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Effects of GJB2 genotypes on the audiological phenotype: Variability is present for all genotypes

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KEYWORDS
Audiological features; Deafness; Genotype—phenotype; GJB2; Variability

Summary

Background and aim: Recent studies have revealed a genotype—phenotype correlation for mutations in the GJB2 gene. Since ethnic difference may have an effect for the degree of hearing loss due to background genes, we aimed to search for confirmation of previously suggested genotype—phenotype correlation in GJB2 deafness in the Turkish population.

Methods: Pure tone audiograms of 63 unrelated probands with GJB2-associated hearing loss having 15 different mutations were obtained and evaluated for correlation between the degree of hearing loss and genotypes.

Results: Three GJB2 genotypes identified in more than one family were homozygous c.35delG (44 probands), homozygous p.E120del (four probands) and c.[35delG] + [IVS1 + 1G > A] (two probands). No statistical difference for the degree of hearing loss was observed when the genotypes were compared individually or grouped according to their effects on the protein. The most likely explanation for this result is the relatively small size of the studied population. Degree of hearing loss was variable in c.35delG and p.E120del homozygotes. Intra-familial phenotypic variability was present for some genotypes. The detailed audiological data for homozygous p.E120del and c.[35delG] + [328delG] genotypes are reported for the first time in this study.

Conclusion: Previously reported genotype—phenotype correlations for the GJB2 deafness should be cautiously interpreted during the clinical counseling since variability in the degree of hearing loss is present for all GJB2 genotypes.

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1. Introduction

Hearing loss is the most common sensory defect affecting 1–2 per 1000 infants. Genetic causes are believed to be involved in at least half of these children. Among the genetic causes those with autosomal recessive inheritance are responsible in 70–80% of affected children [19]. Most of these children do not have any clinical manifestations other than hearing loss and considered to be affected by non-syndromic deafness. Although mutations in 21 different genes have thus far been identified to result in non-syndromic autosomal recessive deafness [30], pathogenic alterations in one gene, GJB2 (encoding connexin 26 protein), are detected in up to 50% of recessive cases in a large part of the world [10]. More than 90 sequence changes have been reported in this gene [1], whereas the c.35delG mutation is by far the most common pathogenic allele in Caucasians for which a single origin has been demonstrated [21,28,31]. Its carrier frequency among hearing individuals from different Caucasian populations ranged from 1% to 4% [10].

Since the initial reports revealing that pathogenic mutations in GJB2 cause deafness, variability in the degree of hearing loss in persons with mutations in this gene has been evident [4,6,17]. A genotype–phenotype correlation has recently been reported [4,13,14]. Hearing impairment associated with homozygous c.35delG mutation has been described by many investigators as severe to profound, symmetrical, sensorineural hearing loss with little or no progression [4,7,16,17]. However, the degree of hearing loss associated with all reported genotypes has not been clearly demonstrated. We and others have previously shown that the GJB2 mutation spectrum in Turkey comprises both oriental as well as Caucasian alterations [9,28,29]. In this study, we demonstrate the audiological characteristics of Turkish individuals with biallelic GJB2 mutations.

2. Materials and methods

Our study was approved by the Ethics Committee of Ankara University in 2001 (10-2001/141). Informed consent forms were signed by each participant or parent prior to study.

A total of 63 probands coming from unrelated Turkish families with non-syndromic autosomal recessive deafness, carrying biallelic GJB2 mutations were included in this study. Probands were ascertained through schools for the hearing impaired located in five cities (Afyon, Amasya, Ankara, Denizli, and Isparta) and from the pediatric genetics clinic of Ankara University School of Medicine. Schools for the hearing impaired admit children with early-onset severe to profound hearing loss. However individuals with all degrees of congenital, pre- and post-lingual onset sensorineural hearing loss are evaluated for the presence of genetic factors at the outpatient pediatric genetics clinic of Ankara University School of Medicine. All probands were initially evaluated for the presence of environmental causes (such as neonatal hyperbilirubinemia, ototoxic medication exposure, meningitis, and acoustic trauma) and syndromic forms of deafness were excluded from this study if such a factor was present. Molecular investigations for identification of GJB2 mutations have been previously published [26,28]. Mutations were described according to the nomenclature for the description of sequence variations published by den Dunnen and Antonarakis [5].

The Speech Reception Threshold Test was performed using a three-syllable word list and The Word-recognition Psychometric Function Test was performed using a mono-syllable phonetically balanced word list. Pure tone hearing thresholds were obtained in a sound-proof room. For air conduction, frequencies between 250 and 6000 or 8000 Hz were assessed, whereas frequencies between 250 and 4000 Hz were used for bone conduction. Sound field visual reinforcement audiometry and play audiometry were used to evaluate four probands who could not otherwise be assessed using standard audiometry. Pure tone average values for frequencies at 500–2000 Hz were calculated in the better ear for each individual [PTA (0.5–2.0 kHz)].

Genetic and audiological data were described according to recommendations of the GENDEAF study group on genotype–phenotype correlations [15]. According to these guidelines following groups were recognized: mild hearing loss: 20–40 dB; moderate hearing loss: 41–70 dB; severe hearing loss: 71–95 dB; profound hearing loss: >95 dB. Audiometric configurations were determined for each ear as follows: low frequency ascending: >15 dB hearing level (HL) from the poorer low frequency thresholds to the higher frequencies; mid frequency U-shaped: >15 dB HL difference between the poorest thresholds in the mid-frequencies, and those at higher and lower frequencies; High frequency (a) gently sloping: 15–29 dB HL difference between the mean of 0.5 and 1 kHz and the mean of 4 and 8 kHz, (b) steeply sloping: >30 dB HL difference between the above frequencies; flat: <15 dB HL difference between the mean of 0.25, 0.5 kHz thresholds, the mean of 1 and 2 kHz and the mean of 4 and 8 kHz. Hearing loss with >10 dB hearing level difference between the ears in at least two frequencies is determined as asymmetrical (the PTA0.5–2 kHz of the better ear should be worse than 20 dB HL).
Comparisons by hearing impairment classification (mild, moderate, severe, profound) between various c.35delG/non-c.35delG, inactivating/non-inactivating genotype groups and between specific genotypes were made using $\chi^2$-test and Student t-test, respectively. The level of significance used was $p = 0.05$.

Some of our data have been used in a recently published multicenter study by Snoeckx et al. [25]. Audiological data of a family including individuals with c.35delG + p.[L90P] genotype have been previously published [27]. However, for the purpose of this study only one individual from this family (with this genotype) was considered proband and included.

3. Results

3.1. GJB2 genotypes

Fifteen different mutations and 16 different genotypes were detected (Table 1). Ages of the probands (44 males and 19 females) ranged from 2 to 26 years (mean ± S.D. = 10.78 ± 5.52 years). The most common genotypes were homozygous c.35delG (69.8%), followed by homozygous p.120delE (6.3%), and c.35delG + [IVS1 + 1G > A] (3.1%). Remaining 13 genotypes were identified in single probands (1.5%), eleven of which were compound heterozygous for c.35delG with c.333_334delAA, c.236_239del4ins7, p.Y155X, c.299_300delAT, c.328delG, p.T55N, c.310_323del14, p.R143W, p.E120del, c.167delT, and p.L90P. Two probands were homozygous for p.R184P and c.511_512insAACG.

When the mutations were classified according to their functional effect on the connexin26 protein, identified DNA alterations included 10 inactivating (leading to an absent or truncated protein) and five non-inactivating mutations. Fifty-four of 63 (85.7%) probands had two inactivating mutations, five (7.9%) had two non-inactivating mutations and four (6.3%) had an inactivating and a non-inactivating mutation (Table 1).

We also classified the GJB2 genotypes as homozygous c.35delG (69.9%), c.35delG + [non-c.35delG] (20.6%) and [non-c.35delG] + [non-c.35delG] (9.5%).

3.2. Audiological features of GJB2 genotypes

All degrees of hearing impairment (HI) were seen in our probands. The HI was profound in 43 (68.2%), severe in 14 (22.2%), moderate in 5 (7.9%) and mild in 1 (1.6%) probands. The mean PTA$_{0.5—2\;\text{kHz}}$ value for GJB2 deafness in our sample was 98.8 dB.

Although all probands with severe to profound HI were of congenital or pre-lingual onset, time of onset of hearing loss could not be precisely assessed in probands with moderate or mild HI.

The only mildly affected proband and three of the five moderately affected probands were homozygous for c.35delG, reflecting the phenotypic heterogeneity for this genotype. The genotypes of other

<table>
<thead>
<tr>
<th>GJB2 genotype</th>
<th>n</th>
<th>Effect on protein</th>
<th>Mean PTA$_{0.5—2;\text{kHz}}$</th>
<th>Audiogram shape</th>
<th>Symmetry of HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.[35delG] + [35delG]</td>
<td>44</td>
<td>i/l</td>
<td>97.4</td>
<td>f/f (22), s/s (1), f/s (8), s/ss (3), s/ss (1)</td>
<td>S(41), A(3)</td>
</tr>
<tr>
<td>p.[E120del] + [E120del]</td>
<td>4</td>
<td>ni/ni</td>
<td>88.33</td>
<td>s/s (2), f/f (1), f/s (1)</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + [IVS1 + 1G &gt; A]</td>
<td>2</td>
<td>i/l</td>
<td>114.66</td>
<td>f/f (2)</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + [333_334delAA]</td>
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<td>i/l</td>
<td>120.00</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + [236_239del4ins7]</td>
<td>1</td>
<td>i/l</td>
<td>118.33</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>c.[511_512insAACG] + [511_512insAACG]</td>
<td>1</td>
<td>i/l</td>
<td>116.66</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>p.[R184P] + [R184P]</td>
<td>1</td>
<td>ni/ni</td>
<td>116.00</td>
<td>s/s</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + p.[Y155X]</td>
<td>1</td>
<td>i/l</td>
<td>115.00</td>
<td>s/s</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + [299_300delAT]</td>
<td>1</td>
<td>i/l</td>
<td>113.33</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + [328delG]</td>
<td>1</td>
<td>i/l</td>
<td>111.66</td>
<td>f/u</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + p.[T55N]</td>
<td>1</td>
<td>i/ni</td>
<td>110.00</td>
<td>s/ss</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + [310_323del14]</td>
<td>1</td>
<td>i/l</td>
<td>101.66</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + p.[R143W]</td>
<td>1</td>
<td>i/ni</td>
<td>101.66</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + p.[E120del]</td>
<td>1</td>
<td>i/ni</td>
<td>95.00</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + [167delT]</td>
<td>1</td>
<td>i/l</td>
<td>85.00</td>
<td>f/f</td>
<td>S</td>
</tr>
<tr>
<td>c.[35delG] + p.[L90P]</td>
<td>1</td>
<td>i/ni</td>
<td>53.33</td>
<td>f/s</td>
<td>A</td>
</tr>
</tbody>
</table>

PTA: pure tone audiometry; i: inactivating; ni: non-inactivating; f: flat; s: gently sloping; ss: steeply sloping; u: u-shaped; HL: hearing loss; S: symmetric; A: asymmetric. PTA values, audiogram shapes and symmetry of HL were not found to be significantly different for specific GJB2 genotypes.
proband with moderate HI were c.[35delG] + p.[L90P] and homozygous p.E120del. Audiological features of each GJB2 genotype are summarized in Table 1 and distribution of the probands according to their mean PTA_{0.5—2 kHz} values is shown in Fig. 1.

Several genotypes were associated with a lower (homozygous p.E120del, compound heterozygotes for c.35delG and c.167delT, p.E120del, p.L90P) or higher (homozygous c.511_512insAACG and p.R184P, compound heterozygotes for c.35delG and c.IVS1 + 1G > A, c.236_239del4ins7, p.Y155X, c.299_300delAT, c.328delG) PTA_{0.5—2 kHz} value when compared to homozygous c.35delG. However, the difference was not found to be statistically significant for any genotype (Student t-test).

Phenotypic variability was striking in the most common two genotypes: homozygous c.35delG and homozygous p.E120del. Three probands of homozygous p.E120del were profoundly affected while above mentioned one proband had moderate HI.

![Fig. 1](image1) Distribution of probands according to their GJB2 genotypes and mean PTA_{0.5—2 kHz} values for the better ear.

![Fig. 2](image2) Distribution of the four classes of hearing impairment over the inactivating/non-inactivating (A) and c.35delG/non-c.35delG genotypes (B) (p-values for inactivating/inactivating vs. non-inactivating/non-inactivating and inactivating/inactivating vs. inactivating/non-inactivating were 0.368 and 0.310, respectively. p-values for c.[35delG] + [35delG] vs. [non-c.35delG] + [non-c.35delG] and for c.[35delG] + [35delG] vs. c.[35delG] + [non-c.35delG] were 0.487 and 0.404, respectively).
The distribution of hearing impairment in various inactivating/non-inactivating and c.35delG/non-c.35delG genotype categories is shown in Fig. 2. No statistical significance was observed for any of the compared groups ($\chi^2$-test).

Audiogram shapes were mainly flat (59.5%) and sloping (39.6%) when determined for 126 ears separately. None of the probands had low frequency ascending phenotype. Similar to the variability of the degree of hearing loss, audiogram shapes of c.35delG homozygotes were also variable (Table 1 and Fig. 3A). Two of profoundly affected p.E120del homozygotes had gently sloping audiograms, however the proband having moderate HI presented with a flat audiogram (Figs. 3B and 4A). Audiograms of the third common genotype c.[35delG] + [IVS1 + 1G > A], were flat for both ears resulting in profound HI (Fig. 3C).

The only U-shaped audiogram was seen for the right ear of c.[35delG] + [328delG] proband who had...
symmetric HI mainly affecting the mid-frequencies profoundly. The proband’s sister with the same genotype (not included in this study) has moderate HI and a gently sloping audiogram bilaterally (Fig. 4B and C).

Ninety-three percent of the probands had symmetrical hearing loss. The proband with c.[35delG] + p.[L90P] was associated with asymmetric hearing loss, being moderate with a gently sloping audiogram on the right ear and profound with a flat audiogram on the left ear. Interestingly the brother of this proband with the same genotype has symmetric, moderate HL and a gently sloping audiogram [27]. Three of homozygous c.35delG probands had also asymmetric HI.

4. Discussion

Previously published genotype–phenotype correlation studies from different regions reported that, several mutation combinations including c.[35delG] + p.[L90P], c.[35delG] + p.[E120del], c.[35delG] + [IVS1 + 1G > A] and c.[35delG] + p.[N206S] result in less severe HI compared to c.35delG homozygotes and emphasize the major impact of GJB2 genotype on the degree of hearing impairment [4,11,14]. They also described a correlation between the frequency of mild or moderate hearing loss and the number of non-inactivating mutations. The results of a recently published multicenter study of 1531 probands that includes some of the previously reported data, were also concordant with these findings [25]. However, our results show that the degree of hearing loss is not significantly different between individuals with various GJB2 genotypes observed in Turkey, even when the genotypes are grouped according to their effect on the protein. A comparison of our data and two previously reported large series is shown in Table 2. Two Turkish c.[35delG] + [IVS1 + 1G > A] compound heterozygous probands were profoundly affected which was consistent with the effect of the mutation combination on connexin 26, but inconsistent with the literature. The most plausible explanation for this discrepancy is that the sample size in our study is relatively small. However, our study indicates that a clear genotype–phenotype correlation could be observed only when very large series are compared, but a generalized conclusion suggested by these large genotype–phenotype correlation studies should be used cautiously during counseling.

Distribution of PTA0.5—2 kHz values in 44 unrelated homozygotes for c.35delG showed a spectrum ranging from mild to profound degree of hearing loss. However, there was accumulation around 100 dB. This distribution of hearing loss associated with homozygous c.35delG mutation is quite similar to those described earlier.

Nine p.E120del homozygotes have been previously reported worldwide; four Iranian [3,18], two Turkish probands [9,29] and single probands from Pakistan [23], France [14] and Germany [8], although detailed audiological features were not provided in most cases. When combining our data with previously described characteristics, a variability for the degree of hearing loss is apparent for this genotype with four profoundly, two severely and two moderately affected individuals. Compound heterozygous states of p.E120del (with c.35delG, p.R148P, c.299_300delAT and p.L90P) have also been reported with varying degrees of HI [4,6,8,14,20,22,24,32].

The variability of hearing loss is not only inter-familial that we describe in the previous paragraphs for c.35delG and p.E120del homozygotes, but also intra-familial as we see different degrees of hearing loss.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Our data (n = 63)</th>
<th>Cryns et al., 2005 (n = 277)</th>
<th>Martin et al., 2005 (n = 256)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.[35delG] + [35delG]</td>
<td>104</td>
<td>mi(1), mo(3), se(11), pr(29)</td>
<td>102</td>
</tr>
<tr>
<td>p.[E120del] + [E120del]</td>
<td>96.6</td>
<td>mo(1), se(1), pr(2)</td>
<td>–</td>
</tr>
<tr>
<td>c.[35delG] + [IVS1 + 1G &gt; A]</td>
<td>109</td>
<td>pr(2)</td>
<td>62</td>
</tr>
<tr>
<td>c.[35delG] + p.[E120del]</td>
<td>95</td>
<td>Pr</td>
<td>71</td>
</tr>
<tr>
<td>c.[35delG] + p.[L90P]</td>
<td>53.3</td>
<td>mo/pr (1)</td>
<td>44</td>
</tr>
<tr>
<td>c.[35delG] + p.[R143W]</td>
<td>101.6</td>
<td>Pr</td>
<td>110</td>
</tr>
<tr>
<td>c.[35delG] + [167delT]</td>
<td>85</td>
<td>Se</td>
<td>81</td>
</tr>
</tbody>
</table>
loss and different audiogram shapes in two siblings with the c.[35delG]+[328delG] and c.[35delG]+p.[L90P] genotypes. Despite recent discoveries suggesting a genotype—phenotype correlation for GJB2 deafness, we have not move much forward from the interpretation made by Denoyelle et al. in 1999 [7], which emphasized that the severity of hearing loss due to DFNB1 was extremely variable and could not be predicted even within families. Why persons with the same GJB2 mutations may have different degrees of hearing impairment has remained unknown; environmental factors or modifier genes are potential explanations.

The typical audiogram shape of GJB2 deafness has been reported as being sloping (two-thirds of cases) or flat (one-third of cases) [2,6,12,13,16]. Table 3 shows a comparison of audiogram shapes observed in our study with a recently published study by Liu et al. [13]. The detailed audiological data of homozygous p.E120del and c.[35delG]+[328delG] genotypes are reported for the first time in this study. However the observed difference on the audiogram shapes between our data and those reported by Liu et al. [13] does not appear to be due to different frequencies of rare mutations.

In conclusion, previously reported genotype—phenotype correlations in GJB2 deafness should be cautiously interpreted during clinical genetic counseling of Turkish patients due to variability of hearing loss with the same genotypes. Future research to uncover the basis of this variation is necessary to provide better counseling to families with GJB2-related hearing loss.

Acknowledgement

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Table 3 Comparison of our data and literature for audiogram shapes

<table>
<thead>
<tr>
<th>Audiogram shapes</th>
<th>Our data</th>
<th>Liu et al., 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (ears)</td>
<td>n (probands)</td>
</tr>
<tr>
<td>Flat</td>
<td>75</td>
<td>48</td>
</tr>
<tr>
<td>Sloping</td>
<td>50</td>
<td>136</td>
</tr>
<tr>
<td>U-shaped</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Ascending</td>
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<tr>
<td>Total</td>
<td>126</td>
<td>194</td>
</tr>
</tbody>
</table>

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[8] H. Gabriel, P. Kupsch, J. Sudendey, E. Winterhager, K. Jahnke, J. Lautermann, Mutations in the Connexin 26/ GJB2 gene are the most common event in non-syndromic hearing loss among the German population, Hum. Mutat. 17 (2001) 521—522.