Carrier Rates in the Midwestern United States for GJB2 Mutations Causing Inherited Deafness

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Context Mutations in the GJB2 gene are the most common known cause of inherited congenital severe-to-profound deafness. The carrier frequency of these mutations is not known.

Objectives To determine the carrier rate of deafness-causing mutations in GJB2 in the midwestern United States and the prevalence of these mutations in persons with congenital sensorineural hearing loss ranging in severity from moderate to profound, and to derive revised data for counseling purposes.

Design Laboratory analysis, performed in 1998, of samples from probands with hearing loss for mutations in GJB2 using an allele-specific polymerase chain reaction assay, single-strand conformation polymorphism analysis, and direct sequencing.

Setting and Subjects Fifty-two subjects younger than 19 years sequentially referred to a midwestern tertiary referral center for hearing loss or cochlear implantation, with moderate-to-profound congenital hearing loss of unknown cause, parental nonconsanguinity, and nonsyndromic deafness with hearing loss limited to a single generation; 560 control neonates were screened for the 35delG mutation.

Main Outcome Measure Prevalence of mutations in the GJB2 gene by congenital deafness status.

Results Of 52 sequential probands referred for congenital sensorineural hearing loss, 22 (42%) were found to have GJB2 mutations. The 35delG mutation was identified in 29 of the 41 mutant alleles. Of probands’ sibs, all homozygotes and compound heterozygotes had deafness. Fourteen of 560 controls were 35delG heterozygotes, for a carrier rate expressed as a mean (SE) of 2.5% (0.66%). The carrier rate for all recessive deafness-causing GJB2 mutations was determined to be 3.01% (probable range, 2.54%-3.56%). Calculated sensitivity and specificity for a screening test based on 35delG mutation alone were 96.9% and 97.4%, respectively, and observed values were 94% and 97%, respectively.

Conclusions Our data suggest that mutations in GJB2 are the leading cause of moderate-to-profound congenital inherited deafness in the midwestern United States. Screening of the GJB2 mutation can be offered to individuals with congenital deafness with high sensitivity and specificity by screening only for the 35delG mutation. A positive finding should establish an etiologic diagnosis and affect genetic counseling.
triplex (multiple cases) families from France, Britain, Tunisia, and New Zealand. This finding makes DFNB1-related deafness the most common type of hereditary congenital hearing loss in those countries. The GJB2 gene encodes the connexin 26 (Cx26) component of gap junctions, and in approximately two thirds of Cx26 alleles from persons with DFNB1-related deafness, a single mutation is found. This mutation, the deletion of 1 of 6 contiguous deoxyguanosines, is referred to as either 35delG (preferred nomenclature as recommended by the Nomenclature Working Group) or 30delG.

In this article, we report the carrier frequency of the 35delG mutation in the midwestern United States and estimate the prevalence of GJB2 mutations in persons with congenital sensorineural hearing loss. These data are used to assess the impact of GJB2 as a cause of autosomal recessive nonsyndromic hearing loss.

METHODS

Subject Accrual

Subjects were younger than 19 years and had congenital sensorineural hearing loss. They were sequentially accrued from hearing loss and cochlear implant referrals to an otolaryngology clinic in the midwestern United States. Excluded from this study were persons from consanguineous populations and those with syndromic, mild, unilateral, acquired, or dominant types of hearing loss. Less than 20% of the referral population was excluded. Evaluation included a complete history taking and physical examination, audiometry, and, in 90% of cases, a temporal bone computed tomographic scan. Hearing loss ranged from moderate to severe at some frequencies (33%), but was most often severe to profound at all frequencies (67%).

The control population consisted of 560 randomly selected neonates born in the midwestern United States in 1995. As part of the Iowa Neonatal Screening Program, blood was obtained and placed on filter-paper cards for each child born in Iowa. These cards were rendered anonymous, assigned a random number, and used for DNA extraction. We used only those DNA samples that had served successfully as an amplification template in an unrelated project (representing 76.5% of neonatal cards).

The population demographics of the control sample reflect those of the state of Iowa: 94.8% non-Hispanic white, 1.9% black, 1.7% Hispanic, 1.3% Asian and Pacific Islander, and 0.3% American Indian. The white population is largely of northern European origin; approximately 0.2% of the population is Ashkenazi Jewish. All procedures were approved by the University of Iowa Human Subjects Committee.

GJB2 Screening

All DNA samples were screened for the 35delG mutation using an allele-specific polymerase chain reaction (ASPCR). The 35delG homozygotes were diagnosed as having GJB2-related hearing loss and no other studies were performed. The 35delG heterozygotes were screened by single-strand conformational polymorphism (SSCP) analysis and direct sequencing (if shifts were observed) to determine whether a second mutation was present. If no 35delG alleles were identified by ASPCR, the GJB2 coding sequence was screened by SSCP and sequenced if SSCP shifts were observed. In all cases with only a single coding sequence mutation, the noncoding exon of GJB2 (exon 1) was sequenced.

The ASPCR was performed using 40 ng of human DNA in an 8–µL PCR reaction containing 1.25 µL of PCR buffer (100 mmol of tris-hydrochloride, pH 8.8, 500 mmol of potassium chloride, 15 mmol of magnesium chloride, 0.01% wt/vol gelatin); 200 µmol each of dATP, dCTP, dGTP, dTTP; 25 pmol each of either normal or mutant primer and the common primer; and 15 pmol each of control primer A, 5′-CCACCTTCCGCTTCTCTTCAGGCAATGGG-3′ and control primer B, 5′-GGGCTTCAAGTTTACTTACAGGATGC-3′. For 35delG ASPCR the normal, mutant, and common primers have been described previously. For M34T, the primers were: normal = 5′-GCTACCCGTCTTCTTTTTTCGATTCT-3′, mutant = 5′-GCTACCGTGCTTCTTTTTCCGATTTC-3′, and common = 5′-TAGAGCGCTGGGTGACAGAAGATCG-3′. Samples were denatured at 95°C for 5 minutes, followed by 32 cycles at 95°C for 40 seconds, 60°C for 30 seconds, and 72°C for 1 minute. The PCR products were analyzed by electrophoresis in a 1.5% agarose gel containing ethidium bromide. The control primers amplified a 360–base pair (bp) product, which served as an internal control. The 35delG and M34T products were 202 bp and 199 bp in length, respectively. For each ASPCR run, sequence-tested 35delG-positive and 35delG-negative DNA samples were examined as controls. Sequencing of 14 positive and 9 negative samples demonstrated complete concurrence between ASPCR results and DNA sequencing; this number of samples was considered to be adequate confirmation of ASPCR sensitivity.

The SSCP was carried out in 3 reactions using primer pairs Cx26-1/Cx26-6, Cx26-10/Cx26-15, and Cx26-5/Cx26-6, as noted earlier. Amplification of exon 1 was performed with primers 5′-TCTTTTCGAGGCAAAACCGC-3′ and 5′-GGGCAATGCGTTAAAACGTC-3′ using Advantage GC Genomic Polymerase Kit (Palo Alto, Calif) according to the manufacturer’s instructions. The PCR products were cleaned using a QIAquick PCR purification kit (Qiagen, Chatsworth, Calif) and directly sequenced. Control samples were screened for 35delG by ASPCR.

Statistical Analysis

Statistical comparison of results was performed using the 1-sided Fisher exact test. Since it is only meaningful to determine whether a mutation is statistically more common in the deaf population in comparison with the hearing population, the 1-sided, rather than the 2-sided, P value was determined in power analysis (described below). To determine whether there were
any statistically significant differences in the 35delG carrier rates between studies, the Fisher exact test was used due to the small sample sizes of the other studies (discussed below). There are a number of conflicting theories on how to calculate the 2-tailed P value of a Fisher exact test; of these, an accepted theory is to double the 1-sided P value. Since none of our comparisons was statistically significant in a 1-tailed comparison, and since all 2-tailed P values are greater than the corresponding 1-tailed P values, 2-tailed values are not given.

The power was computed via Monte Carlo simulations. We generated replications of the data as binomial random variables, C for binomial (128, 0.0234) corresponding to the control sample and D for binomial (52, 0.117) corresponding to the deaf sample, with the assumption of a 5-fold increase in the control prevalence rate. For each simulation, we computed the 1-sided tail area of the Fisher exact test and calculated the power as the percentage of 1-sided rejections in 50,000 replications.

The quantities q and r, sensitivity, and specificity (defined below) are functions of 3 binomial random variables. We computed the probable error (median error) of sensitivity, specificity, and the quantities of $2r(q + r)^2$, $2(q + r)$, and $q^2 + 2qr$ (defined below) by generating 10,000 replications of the 3 underlying binomial random variables. For each replication, we calculated the values of the dependent variables, producing 10,000 replications of each. Positive and negative probable errors then were calculated from the set of replications as the deviations of the first and third quartiles of each function from its median and used to determine the upper and lower limits of the probable range. Probable error for R_{Other} and R_{Overall} (defined below) were computed by the same method. The variance of an estimated proportion ($p$) from a sample population ($n$) is $p(1-p)/n$. The SE is the square root of this variance.

### RESULTS

Screening of 52 sequential probands referred for congenital sensorineural hearing loss identified 22 individuals with $GJB2$ mutations (19 with mutations of both alleles; the individual with the M34T/H100Y genotype was not considered to have $GJB2$-related deafness as described below). The 35delG mutation was found in 29 of the 41 mutant alleles; 2 mutations, M34T and 167delT, were identified twice; 8 other mutations occurred once (TABLE 1).

TABLE 2 presents $GJB2$-related deafness prevalence in several subpopulations with deafness. Mutations of $GJB2$ were present on both alleles in 7 of 12 deaf sibships we studied. Examination of the sibs of probands revealed that all homozygotes and compound heterozygotes had hearing loss. The degree of loss could not be predicted by the $GJB2$ genotype, as demonstrated in 1 family in which one child had moderate and the other child profound hearing loss. Whether some genotypes are associated with profound hearing loss more consistently than other genotypes is not known.

Three persons carried only a single $GJB2$ mutation (genotypes 35delG/*, M34T/*, and R98Q/*). A second mutation could not be demonstrated by sequencing both exons in these individuals. The 35delG carrier was found to have dilated vestibular aqueducts, a feature seen in approximately 10% of the referral population but in none of the other $GJB2$ mutation carriers. These 3 persons were not considered to have $GJB2$-related deafness. The other person carrying $GJB2$ mutations who did not have $GJB2$-related deafness was the individual with the M34T/H100Y genotype (TABLE 1).

Fourteen 35delG heterozygotes were identified in the control population among 560 individuals, for a 35delG carrier rate of 2.3% ± 0.66% (SE). The M34T polymorphism was identified in 3 (2.3%) of 128 unrelated Centre d’Etude du Polymorphisme Humain controls (standard reference group composed primarily of persons of northern European ancestry22), which is not significantly different from the prevalence in the deaf population (2/52; $P = .85$, 1-sided Fisher exact test).

Despite the comparable prevalence of the M34T and 35delG mutations in the general population, no M34T homozygotes or M34T/35delG compound heterozygotes were identified. A 5-fold difference in M34T carrier rates between the deaf and control population at $P = .05$ can be
detected with the sample sizes of our populations with a power of 0.68. Combining our data with similar data (derived from individuals in the midwestern United States with moderate-to-profound deafness [W. J. Kimberling, oral communication, May 20, 1999]) obtained by Kelley et al increases the power to 0.96 with Monte Carlo error of about 0.5%. Thus, the M34T mutation was not considered to cause deafness.

These data were used to estimate the proportion of the population in the midwestern United States with moderate-to-profound congenital GJB2-related deafness. Based on the Hardy-Weinberg law, this proportion, N_{GJB2}, can be represented as \((q + r)^2 = q^2 + 2qr + r^2\), in which \(q\) equals the chromosomal rate of the 35delG mutation and \(r\) equals the chromosomal rate of all other GJB2 mutations that cause hearing loss in the compound heterozygote.

We determined the 35delG chromosomal rate experimentally to be 14 of 1120, and although \(r\) cannot be calculated similarly since all other deafness-causing alleles of GJB2 are not known, it can be estimated. If \(N_{Other}\) represents the proportion of the population in the midwestern United States with deafness due to other causes (GJB2-unrelated deafness), then the proportion of these individuals who are, coincidentally, 35delG carriers is approximately \(2q \times N_{Other}\). Based on these definitions, the ratio of 35delG homozygotes to 35delG heterozygotes among deaf individuals, \(h\), can be represented as \(q^2/2qr + 2q(N_{Other})\). Since the ratio of the proportions, \(N_{GJB2}/N_{Other}\), equals the ratio of individuals with GJB2-related deafness to individuals with GJB2-unrelated deafness (18 and 34, respectively, in our population), \(N_{GJB2}/N_{Other} = 18:34\).

By substitution, \(h\) can be expressed as \(q^2/2qr + 2q(34:18)(N_{GJB2})\). When data reported by Kelley et al are combined with ours, \(h\) is 25:12 (Table 3). Therefore, a ratio of 25:12 = \(q^2/2qr + 2q(34:18)(q + r)^2\). Solving this equation with \(q = 14:1120\) gives a value for \(r\) of 0.00257. This value makes the carrier rate for non-35delG GJB2 recessive deafness-causing mutations approximately 0.51% (probable range, 0.38%-0.68%).

The carrier rate for all GJB2 recessive deafness-causing mutations is the sum of the carrier rates for the 35delG mutation and all other deafness-causing mutations, or 0.31% (probable range, 0.25%-0.56%). Based on values for \(q\) and \(r\), the proportion of the population with GJB2-related congenital deafness, \((q + r)^2\), is 22.7 (probable range, 15.1-31.9) per 100 000; the proportion with congenital deafness due to other causes, 0.66; and the proportion with congenital deafness not coincidentally carrying the 35delG mutation divided by the population with GJB2-unrelated deafness not coincidentally carrying the 35delG mutation divided by the population with GJB2-unrelated deafness (41.8/42.9 = 97.4%),

### Table 3. Prevalence of 35delG Mutation Internationally*

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Country (Race)</th>
<th>No. of Deaf and Homozygous for 35delG</th>
<th>No. of Deaf and Heterozygous for 35delG</th>
<th>35delG Carrier Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denoyelle et al, 1997</td>
<td>Tunisia, France, United Kingdom, New Zealand (white)</td>
<td>19</td>
<td>18</td>
<td>0/119†</td>
</tr>
<tr>
<td>Estivill et al, 1998</td>
<td>Italy, Spain (white)</td>
<td>44</td>
<td>14</td>
<td>9/280</td>
</tr>
<tr>
<td>Lench et al, 1998</td>
<td>United Kingdom, Belgium (white)</td>
<td>3</td>
<td>3</td>
<td>...</td>
</tr>
<tr>
<td>Scott et al, 1998</td>
<td>United States (white)</td>
<td>...</td>
<td>...</td>
<td>1/100</td>
</tr>
<tr>
<td>Morell et al, 1998</td>
<td>United States (white)</td>
<td>...</td>
<td>...</td>
<td>1/173</td>
</tr>
<tr>
<td></td>
<td>(black)</td>
<td>...</td>
<td>...</td>
<td>0/173</td>
</tr>
<tr>
<td></td>
<td>(Asian)</td>
<td>...</td>
<td>...</td>
<td>0/53</td>
</tr>
<tr>
<td></td>
<td>(Ashkenazi Jewish)</td>
<td>...</td>
<td>...</td>
<td>4/551</td>
</tr>
<tr>
<td>Kelley et al, 1998</td>
<td>United States (white)</td>
<td>14</td>
<td>5</td>
<td>2/96</td>
</tr>
<tr>
<td>Present study</td>
<td>United States (white)</td>
<td>11</td>
<td>7</td>
<td>14/560</td>
</tr>
<tr>
<td>Total</td>
<td>United States†</td>
<td>25</td>
<td>12</td>
<td>...</td>
</tr>
<tr>
<td>Worldwide</td>
<td>91</td>
<td>47</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

*Levels of deafness described as moderate-to-profound, congenital deafness of different degrees, sensorineural deafness, and moderate-to-profound deafness (W. J. Kimberling, oral communication, May 20, 1999). †Ellipses indicate data not applicable.

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probable range, 97.0%-98.0%), which also approaches 1 - 2q/q since q is small. The observed sensitivity (17/18 = 94%) and specificity (33/34 = 97%) are comparable with these calculated values. Inclusion of data from international sources12-15 does not substantially alter these results.

These calculations assume the population is randomly mating with respect to GJB2. The existence of population substructure, particularly endogamous subpopulations, results in a decreased proportion of heterozygotes (Wahlund effect) and an undercalculation of r with an overestimation of test sensitivity for the population as a whole. Other assumptions made in these calculations include complete penetrance and lack of ascertainment bias (ie, equal referral rates regardless of genotype), and negligible heterozygote selection advantage, spontaneous mutation rate, and migration effects. Deviation of the actual population from Hardy-Weinberg equilibrium due to these factors is likely to be minimal and does not affect the order of magnitude of the figures obtained with the possible exception of assortative mating among the deaf, which is discussed below.

Bayesian analysis using these data permits delineation of the recurrence risk for heritable deafness. The overall chance of having a second deaf child (R_{overall}) is dependent on the proportion of that population with GJB2-related hearing loss (N_{GJB2}), the proportion of that population with GJB2-unrelated hearing loss (N_{other}), and the individual recurrence risks for GJB2-related deafness (R_{GJB2}) and GJB2-unrelated deafness (R_{other}). Assuming near-complete penetrance, R_{GJB2} is approximately 25%. Therefore, R_{overall} = \frac{N_{GJB2}}{N_{total}} \times R_{GJB2} + \frac{N_{other}}{N_{total}} \times R_{other} = \frac{N_{GJB2}}{N_{total}} \times 25% + \frac{N_{other}}{N_{total}} \times R_{other}.

This study has determined the proportions of sequential deaf probands with GJB2-related (N_{GJB2} = 18/52) and GJB2-unrelated deafness (N_{other} = 34/52). The ratio, S, of GJB2-related to GJB2-unrelated deafness among deaf siblings is dependent on the relative frequencies, proband frequencies, and recurrence chances of couples with a deaf child. Assuming no differences in fecundity, S = N_{GJB2} \times R_{GJB2}/N_{other} \times R_{other}. Since S = 41/42 (Table 2), and N_{GJB2}, N_{other}, and R_{GJB2} are known, R_{other} can be shown to be 13.6% (probable range, 10.6%-17.2%). The a priori chance, R_{overall}, is then 17.5% (probable range, 15.0%-20.4%).

For a hearing couple, assortative mating among the deaf does not affect R_{overall}, which is calculated exclusively from values determined for hearing couples. For a deaf couple, recurrence chances must be calculated independently and may vary from 0% to 100%, depending on the etiologies of deafness and carrier statuses for deafness-causing mutations. (Assortative mating results in increased carrier rates of deafness genes among deaf persons who have multiple deaf progenitors in comparison with deaf persons who do not have deaf progenitors [ie, a deaf man with congenitally deaf parents and grandparents is more likely to carry deafness genes], including genes unrelated to the etiology of his own deafness.) Additionally, the recurrence chance for a couple in which 1 spouse is deaf or has nonpenetrant deafness is higher. Since the carrier rate of 35delG (2.5%) is much greater than the rate of congenital hereditary deafness from assortative couplings (>0.1% of the general population), the calculation of carrier rates is not affected substantively by assortative mating.

**COMMENT**

Mutations in GJB2 are the most common cause of moderate-to-profound congenital inherited deafness in the midwestern United States. This deafness etiology (requiring deafness-causing mutations of both alleles) was found in 18 (35%) of 52 probands evaluated for congenital, moderate-to-profound sensorineural hearing loss (Table 1) and in more than 50% of multiple sibships. Although numerous deafness-causing mutations of this gene occur, a single mutation, 35delG, predominates. Our data are consistent with other national and international data in showing that 60% of persons with GJB2-related deafness are homozygous for the 35delG allele.13,14,16 The carrier rate as a mean (SE) in the general population for this allele is 2.5% (0.66%) (Table 3). The total carrier rate for all GJB2 deafness-causing mutations is 3.01% (probable range, 2.54%-3.56%). The corresponding predicted prevalence of GJB2-related congenital hearing loss is 22.7 (probable range, 15.1-31.9) per 100,000 births. More than two thirds of these individuals have profound or severe-to-profound hearing loss, although there can be phenotypic variability in the degree of loss even within the same family. The lower 35delG carrier rates noted by Scott et al21 (1/100; P = .31) and Kelley et al16 (2/96; P = .58) (P values represent comparison with our results) are consistent with stochastic variances due to smaller sample sizes. The study by Morell et al23 showed a carrier rate of 1 in 173 among white college students. Although the difference between this result and our results does not achieve statistical significance (P > .09), it is important to note that the predicted prevalence of 35delG homozygosity corresponding to this carrier rate would be 0.8/100,000, a value inconsistent with observed data.13,14,16

Data used to provide information for genetic counseling and estimates of recurrence risks for hearing couples with a deaf child are taken from segregation analyses of demographic data from the 1800s and from large family surveys.24 Analyses using these sources provided estimates of the chance of recurrence under these conditions at 9.8%.25 Using the results derived from the sequentially accrued probands in our study, we estimate the chance for a normal-hearing couple to have a second deaf child to be 17.5% (probable range, 15.0%-20.4%) provided the first deaf child meets the criteria for inclusion in this study. This value is comparable with a population-based estimate from Canada of 16% ± 3%.2

Several factors have contributed to this change in recurrence-risk estimates, including an improved ability to identify syndromic forms of deafness characterized by variable expressivity.
and a decrease in congenital-acquired hearing loss most recently reflected in the effect of rubella immunization on rubella-induced deafness.26 These factors, and an increase in population mobility leading to a loss of segregating communities (thereby decreasing contributions from rare genes), act to increase the proportion of deafness due to GJB2 mutations both in the hearing and Deaf communities.

The sensitivity of a genetic screen for GJB2-related deafness can be enhanced by examination for mutations beyond 35delG that are known to cause deafness. In particular, the 167delT mutation appears to be relatively common among Ashkenazi Jews, with a measured prevalence of 4%, despite being rare in the general population.23 Screening for 167delT to identify GJB2-related deafness should be considered for populations containing a sizable Ashkenazi Jewish subpopulation. After identification of a single mutation in GJB2, more exhaustive methods, such as SSCP and sequencing, are recommended for screening for the presence of a second mutation.

These data support the future use of a genetic test, such as ASPCR, designed to identify the 35delG GJB2 mutation, as a valuable complement to audiometric screens to identify neonates with hereditable congenital hearing impairment in nonendogamous white populations of the Midwest and other ethnically similar populations. Use of this test may facilitate earlier habilitation in a substantial percentage of deaf infants and ultimately may provide parents with valuable prognostic and therapeutic information.

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REFERENCES