Short Communication

Mutation analysis of connexin 26 gene and del(GJB6-D13S1830) in patients with hereditary deafness from two provinces in Iran

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Abstract
Mutations in the connexin 26 (Cx26) gene at the DFNB1 locus on chromosome 13q12 are associated with autosomal recessive non-syndromic hearing loss (ARNSHL). There are many known mutations in this gene that cause hearing loss. A single frameshift, at position 35 (35delG) accounts for 50% of mutations in the Caucasian population with carrier frequencies of 1.5-2.5%. In this study we investigated the prevalence of Cx26 gene mutations by directly sequencing the coding exon of this gene belonging to ARNSHL individuals from 53 families in Qom and Markazi provinces of Iran. Seven different Cx26 variants were identified. Five Cx26 mutations including 35delG, 233delC, 176del16, W24X, L90P were found in 10 of 53 families (18.87%). One polymorphism V153I was also found. One variant A171T with unknown effects was also detected. Six of the 53 families were observed to have GJB2 mutations in both alleles (11.32%). The most common mutation was 35delG. Three out of 10 families (30%) with GJB2 variants contained 35delG mutation in both alleles and the frequency of 35delG allele was 0.50 among 10 out of 53 families. Also screening for the 342-kb GJB6 deletion mutant did not reveal any large deletion among families studied. Thus, in the two provinces, contribution of GJB2 (Gap Junction Protein Beta 2) mutations to familial deafness appears to be less significant. This necessitates further assessment of the other known genes regions as well as a search for new genetic factors in hereditary deafness in the Iranian population.

Keywords: Hereditary Deafness; Iranian; Connexin 26; del(GJB6-D13S1830); GJB2

Congenital deafness is a frequent disorder that affects 1 in 1000 neonates with approximately 50% caused by inheritance. Almost 80% of familial hearing loss is non-syndromic, with a predominant autosomal recessive mode of inheritance (Cryns et al., 2004; Marlin et al., 2005). More than 51 autosomal recessive hearing impairment loci have been reported (Hereditary Hearing Loss Homepage, http://webhost.ua.ac.be- hh). Mutation in the connexin 26 gene (Cx26) is the predominant genetic cause of sensorineural hearing loss in many populations (Cryns et al., 2004; Marlin et al., 2005). Although a single mutation, known as 35delG, is a common mutation among Caucasians, it is less frequent in other populations (Shahin et al., 2002; Ghosh et al., 2004; Mustafa, 2004; Kalay et al., 2005). The other prevalent mutations of this gene are 235delC among the Japanese and Korean populations (Abe et al., 2000), the 167delT in the Ashkenazi Jews (Sobe et al., 1999), R143W in an African village (Brobbey et al., 1998) and W24X in the India population (Ghosh et al., 2004).

Cx26 encodes a gap junction channel protein, called GJB2. This protein is widely expressed throughout non–sensory epithelial and connective tissue cells and is thought to be important in maintaining endocochlear potential (Kalay et al., 2005). A large deletion (342 kb) involving the GJB6 gene encoding connexin 30, which is also located at the DFNB1 locus, del(GJB6-D13S1830), has been reported to cause ARNSHL in homozygote or compound heterozygote individuals carrying deafness-causing allele variants of the GJB2.
on the opposite allele (Frei et al., 2004; Riazalhosseini et al., 2005). This large deletion is found in many populations, with higher frequencies observed in France and Spain where the percentage of unexpected GJB2 heterozygotes fell to 16.0-20.9% after screening for the del(GJB6-D13S1830) mutation (Castillo et al., 2003).

In this study, we investigated the GJB2 gene mutations and the del(GJB6-D13S1830) mutation in 53 families with ARNSHL from Qom and Markazi provinces. 79.24% of these families had 3-10 deaf individuals and the remaining 20.76% had 2 affected children. All affected members of the families studied suffered from a prelingual hearing loss with no other associated clinical findings (non-syndromic) and no suggestive history of any other possible etiology. Informed consent was obtained from all of the participating subjects (in case of individuals under 18 years old, consent was obtained through their parents). Blood samples were taken from all individual belonging to the 53 families.

DNA was extracted from peripheral blood samples using the phenol/chloroform standard procedure (John et al., 1991). The coding region of Cx26 gene (Genbank accession # M86849) was amplified using primers: forward: 5′-GTCTCCCTGTTCCTCGTCTCTA-3′ and reverse: 5′-TCTAACAACTGGGCAATG-3′. The amplification resulted in a fragment of 743 bp containing the entire coding sequence and right splice site. After a quality check of the PCR products by electrophoresis on 1.5% agarose gel, sequencing was carried out using a capillary automated system 3700 ABI sequencer (Macrogen, South Korea). One affected sample from each family was sequenced initially. Detected mutations were confirmed by sequencing the reverse strand. Alternatively the sequencing was carried out on samples from another member of the families.

In order to analyze the 342-kb deletion in all the probands multiplex PCR was performed. The junction fragment caused by the deletion was amplified using the primer pair: forward: GJB6-1R, 5′-TTTAGGGCATGATTGGGTGATT-3′ and reverse: BKR-1, 5′-CACCATGCGTACCTTAACCATT-3′. The resulting amplicon was 460 bp in length. The primer pair: forward, STS-CX636F 5′-TGCCCACCC-CCCAAGTAGAG-3′ and reverse, STS-CX636R 5′-TTTCGTTTCCATTCATTCCCCATATT-3′ used as an internal control, generated a 360 bp fragment. The products of multiplex PCR were electrophoresed on 1% agarose gel and visualized with ethidium bromide staining under ultraviolet light to verify their size and quantity. Control samples for large deletion were a gift from the Center of Medical Genetics, at the University of Antwerp, Belgium.

In this study, 53 families were investigated for seven different genetic variants shown in Table 1. Six out of 53 families had GJB2 mutations in both alleles (11.32%). These six families encompass 35delG/35delG, 35delG/W24X, 233delC/233delC and 176del16/176del16 variants. Five GJB2 mutations including 35delG, W24X, L90P, 233delC and 176del16 were found in 10 of 53 families (18.87%). One polymorphism variant, V153I was found in 2 families. A171T variant was found in one family as heterozygote. The most common mutation was 35delG. Three out of 10 (30%) families that had GJB2 variations containing 35delG mutation in both allele. The frequency of 35delG allele was 0.50 among 10 out of 53 families. Five out of 53 families were homozygous for one mutation in GJB2 gene as shown in Table 1. None of the families, including those who carried one mutation in the coding region of the Cx26 gene, revealed the 342-kb GJB6 large deletion mutation (Fig. 1).

All families were the result of consanguineous mat-
ing with autosomal recessive inheritance of the disease. GJB2-related deafness was found in 6 of the 53 ARNSHL studied families (11.32%) and revealed that the rate of mutation in Cx26 gene in the two populations is lower than that in the European populations (Cryns et al., 2004; Marlin et al., 2005). All of the mutations have been previously described in the Iranian population except 176del16 which was initially reported by Kudo et al. (2002). 35delG and L90P heterozygosity were found in 3 and 1 families, respectively and since these mutations were found in another affected member of the family therefore, they may not be due to polymorphism.

The results of this study on the frequency of Cx26 gene mutations support the previous reports on Iranian hereditary deafness (Hashemzadeh et al., 2005; Najmabadi et al., 2005; Hosseinipour et al., 2005). Similar studies in Egypt (Mustafa, 2004), Turkey (Kalay et al., 2005), Palestine (Shahin et al., 2002) and India (Ghosh et al., 2004) demonstrated a lower rate of mutation in this gene in comparison to those in Caucasians. This study shows 35delG is the most common deafness-causing mutation in the Iranian population that confirms the previous studies in Iran (Hashemzadeh et al., 2005; Najmabadi et al., 2005; Hosseinipour et al., 2005). Our data and those by other researchers show that GJB2-related deafness in the Iranian population is due to the common mutation (35delG) similar to that in the Caucasian population, with a mutation rate resembling that in the Asian population.

Absence of the 342 kb GJB6 variant is one of the most important findings of this study. Previous reports on the same population also presented the same finding (Riazalhosseini et al., 2005). The families with no mutation in the Cx26 gene will need to be investigated further to look for the presence of mutations in other known deafness-causing genes, and new deafness-causing loci.

The Iranian population is an important resource for research on ARNSHL due to the availability of large, extended and highly consanguineous pedigrees. It should be note that the rate of familial marriage in Iran is relatively high (38.6%) Saadat et al. (2004), therefore an expected higher frequency of hearing loss, with more than 1 in 1000 newborn is to be expected.

In conclusion, identification of the spectrum and frequency of GJB2 mutations and other genes in different ethnic groups of Iran can eventually help and facilitate with detection and prevention of deafness.

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References

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