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ORIGINAL ARTICLE

A Novel Dominant Missense Mutation, I20F, in the GJB2 Gene Causes Sporadic Nonsyndromic Sensorineural Hearing Loss

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ABSTRACT
The most common cause of hereditary hearing loss (HL) is mutations in the Connexin 26 (Cx26 or GJB2) gene. We report here a novel missense mutation in GJB2 (c.58A>T), observed in an Iranian patient with bilateral hearing loss. The mutation was a novel heterozygous -A to -T transversion at codon 20 (ATT→TTT) of the GJB2 gene which leads to the substitution of isoleucine (I) by phenylalanine (F) residue (I20F). This dominant mutation was recorded in NCBI GenBank with accession number BankIt1688072 Seq1 KJ021927. It is predicted that the mutation affects the N-terminal domain of connexin 26 by substitution of its much conserved isoleucine residue. The N-terminal domain is important for membrane integration and hexamer aggregation as well as voltage gating response. Genomic DNA was extracted by rapid genomic DNA extraction (RGDE) method and Cx26 gene was studied by PCR and sequencing methods. The I20F mutation has not previously been described in affected or control samples from other populations.
Keywords: *Connexin 26, GJB2, Hearing loss,Novel mutation*

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INTRODUCTION

Hearing loss (HL) is the common genetically heterogeneous neurosensory disorder that affects about 1 in 1000 newborns [1]. HL can occur because of either genetic or environmental causes or a combination of both [2]. In more than 50% of HL cases changing of genetic factors are observed [3-5]. Nonsyndromic sensorineural hearing loss could follow autosomal dominant, autosomal recessive, X-linked HL, and maternal inheritance which among them 80% are of due to autosomal recessive conditions [3, 6].

Up to now, several different loci and genes have been identified that their mutations are involved in HL [7]. Connexin 26 (*GJB2*) gene (Gen Bank M86849, MIM 121011) with chromosomal location 13q11-12 has been known to be the major cause of autosomal recessive non-syndromic sensorineural deafness (ARNSD) [8-10]. *GJB2* has two exons, of which only one contains the coding region with 681bp length that encodes a gap-junction protein with 226 amino acids [11]. GJB2 -a member of gap junction proteins family named connexin family- makes a channel in order to facilitate intercellular communication and directly links the cytoplasm of adjacent cells [12, 13].

It has been reported that more than 100 different mutations in connexin 26 gene are associated with hearing loss [14]. The prevalence of these mutations differs among ethnic groups. The c.35delG is common mutation in many populations, especially in the European and Mediterranean regions, where it

accounts for approximately 60% of *GJB2* mutated alleles. The frequency of 35delG mutation in other ethics is low and common *GJB2* mutations are different. These include 235delC in the Japanese and Korean, 167delT in the Ashkenazi Jews [15]. It means that other regions could have other common mutations.

In the present study, we report a novel dominant mutation, I20F, in an Iranian patient with bilateral non-syndromic sensorineural hearing loss using direct sequencing technique for coding region of the gene in both directions.

MATERIALS AND METHODS

Patient history and Sampling

The study was conducted on a 21-year-old woman with bilateral non-syndromic hearing loss (with profound degree) that was confirmed by physical and autoscopic and audiological tests (Figure 1). The proband (Figure 2) was a girl, born to nonconsanguineous clinically normal parents. The parents were not affected by hearing loss. No other cases of hearing loss were reported in the family. The patient was referred to welfare organization of Marand, Iran.

After filling in written informed consent form, her whole blood sample was obtained in order to molecular analysis of her deafness condition. This study has been approved by ethical committee in Marand Branch, Islamic Azad University, Marand, Iran. In order to molecular analysis, 5-ml blood sample was drawn into coded EDTA-treated tubes. Then, genomic DNA was extracted from 1 ml whole blood by rapid genomic DNA extraction (RGDE) method [16] and used for PCR analysis.

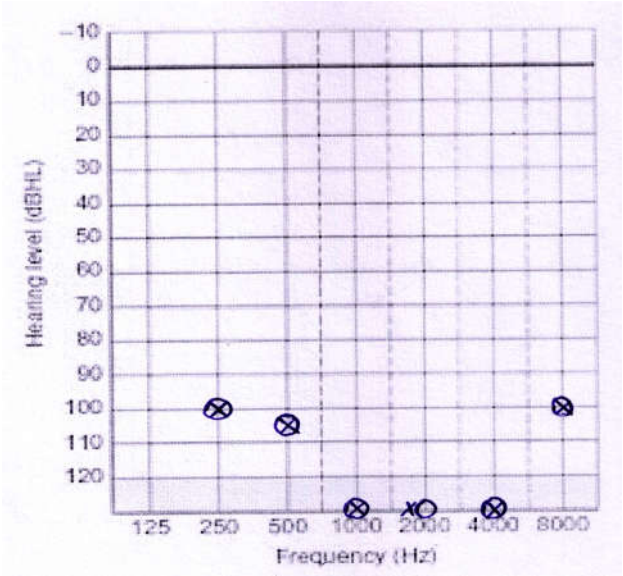

Fig. 1.Behavioral audiogram for the patient. Right ear (O) and left ear (X) thresholds are shown.

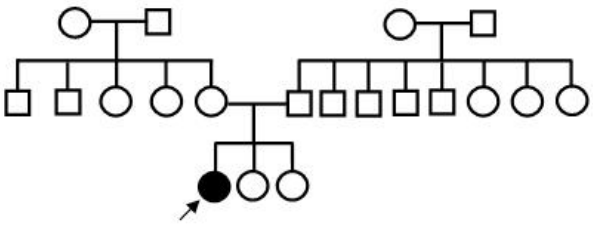

Fig. 2.Pedigree of the family carrying the I20F (c.58A>T) mutation of the GJB2 gene. The arrow denotes the proband.

AMPLIFICATION AND SEQUENCING OF GJB2 CODING SEQUENCE

To identify *GJB2* mutations, the purified DNA was analyzed by Nanodrop ND-3000 and its purity and concentration was measured. A DNA fragment containing the entire coding region of *GJB2* was amplified using cx26F: 5'-tct tttcca gag caaacc gc-3' as a forward and cx26R: 5'-tgg gcaatgcgttaa act ggc-3' as a reverse primers by Polymerase Chain Reaction (PCR). PCR reactions were done in 25 µl reaction mixture

as final volume including 1X PCR buffer, 1.5 mM MgCl2, 0.2 mMdNTP, 10 pmoles of forward and reverse primers, 0.5 U of Taq DNA polymerase and 1µg of genomic DNA on a SENSOQUEST (Labcycler/Germany) Thermal Cycler. The program for PCR reaction was as follows; Initial denaturation at 95°C for 5 minutes, followed by 34 cycles of denaturation at 95°C for 45 seconds, annealing at 59°C for 60 seconds and extension at 72°C for 60 seconds and a final extension for 5 min at 72 °C followed.
PCR products were electrophoresed on 1.5% agarose gel, stained using DNA safe stain dye and visualized by gel documentation system. Furthermore, PCR products were sequenced bi-directionally and the sequences analyzed by sequencing-analysis ChromasLite 2.1 software. To find casual probable mutation(s), the sequences were compared with the wild type sequence.

RESULTS AND DISCUSSION

A 724 bp length fragment of the genomic DNA of the patient, encoding *GJB2* gene, was amplified by PCR and electrophoresed on 1.5% agarose gel where then visualized by gel documentation system (Figure 3). The PCR products were sequenced bi-directionally and analyzed. Sequence analysis revealed a novel dominant missense mutation in *GJB2* gene (c.58A>T) which was in heterozygote state in the patient (Figure 4). The mutation was an -A to -T transversion at codon 20 (ATT→TTT) of the *GJB2* gene which leads to the substitution of isoleucine (I) by phenylalanine (F) residue (I20F). Searching the CX26-deafness database [17] revealed that this mutation has not previously been reported. So we recorded the mutation in the NCBI GenBank with accession number BankIt1688072 Seq1 KJ021927.

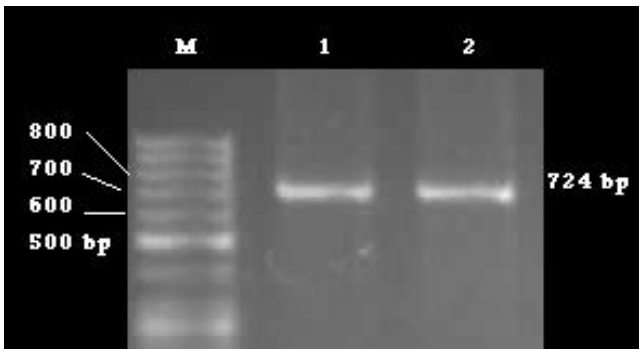

Fig. 3. Safe dye-stained 1.5% agarose gel showing PCR products corresponding to a 724 bp amplification of coding region of the *GJB2* using cx26F and cx26R primers. Lanes from left to right are: M: Molecular weight standard Marker, 1: patient and 2: normal.

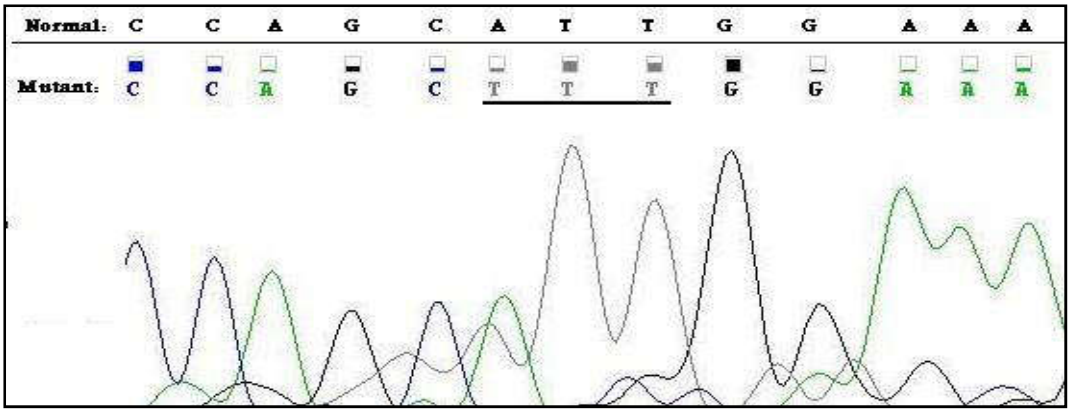

Fig. 4.PCR product partial sequence of the *GJB2* coding exon showing the c.58A>T missense mutation. (A) Forward sequence; the position of mutation indicated with underlying.

GJB2 or *Cx26* is the most important gene that its mutations are related to hearing loss. More than 100 mutations in *Cx26* (*GJB2*) gene are responsible for 30%–40% of hereditary hearing loss in deaf subjects. Moreover Cx26 mutations causing non-syndromal, sensorineural deafness have arisen independently in geographically and ethnically distinct populations [11]. However, many studies showed that 35delG mutation in *GJB2* gene is the most prevalent cause of nonsyndromic neurosensorinal hearing loss in populations of northern European, Spanish, Italian and Israeli [15].

It has been reported that there are more than 14 different types of connexins and each connexin has four transmembrane domains(TM), two extracellular domains (EC), one cytoplasmatic loop (CL), and N- and C- cytoplasmic termini (NT-CT). The N-terminal domain not only is involved in the process of membrane integration and hexamer aggregation to make connexon [18, 19] but also together with the first transmembrane domain, determines voltage gating. The connexon-connexon interactions are regulated by the extracellular loops. Each loop contains three conserved cysteine residues that form essential intramolecular disulfide bonds [20]. The intracellular loop and C-terminal domains regulate pH gating [19]. The TM domains are important for protein folding. Because the mutation reported here occurs in the N-terminal domain of GJB2, it is predicted that the mutation might interfere with the membrane integration, hexamer aggregation or voltage gating processes [18, 19].

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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