Spectrum and frequency of GJB2 mutations causing deafness in the northwest of Iran

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Objective: Mutations in GJB2 and GJB6 which comprise DFNB1 locus cause up to half of all cases of the prelingual autosomal non-syndromic hearing loss (ARNSHL) worldwide. This study has intended to assess the spectrum and frequency of GJB2/GJB6 mutations in northwest of Iran.

Methods: 508 Patients with presumed ARNSHL were analyzed by applying ARMS-PCR, SSCP, PCR-RFLP and sequencing assays.

Results: Seventy-five (14.7%) different homozygous and eighty-seven (17.1%) different compound heterozygous genotypes were detected in this cohort. Concerning the GJB2 gene, c.35delG was the most prevalent mutation, accounting for 16.4% of the samples. In addition 29 sequence variations other than c.35delG mutation were distinguished in GJB2; namely, delE120, insA 290-291, R143Q, V37I, R32H, Y155X, V271 +T123N, F154F, 167delT, 312del14, 299-300delA, T8M, W24X, E114G + V27I, 235delC, R184P, V153L, S139N, A171T, M163V (unknown mutation), G127V, E147X, R127H, 35insG, R143W, V27I, G160S, E114G and IVS1+1G > A. Moreover, the IVS1 +1G > A was accounted as a second common mutation.

Conclusions: Overall, the frequency of GJB2 mutations (>31%) is in agreement with other white population. These findings highlight the importance of the study of GJB2 gene in the diagnosis to provide early treatment and genetic counseling.

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1. Introduction

Approximately one in 1000 children is affected by severe or profound hearing loss at birth or in the early childhood (prelingual deafness) [1]. In some populations with high level of consanguinity, the frequency of childhood hearing loss is high [2]. Estimates suggest that at least 50–60% of hearing loss is caused by genetic reasons [3]. Approximately 70% of them are non-syndromic and are not associated to other clinical signs or symptoms [4].

Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most frequent form of hereditary hearing loss. The phenotype is usually prelingual and more severe [5–7]. To date about 60 loci and close to 30 genes involvement in ARNSHL have been reported [8]. In developed countries up to 50% of ARNSHL is result from the mutation in GJB2 gene, which is also responsible for a few dominant hearing losses [9,10]. Mutations in GJB2 (Cx26) and GJB6 (Cx30) cause alteration in DFNB1/A3 locus at 13q12 [11]. In the vertebrate cochlea, Cx26 and Cx30 are coexpressed to form homo- or hetero-hexameric channels (connexons). Connexons fuse to shape gap junctions which are crucial for potassium homeostasis [12,13]. Over 110 sequence changes in GJB2 and only 2 mutations in GJB6 have so far been reported (http://davinci.crg.es/deafness). In several studies a sacred frameshift mutation, c.35delG, has been announced as a main mutation among different populations. Some of the GJB2 mutations are very frequent and exhibit ethnic specificity, namely, c.35delG in whites (up to 85%), c.167delT in Ashkenazi Jews (76–81%), c.V37I in Taiwanese and Thai people, c.235delC in Japanese (34–75%), Chinese (58–96%) and Koreans (56%), c.R143W in Ghana (93%) and c.W24X in India (83–96%) and Slovak Romany Gypsies (79%) from European countries [14,15]. However, the patients with GJB2 mutation have single mutated allele, suggest that there could be other mutations outside the GJB2 gene in charge of the ARNSHL. The splice site mutation IVS1 +1G > A also called –3172G > A, is frequent among Czech, Hungarian, Yakut population (Sakha Republic, Russian Federation) and Turkish [16–19]. In contrast to the GJB2, only two large deletions have so far been reported in GJB6 gene. These deletions in homozygous state or heterozygous with each other or in trans mode with GJB2 mutations have resulted in ARNSHL in Spain,
France, UK, Italy, Belgium, USA, Argentina, Brazil and Australia [8,21]. The objective of this study was to find the spectrum of GJB2 mutations (including the coding region and the splice site of GJB2 gene) among over 500 Iranian Azeri ARNSHL patients.

2. Materials and methods

508 sequential unrelated patients with ARNSHL from Iranian Azeri Turkish ethnic group were genetically screened in this study. All patients were of Azeri Turkish origin who had received a clinical diagnosis of prelingual non-syndromic hearing loss. For each patient, an ample pre-designed questionnaire filled out to rule out environmental causes of hearing loss. All families were informed about the study and written informed consent was obtained from participants or their families. Genomic DNA was extracted from peripheral leukocytes in whole-blood samples using standard protocols based on salt extraction.

2.1. Detection of 35delG and del (GJB6-D13S1830) with multiplex amplification refractory mutation system

Genetic testing of 35delG and del (GJB6-D13S1830) was carried out by using multiplex amplification refractory mutation system (ARMS). This screening was accomplished using two polymerase chain reactions for normal and mutant primer sets of 35delG and Cx30 according to the protocol published previously [13]. No further testing was fulfilled on homozygous for 35delG and the diagnosis of DFNB1 hearing loss was made.

2.2. Confirmation and analysis of the IVS1 + 1G > A by restriction fragment length polymorphism procedure

The heterozygote and negative samples for 35delG were tested for c.IVS1 + 1G > A mutation using polymerase chain reaction restriction-digestion testing procedure (PCR-RFLP). The occurrence of c.IVS1 + 1G > A mutation wipes out Hph1 recognition site on PCR amplicon amplified with primers flanking exon 1 and its exon-intron boundaries (Denoyelle, 1999). The PCR amplicon, 360 bp, was digested into two fragments of 242 bp and 118 bp for wild-type allele whereas the mutant allele remains uncut. All PCR products and restriction enzyme-digested fragments were electrophoresed in an agarose and polyacrylamide gel respectively and visualized using ethidium bromide.

2.3. Single-strand conformation polymorphism and direct sequencing analysis

Finally, the samples, which were remained unsolved, the negative or heterozygous samples for 35delG and IVS1 + 1G > A, were further analyzed by the single-strand conformation polymorphism (SSCP) technique and subsequently subjected to automated sequence analysis by dye-terminator reactions for the complete coding region of GJB2 gene, using primers described previously[12].

3. Results

In the current study, mutations in GJB2 and the delGJB6 (D13S1830) were analyzed in 508 patients with presumed ARNSHL. The age of patients varied from 1 month to 45 years (mean: 22 years) and the consanguinity was discovered in about 59% of the families. There were 249 (49%) males and 259 (51%) female in this group. The mutations in this cohort comprise seventy-five different homozygous genotypes and eighty-seven different compound heterozygous genotypes [154/508 families (≥31%)] (Fig. 1). One hundred and four (62 homozygous and 24 heterozygous or 18 compound heterozygous associated with another mutation) families out of the 508 families had the c.35delG mutation (20.5%). Altogether, 30 different GJB2 variants were detected in this study. Only 99 of the 508 probands were found to carry two mutated alleles (19.5%). Concerning the GJB2 gene, c.35delG is the most frequent mutation in this population (16.4%). Moreover, 59.6% of the patients with c.35delG were detected in homozygous mode. The second prevalent mutation which detected in this ethnic was c.IVS1 + 1G > A with 1.9% of the all patients. Another fourteen described mutations/polymorphism were present in more than one allele in the population studied (Fig. 1). Moreover, further studies carried out on parents of positive cases. The allelic (GJB6-D13S1830) which cause hearing loss in either homozygous or heterozygous state in trans mode with GJB2 mutations was not present in this cohort.

4. Discussion

Hearing loss is the most common inherited sensory disorder [22]. Despite the high heterogeneity, up to 50% of early prelingual autosomal recessive non-syndromic hearing loss (ARNSHL) is attributed to GJB2 mutations which are located at DFNB1 locus (13q12) in many countries or even within a particular ethnic group throughout the world. So it is necessary to determine the frequency of GJB2 mutations in any population. Several studies have mainly presented that c.35delG mutation accounts as the most frequent mutation in GJB2 gene [23].

This study revealed the frequency of 25.7% for GJB2 and the c.35delG mutation was found in more than 16.4% of ARNSHL patients in this ethnic group. These results are comparable with the results of close ethnicity from Turkey.

The low contribution of GJB2 mutations and the presence of heterozygous for GJB2 emphasize the necessity of extending the investigations into the other regions of DFNB1 locus outside the coding region. Presence of the near to 50% of the studied individuals with 35delG mutation belong to non-consanguineous marriages may point the rate of the 35delG mutation in hearing population is quite high.

The c.IVS1 + 1G > A was the second most prevalent mutation (1.9%). This should be noted that different results have been reported in various populations [8,10,16–20] and in some populations like Yakut population the large number of the patients were homozygous for c.IVS1 + 1G > A mutation. The frequency that we found was similar to Turkish population (1.4%) [17] and significantly lower than that reported in Hungry, Czech, Yakut population in Sakha republic (Russian federation) and India [16,18,19]. However the c.IVS1 + 1G > A mutation in homozygous state is not prevalent in many studied populations [20]. One of our patients was found to be homozygous for this mutation.

With respect to the less common mutations, the third significant mutation in our cohort was c.delE120 which presents in 1.3% of the pathogenic alleles. This mutation was found in 8 patients (4 homozygous and 4 heterozygous states). This frequency was lower than that reported in Turkey (2.1%), the neighbor country which is closely related to our studied ethnic. Moreover, the prevalence of c.delE120 mutation is comparable to prior report in Iran (1%) [24]. The other widespread mutations c.167delT, V37I, 235delC, W24X, R143W and V27I were present in 6.8% of the positive genotypes. The TBM mutation was distinguished in a patient with the V153I which is similar to the result of previous study in Argentina. This result suggests the association of these two variants. Furthermore, our finding revealed four mutated patients for both p.V27I and p.E114G changes, demonstrating that these two mutations take place in cis. This result hints that the p.V27I is on the same chromosome which carries the p.E114G. Moreover, existence of another mutation in trans is necessary to accompany with p.V27I + p.E114G that lead to significant impairment [22].
Fig. 1. Connexin 26 mutations in screening of 508 patients with presumed autosomal recessive non-syndromic hearing loss in northwest of Iran.

There are several reasons to explain the remaining negative genotypes: (1) existence of the unidentified mutations in non-coding regions such as the promoter region or non-coding exon 1 and (2) it is likely that the mutated allele may be present in a different gene.

Discovery of the large deletions which involved the GJB6 gene explained up to 50% of the heterozygous for GJB2 mutations in different population [8]. Although there were no del(GJB6-D13S1830) mutations among Iranian patients, this means that our findings support the previous publications from Iran [13,24,25] indicating that the del(GJB6-D13S1830) mutation is confined to some certain population and could be due to a founder effect.

Assuming near-complete penetrance, GJB2 mutations considered being involved in 31% of the Iranian patients with ARNSHL. Nevertheless, the origin of the remaining 69% has stayed evasive which should be found in next studies.

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References


