

Analysis of GJB2 (Connexin 26) Mutation in Patients with Congenital Non-Syndromic Sensorineural Hearing Loss

Original Investigation

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Abstract

Objective: This study was performed to investigate the GJB2 (connexin 26) gene mutations that are the most frequent cause of sensorineural deafness in patients with congenital non-syndromic sensorineural hearing loss in our region.

Methods: Sixty patients [35 males (58.3%) and 25 females (41.7%)] between the age of 2-43 years (12.11±9.03) diagnosed with congenital non-syndromic sensorineural hearing loss were included in the study. The control group consisted of 60 individuals with similar demographic features having no hearing problems. 35delG, 167delT, delE120 and 235delC of GJB2 gene mutations and GJB6 gene mutations, and the presence of new mutations were also investigated by analysis of DNA sequences in all individuals.

Results: Mutations were identified in 6 (10%) of the 60 patients in the study group. Five of these (8.3% of total)

Introduction

Hearing loss is a disorder that affects not only social, educational, and intelligence development but also speech, expressing, comprehension, and psycho-social growth of the individual in a negative way. The incidence rate of cases with congenital hearing loss is 0.4-1.1 per 1000 births in the United States of America and 2 per 1000 births in our country (1, 2).

Congenital hearing losses are thought to occur due to genetic factors in about one-half of cases and environmental factors in the other half of cases (3). The cases definitely with a genetic origin are put into two groups as syndromic and non-syndromic sensorineural hearing losses (NSSNHL). About 80% of non-syndromic sensorineural hearing losses are autosomal recessive, 15%-20% is autosomal dominant, and 1%-2% is X-linked inherited. In addition, it has been reported that NSSNHL differs depending on ethnicity, and 1%-5% of cases are mitochondrially inherited (4).

The products of the genes that are effective in hearing mechanism include connexin proteins, which have a role in ionic hemostasis, transcrip-

tion factors, extracellular matrix proteins, cytoskeleton proteins, and many proteins with incomplete function (5).

Among connexin genes, the mutations of especially connexin 26 (GJB2) and less frequently the connexin 30 (GJB6) and 31 (GJB3) genes are important for NSSNHL (4).

Conclusion: This is the first study which investigated the GJB2 gene mutation in our region, and our results indicate that 35delG mutation was the most frequent. We believe that our results are noteworthy for the identification of heterozygous or homozygous individuals and the genetic counseling of patients with congenital non-syndromic sensorineural hearing loss and their family.

Key Words: Congenital hearing loss, mutation, GJB2, 35delG, 167delT

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The GJB2 gene encodes connexin 26 protein (Cx26), which functions in the formation of gap-junction channels, providing intercellular diffusion of small molecules and ions (4, 6). Cx26 protein plays a highly important role in the movements of potassium ions (K⁺), which are functional in hearing mechanism, throughout hair cells and endolymph fluid (4).

About 50% of autosomal recessive NSSNHL results from the mutations in the GJB2 gene. More than 90 mutations have been defined in the GJB2 gene so far (7). It has been determined that of the defined mutations, the 35delG mutation occurs due to the deletion of one of 6 successive guanine bases, and it is responsible for about half of the Mediterranean, North American, and North and

South European cases with autosomal recessive inherited hearing loss (4,8). The frequent incidence of the 35delG mutation requires analyzing both affected individuals and parents (in terms of being carriers) for genetic counseling.

The aim of this study was to determine the frequency of GJB2 gene mutations in patients with congenital NSSNHL and to investigate new mutations of the GJB2 gene in our region.

Methods

Study and Control Groups

After receiving approval from the Clinical Investigation Ethics Committee, the study was conducted with 60 patients admitted to our clinic and diagnosed with congenital and bilateral sensorineural hearing loss (SNHL) and also 60 cases admitted to our outpatient clinic with another disorder other than ear diseases and found to have normal hearing in the audiological examination between the dates of December 2009 and December 2010.

All patients who participated in the study were given information about the study verbally, and their written informed consents were obtained. For patients younger than 18 years, written informed consents were obtained from their parents.

Medical histories of all patients involved in the study were provided in detail, and they underwent a physical examination. Pediatric patients were referred to the pediatrics clinic for being evaluated in terms of syndromic diseases, and additional tests (ophthalmologic examination, thyroid function tests, renal ultrasonography, and electrocardiography) were performed when necessary. Patients with syndromic hearing impairment, with suspicion or evidence of environmental factor-induced hearing impairment, chronic otitis, or similar disease-induced hearing loss were excluded from the study. Also, patients identified to be in this or similar conditions were excluded.

Evaluation of Hearing

Hearing levels of the patients who participated in the study were determined using one or more of the tests, including pure tone audiogram, otoacoustic emission, and brainstem auditory-evoked response (BAER) tests. Patients with moderate and severe bilateral SNHL were involved in the study according to Goodman's classification (8).

Identification of GJB2 and GJB6 Mutations

Peripheral venous blood (3 mL) was taken from each patient in the study and control groups. Blood samples were stored at +4°C until DNA isolation was performed. Then, the samples were exposed to gene mutation analysis in the Department of Medical Biology and Genetics.

DNA isolation was performed by using the Wizard Genomic DNA Isolation Kit (Promega Corp, Madison, USA) on the pe-

ripheral blood samples taken from the patients, in accordance with the manufacturer's instructions. Restriction fragment length polymorphism (RFLP) was used to determine GJB2 mutations, and the allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) method was used to determine GJB6 mutations. The products obtained after polymerase chain reaction (PCR) were kept in a water bath with mutation-specific restriction enzymes for 16 hours. PCR products generated after the cutoff were conducted in a 3.5% gel. Mutations were detected according to the sizes of the bands after being examined under ultraviolet light using a gel imaging system (9-13).

GJB2 sequence analysis was performed using an ABI 310 DNA sequencer (Refgen, Ankara, Turkey). The results were compared to the Fasta genome database, and then, mutations were detected.

Statistical Analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) for Windows (version 15.0) (SPSS Inc. Chicago IL, USA). Pearson's correlation analysis was used to find correlations between the parameters. Genotype and allele frequency distributions of the cases were determined via chi-square analysis. Mann-Whitney U-test was used to assess the differences between the groups. The value of $p < 0.05$ was considered statistically significant during evaluation.

Results

A total of 60 patients [35 males (58.3%) and 25 females (41.7%)] who were in the age range of 2 to 43 years (12.11 ± 9.03) and diagnosed with congenital non-syndromic sensorineural hearing loss were involved in the study. The control group included 60 patients [35 males (58.3%) and 25 females (41.7%)] who were in the age range of 8 to 50 years (20.38 ± 9.64) and had normal hearing level (Table 1). It was revealed that hearing loss level was moderate to severe in 3.3%, severe in 40%, and too severe in 56.7% of the patients in the study group, while hearing levels were found to be normal for all patients in the control group (Table 1). A statistically significant difference was found between the study and control groups in terms of hearing loss ($p = 0.001$).

The parents of 11 patients (18.3%) in the study group had a history of SNHL, but none of the parents did in the control group. The difference between the two groups was statistically significant ($p = 0.001$).

Gene mutation was detected in six of the patients (10%) in the study group. Five of them had 35delG (8.3%) and one (1.7%) had delE120 gene mutations. In the control group, no gene mutation was observed in any patient (Table 1). Although five patients in the study group had the 35delG gene mutation, compared to the control group, no statistically significant difference was found between these groups regarding this point ($p = 0.057$). An agarose gel electrophoresis image of PCR products for 35delG mutations is demonstrated in Figure 1.

The DelE120 mutation (homozygote) was detected in one of the patients (1.7%) in the study group, but it was not found in any of the patients in the control group. In terms of delE120 mutation frequency, there was no statistically significant difference between the groups (p=1.000). An agarose gel electrophoresis image of PCR products for the delE120 mutation of the GJB2 gene is shown in Figure 2. The DNA sequence of a patient for whom no mutation was observed in the DNA sequence for the delE120 mutation of GJB2 is demonstrated in Figure 3. Moreover, the DNA sequence of a patient with a delE120 homozygote mutation and the location of the mutation are shown in Figure 4. All patients in both the study and control groups were evaluated for 235delC, 167delT, and GJB6 mutations, but these mutations were not seen in any case.

In the study group, the relationship between the familial history of SNHL and 35delG mutation was evaluated, and it was found that the 35delG mutation was detected in 27.3% of patients with a familial history of SNHL, while this rate was 4.1% for patients without a familial history of SNHL. When the relationship between the familial history of SNHL and delE120 mutation was examined in the study group, it was seen that 9.1% of the patients with a familial history of SNHL had the delE120 mutation.

There was no statistically significant relationship between the presence of the 35delG and delE120 mutations and hearing findings and genders for patients in the study group (p>0.05). In the parents of patients in the study group, a weak positive relationship was found between the presence of SNHL familial history and the presence of the 35delG mutation (r=0.325;

p=0.011). Similarly, there was a weak positive relationship between the presence of SNHL history in the family and the presence of the delE120 mutation (r=0.275; p=0.034).

Discussion

Of the genetic factors-induced hearing losses, 70% are nonsyndromic and 30% are syndromic hearing losses (14). The clinical course of autosomal recessive NSSNHL is usually with prelingual onset, non-progressive, and severe hearing losses. Autosomal dominant NSSNHL is usually seen with post-lingual onset, progressive, and moderate-to-severe hearing losses (4). Nearly 50% of nonsyndromic sensorineural hearing losses comprise

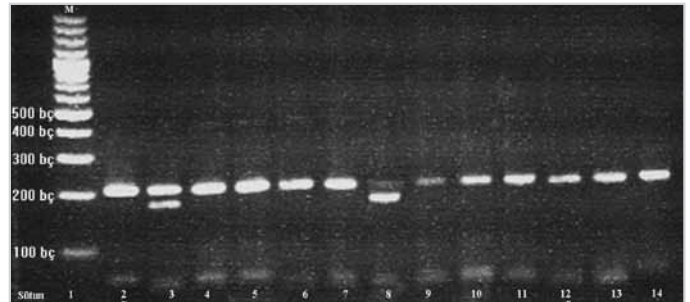


Figure 1. Agarose gel electrophoresis image of PCR products for 35delG mutation (DNA size marker of 100 bc in column 1, wild-type allele in column 2 (234 bc), heterozygote mutation (234 bc+211 bc+23 bc) in column 3, and homozygote mutation (211bc+23bc) in column 8 are seen)

PCR: polymerase chain reaction

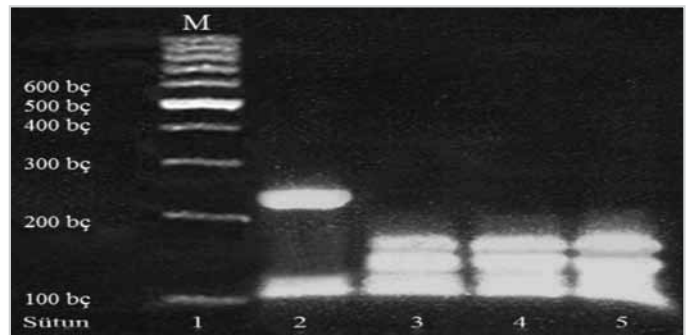


Figure 2. Agarose gel electrophoresis image of PCR products for delE120 mutation (DNA size marker of 100 bc in column 1, 120delE homozygote mutation (243 bc) in column 2, and wild-type allele (127 bc+116 bc) in other columns (3-6) are seen.)

PCR: polymerase chain reaction

Table 1. Demographic features of the patients and results

Features	Study group	Control group
Number of patients (n)	60	60
Gender		
Female	25 (41.7%)	25 (41.7%)
Male	35 (58.3%)	35 (58.3%)
The Mean Age (Year±SD. age interval)	12.11±9.03 (2-43)	20.38±9.64 (8-50)
Level of hearing loss		
Normal hearing (0-25 dB)	-	60 (100%)
Moderate to severe (56-70 dB)	2 (3.3%)	-
Severe (71-90 dB)	24 (40.0%)	-
Too severe (≥91 dB)	34 (56.7%)	-
GJB2 mutations		
35delG	5 (8.3%)	-
delE120	1 (1.7%)	-
235delC	-	-
167delT	-	-
GJB6	-	-

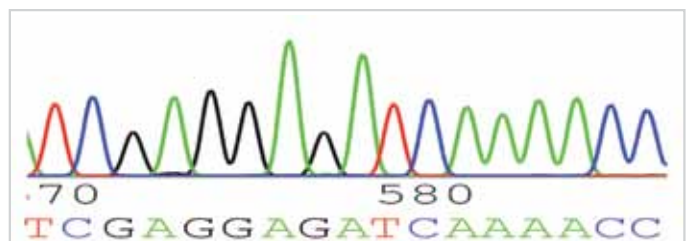
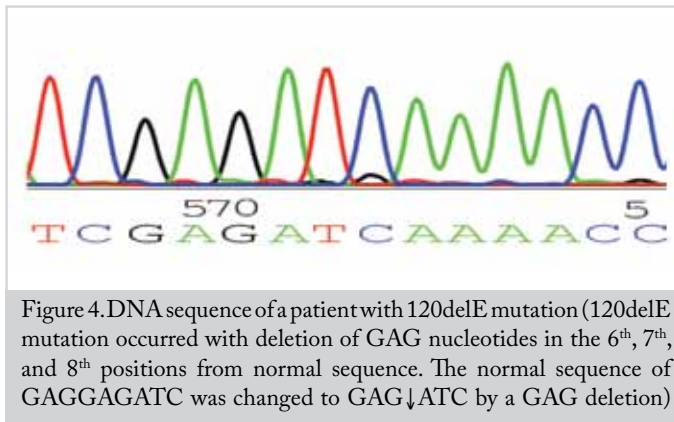


Figure 3. DNA sequence for 120delE mutation belonging to a wild-type sample



GJB2 mutations. It was reported that mutations in the GJB2 gene encoding the expression of connexin 26 protein disrupted the recycling of K⁺ ions back to the endolymph and led to permanent damage in the organ of Corti (15).

GJB2 mutations vary, apparently depending on the ethnicity of the community. 35delG is the most common mutation of this gene. Nearly 70% of the pathological GJB2 mutations seen in Europe, North America, and Mediterranean societies comprise 35delG (16). It has been reported that the incidence frequency of the 35delG mutant allele is in the range of 5% to 53% in different cities of our country (17, 18). In studies conducted, GJB2 mutations, other than 35delG, were found at high frequency for different ethnic groups. For example, the 167delT mutation in Ashkenazi Jews, 235delC mutation in Far East populations, especially in Japanese society, and the R143W mutation in African populations are some of them (19–21). In our study, we investigated the 35delG, 167delT, delE120, and 235delC mutations of the GJB2 gene and also the mutations of the GJB6 gene.

In various studies performed in different countries around the world, the rates of 35delG homozygous mutation differ. Even big differences may be seen in studies conducted with different populations living in the same country. In a multicenter study performed by Minrik et al. (22), the rate of homozygous 35delG mutation was found to be 1.9% in the Romani population of Eastern Slovakia and 40% in Slovakia. On the other hand, some studies reported no homozygous 35delG mutation (4, 23). Tekin et al. (18) evaluated patients with prelingual-onset NSSNHL, who were educated in schools for hearing-impaired students in Ankara, Afyon, Amasya, and Denizli, and they revealed that 15% of them had a homozygous 35delG mutation and 7.81% had a heterozygous 35delG mutation. Kalay et al. (24) reported 21.5% homozygous and 4.3% heterozygous 35delG mutations in their study. In our study, the rate of 35delG mutation was 8.3%. Of these patients with a 35delG mutation, 3 (4.98%) had a homozygous mutation and 2 (3.32%) had a heterozygous mutation. Compared to other studies in our country, the mutation rates of 35delG were found to be lower. As in the literature, the frequency of 35delG mutation may differ depending on the region and society. When the 35delG mutation of the GJB2 gene is mutant in both alleles, it causes SNHL. In NSSNHL

patients with familial autosomal recessive inheritance, the rate of 35delG homozygosity was reported as 17.5%–21.7%, and the rate of heterozygous mutation was reported as 1.9%–4.3% (18, 25, 26). Regarding the familial history of patients in the study group of our study, congenital NSSNHL was determined at least in one person in the families of 18.3% of patients. In terms of the relationship between the familial history of SNHL and 35delG mutation, the 35delG mutation was detected in 27.3% of patients with a familial history of SNHL (18.1% homozygote, 9.2% heterozygote), while this rate was 4.1% for patients without a familial history of SNHL. This difference was found to be statistically significant. The rate of 35delG homozygous mutation was 18.1% for patients with familial autosomal recessive NSSNHL, which was a higher value than the general mutation rate (8.3%) of the study group. This suggests that familial medical history is important for patients with NSSNHL, which is consistent with the literature. The carrier frequency of 35delG mutation was identified to be between 0% and 4% in different regions of the world (7). In our country, this rate was reported to be in the range of 0.8% to 2.7% by various studies (7, 25, 26). The mutant allele was not observed in any of the 60 patients who participated in our study. This might have resulted from the differences among the regions or the low number of patients evaluated in the control group.

The carrier frequency of the 167delT mutation, which is the most common mutation in Ashkenazi Jews (84%) and considered to have generated from only one origin, was reported as 4.03% (19, 27). In a study performed in our country, it was notified that the 167delT mutation was seen in only one allele (0.3%) (17). This mutation, which is seen rarely except in Ashkenazi Jews, was not found in our study, too. The delE120 mutation, which results from the deletion of a glutamine amino acid at the 120th position of the connexin 26 gene, is another mutation investigated in our study. The frequency rate of this mutation was reported to be in the range of 1.07% to 1.66% in our country (18, 24, 25). In our study, one patient in the study group (1.66%) had a homozygous delE120 mutation, but none of the patients in the control group had this mutation. Our findings are also consistent with the literature. For the patient with the delE120 mutation in the study group, the familial history was also found to be positive. When the relationship between the familial history of SNHL and delE120 mutation was examined in the study group, it was seen that 1 of 11 patients (9.1%) with a familial history of SNHL had the delE120 mutation, while the delE120 mutation was not detected in patients without a familial history of SNHL ($p=0.034$).

In Japan, a high rate of 235delC mutation was observed in patients with congenital NSSNHL (44.8%). 235delC is formed as a frame-shift mutation due to the deletion of a cytosine amino acid at the 235th position (20). As in our study, the 235delC mutation was not seen in other studies conducted in our country (24–26). In these studies, del (GJB6-D13S1830) mutations were not been reported for patients with congenital NSSNHL

(24, 25). Our study did not detect the GJB6 mutation, which is consistent with other studies in our country.

This is the first study that has investigated GJB2 gene mutations in our region. Our results were generally found to be below the average of Turkey. This may be due to the fact that the rates of mutation differ depending on the region. Limitations of the study are the low number of patients and the absence of previous data that can be used to compare the rates of mutations in the region. Therefore, this study should be supported with a series including a larger sampling.

Conclusion

Increased social awareness on genetic diseases can also increase the participation rate of patients with hearing loss and their parents to tests conducted for detecting the genetic causes of the disease. Patient-based genetic counseling is highly important for determining the etiology of hearing loss and identifying heterozygote and homozygote individuals through genetic tests. With identification of these individuals, genetic counseling services about possible pregnancy risks, what to do in the pre- and post-pregnancy periods, the course of the disease, treatment methods, and their results can be provided for carriers of the GJB2 mutation.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Elazığ Clinical Investigation of General Directorate for Pharmaceuticals and Pharmacy in 2009.

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

Peer-review: Externally peer-reviewed.

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