

Short Communication

## Contribution of *GJB2* mutations and Four common DFNB loci in autosomal recessive non-syndromic hearing impairment in Markazi and Qom provinces of Iran

Abdorrahim Sadeghi<sup>1,2,3</sup>, Mohammad Hossein Sanati<sup>\*2</sup>, Fatemeh Alasti<sup>2</sup>, Morteza Hashemzadeh Chaleshtori<sup>4</sup>, Saeid Mahmoudian<sup>5</sup>, Mitra Ataei<sup>2</sup>

<sup>1</sup>Department of Genetics, Faculty of Basic Sciences, Tarbiat Modarres University, P.O. Box 1411-5317, Tehran, I.R. Iran <sup>2</sup>Department of Medical Genetics, National Research Institute of Genetic Engineering and Biotechnology, P.O. Box 14965/161, Tehran, I.R. Iran <sup>3</sup>Medical and Molecular Research Center, Arak University of Medical Sciences, P.O. Box 646, Arak, I.R. Iran <sup>4</sup>Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, P.O. Box 571, Shahrekord, I.R. Iran <sup>5</sup>Research Center of ENT-HNS, Iran University of Medical Sciences, P.O. Box 14455-364, Tehran, I.R. Iran

### Abstract

This study aimed to investigate the contribution of four common DFNB ("DFN" for deafness and "B" for autosomal recessive locus) loci and *GJB2* gene mutations (exon 2) in hearing impairment in individuals living in Markazi and Qom provinces of Iran. Forty consanguineous Iranian families with at least three affected individuals in family or pedigree who suffer from an autosomal recessive non-syndromic congenital hearing impairment were the subjects of this study. Blood samples were taken from both hearing and non-hearing individuals, DNA was extracted and amplified by using specific primers for the coding region of *GJB2* gene (exon 2). The PCR product of *GJB2* gene was then sequenced. Also short tandem repeat (STR) markers amplified by using specific primers for loci DFNB2, DFNB3, DFNB4 and DFNB21. At least 2 microsatellite markers (STR) for each DFNB locus exceeding to 4-6 markers for the linked families were used. The amplified markers were analyzed by conventional Polyacrylamide Gel Electrophoresis followed by silver staining. Six families were homozygous or compound heterozygous for *GJB2* mutations and were excluded from further studies. Linkage analysis was carried out for the remaining 34 families by genotyping the flanked STR markers of DFNB2, DFNB3, DFNB4 and DFNB21 loci. Six families showed linkage; includ-

ing one family to DFNB2, two families to DFNB3 and three families to DFNB4 locus while no family showed linkage to DFNB21 locus. Undoubtedly, the best understanding of the genetic basis of hearing loss in Iranian population will be achieved by performing similar experiments in other provinces and also by analyzing more loci.

**KeyWords:** Hearing impairment; Autosomal recessive non-syndromic hearing loss (ARNSHL); *GJB2*; Linkage analysis; Short Tandem Repeat (STR)

Congenital deafness is the most prevalent sensorineural disorder that affects one in 1000 neonates with 50% genetic basis (Kalay *et al.*, 2005; Ramshankar *et al.*, 2003). Hereditary deafness is a genetically heterogeneous disorder that is classified as non-syndromic (70%) and syndromic. Non-syndromic hearing impairment can be further subdivided by the mode of inheritance. The majority of the non-syndromic cases (77%) show autosomal recessive inheritance while 22% are autosomal dominant and only 1% is X-linked or due to mitochondrial mutations (Petersena and Willemsb 2006; Mukherjee *et al.*, 2003). More than 100 genes are estimated to be involved in hearing impairment and to date about 130 loci have been described in previous studies and 47

\*Correspondence to: Mohammad Hossein Sanati; ph.D.  
Tel/Fax: +98 21 44580346  
Email: m-sanati@nigeb.ac.ir

relative genes have so far been mapped (<http://webh01.ua.ac.be/hhh/>). The different loci for numerous forms of deafness have been called DFN (for deafness) and are numbered in chronological order of discovery. Autosomal dominant forms have been designated as DFNA, autosomal recessive loci as DFNB and X-linked as DFN (Mukherjee *et al.*, 2003).

Despite this heterogeneity, up to 50% of prelingual recessive non-syndromic hearing loss (ARNSHL) can be attributed to mutations in *GJB2* in many populations (Petersena and Willems, 2006). Consanguinity in the Iranian population is highly prevalent (Saadat *et al.*, 2004), so research on ARNSHL is much recommended (Hashemzadeh Chaleshtori *et al.*, 2007; Najmabadi *et al.*, 2005). The main purpose of this study was to identify the contribution of 4 common DFNB loci (DFNB2, DFNB3, DFNB4 and DFNB21) and *GJB2* gene mutations (exon 2) in hearing impairment of the Markazi and Qom provinces of Iran. Questionnaires were distributed in deaf schools, hearing loss centers and rehabilitation centers in Qom and Markazi provinces and were collected after being filled by the parents. Forty families were selected for this study. All of the studied families in this research have consanguineous marriages with the autosomal recessive mode of inheritance. These families have at least 3 deaf individuals in their pedigree. All affected individuals of this study suffer from a prelingual autosomal recessive hearing impairment with no other

associated clinical findings and no history suggestive of any other possible etiology.

Informed forms were provided for each adult individual of this study (for the individuals under 18 years old, permission was taken from their parents). DNA was extracted from peripheral blood samples using the phenol/chloroform standard procedure (John *et al.*, 1991).

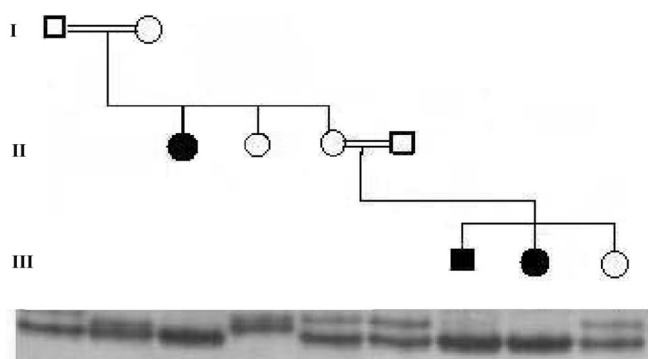
The coding exon of *GJB2* (Genbank accession no. M86849) was amplified using the following primer pair; Forward: 5'-GTCTCCCTGTTCTGTCTA-3' and Reverse: 5'-TCTAACAACTGGGCAATG-3'. The amplified DNA fragment of 743 bp contains the coding sequence (681bp). The PCR products of the *GJB2* gene were quality controlled on the 1.5% Agarose gel and then sequenced. Sequencing the whole coding region of this gene was carried out using a capillary automated system 3700 ABI sequencer (Macrogen, South Korea). One affected sample in each family was sequenced for both strands initially and then mutations were confirmed by sequencing another affected member of these families. DNA samples from the families without a mutation in the coding region of *GJB2* were amplified by using specific primers (Table 1). At least 2 microsatellite markers were used for each locus (Alasti *et al.*, 2008). The PCR products of the STR markers were analyzed by 12% (29:1, acryl:bis) polyacrylamide gel electrophoresis (PAGE) followed by silver staining (Fig. 1).

Altogether 354, questionnaires containing complet-

**Table 1.** The specific primers used for amplification of STR markers.

Locus	Marker	Forward (5' → 3')	Reverse (5' → 3')	Heterozygote rate	Variability in allele size (bp)
DFNB2	D11S911	CTTCTCATGCTTGACCATT	CTTCTGAACAATTGCCACAT	0.86	159-203
	D11S1789	ACCAGGAAATTGAGAACCA	TCTGGCCCAACAGAAGT	0.69	208-252
	D11S4186	CCCAGCCTTACATATTCC	GCTGATGAGCAGAGGTAG	0.79	154-175
	D11S4079	CAGCAAGATCCTGTCTCAA	CTCCTTAAAGTGGGGGAGTT	0.75	257-265
DFNB3	D17S2196	CCAACATCTAGA ATTATTCAGAATC	ATATTTCAATATTGTAACCAGTCCC	0.83	139-163
	D17S1794	GGTAGAGATGGGTTTCACCA	GTGTGTCCAGACTTTGACGA	0.66	179-189
	D17S2207	TATTCTTACCACCTCCCCTG	CAGGACCTGCTAGTGCAGG	High	256-267
	D17S2206	CTGCCTGTCCCTCCACCCACAC	CCTCCCTCCTGGACGCTCTTG	High	141-165
DFNB4	D7S501	CACCGTTGTGATGGCAGAG	ATTTCTTACCAGGCAGACTGCT	0.82	163-179
	D7S2420	CCTGTATGGAGGGCAAACCTA	AAATAATGACTGAGGCTCAAAACA	0.81	240-292
	D7S496	AACAACAGTCAACCCACAAT	GCTATAACCTCATAANAAACCAAAA	0.76	129-141
	D7S2456	CTGGAAATTGACCTGAAACCTT	ACAGGGGTCTCTCACACATATTA	0.64	238-252
	D7S2459	CAGAACTATTATTTAGGAG	TAGTAAAACCCATTTGAAC	0.77	140-152
	D7S1817	CAAATTAATGGCAAAAACCTGC	CCCCCATTGAGGTTATTAC	0.69	125-141
DFNB21	D11S925	AGAACCAAGGTCGTAAGTCCTG	TTAGACCATTATGGGGGCAA	0.85	173-199
	D11S4089	ATTCCTAGTTCCTCATAAACACTG	TAATCAAAGGCTGTAGTGAATTGG	0.75	199-213

Data in above table were extracted from Marshfield clinic (<http://research.marshfieldclinic.org/genetics/>) and Genome Data Bank (GDB) (<http://www.gdb.org>).



**Figure 1.** Genotyping of the D7S496 STR marker showing linkage for DFNB4 locus with 12% (29:1, acryl:bis) Polyacrylamide Gel Electrophoresis (PAGE) followed by silver staining. Affected individuals are homozygous for the D7S496 STR marker. Numbers under the gel represent genotypes.

ed information about hearing loss families were collected. The families studied were the result of consanguineous (59.3%) and non-consanguineous marriages (36.7%). The marriage type was not determined in 4% of the families. Mean of deaf individuals among these families was  $1.8 \pm 1.4$ . By assessing the filled questionnaires and pedigrees and by talking to the parents, the deafness etiology in the studied population was categorized as genetic (70.9%), environmental (9%) and unknown (20.1%). Forty families with ARNSHL were selected for this study. At first, these families

were analyzed for *GJB2* gene mutations (exon 2). Six families were homozygous or compound heterozygous for a mutation in *GJB2* and were excluded from linkage analysis of the four common loci. The mutations found in *GJB2* gene are represented in Table 2.

Linkage analysis using highly polymorphic microsatellite markers was performed on 34 remaining consanguineous families. The families were genotyped for microsatellite markers close to DFNB2, DFNB3, DFNB4 and DFNB21 loci. A linkage was identified in 6 families, including one to DFNB2, two families to DFNB3 and three others to DFNB4 loci, while none of the families showed linkage to the DFNB21 locus (Table 3).

Mutations in *GJB2* have been shown to be the major cause of ARNSHL in many populations (Mustafa, 2004; Bayazit *et al.*, 2003). The results of this study also show that the contribution of *GJB2* mutations is significant in familial deafness in Iran (Hashemzadeh Chaleshtori *et al.*, 2007; Najmabadi *et al.*, 2005). Deafness in 15% of these families is due to mutations in *GJB2*, which is consistent with other studies in the Iranian population (Hashemzadeh Chaleshtori *et al.*, 2007; Najmabadi *et al.*, 2005). However, this needs further assessment of the other known loci as well as searching for factors related to hereditary deafness in the Iranian population.

ARNSHL is the most prevalent type of hereditary

**Table 2.** *GJB2* genetic variants identified in Iranian ARNSHL families (compared to Genbank accession #M86849).

No.	Mutation	Description	Effect	Frequency	Percent (family)
1	35delG/35delG	del of G at 30-35	Truncated protein	3	7.5
2	35delG/W24X	del of G at 30-35/G to A at 71	Truncated protein	1	2.5
3	233delC/233delC	del of C at 233-235	Truncated protein	1	2.5
4	176-191del16*	del 16 nt at 176	Truncated protein	1	2.5

\*This mutation is reported for the first time in the Iranian population.

**Table 3.** Linkage analysis results for four DFNB loci.

No.	locus	Chromosomal location	Most Important Reference	Frequency	Percent (family)
1	DFNB2	11q13.5	Liu <i>et al.</i> , 1997 Weil <i>et al.</i> , 1997	1	2.5
2	DFNB3	17p11.2	Friedman <i>et al.</i> , 1995	2	5
3	DFNB4	7q31	Baldwin <i>et al.</i> , 1995	3	7.5
4	DFNB21	11q	Mustapha <i>et al.</i> , 1999	0	0

These data were extracted from the hearing loss homepage (<http://webh01.ua.ac.be/hhh/>).

hearing loss in non-Caucasian populations with a high frequency of consanguinity (Petersena and Willemsb, 2006; Kalay *et al.*, 2005; Mukherjee *et al.*, 2003). The consanguinity in the Iranian population is highly prevalent (Saadat *et al.*, 2004), so further research on ARNSHL is highly recommended in Iran (Hashemzadeh Chaleshtori *et al.*, 2007; Najmabadi *et al.*, 2005; Sadeghi *et al.*, 2005).

Fifteen percent of these families showed linkage to 3 ARNSHL loci. Hearing loss in the linked families is likely due to mutation in the known deafness genes in these 3 loci (*Myosin 7A*, *Myosin 15A*, *SLC26A*). Undoubtedly, the best results on the contribution of different loci to Iranian hearing loss will be achieved by arranging these experiments in other provinces and also analyzing some other loci.

Mutation in the *GJB2* gene and linkage to 3 analyzed common loci were found in 30% of studied families. Because, 59 loci for ARNSHL have already been reported (<http://webh01.ua.ac.be/hhh/>), it is likely that the linkage to one of these known loci as well as the involvement of a new genomic region can contribute to deafness in the remaining families. The next step of this work would be sequencing the known deafness genes which have already been mapped in those loci in the linked families, so as to identify the pathogenic variants in these families.

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