

Deafness –Associated Connexin 26 Gene (GJB2) Mutations in Iranian Population

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Abstract

Mutations in the GJB2 gene at the DFNB1 locus on chromosome 13q12 are associated with autosomal recessive non syndromic hearing loss (ARNSHL) in many populations. 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 this study we have investigated the prevalence of the GJB2 gene mutations using direct sequencing in 43 presumed ARNSHL subjects from 34 families in an Iranian population. Eleven different genetic variants were identified. GJB2-related deafness mutations (35delG, 235delC, W24X, R184P and IVS1+1G>A) were found in 9 of 34 families (26.5%). The 35delG was the most common mutation found in 5 of 34 families (14.7%). We found one novel variant (-3517G>A) in the upstream region to the gene. The mutation frequency found in this study is lower than other ethnic groups with European ancestry, but it is indicating that mutation in GJB2 in Iranian population has contribution to ARNSHL. We have also developed a simple and accurate nested PCR assay to screen the 35delG mutation in 250 unrelated unaffected Iranian individual (controls). No 35delG heterozygous was found in the control population.

KEY WORDS: Connexin 26, GJB2, Deafness, Iran, Autosomal recessive non syndromic hearing loss

INTRODUCTION

Congenital deafness is a frequent disorder that affects 1 in 1000 neonates of which 50% is the result of genetic factors (15, 17). About 80% of the hereditary deafness cases are non-syndromic and are inherited in an autosomal recessive mode (25). Mutations in the connexin 26 (GJB2) genes (GJB2; MIM#121011) at the DFNB1 locus (DFNB1; MIM#220290) on chromosome 13q12 are associated with ARNSHL in many populations (6, 10, 16). 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 (7, 8, 10, 11). The 35delG mutation is less frequent or even absent in other ethnic groups with other common mutations prevailing such as 235delC in the Japanese and Korean (1, 13, 20) 167delT in the Ashkenazi Jews (16, 26) and R143W mutation in an African village (4). In the present study we have identified the spectrum and prevalence of connexin 26 mutations using direct sequencing technique of both coding and non-coding exons of this gene. We have also developed a simple and accurate method to screen the Iranian population for the 35delG mutation.

MATERIALS and METHODS

Two groups of individual containing 250 anonymous blood donors (controls) and 43 patients with presumed ARNSHL from 34 families were studied. Medical history and pedigree information were obtained by a questionnaire. The patients were students attending a school for hearing-impaired in Tehran and their siblings between age of 3 to 32 (mean: 14.5 years). All parents had normal hearing with one or more affected

children. There was no evidence of any obvious syndrome or dominant family history. All patients had mild to profound sensorineural hearing loss. A relatively high level of consanguinity (73%) was seen in the families studied. All the families were informed and consent was obtained in all cases. Blood samples from patients and controls were prepared in dried blood spots. DNA was extracted following a standard phenol chloroform procedure. Control genomic DNA samples containing the 35delG mutation were kindly provided by Rohan Taylor (S. W. Thames Regional Genetic Services). Using a nested PCR procedure, the one base pair deletions of 35delG were screened in 250 controls and all the members of 34 patient's families. The entire coding sequence of cx26 gene (Genbank accession#M86849) was amplified using primers CX148F2 5'CCTGTGTTGTGTGCATTTCGTC3' / CX929R3 5'CTCATCCCTCTCATGCTGTC3' (782 bp) at an annealing temperature of 59°C. The amplified product was then diluted and used as a template for a second round of PCR using primers CX210F4 5'CACGCTGCAGACGATCC3' / CX252R4 5'GGTGGAGTGTTCAC3'(43 bp) at an annealing temperature of 54°C. The amplified products were then separated by electrophoresis on a 15% Polyacrylamide gel (40% 19:1 Acrylamide: Bisacrylamide) at 35 mA for 2:30 hours and the products were then visualised by silver staining. The 35delG mutations were detected by identification of two separate bands of 43 bp for the wild type and 42 bp for the mutant allele. Two sets of primers were used to produce the template for sequencing in both coding and non-coding (Genbank accession#U43932) regions. The first pair of primers CX148F2 / CX929R3 (as described above) was used to amplify the entire coding region of the gene. The second pair of primer CX1197F1 5'AGGCGGCGCTCGGGGTAAC3' / CX1679R1 5'TCCCCGCGCCAGGTTCTG3' (483 bp) was used to

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amplify the non-coding, the flanking donor splicing site and upstream region to the gene at annealing temperature of 64°C. The same primers were used for sequencing of the gene in both directions. In addition two internal primers CX586R2 5'CTTCGATGCCGACCTTCTGG3' and CX482F3 5'TGGCCTACCGGAGACATGAG3' were used for sequencing of the coding region. Sequencing was carried out using an ABI Big Dye Terminator on an ABI 377 automated sequencer.

RESULTS

The initial screening using a nested PCR found no 35delG heterozygous mutation in the control subjects, but identified this mutation in 5 out of 34 ARNSHL families studied. All of the detected 35delG mutations then were confirmed by sequencing. To investigate other mutations in the GJB2 gene, sequencing of the whole coding and non-coding region of the gene was carried out and altogether 11 different genetic variants were identified (Table 1). All of these variants except -3517G>A (Fig. 1) have been described elsewhere (21, 24). GJB2-related deafness mutations (35delG, 235delC, W24X, R184P and IVS1+1G>A) were found in 9 ARNSHL families (26.5%). Three GJB2-related deafness mutations including 35delG, 235delC and R184P were homozygous in 7 families (20.6%). The homozygous 35delG was the most common mutation accounting for 55.5% of all GJB2-related deafness mutations found in 5 out of 34 families (14.7%). In addition one

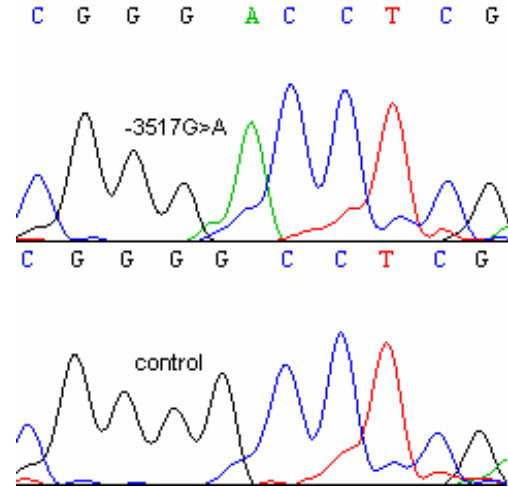


Figure 1. Nucleotide sequence of the novel variant (-3517G>A) compare to control.

genetic variant with unknown relation to the deafness (M163V) and four polymorphisms (V153I, S86T, R127H, and -3558T>C) were found in this study. We also found one novel variant (-3517G>A) in upstream region to the gene. Eight samples of the control population fully sequenced. All showed S86T variant, 37.5% (3/8) showed -3558T>C variant and 12.5% (1/8) showed the -3517G>A variant.

Table 1 Genetic variants in the GJB2 gene identified in Iranian ARNSHL families

Name of variant	Nucleotide change	Second variant ^a	Chromosome n (%) ^b	Family No
-3558T>C	T to C at -3558	Yes	21(31)	15
-3517G>A	G to A at -3517	Yes	4(6)	3
IVS1+1G>A	G to A at +1 from exon 1	No	1(1.5)	1
35delG	Deletion of G at 35	Yes	10 (15)	5
W24X	G to A at 71	No	1(1.5)	1
235delC	Deletion of C at 235	Yes	2(3)	1
S86T	GC to CG at 257-8	Yes	68 (100)	34
R127H	G to A at 380	No	1(1.5)	1
V153I	G to A at 457	Yes	6(9)	5
M163V	A to G at 487	Yes	2(3)	1
R184P	G to C at 551	Yes	2(3)	1

^aSecond variant identified in other allele of cx26 at least in one patient.

^bNo and percentage of chromosomes from 34 ARNSHL families (68 chromosomes).

DISCUSSION

We have developed a nested PCR to determine the frequency of 35delG mutation in Iranian population. Using this procedure both the mutant (42 bp) and wild type (43bp) alleles were separated and visualised in a polyacrylamide gel. The 35delG mutation is a deletion of a single guanine residue within a stretch of six Guanines between nucleotide position of 30-35 in the coding region of GJB2 gene. The 43-bp sequence of the nested PCR containing a stretch of eight nucleotides (TGGGGGGT) in the position of 29-36 between the position of two forward and reverse primers. Using nested PCR, 35delG mutation was detected in the stretch of six Gs plus two flanking Ts. It means that, the deletion of the flanking T in the stretch of eight nucleotides (TGGGGGGT) can be mistaken with the 35delG mutation and decreases the specificity of the method. However, no 29delT and 36delT have been reported so far. The same stretch of 8 nucleotides has been used as a target sequence for PCR-mediated site-directed mutagenesis followed by BsiYI digestion to detect the 35delG mutation (27). We found the nested PCR suitable to detect 35delG in different source of DNA particularly spotted blood (data not shown). The sensitivity of the nested PCR method is also very high (14) and there is no probability of missing a mutation due to non amplification. However, we found nested PCR a very accurate and reliable method to detect the 35delG mutation and may find widespread use in DNA diagnostics of ARNSHL subjects. This screening method determined 35delG mutation in 5 families (14.7%) and all were confirmed by direct sequencing.

Mutation in 35delG is responsible for 10% of all childhood hearing loss and for 20% of all childhood hereditary hearing loss in Caucasian American originated from northern and southern Europe (12). Our finding represented a lower rate of 35delG mutations. No 35delG heterozygote carriers were identified in the control group of 250 blood donors. A low frequency or absence of the 35delG carriers have been reported in Omani, Pakistani, Turkish, British Asian, Palestinian and Japanese populations (1, 3, 5, 22, 23, 24). The rare carrier frequency of 35delG could be due to the decreased prevalence of this mutation outside the European ancestry and the contribution of other mutations depending on the ethnic origins. It could be also due to ascertainment and relatively small size of the control population studied.

Eleven different genetic variants were identified, all of which, except (-3517G>A) have been described previously. Among them the GJB2-related deafness mutations have been determined as 35delG, 235delC, W24X, R184P and IVS1+1G>A. Also four polymorphisms (V153I, S86T, R127H, and -3558T>C) and one variant with unknown relation to the deafness (M163V) were determined in this study. We also found one novel variant (-3517G>A) in the upstream region to the gene. The -3517G>A variant was found in 1 of 8 controls studied and comparing this variant with Genbank (2). The same sequence was found in ref / NT_009917.8 / Hs13_10074 which makes it likely to be a polymorphism in this nucleotide position.

Najmabadi and coworkers have recently analysed GJB2 gene mutations in 168 Iranian ARNSHL subjects from 83 families.

They used an allele-specific PCR to screen the 35delG and pre screening method of SSCP followed by sequencing to screen the other variants in the GJB2 gene. They found 9 different variants and 9 of 83 families (11%) with homozygous and compound heterozygous GJB2-related deafness mutations. They identified 55% of homozygosity in GJB2-related deafness mutations (19). Using a direct sequencing strategy in the present study, we found a higher rate of 11 variants and 7 of 34 families (20.6%) with homozygous GJB2-related deafness mutations. Our study identified 60% of homozygosity in GJB2-related deafness mutations. The high level of homozygosity in this study could be due to the consanguinity or ascertainment in the population studied. The present study was carried out on a population with consanguinity of 73%. This rate of consanguinity is relatively high, compare to the rate of 37.3% consanguinity reported in the Iranian population (9). However no data of consanguinity has been given by Najmabadi and coworkers.

The frequency of congenital deafness is 1 in 1000 neonates of which 50% is the result of genetic factors (15, 17) and about 80% of the hereditary deafness cases are recessive non-syndromic (25). Our finding indicated GJB2-related deafness in 9 of 34 ARNSHL families (26.5%). These data suggest that about 13% of all congenital hearing loss is caused by mutation in GJB2. The contribution of the GJB2 gene in ARNSHL in Iran (26.5) is lower than many populations studied, such as 33% in Lebanon (18) 40% in USA (12) and 49% in Italy and Spain (8). However, it is indicated that mutations in GJB2 in Iranian population contribute to recessively inherit non-syndromic hearing loss. The reason of deafness for the remaining 73.5% ARNSHL families in Iran remains to be studied. The Iranian population is composed of several ethnic groups and more work on the ethnic population basis is needed. Finally, since there is a contribution of mutation in GJB2 in Iranian population, screening of the GJB2 mutations particularly 35delG can be offered to individuals with congenital deafness.

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