# INVESTIGATION OF THE 35delG MUTATION IN THE GJB2 (CONNEXIN 26) GENE-RELATED FAMILY IN MANISA AND VICINITY IN TURKEY
# Türkiye'de Manisa ve Yöresinde Akraba Evliliği Yapmış Ailelerde GJB2 (Connexin 26) Genindeki 35delG Mutasyonunun Araştırılması

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***Summary :*** *Mutations in connexin 26 gene (GJB2) lead to a significant proportion of non-syndromic autosomal recessive congenital hearing loss in all populations studied so far. Eight patients with non-syndromic hearing loss out of 14 related family (marriage couple) were screened to determine hearing loss attributed to connexin 26 gene and the types of mutations living in Manisa and vicinity, Turkey. The 35delG mutations were detected by Polymerase Chain Reaction (PCR) with Competitive Amplification Refractory Mutation System (C-ARMS). Seven patients were detected as homozygous for the 35delG mutation and one patient detected as heterozygous. Our results indicate that 35delG mutation in the connexin 26 gene was the cause of non-syndromic congenital hearing loss of the patients in Manisa and vicinity in Turkey.*

***Keywords:*** *35delG, connexin 26, GJB2 , autosomal recessive non-syndromic congenital hearing loss*

***Özet:*** *Günümüze kadar tüm popülasyonlarda yapılan çalışmalarda Connexin 26 (GJB2 ) genindeki mutasyonlar non sendromik otozomal resesif konjenital işitme kaybında önemli bir yere sahiptir. Connexin 26 geni ve mutasyon tipleriyle ilişkilendirilen işitme kaybını belirlemek için Türkiye'de Manisa ve yöresinde yaşayan 8 non-sendromik işitme kayıplı birey, akraba evliliği yapmış 14 aile (evli çiftler) çalışmaya alınmıştır. 35delG mutasyonları polimeraz zincir reaksiyonu (PZR) ve kompetitif amplifikasyon refrakter mutasyon sistemi (C-ARMS) ile belirlenmiştir. Yedi hasta 35delG mutasyonu için homozigot, bir hasta heterozigot olarak saptanmıştır. Sonuçlarımız, Türkiye'de Manisa ve çevresindeki hastalarda non sendromik konjenital işitme kaybına Connexin 26 genindeki 35delG mutasyonunun neden olduğunu ortaya koymuştur.*

***Anahtar kelimeler:*** *35 delG, connexin 26, GBJ2, otosomal resesif non-sendromik işitme kaybı*

Prelingual deafness occurs with a frequency of 1 in 1.000 live births and is divided into syndromic and non-syndromic forms contributing 40 and 60%, respectively. Autosomal recessive non-syndromic hearing loss (ARNSHL) is responsible for 80% cases of childhood deafness. Nearly all genes localized for ARNSHL cause prelingual, severe to profound, sensorineural hearing impairment (1).

Congenital deafness occurs in approximately 1 in 1.000 live births and 50% of these cases are hereditary. The majority (70%) are nonsyndromic, and the remaining cases (30%) exhibit syndromic hearing loss. Approximately 80% of nonsyndromic hereditary hearing loss is inherited in an autosomal recessive manner, whereas 15–20% of cases exhibit autosomal dominant inheritance, and the remaining cases exhibit X-linked or mitochondrial inheritance. Hereditary nonsyndromic sensorineural hearing loss is transforming silence to sound (2).

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Most non-syndromic hearing losses are caused by mutations in the GJB2 gene, and studies have revealed that the forms and frequencies of these mutations are largely dependent on ethnic origin (3). Mutations in Connexin 26 gene (GJB2) are the most common cause of hearing loss in different populations (4).

The aim of this study was to investigate the hearing loss attributed to the 35delG mutation in the connexin 26 gene of the patients with nonsyndromic hearing loss who were parental relative families, but healthy people living in Manisa and vicinity, Turkey.

## MATERIAL AND METHOD

### Patients

The study included 8 patients with nonsyndromic hearing loss (4 female and 4 male, between 10-to-20 years of age) who were parental relative families (14 family; 28 individuals), but healthy people. Pedigree analysis and audiological examination revealed in 8 cases. All the families were informed about the study and consent was obtained from patients and when needed from parents. The study protocol was approved by the local ethical committee.

### Audiology

Hearing levels of probands carrying 35delG mutation were measured by pure-tone audiometry, timpanometry and acustic reflex test. Values between 0-20 dB was accepted as normal.

### Detection of 35 delG with C-ARMS

Genomic DNA was extracted from peripheral blood lymhocytes according to standard protocols (5). To identify 35delG mutant and normal GJB2 alleles in a single polymerase chain reaction (PCR) with competitive amplification refractory mutation system (C-ARMS), a wild-type allele specific primer 5'-GTA GTG TTT GAT CAT ACG CCC C-3` and a 35delG specific primer 5'-A GTA TTT GTA CAC ACC CCC A-3'. A reverse primer 5'-CAT TCG TGT TTT CGA GAG CA-3' was used (6). PCR of C-ARMS was performed in a final volume of 25µl containing 200ng genomic DNA, 50mM KCL, 10mM Tris HCL ( pH 9.0), 0.1 % (v/v) Triton X -100, 1,2 mM $MgCl_2$, 16 µM of each dNTPs, 5 pmol from each primer, and 0,5 U of Taq polymerase (Promega, Leiden, The Netherlands) in a Genius thermal cycler (Techne, Cambridge, England). First the samples were denaturated at $94^0$ C for 7 min. Subsequently, 35 cycles of denaturation were performed at $94^0$ C for 35 s, annealing at $64^0$C for 3 min, and elongation at $72^0$ C for 35 s, followed by a final extension step of $72^0$ C for 5 min. The complete PCR mix was added to 5µl loading buffer, 0.1% (w/v) bromophenol blue, 0.1 % (w/v) xylene cyanol, 50% (w/v) sucrose, and 50mM $Na_2EDTA$ ( pH 8.0 ) . Fifteen microliters of the PCR products were electrophoresed in a 10% native polyacrylamide gel at 350 V and 29 mA for 5 h. DNA fragments were visualized using silver staining (7).

## RESULTS

Eight patients with nonsyndromic hearing loss were included in this study and pedigree analysis revealed in these patients ( Figure 1).

Hearing levels of the patients carrying 35delG mutation were measured by pure-tone audiometry, timpanometry and aqustic reflex test. Values between 0-20 dB was calculated as normal.

In audometric analysis of patients ;bilateral severe hearing loss was detected in one patient, bilateral total hearing loss in three patients, severe hearing loss in two patients, and bilateral severe hearing loss in one patient (Table I).

GJB2 was analyzed in 8 patients who were parental relative of the families, but healthy people. The presence of the 35delG mutation was detected by C -ARMS in a single PCR. Seven patients were homozygous for the 35delG mutation and one patient was heterozygous.

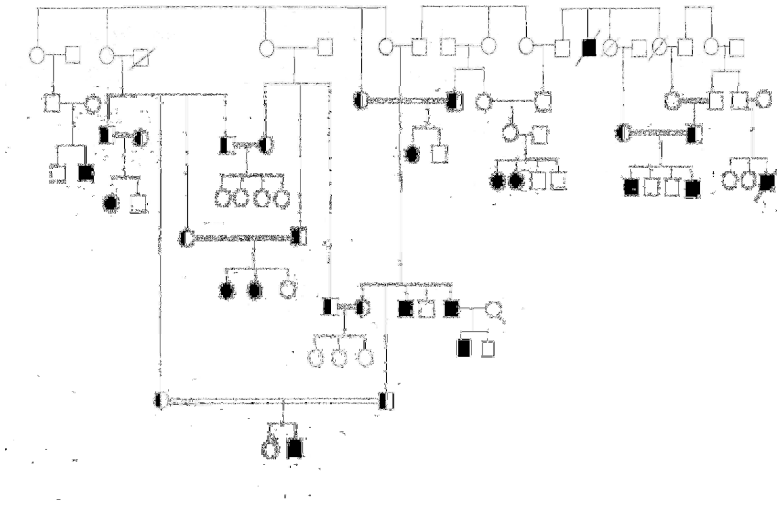

**Figure 1**. Pedigree analysis of 28 individuals in related family with non-syndromic hearing loss.

**Table I.** Audometric results of 8 patients with non-syndromic hearing loss

| Patient No. | Audiometer | Timpanometer | Acoustic reflex and thresold |
|---|---|---|---|
| 1 | Bilateral<br>103 dB NSHL | N/N | Φ / Φ<br>110 dB/110 dB |
| 2 | 113 dB / 95 dB<br>NSHL | N/N | Φ / Φ<br>110 dB/110 dB |
| 3 | Bilateral<br>Total SHL | N/N | Φ / Φ<br>110 dB/110 dB |
| 4 | Bilateral<br>100 dB NSHL | N/N | Φ / Φ<br>110 dB/110 dB |
| 5 | 110 dB / 90 dB<br>NSHL | N/N | Φ / Φ<br>110 dB/110 dB |

## DISCUSSION

Baris et al., (8) screened a total of 235 unrelated hearing-impaired children and found 48 of the subjects to be homozygous for the mutation, including 27 of 83 familial cases, 15 of 101 singletons, 4 of 9 subjects born to assortative marriages (deaf married to deaf), and 2 of 42 subjects for whom the parents claimed an environmental factor as the etiology of the condition. The high ratio of individuals homozygous for the mutation indicated that the 35delG mutation in the Connexin gene accounts for more than 90% of the mutations at this locus.

Bayazit et al. (9), identified fourteen families who had at least two prelingually deaf children per family that were included in their study. One affected child from each of the 14 families was selected for Single-Stranded Conformational Polymorphism (SSCP) analysis. Six of the 14 representative family members (42.9%) demonstrated shifts on SSCP and were subsequently sequenced for Exons 1 and 2 of GJB2 and were tested for the 432 kb upstream deletion. No mutations were found in Exon 1 and no 432 kb deletions were noted. Three different GJB2 mutations were found in Exon 2 of the probands, which were 35delG, 299-300delAT, and 487G > A (M163V). GJB2 mutations were detected in 21.4% of the families. Two patients were homozygous for 35delG and 299-300delAT mutations, and were given a diagnosis of DFNB1 deafness (14.3%). Two different polymorphisms, 457G > A (V153I) and 380G > AG (R127H) were also found. Although GJB2 mutations were detected in 21.4% of the families tested, only 14.3% of subject representatives were homozygous and therefore deafness caused by Cx26 mutation was segregated with DFNB1.

The frequency of the mutant chromosomes having the 2-6-4 haplotype was compared with the Eastern Black Sea region and the other regions of Turkey and the significant difference was detected (Chi square = 5.13/df = 1/p = 0.023) by Balci et al (10). Also, when constituted the majority of the GJB2 mutations in Yılmaz et al.'s study group. Similar findings have been observed in other populations. The 35delG mutation accounts for 95.2% of mutations in Greece, 93.1% in Slovakia, 86.7% in Finland, and 82.8% in the Czech Republic (3).

Relatives marriage is about 20% of the Turkish population (11,12)**.** Our results were comparable and indicated that GJB2 mutations account for about consanguineous marriages of Turkish patients with ARNSHL. As a result; our study support previous findings concerning contributions of GJB2 to hearing loss. It shows that the 35delG mutation in Connexion 26 gene is the most frequently mutation in congenital non-syndromic deafness in Manisa and vicinity, Turkey. These findings suggest that it will be beneficial to consult the families with hearing anomalies about the risk of deafness by evaluating their genetic encoding. And it also will get more successful results by confirming the patients with hearing loss earlier.

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