

A Novel Compound Heterozygous Mutation (35delG, 363delC) in the Connexin 26 Gene Causes Non-Syndromic Autosomal Recessive Hearing Loss

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Abstract- Mutations in the Connexin 26 (Cx26) gene are a common cause of hereditary hearing loss in different populations. In the present study, an Iranian patient with bilateral hearing loss underwent molecular analysis for the causative mutation. DNA studies were performed for the Cx26 gene by PCR and sequencing methods. We describe a novel compound heterozygous mutation (35delG, 363delC) in the Cx26 gene that is strongly associated with congenital non-syndromic hearing loss (NSHL).

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Introduction

The most common congenital sensory disability is hearing loss (HL). Its prevalence is 1 in 1000 neonates and 50% of them are because of genetic factors (1-6). Non-syndromic HL is main type of hereditary HL and it is approximately 70% of hereditary HL. Non-syndromic HL subdivided to autosomal dominant (20%), autosomal recessive (75%), X-linked HL (1%), and maternally-inherited HL associated with the mitochondrial DNA mutation (2,7). In spite of the contribution of several different genes as causative agents of deafness, mutations in one gene encoding Connexin 26 (GJB2) (GenBank M86849, MIM 121011) with chromosomal location 13q11-12 known as DFNB1 (MIM 220290) responsible for half of severe to profound autosomal recessive non-syndromic deafness in many populations (1,3,8,9,10). GJB2 as a small gene has 5500bp length. It contains two exons, between them only one contains the coding region with 681bp that encodes a gap-junction protein with 226 amino acids (8). One of important members of connexin family producing gap junction proteins, is Cx26 that encode channels and facilitate intercellular communication by directly linking the cytoplasm of adjacent cells (11,12). A single mutation, at position 35 (35delG), accounts for approximately 30-63% of mutations in white populations with a carrier

frequency of 1.5-2.5% in most European, North American and Mediterranean populations. In other ethnic groups there is other common mutations prevailing such as 235delC in the Japanese and Korean, 167delT in the Ashkenazi Jews than 35delG (1). In this article, we have reported a novel compound heterozygous mutation of connexin 26 gene using direct sequencing technique of coding region of the gene in both directions.

Case Report

A 20-year-old woman with bilateral hearing loss (with a profound degree) was referred to welfare organization of Marand, Iran, for molecular analysis of deafness. This research project has been approved by ethical committee. Genomic DNA was extracted from 1 ml of EDTA anticoagulated peripheral blood by rapid genomic DNA extraction (RGDE) method and assessed the causal mutation (13). Polymerase chain reaction of coding region of the Cx26 gene was performed using cx26F: 5'-tct ttt cca gag caa acc gc-3' as a forward primer and cx26R: 5'-tgg gca atg cgt taa act ggc-3' as a reverse primer. PCR reactions were carried out in 25 µL reaction mixture as final volume containing 0.2 mM dNTP, 10 pmoles of each primer, 1.5 mM MgCl₂, 0.5 U of Taq DNA polymerase, 1× PCR buffer, and about 1µg

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of genomic DNA on a SENSOQUEST (Labcyler/Germany) Thermal Cycler. Initial denaturation for 5 min at 95°C was done then 34 cycles of 45 s at 95°C, annealing for 60 s at 59°C and extension for 60 s at 72°C were run and followed by 1 cycle as a final extension for 5 min at 72°C. The amplified fragments were run on 1.5% agarose gel by safe dye staining (Figure1). Expected PCR products with 724bp length were subjected to direct sequencing in both directions. Sequences were analyzed by sequencing-analysis Chromas Lite 2.1 software. The sequences were compared with the wild type (Figure2).

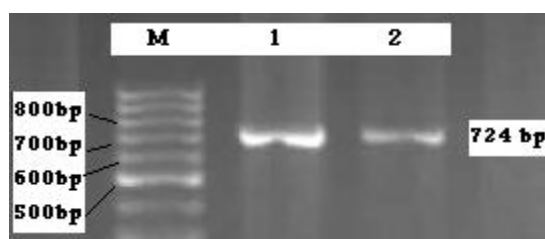


Figure 1. PCR product with 724bp length of coding region of the GJB2 gene has been shown by safe dye-staining on 1.5% agarose gel. Lanes from left to right are: M: Molecular weight standard Marker, 1: patient and 2: normal

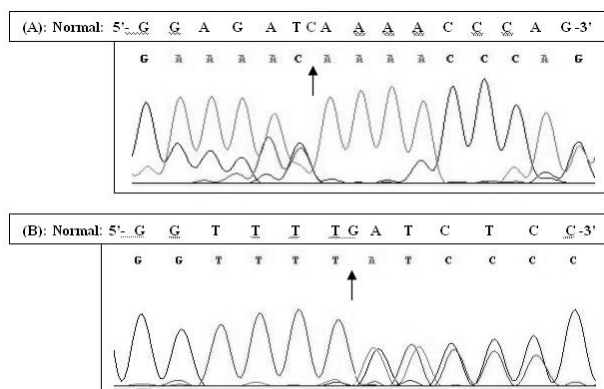


Figure 2. The GJB2 coding exon DNA sequencing result for the 363delC mutation. (A) Forward sequence; (B) Reverse sequence.

Arrows indicate the position of the mutation.

Discussion

Hearing loss is genetically highly heterogeneous diseases and more than 100 mutations in Cx26 (GJB2) gene are reported to be responsible for 30%–40% of hereditary hearing loss in deaf subjects (8). In the present study, DNA sequencing revealed a compound heterozygous mutation. One allele showed a 35delG corresponding to frameshift and premature termination codon at 13. The most common variant of GJB2 is

35delG allele in the population of northern European ancestry. Also, the 35delG mutation has found with very high frequency in Italian, Spanish, and Israeli patients (11). This relieved that 35delG mutation as a deletion mutation has spread in Middle East and Europe. But, the 35delG mutation was not found in the Chinese, Japanese, and Taiwanese populations (10,14,15).

The other allele showed a 363delC mutation, which causes a frameshift at codon 121 resulting in a premature stop codon at codon 167 corresponding to a truncated polypeptide (16). The large cross-sectional analyses of GJB2 genotype-phenotype correlation data suggest that the severity of HL associated with biallelic truncating mutations is significantly more severe than that associated with biallelic non-truncating mutations (4). Therefore, this novel mutation might be has prevalence in sever deafness patients in Marand region. So, study of causative mutations of deafness in the region of Marand is necessary.

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