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Two Novel Mutations and Predominant 35delG Mutation in the Connexin 26 Gene (GJB2) in Iranian Populations

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Abstract

Mutations in the GJB2 gene encoding Connexin 26 (Cx26) protein are a major cause for autosomal recessive non syndromic and sporadic deafness in many populations. In this study we have investigated the prevalence of the GJB2 gene mutations using nested PCR pre screening strategy and direct sequencing method. Two hundred and sixty autosomal recessive non syndromic and sporadic deaf subjects from 199 families in two provinces of Iran (Gilan and Khorasan) were studied. Altogether 14 different genetic variants were identified from which 2 were novel variant (327delG+G109G and 431insC). Eight GJB2 mutations including 35delG, 235delC, W77X, R127H, M34T, V27I+E114G, L90P and delE120 were also found in 54 of 199 families (27%). Four polymorphysms V27I, S86T, V153I and G160S also were detected. Thirty two of 199 families were observed to have GJB2 mutations in both alleles (16%). The most common mutation was 35delG so that 43 out of 55 GJB2 mutations (78.2%) contained 35delG mutation.

Keywords: *Connexin 26, GJB2, Deafness, ARNSHL, Iran*

Introduction

Hearing impairment is one of the most common disorders that affect 1 infant per 1000 born with at least 60% of inherited cases (1-3). The most frequent form of hearing loss is autosomal recessive non-syndromic hearing loss accounting for about 80% of the hereditary deafness cases (4, 5). It is estimated that more than 100 genes to be involved in hearing loss (6, 7). While it was assumed that these large number of genes had the equally rare role in causing genetic deafness, surprisingly mutations in one gene (GJB2) showed to be responsible for about 50% of genetic cases in many populations. Mutations in the connexin 26 (GJB2) gene (GJB2; MIM# 121011) at the DFNB1 locus (DFNB1; MIM#220290) on chromosome 13q12 are associated with autosomal recessive and sporadic non- syndromic hearing loss in different populations (8-11). 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(6, 10,12,13).

However there are other mutations other than 35delG which are prevailing in different ethnic groups (5, 8, 14-18). In the present study we have identified the spectrum and prevalence of

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connexin 26 mutations in 260 autosomal recessive and sporadic non syndromic deaf subjects from 199 families in two provinces of Iran (Gilan and Khorasan) using nested PCR prescreening strategy and direct sequencing technique of coding exon of the gene.

Materials and Methods

The patients were students attending schools for hearing-impaired and their siblings between age of 2 to 35 (mean: 13.8 years) in 2 provinces, Gilan in north and Khorasan in east of Iran. The subjects with presumed autosomal recessive and sporadic non- syndromic hearing loss from 199 families were studied. Medical history and pedigree information were obtained by a questionnaire.

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 moderate to profound sensorineural hearing loss. A relatively high level of consanguinity (70%) was seen in the families studied.

All the families were informed and consent was obtained in all cases. Five mls of peripheral blood samples from patients were obtained in EDTA. DNAs were extracted following a standard procedure.

Pre screening of 35delG mutation and direct sequencing of Cx26 coding exon were performed according to the previously described procedure (19).

Results

Two hundred and sixty autosomal recessive and sporadic non syndromic deaf subjects from 199 families in two provinces of Iran (Gilan and Khorasan) were studied. The pre screening strategy using a nested PCR found mutation of 35delG in 43 out of 199 autosomal recessive and sporadic non- syndromic deaf families. All of the detected 35delG mutations then were confirmed by sequencing. To investigate other mutations in the GJB2 gene, sequencing of the whole coding region of the gene was carried out and altogether 14 different genetic variants were identified (Table 1).

Table 1: Genetic variants in the GJB2 gene identified in Iranian autosomal recessive and sporadic non syndromic deaf families (compare to Genbank accesion#M86849)

Genotype	Gilan families No. (%)	Khorasan families No. (%)	Total
35delG/35delG	18(20.7)	11(10)	29
35delG/wt	11(12.6)	3(2.7)	14
V27I/wt	1(1.2)	5(4.5)	6
V27I+E114G/wt	1(1.2)	3(2.7)	4
M34T/wt	-	1(0.9)	1
W77X/W77X	-	1(0.9)	1
235delC/235delC	-	1(0.9)	1
S86T/S86T	87(100)	112(100)	199
L90P/ DelE120	-	1(0.9)	1
327delG+G109G/ 327delG+G109G	1(1.2)	-	1
R127H/wt	-	3(2.7)	3
431insC/431insC	1(1.2)	-	1
V153I/wt	4(4.6)	5(4.5)	9
G160S/wt	1(1.2)	-	1

Eight GJB2 mutations including 35delG, 235delC, W77X, R127H, M34T, V27I+E114G, L90P and delE120 were found in 54 of 199 families (27%). Four polymorphysms V27I, S86T, V153I and G160S also were found. Two novel variants (327delG+G109G and 431insC) were also detected (Fig 1). Thirty two of 199 families were observed to have GJB2 mutations in both alleles (16%). The most common mutation was 35delG so that 43 out of 55 GJB2 mutations contained 35delG mutation (78.2%).

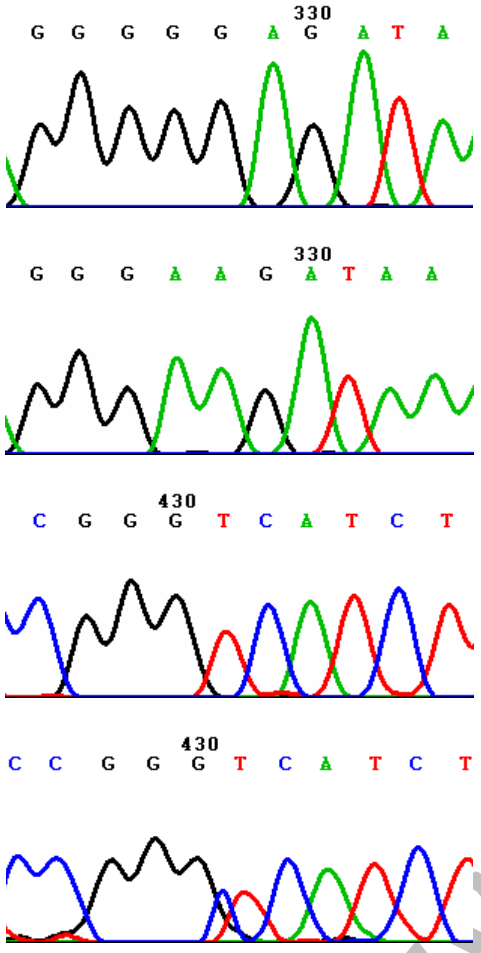

Fig. 1: Nucleotide sequence of the novel variants (327delG+G109G and 431insC) compare to controls

Discussion

Eighty seven deaf families from Gilan province of northern Iran were investigated for mutations of Connexin 26.Two GJB2 mutations including 35delG and V27I+E114G and four polymorphysms V27I, S86T, V153I and G160S were found. Surprisingly only one GJB2 mutation including 35delG was detected in 27% of deaf familie's chromosomes.

We also found 2 novel homozygote variants 327delG+G109G and 431insC in that population. The first variants, a 327delG + G109G con- taining 2 homozygous change occurs in the second intracellular domain (IC2) of the Cx26 protein. The second variant, 431insC occurs in third transmembrane domain (TM3). Both of the novel variants are homozygous, result in frame shifts and may be disease related. However a variant is supposed to be a recessive inherited mutation if there is either homozygosity or a second mutation in the other GJB2 allele noted. The nature of the amino acid changes, the degree of evolutionary conservation and observed segregation in deaf families with hearing loss could give a first indication referring to the importance of a detected mutation (12, 20).

Eleven different genetic variants were identified from Khorasan province of eastern Iran. Eight GJB2 mutations including 35delG, 235delC, W77X, R127H, M34T, V27I+E114G, L90P, delE120 and 3 polymorphysms V27I, S86T, V153I were detected. The most common mutation was also 35delG but in 11.2% of deaf families chromosomes of Khorasan.

Altogether 14 different genetic variants were identified from which 2 were novel variant (327delG+G109G and 431insC). Eight GJB2 mutations including 35delG, 235delC, W77X, R127H, M34T, V27I+E114G, L90P and delE120 were found in 54 of 199 families (27%).Four polymorphisms V27I, S86T, V153I and G160S also were detected. Thirty two of 199 families were observed to have mutations in both alleles (16%) of Connexin 26. The most common mutation was 35delG so that 43 out of 55 GJB2 mutations contained 35delG mutation (78.2%). 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 (21). Our finding partly represented a lower rate of 35delG mutations accounting for 11.2% of the deaf families chromosomes in Khorasan province. This lower rate of 35delG mutations is in agreement with the data having been reported previously in some populations (15, 22- 26). A part of our finding represented a higher rate of 35delG mutations accounting for 27% of the deaf families chromosomes and about 100% of GJB2 mutations in Gilan province. We suggest

that this high rate of allele frequency for the 35delG in Gilan province is approximately the same as most European, North American and Mediterranean populations (6, 10, 12, 13). According to my knowledge this is the highest rate of 35delG mutations reported from Iran so far (19, 27).

In our previous study, we have analysed GJB2 gene mutations in 43 Iranian autosomal recessive non syndromic hearing loss subjects from 34 families. Using the same methodology (nested PCR pre screening strategy and direct sequencing method) in coding and non coding region of the gene, we found 11 different variants and 7 of 34 families (20.6%) with homozygous GJB2-related deafness mutations. We identified 60% of homozygosity in GJB2-related deafness mutations (19).

In the present study we found a lower rate of 16% (32 0f 199 families) with homozygous and compound heterozygous GJB2-related deafness mutations. The fact that only coding region of the Cx26 gene has been analysed in this study might account for the lower rate of mutations in both alleles. Our study identified about 59.3% 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 70%. This rate of consanguinity is relatively high, compare to the rate of 37.3% consanguinity reported before (19, 28). The frequency of congenital deafness is 1 in 1000 neonates of which 50% is the result of genetic factors (1, 2) and about 80% of the hereditary deafness cases are recessive non-syndromic (5). Our finding indicated GJB2-related deafness in 54 of 199 deaf families (27%). These data suggest that about 13.5% of all congenital hearing loss is caused by mutation in GJB2. The contribution of the GJB2 gene in autosomal recessive and sporadic non syndromic hearing loss in Iran is lower than many populations studied (13, 21, 29). In agreement with our previous work, it is indicated that mutations in GJB2 in Iranian population contribute to recessively inherit non-syndromic hearing loss. 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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