



Original Article

The Prevalence of Gap Junction Protein Beta 2 (GJB2) Mutations in Non Syndromic Sensorineural Hearing Loss in Çukurova Region

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OBJECTIVE: To date, studies in all populations showed that mutations in the gene of Gap junction protein beta 2 (GJB2) play an important role in non-syndromic autosomal recessive congenital hearing loss. The aim of this study was to evaluate GJB2 gene of patients with hearing loss in our region using deoxyribonucleic acid (DNA) sequencing method and to demonstrate region-specific mutation and polymorphism distribution.

MATERIALS and METHODS: Patients who had bilateral severe sensorineural non-syndromic hearing loss identified by audiologic evaluation were included. Peripheral blood samples were collected and the GJB2 gene exon1 and exon 2 regions were amplified by polymerase chain reaction (PCR). Obtained PCR products were sequenced by the DNA sequence analysis method (SeqFinder Sequencing System; ABI 3130; Foster City, CA, USA) and analyzed using the SeqScape software.

RESULTS: Of the 77 patients, 16 had homozygous or heterozygous mutation.

CONCLUSION: The mutation of 35delG, which is known as the most frequent mutation of GJB2 gene, was also the most frequently seen mutation at a ratio of 5.5% in patients with hearing loss in our region; this was followed by the V27I mutation. As this is the first study conducted by sequence analysis in our region, it was worth to be presented in terms of showing the distribution of mutation.

KEYWORDS: Non-syndromic, autosomal recessive, deafness, 35delG, V27I

INTRODUCTION

Hearing is the most important mode of communication and is crucial for leading an independent life in social environment. Hearing loss, whether congenital or acquired during childhood, affects the education of individuals and leads to challenges when communicating with other individuals in the social environment. Hearing loss is seen in every 1000 live births because of genetic conditions (50%-60%), infection, trauma, and premature birth^[1,2]. However, these rates in our country differ, and they are 76.8% for genetic reasons and 23.2% for environmental reasons^[3].

Hereditary hearing loss may be non-syndromic (60%-70%) or syndromic (30%-40%). Of the non-syndromic hearing losses, 75%-80% are autosomal recessive, 15%-20% are autosomal dominant, 5% are X-dependent, and 1% are inherited by mitochondrial genes^[2,4-8]. Because kin marriage is common in our country and because families live in isolation in certain regions, the frequency of rare genes responsible for autosomal recessive hearing loss is enhanced. Therefore, these rates, in our country, are 93.4% for autosomal recessive heritage and 6.6% for autosomal dominant heritage^[3].

At present, at least 39 genes were described to be responsible for non-syndromic autosomal recessive hearing losses. Among the autosomal recessive genes, Gap junction protein beta 2 (GJB2) gene is the most important that is located in 13q.12 and encodes Connexin 26, a protein with 226 amino acids that is the structural component of intercellular gap junctions^[9-11].

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In many communities, most of the genetic hearing losses are explained by GJB2 gene mutations located in Deafness B (DFNB) locus^[12]. The mutations of this gene comprise 50% of genetic hearing losses^[13]. Similarly, GJB2 mutations are the most frequent cause of hearing loss at the rate of 18.9% in our country^[3]. Although more than 100 mutations have been described for this gene, the c.35delG mutation comprises 60%-70% of all mutations in Caucasians. The rate of carriers for this mutation in healthy Turkish population is 1.8%, whereas it is 2%-4% in European population^[14-15].

MATERIALS and METHODS

Patients

This study included 77 patients (males/females; 45/32) who were admitted to the ENT outpatient clinic of the Adana Numune Training and Research Hospital with the complaint of hearing loss. Audiologic evaluation revealed non-syndromic bilateral severe sensorineural hearing loss (over 95 dB) in all patients. The mean age was 12.7 (range, 1-16) years. All patients were assessed by a clinical geneticist to eliminate syndromic and environmental causes of hearing loss. Patients with conductive-type hearing loss, history of prenatal TORCH infection, prematurity, low birth weight, hyperbilirubinemia, meningitis, mumps, and other infections were excluded. Syndromic patients were also excluded. Written informed consents from the parents of all patients were obtained following audiologic and clinical evaluations. The approval of the Local Ethics Committee was also obtained.

Molecular Analysis

Peripheral blood samples (2 cc) of patients were collected into EDTA tubes. Deoxyribonucleic acid (DNA) isolation from collected blood samples was performed manually by a commercial kit (Invitrogen CA, USA). Exon 2, the only encoding exon of GJB2 gene, and non-coding exon 1 regions were amplified by using DNA samples according to THE GML® kit protocol (GML AG, Wollerau, Switzerland). Amplified polymerase chain reaction (PCR) products sequenced by DNA sequence analysis in a genetic analyzer device (SeqFinder Sequencing System; ABI 3130; Foster City, CA, USA). Data were analyzed using the SeqScape software in accordance with the literature^[1, 16].

RESULTS

The overall rate of hearing loss-related mutation was 20.78%. Presence of mutation related with hearing loss was determined in 12 of 45 male patients (26.6%) and 4 of 32 female patients (12.5%). Pedigree analysis showed that, of the 77 subjects, 55 probands (71%) were simplex cases (single affected individual) and 22 (29%) were multiplex cases (more than one affected family member).

Sixty-seven patients had rs3751385:C>T polymorphism (87%). Of the patients who were admitted with hearing loss, 16 (20.8%) showed eight mutations that contained 9 genotypes. Of the 16 patients who had a mutation, seven (43.7%) were multiplex, whereas nine (56.3%) were simplex cases. Seven of the 22 multiplex patients (31.9%) had at least one mutant allele; on the other hand, the rate of mutation was 16.4% in nine of the 55 simplex cases.

The most frequently observed mutation in our region was 35delG (30.8%, eight of 26 mutant alleles) followed by V27I mutation (23.1%, six of 26 mutant alleles). E114G exchange was observed in three al-

Table 1. Allele distribution and mutations found in patients

Allele	Number of alleles	Percentage of mutations (%)	Percentage of patients (%)	Mutation type
35delG	8	30.8	5.5	Deletion-frameshift
V27I	6	23.1	4.2	Missense
E114G	3	11.5	2.1	Missense
IVS1-1G>A	2	7.7	1.4	Splice site
Val153Ile GTC>ATC	2	7.7	1.4	Missense
delE120	2	7.7	1.4	Deletion
R184P	2	7.7	1.4	Missense
T123N	1	3.9	0.7	Missense

Table 2. Genotype distribution of mutations

Genotype	Patient	Percentage of patients (%)
V27I / E114G	3	3.9
35delG / 35delG	3	3.9
V27I / Wt*	2	2.6
V153I / Wt*	2	2.6
35delG / Wt*	2	2.6
V27I / T123N	1	1.3
delE120 / delE120	1	1.3
R184P / R184P	1	1.3
IVS1+1G>A / IVS1+1G>A	1	1.3

*Wt: Wild type

leles, and its prevalence was 11.5% among the mutations. IVS1-1, Val153Ile, delE120, and R184P mutations (7.7%) were found in two alleles, and T123N mutation (3.9%) was found in one allele (Table 1). In three patients, 35delG mutation was homozygous, whereas V27I and E114G were both heterozygous. The mutations that were found were summarized in Table 1 as allele distribution and in Table 2 as genotype distribution.

DISCUSSION

GJB2 gene mutations comprise 50% of all non-syndromic hereditary hearing losses. Mutations of this gene mostly (40%-90%) result from 35delG that leads to a frameshift mutation^[15, 17-19]. In 2003, Tekin et al.^[20] reported that the frequency of 35delG mutation varies between 5% and 50% across regions in our country. In our study, 16 (20.8%) patients showed genetic variations related with hearing loss. Five patients had 35delG mutations, of whom three were homozygous, and two were heterozygous. This was 5.5% of patients who were admitted with hearing loss in terms of allele frequency, and 30.8% of patients who had GJB2 gene mutation.

In this study, V27I mutation was found in six patients; this mutation is caused by the exchange of amino acid 27 from valine to isoleucine. Of the six patients with this mutation, three were also carriers for E114G change at the same time. There are studies that also showed that these two mutations were present on the same allele as "cis"

form and have a tendency to be inherited together ^[21, 22]. Although the E114G change that was found in three patients have previously been described as a polymorphism in the literature, some other studies described it as a mutation because of amino acid exchange ^[23]. In general, V27I mutation that was found together with E114G exchange has been discussed as a polymorphism because it is also found in the control groups of some studies, despite having found to be effective in in vitro studies ^[22, 24].

In our study, one patient had heterozygous Thr123Asn together with V27I and rs3751385 exchanges. It has been reported that Thr123Asn mutation may lead to a missense change and was associated with hearing loss ^[24].

V153I exchange in GJB2 gene was described as polymorphism, and in general, it was seen together with 35delG ^[25]. In our study, we found a patient having V153I exchange; however, besides this polymorphism, there was no other mutation, such as 35delG, which may explain hearing loss in this patient.

In one patient, we found homozygous delE120 mutation that leads to the deletion of codon 120 and glutamate loss. It was first described in 1999, and in a study, its frequency was reported to be approximately 1% ^[26]. In two studies from our country, it was reported that delE120 mutation is the second most common mutation following 35delG, and that its allele frequency was approximately 2% ^[27, 28]. In our study group, frequency of this mutation was 1.4%.

Various references reported that IVS1+1G>A mutation, also known as -3170 G>A splice-site mutation, leads to hearing loss ^[29-30]. The frequency of this mutation varies between 1.85% and 9.4%, and it may be accompanied with other mutations. However, in our study, only one patient had homozygous mutation, and the allele frequency was 1.4%.

R184P is a rare mutation that leads to the exchange of amino acid 184 from arginine to proline. Although this mutation is usually found together as heterozygous with another mutation, in this study, one patient had homozygous mutation ^[30, 31]. The functional studies revealed that R184P mutation causes complete absence of hemichannel formation. This mutation takes place in the second loop of the Connexin 26 protein and shows only monomeric Connexin 26 protein ^[32].

Most of the patients had homozygous or heterozygous rs3751385:C>T exchange. This exchange did not lead to an alteration in the structure of the protein and is classified as a polymorphism ^[33]. This polymorphism may pose a tendency to cause other disorders, but it is not associated with hearing loss ^[34].

In GJB2 gene mutation, genotype-phenotype relationship may be different. In our study, we only evaluated non-syndromic bilateral severe sensorineural hearing loss, and we did not perform a detailed clinical evaluation of patients and phenotype reflection of this mutation.

In conclusion, the mutation of 35delG, which is known as the most frequently seen mutation of GJB2 gene, was the most frequently seen mutation at a 5.5% ratio in patients with hearing loss in our region that was followed by V27I mutation. Although DNA sequencing

analysis is an effective mutation screening method, microarray and next generation sequencing technologies enable mutation screening in more genes. Description of the new genes that are involved in hearing loss may enable us to perform more sophisticated genetic tests and increase the sensitivity, eventually aiding in the early diagnosis of this condition.

Ethics Committee Approval: Ethics committee approval was received for this study from the local institutional review board (2013/29).

Informed Consent: Written informed consent was obtained from the parents of all participants who participated in this study.

Peer-review: Externally peer-reviewed.

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