RESEARCH ARTICLE

GJB2 and mitochondrial A1555G gene mutations in nonsyndromic profound hearing loss and carrier frequencies in healthy individuals

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Abstract

This study aimed to assess mutations in *GJB2* gene (connexin 26), as well as A1555G mitochondrial mutation in both the patients with profound genetic nonsyndromic hearing loss and healthy controls. Ninety-five patients with profound hearing loss (>90 dB) and 67 healthy controls were included. All patients had genetic nonsyndromic hearing loss. Molecular analyses were performed for connexin 26 (35delG, M34T, L90P, R184P, delE120, 167delT, 235delC and IVS1+1 A → G) mutations, and for mitochondrial A1555G mutation. Twenty-two connexin 26 mutations were found in 14.7% of the patients, which were 35delG, R184P, del120E and IVS1+1 A → G. Mitochondrial A1555G mutation was not encountered. The most common *GJB2* gene mutation was 35delG, which was followed by del120E, IVS1+1 A → G and R184P, and 14.3% of the patients segregated with DFNB1. In consanguineous marriages, the most common mutation was 35delG. The carrier frequency for 35delG mutation was 1.4% in the controls. 35delG and del120E populations, seems the most common connexin 26 mutations that cause genetic nonsyndromic hearing loss in this country. Nonsyndromic hearing loss mostly shows DFNB1 form of segregation.

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Introduction

Profound hearing loss affects almost one in 1000 newborns, and more than 50% of these are caused by genetic factors (Lalwani and Castelein 1999). More than 120 independent genes that cause hearing loss have been identified.

GJB2 gene on chromosome 13q12-13 that codes for a gap junction protein called connexin 26 is responsible for the majority of genetic nonsyndromic hearing losses. This transmembrane protein forms connexons in the cochlea that functions in potassium recycling in the hair cells. Mutations in the *GJB2* gene that cause abnormal connexin synthesis result in impaired potassium cycle, and in turn hearing loss. There are numerous *GJB2* mutations, the frequencies of which vary among different populations. The most common mutations are 35delG, 167delT, and 235delC, which are frequent in the Caucasian, Jewish and Asian populations, respectively (Morell *et al*. 1998; Abe *et al*. 2000). The 35delG mutation constitutes almost 50% of all *GJB2* mutations in the Caucasians (Zelante *et al*. 1997; Estivill *et al*. 1998; Kelley *et al*. 1998; Scott *et al*. 1998; Topol *et al*. 1998).

Mitochondrial hearing loss constitutes less than 1% of all hereditary hearing losses (Bayazit and Yilmaz 2006). A mi-

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tochondrion has 16,569-bp genome containing 22 tRNA and 2 rRNA, which codes for 13 proteins. The mutations of 12S ribosomal RNA may result in hearing loss in the phenotype, and increases the susceptibility to aminoglycoside ototoxicity (Fischel-Ghodsian *et al*. 1997). In bacteria, 1555A is the binding site for aminoglycoside antibiotics, which may increase aminoglycoside ototoxicity in addition to hearing loss.

The objective of this study is to assess mutations in *GJB2* gene as well as A1555G mitochondrial mutation both in the patients with profound genetic nonsyndromic hearing loss and healthy controls.

Materials

Ninety-five patients (29 females; 66 males; aged from 2 months to 35 years, mean age 10.3 years), who had profound hearing loss (>90 dB), and 67 healthy controls (37 females; 30 males; aged from 18 to 48 years, mean age 30.9 years) were included in this study. This study was approved by the ethical committee of the medical faculty. There were sporadic and familial cases. One proband was considered to be representative for the family in familial cases.

All patients had genetic nonsyndromic hearing loss. Diagnostic work up included history including perinatal history, physical examination including otorhinolaryngologic examination, audiologic assessments (pure tone and speech audiometry, behavioural audiometry, evoked response auditometry and otoacoustic emission testing as indicated), and computed tomography of the temporal bone as indicated.

Methods

Molecular analyses

Genomic DNA was extracted from blood of the patients with an EZ-DNA Genomic DNA isolation reagent (Biological Industries, Israel), according to the procedures of the manufacturer. PCR amplification was performed for 680 bp region that codes for connexin 26 (for 35delG, M34T, L90P, R184P, 167delT and 235delC mutations), 380-bp region in connexin 26 promoter (for IVS1+1 A → G mutation), and 180-bp region that codes for mitochondrial DNA (for A1555G mutation). Nanogen® Genetic hearing loss amplification Kit was used according to the manufacturer's guidelines. The genotypes were obtained using Nanochip®, Molecular Biology Study Station, Data Analysis programme. The delE120 mutation was detected by amplification of a 308-bp DNA fragment with the previously used primers (Tekin *et al*. 2003a) and subsequent digestion with 2 units of BseRI (New England Biolabs, UK) restriction enzyme at 37°C overnight. The results were evaluated with two blind observers after electrophoresed in a 2% agarose gel. In the absence of mutation, the PCR product is digested by BseRI in fragments of 194 bp and 114 bp. When the delE120 mutation is present, the PCR product is not digested. The presence of the delE120 mutation was also confirmed by sequencing as described below.

Heterozygote mutation carriers were then screened by DNA sequencing using an ABI310 sequencer with BigDye Terminator v3.1 kit. For the amplification of the entire coding region of the *GJB2* gene previously described primer pairs were used. PCR products were sequenced on both strands.

Results

Genotypes

There were 63 (66.3%) sporadic and 32 (33.7%) familial cases with hearing loss. Twenty-two mutations were found in 14 out of 95 (14.7%) patients. These were 35delG, R184P, del120E and IVS1+1 A → G mutations of the *GJB2* gene while M34T, L90P, 167delT and 235delC were not encountered. Mitochondrial A1555G mutation was not encountered as well.

The most common *GJB2* gene mutation was 35delG, which was followed by del120E, IVS1+1 A → G and R184P. 35delG mutation was detected in 8 out of 95 (8.4%) patients, of whom three were heterozygous and five were homogenous for the mutations. R184P mutation was detected in 2 out of 95 (2.1%) patients, who were heterozygous for the mutation. del120E was found in 2 (2.1%) patients, who were homozygous for the mutation. IVS1+1 A → G mutation was detected in 2 (2.1%) patients (one compound heterozygous with 35delG and one homozygous) (table 1).

Allele frequencies

There were 190 alleles of 95 patients. Among these alleles, 35delG was the most frequent mutant allele for both familial

Table 1. Mutations detected in 148 patients.

Genotypes	Sporadic (n = 63)	Familial (n = 32)	Total (n = 95)
35delG/35delG	2 (3.2)	3 (9.4)	5 (5.3)
35delG/N	1 (1.6)	1 (3.1)	2 (2.1)
35delG/IVS1 + A → G	-	1 (3.1)	1 (1.1)
del120E/del120E	2 (3.2)	-	2 (2.1)
IVS1+ A → G/ IVS1+ A → G	1 (1.6)	-	1 (1.1)
R184P/N	2 (3.2)	-	2 (2.1)

Table 2. The frequency of mutant allelels among all the alleles.

	Frequency		
Mutant allele	Sporadic (n = 126 alleles)	Familial (n = 64 alleles)	Total (n = 190 alleles)
35delG	5 (3.9)	8 (12.5)	13 (6.8)
120delE	4 (3.2)	-	4 (2.1)
IVS1+1 A → G	2 (1.6)	1 (1.6)	3 (1.6)
R184P	2 (1.6)	-	2 (1)

and sporadic cases. R184P and del120E, were encountered only in sporadic cases. The frequency of IVS1+1 A → G mutation was equal between sporadic and familial cases (table 2).

There were totally 22 mutant alleles. 35delG was the most common mutant allele, and its frequency was higher in familial cases than sporadic cases. del120E was the second most common mutant allele, which was seen only in sporadic cases (table 3).

Consanguinity

There were 12 (12.6%) consanguineous marriages among 95 subjects. The most common mutation was 35delG, that constituted 80% of all mutant alleles. The IVS1+1 A → G was the second most common mutation, which constituted 20% of the mutant alleles in the presence of consanguinity.

Inheritance: Evaluation of the pedigrees of 32 familial cases revealed autosomal recessive, autosomal dominant and X-linked inheritance in 28 (87.5%), 3 (9.4%) and 1 (3.1%) of the patients, respectively. There was no pedigree suggesting mitochondrial inheritance. Of 28 patients with autosomal recessive inheritance, four had *GJB2* gene mutation, and these patients segregated with DFNB1 (14.3%).

Control group

35delG mutation was the only mutation detected in 2 of 67 individuals who were heterozygous for the mutation (mutation frequency of 1.4%). The other mutations were not encountered in the control group.

Discussion

It was reported that *GJB2* mutations are responsible for about 25% of autosomal recessive nonsyndromic hearing losses in Turkish population (Kalay *et al*. 2005). Another study found *GJB2* mutations in 21.4% of the families in this country (Bayazit *et al*. 2003). In this study, *GJB2* gene mutations were responsible for 14.7% of genetic nonsyndromic hearing losses and 12.5% of the familial cases. These results are lower than in the previous reports where the patient selection criteria may play a role. In addition, all of the patients included in our study had profound hearing loss rather than having a wide range of hearing spectrum from mild to profound.

The frequency of *GJB2* mutations varies among different populations. In Caucasians, 35delG is the most frequent mutation causing hearing loss (Carrasquillo *et al*. 1997; Brobby *et al*. 1998; Rabionet *et al*. 2000). 167delT, 235delC and R143W are the most common mutations in Askenazi Jewish, Asian and African populations, respectively (Denoyelle *et al*. 1997; Estivill *et al*. 1998; Lench *et al*. 1998; Fuse *et al*. 1999; Hammelmann *et al*. 2001). In our study, the most common *GJB2* gene mutation was 35delG, which is similar to Caucasians, whereas 167delT and 235delC were not encountered at all. The frequency of 35delG mutation ranges from 28% to 63% in otosomal recessive inheritance, and 10% to 33% in sporadic cases (Jaber *et al*. 1992; Abe *et al*. 2001; Gabriel *et al*. 2001; Uyguner *et al*. 2003; Tekin *et al*. 2005). In a previous study from Turkey, 35delG was found to be the most common mutant allele, and reported to be 73.6% of all *GJB2* mutations (Tekin *et al*. 2001). In our study, 35delG mutation constituted 59.1% of all mutant alleles, and it was significantly more common in familial cases (88.9%) than in sporadic cases (38.5%).

It was reported that the second most common mutation in Turkey is delE120 (Tekin *et al*. 2005). Similarly, the second most common mutation was delE120 in our study as well. There has been only one reported homozygote individual with delE120 from countries other than Turkey (Gabriel *et al*. 2001). Our study includes the largest size of delE120 homozygous as there were two patients homozygous for this mutation. This mutation was more common in sporadic cases

Table 3. The frequency of mutations among mutant alleles.

	Frequency		
Mutant allele	Sporadic (n = 13 alleles)	Familial (n = 9 alleles)	Total (n = 22 alleles)
35delG	5 (38.5)	8 (88.9)	13 (59.1)
120delE	4 (30.7)	-	4 (18.2)
IVS1+1 A → G	2 (15.4)	1 (11.1)	3 (13.6)
R184P	2 (15.4)	-	2 (9.1)

than familial cases. IVS1+1 A → G mutation has not been reported in Turkish population to date, and this is the first report of such incidence in this country. It was reported that compound heterozygosity of IVS1+1 A → G and 35delG mutations leads to a milder hearing loss compared to 35delG homozygous (Jaber *et al*. 1992). However, the patient who was compound heterozygous in our study had profound hearing loss.

There was consanguineous marriage in 12.6% of the families. 35delG and IVS1+1 A → G were the most common mutant alleles (80% and 20%, respectively) in the presence of consanguinity. In familial cases, the most common pattern of inheritance was autosomal recessive (87.5%), which was followed by autosomal dominant (9.4%) and X-linked inheritance (3.1%). There was DFNB1 form of segregation in 14.3%. In our previous study, a diagnosis of DFNB1 deafness was given in 14.3% as well (Bayazit *et al*. 2003).

A1555G mutation is the most common mitochondrial mutation causing hearing loss (Abe *et al*. 2001). In Asian countries, the mutation frequency ranges between 7.7% and 10% (Pandya *et al*. 1999; Usami *et al*. 2000). In Turkey, the frequency of this mutation was reported to be 2% among 168 patients screened (Tekin *et al*. 2003b). However, none of the patients and the controls had this mutation in our study.

In a multicenter study in Europe, the carrier frequency for 35delG was found to be 1/35 for South Europe, 1/79 for middle and North Europe (Gasparini *et al*. 2000). In our study, 35delG was the only mutation found in the controls, who were carriers for the mutation with a frequency of 1/34 or 1.4%, which is comparable to the frequencies in Europe. In a previous study in this country, a carrier frequency of 1.78% was found for 35delG mutation (Tekin *et al*. 2001).

In conclusion, 35delG and 120delE seems the most common connexin 26 mutations that cause genetic nonsyndromic hearing loss in this country. Nonsyndromic hearing loss mostly shows DFNB1 form of segregation.

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