tein product with partial functional activity may be made.
Therefore, the difference in the presenting audiogram be-
tween truncating and nontruncating mutations makes
sense. However, there was no statistical difference be-
tween truncating and nontruncating groups in terms of
incidence of progression of the hearing loss. In addi-
tion, progression could not be linked to any 1 particular
mutation. Possible explanations include as yet uniden-
tified modifiers to the genotype, including modifier genes
or environmental factors. Length of follow-up may also
be a factor; it is possible that if all patients with biallelic
GJB2 mutations are followed up long enough, they will
exhibit progression. Even this thought, however, does
not explain the variability in the rate of progression.

CONCLUSIONS

Mutations in GJB2 are the most common cause of reces-
sive hearing loss. The hearing losses range from mild to
profound and are usually congenital. Of the two-thirds
of our patients who presented with mild to severe SNHL
owing to biallelic GJB2 mutations, 50% experienced pro-
gressive hearing loss. In most cases, this was a progres-
sive loss that occurred over a period of years; however,
1 patient had precipitous bilateral hearing loss that pro-
gressed from mild to profound in a period of months. The
exact causes of the varying audiologic phenotype and au-
diologic progression remain unclear. Hearing-loss severity may be influenced by genetic factors, such as the degree of preserved protein function in nontruncating mutations, whereas hearing-loss progression may be dependent on factors other than the Cx26 protein. Regardless of the mechanism, genetic counseling for patients with GJB2 mutations needs to include the variability of the audiologic phenotype as well as the possibility of progression.

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