Mutation Analysis of Familial GJB2-Related Deafness in Iranian Azeri Turkish Patients

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Aims: Mutations in the GJB2 gene—encoding connexin 26 protein are the main cause for autosomal recessive nonsyndromic hearing loss worldwide. In this study, we assessed the contributions made by GJB2 and del(GJB6-D13S1830) mutations to the autosomal recessive nonsyndromic deafness genetic load in Iranian Azeri Turkish patients. Results: Probands from 209 different nuclear families were investigated. GJB2 mutations were found in 28% of the patients. Among these patients 44 families had 35delG mutation. The following GJB2 variants, R184P, DelE120, V27I+E114G, W24x, DelE119, R127H, 235DelC, 290-291 insA, Y155x, A171T, E147x, 35insG, G158S, R32H, R143Q, T123N, R143W, H16R, V153I, V27I, M163V, and F154F (a new variant), were identified in 126 of the 418 chromosomes. R143Q mutation was identified as compound heterozygous with 35delG in one profoundly deaf patient. Both parents of this patient were healthy, and one normal sister of this patient was also a carrier for the R143Q, indicating that this mutation has incomplete penetrance. Conclusions: Our results show that GJB2 mutations are responsible for about 28% of the autosomal recessive nonsyndromic hearing loss in this ethnic group. 35delG is the most prevalent GJB2 mutation accounting for 64.5% of the GJB2 mutations.

Introduction

Autosomal recessive nonsyndromic hearing loss (ARNSHL) is a genetically heterogeneous disorder. Despite the contribution of 23 different genes in causing ARNSHL, DFNB1 locus containing GJB2 and GJB6 genes accounts for about 50% cause of this type of hearing impairment (Petersen and Willems, 2006). It has been shown that mutations in the GJB2 gene, which encodes connexin 26 (CX26), are involved in the development of syndromic and nonsyndromic deafness in many populations (Ballana et al., 2009). CXs are important components of gap junctions expressed in the epithelial supporting cells of the inner ear and are necessary for ionic recycling in the endolymph (Angeli et al., 2000). More than 150 different polymorphisms, mutations, and unclassified variations in GJB2 and only two mutations in GJB6 have so far been reported for ARNSHL (Ballana et al., 2009). The most common GJB2 mutation is a frame shift mutation due to deletion of a single guanine at position 30–35 (35delG), which causes a premature stop codon at amino acid 13 (Denoyelle et al., 1997; Zelante et al., 1997). The 35delG mutation is less frequent or even absent in some ethnic groups. However GJB2 mutations are ethnic specific and other mutations such as 235delC (Abe et al., 2000; Kudo et al., 2000; Park et al., 2000; Yan et al., 2003; Dai et al., 2007), 167delT (Morell et al., 1998; Sobe et al., 1999), and R143W (Brobbey et al., 1998) are common in East Asian, Ashkenazi Jews, and African populations, respectively. 35delG mutation has been shown to have highest clinical impact in deafness in homozygous state (Snoeckx et al., 2005).

However, the low contribution of 35delG mutation to ARNSHL in one hand (Petersen and Willems, 2006) and the presence of 10–50% GJB2 heterozygotes within ARNSHL patients in other hand (Kenneson et al., 2002) necessitate the analysis of other mutations in the coding region of the GJB2 gene.

The objective of this study was to find the spectrum of GJB2 mutations in Iranian Azeri Turkish patients.

Subjects and Methods

Patients

Two hundred and seventy-one independent patients from the northwest of Iran (Azeri Turks) were referred to the Medical Genetic Center. Sporadic cases were excluded from
the study. A total of 209 unrelated families were included in the study based on the criteria reported previously (Esmaeili et al., 2007). Each family was informed about the study and consent was obtained from them. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols (Miller et al., 1988).

**Mutation analysis**

Multiplex amplification refractory mutation system (ARMS) polymerase chain reaction. Genetic testing for 35delG and del(GJB6-D13S1830) mutations was accomplished using two polymerase chain reactions for normal and mutant set according to the protocol described previously (Esmaeili et al., 2007).

Single-strand conformation polymorphism and sequencing. Subsequently, the negative 35delG samples and samples heterozygous for the 35delG allele were analyzed by the single-strand conformation polymorphism technique followed by sequencing for the coding region (exon 2) of the GJB2 gene (Kalay et al., 2005).

**Results**

The age range of the patients was 2–45 years (mean 23.5 years) and consanguinity was present in about 58% of the families. Forty-four (32 homozygous and 12 heterozygous) families out of the 209 families had the 35delG mutation. No further testing was performed on persons homozygous for the 35delG, and in this group the diagnosis of DFNB1 deafness was made.

None of the studied chromosomes showed a del(GJB6-D13S1830) mutation in CX30.

In 35delG heterozygotes (12/209) and in negative samples (177/209), single-strand conformation polymorphism analysis followed by sequencing of the coding region of GJB2 was carried out. Altogether, there were 23 different GJB2 variants from which 20 mutations (28%) and 3 polymorphisms (1.9%) were detected (Table 1). We found one unknown mutation, namely M163V (1/418) (Ballana et al., 2009). GJB2 mutations were found in both alleles in 106 of the 418 chromosomes (25.3%). However, 35delG was the most prevalent GJB2 mutation accounting for 76 of 118 (64.5%) of the GJB2 mutations chromosomes and 76 of 418 (18.2%) of all chromosomes studied. The second prevalent GJB2 mutation detected in this ethnic group was R184P with 5% of the GJB2 mutations. One novel variant, F154F (462 C>T, TTC=TTT), in the studied group was detected. This variant was found in one patient who had 35delG mutation in heterozygous state (Fig. 1).

The R143Q was identified in one patient. Further analysis of the family showed that only two sisters of this family had hearing impairment. Both of them had the same mutation, compound heterozygous with 35delG. Parents were healthy (with nonconsanguineous marriage) but they were not available for genetic analysis. One of the healthy members of this family was also a carrier for R143Q.

V27I+ E114G/wt genotype was found in three independent patients. Further genetic analysis of these families confirmed cosegregation of these two alleles.

**Discussion**

Deafness at the DFNB1 locus is the most common cause of ARNSHL among various populations. The high heterogene-

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*Table 1. GJB2 Genotypes in Iranian Azeri Turkish Patients with Autosomal Recessive Nonsyndromic Hearing Loss*

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>35delG/35delG</td>
<td>32</td>
</tr>
<tr>
<td>35delG/wt</td>
<td>5</td>
</tr>
<tr>
<td>V153I/wt</td>
<td>5</td>
</tr>
<tr>
<td>35delG/delE119</td>
<td>3</td>
</tr>
<tr>
<td>R184P/R184P</td>
<td>3</td>
</tr>
<tr>
<td>V27I+E114G/wt</td>
<td>3</td>
</tr>
<tr>
<td>delE120/delE120</td>
<td>2</td>
</tr>
<tr>
<td>235delC/235delC</td>
<td>1</td>
</tr>
<tr>
<td>Y155X/W24X</td>
<td>1</td>
</tr>
<tr>
<td>35delG/R143Q</td>
<td>1</td>
</tr>
<tr>
<td>35delG/R32H</td>
<td>1</td>
</tr>
<tr>
<td>35delG/Y155X</td>
<td>1</td>
</tr>
<tr>
<td>35delG/F154Fa</td>
<td>1</td>
</tr>
<tr>
<td>H16R/R143W</td>
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</tr>
<tr>
<td>W24X/W24X</td>
<td>1</td>
</tr>
<tr>
<td>V27I+/T123N</td>
<td>1</td>
</tr>
<tr>
<td>290-291insA/290-291insA</td>
<td>1</td>
</tr>
<tr>
<td>V27I+=E114G/G/W24X</td>
<td>1</td>
</tr>
<tr>
<td>G158S/wt</td>
<td>1</td>
</tr>
<tr>
<td>delE120/wt</td>
<td>1</td>
</tr>
<tr>
<td>R127H/wt</td>
<td>1</td>
</tr>
<tr>
<td>V27I+/wt</td>
<td>1</td>
</tr>
<tr>
<td>35insG/wt</td>
<td>1</td>
</tr>
<tr>
<td>R127H/wt</td>
<td>1</td>
</tr>
<tr>
<td>E147X/wt</td>
<td>1</td>
</tr>
<tr>
<td>235delC/wt</td>
<td>1</td>
</tr>
<tr>
<td>R127H/wt</td>
<td>1</td>
</tr>
<tr>
<td>M163Vp/wt</td>
<td>1</td>
</tr>
<tr>
<td>A171T/wt</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
</tr>
</tbody>
</table>

*aPolymorphism.  bUnknown mutation.

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**FIG. 1.** Sequence of novel variant. The arrow indicates the location of the base change.
FAMILIAL GJB2-RELATED DEAFNESS IN IRANIAN PATIENTS

ity of GJB2 mutation spectrum has been reported within different populations and ethnic groups (Morell et al., 1998; Park et al., 2000; Liu et al., 2002; Hwa et al., 2003; Ohtsuka et al., 2003; Najmabadi et al., 2005). One GJB2 mutation, the 35delG allele variant, is most common in populations of northern European ancestry (Kelley et al., 1998).

Iran consists of different ethnic groups and the 15–20 million Azeri Turks living in northwestern Iran, ethnically identical to Azeris and closely related to Turks, are believed to constitute 25% of the population. This study carried out on Iranian Azeri Turkish families with ARNSHL patients confirms that GJB2 mutations account for 28% of the mutations, which is only slightly lower than a rate of 31.7% that reported from neighboring ethnic group in Turkey (Uyguner et al., 2007). Of the GJB2 mutations, 35delG variant with the frequency of 64.5% is the most frequent mutation, which is lower than a rate of 76% reported from Turkey. This result is consistent with a Europe-to-Asia gradient (Lucotte and Mercier, 2001; Najmabadi et al., 2005) and is similar to the reported results of close ethnicity from Turkey (Tekin et al., 2003; Kalay et al., 2005), indicating presence of a common ancestor within the European population.

The second prevalent GJB2 mutation detected in our cohort was R184P with 5% of the GJB2 mutations. This mutation was found in three independent patients in homozygous state. The frequency of this mutation is reported to be low in Turkey.

The W24X has been reported to be one of the most frequent mutations in Turkey (Kalay et al., 2005). However, this mutation was the third prevalent GJB2 mutations detected in our ethnic group, accounting for 3% of the GJB2 mutations, which is less than that reported from Turkish population. This mutation was originally reported in two unrelated Pakistani families (Kelsell et al., 1997) and it has been suggested that this mutation may be originated from Asian population.

The R143Q mutation segregates with dominantly inherited hearing loss sloping from normal or mild to moderate or severe (Lofller et al., 2001). In our study group, this mutation was identified as compound heterozygous with 35delG in one profoundly deaf girl. Analysis of the family showed that both parents were healthy (with nonconsanguineous marriage) and there were two affected patients with the similar genotypes in this family. Further molecular analysis of this family showed that one normal sister of this patient was a carrier for the R143Q mutation indicating that this mutation has incomplete penetrance. Observing less penetrance of R143Q in this family could be due to some other possible modifier genes cosegregating with the mutation.

In this cohort, one novel variation (F154F (462 C>T, TTC/TTT) and one unknown mutation (M163V) were also identified. The frequency of each of these variations was 1/418 chromosomes. F154F and M163V variations are located in transmembrane domain 2 and extracellular domain 2 of the CX26 protein, respectively.

The M163V mutation was found in a 5-year-old female with the familial history of deafness and her parents were healthy (with consanguineous marriage). Genetic analysis of this family determined that her mother was also heterozygous for this mutation and therefore she was considered carrier for this mutation.

Our findings also confirmed that there were no del(GJB6-D13S1830) mutation among Iranian Azeri Turkish patients as previously reported (Esmaeili et al., 2007). Absence of this mutation in this ethnic group indicates that the del(GJB6-D13S1830) mutation is restricted to certain populations (del Castillo et al., 2003) and that there is a founder effect concerning this mutation.

The results of our study in the Iranian Azeri Turk ARNSHL patients show that GJB2 mutations are responsible for about 28% of the ARNSHL in this ethnic group. However, the cause of the remaining 72% has remained elusive. Further studies are necessary to see whether there are some genes that account for another important proportion of ARNSHL in the Azeri Turkish population.

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Disclosure Statement
No competing financial interests exist.

References


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