

# Autosomal Recessive and Sporadic Non Syndromic Hearing Loss and the Incidence of Cx26 Mutations in a Province of Iran

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## Abstract

Despite the enormous heterogeneity of genetic hearing loss, mutations in the GJB2 (connexin 26) gene located on “DFNB1” locus (13q12) account for up to 50% of cases of autosomal recessive non-syndromic hearing loss (ARNSHL) in some populations. This study describes the analysis of 100 autosomal recessive and sporadic nonsyndromic hearing loss individuals from 79 families each having at least one deaf child in Chehar Mahal va Bakhtiari province in west of Iran. We have investigated the prevalence of the connexin 26 gene mutations using nested PCR strategy to screen the predominant 35delG mutation and subsequent direct sequencing to detect other Cx26 mutations. Seven different genetic variants were detected from which one novel variant was including 363delC. The 35delG was the most common mutation found in 5 of 79 families (6.3%). Cx26 related deafness mutations (35delG, [V27I; E114G]) and R127H) were found in 12 of 158 chromosomes studied (7.8%). We conclude that the association of Cx26 mutations with deafness in Chehar Mahal va Bakhtiari province is low and looks like most other populations of Iran.

**Keywords:** *Connexin 26, GJB2, Deafness, Autosomal recessive non syndromic hearing loss, Iran*

## Introduction

Hearing loss is a common disorder affecting millions of individuals worldwide with approximately 1 in 1000 newborns and 60% of people over 70 yr of age (1). Hearing loss can be caused due to genetic or environmental factors or combination of them. The genetic hearing loss is highly heterogenous and more than 100 genes are predicted to cause this disorder in human. Recent advances in the genetic of hearing loss have improved our knowledge to detect

the etiology of several different deafness in many cases. In spite of contribution of several different genes as causative agents of deafness, mutations in one gene encoding Connexin 26 (GJB2) (GenBank M86849, MIM 121011) with chromosomal location 13q11-12 known as DFNB1 (MIM 220290) responsible for half of severe to profound autosomal recessive non syndromic deafness in many populations (2, 3). More than 100 different mutations have been reported in connexin 26 gene (4). A single base

deletion (35delG) accounts for about 70% of the Cx26 mutations in white populations (5). Here we have reported the spectrum and frequency of cx26 mutations among 100 patients with autosomal recessive and sporadic non syndromic hearing loss in Chehar Mahal va Bakhtiari province and investigated the association of Cx26 mutations with deafness.

## Materials and Methods

**Subjects** This study was a part of a research project to characterize Cx26 mutations of more than 1000 autosomal recessive and sporadic non- syndromic hearing impaired subjects in 10 provinces of Iran (6-9). Subjects to be included in this study had to meet the following criteria: (1) a pedigree structure consistent with autosomal recessive inheritance (2) both parents have normal hearing (3) one or more deaf children in the family (4) hearing loss in the absence of other clinical features (5) hearing loss was not a result of environmental factors such as infection, trauma, rubella, meningitis, mumps, ototoxic drugs and premature birth. One hundred subjects with mild to profound sensorineural hearing loss were investigated. The subjects were students of schools for deaf and their sibs between 2-30 yr of age (mean 14). Medical history and pedigree information were obtained by the questionnaire. Informed consent was obtained from all subjects or parents of under aged patients.

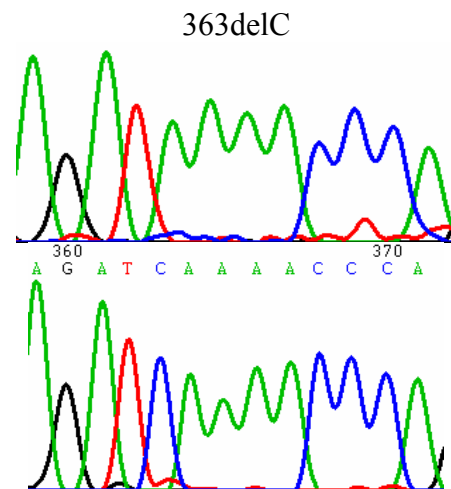
**Mutation detection** Mutation detection of the coding region of the Cx26 gene was performed using nested PCR prescreening and subsequent direct sequencing procedure. PCR and nested PCR amplifications and conditions were performed as previously described by Hashemzadeh and coworkers (8, 10). Briefly, genomic DNA was extracted from peripheral blood following the standard protocols. The whole samples were first screened for the predominant Cx26 mutations (35delG) using nested PCR strategy. Subsequently, the negative 35delG samples and those heterozygotes

for the 35delG allele were sequenced for the coding region (exon 2) of the gene.

## Results

The majority of the probands (81%) were from families with only one deaf child. Fifteen probands (19%) were from families with two or more deaf children. A relatively high level (72%) of consanguineous marriage (first cousins, first cousins once removed and double first cousins) was observed in the deaf families studied, from which first cousins marriage was the frequent one (56%).

One hundred autosomal recessive and sporadic non syndromic deaf subjects from 79 families were investigated. Altogether 7 different variants were detected. Three cx26 recessive mutations including 35delG, [V27I; E114G] and R127H were identified in 12 of 158 chromosomes (7.8%). We found also three polymorphisms (V27I, V153I and S86T) from which two first polymorphisms were detected in 4 chromosomes while S86T was found homozygous in all the cases (Table 1). One novel variant including 363delC was detected in this study (Fig. 1). The most common mutations was 35delG in 10 out of 158 chromosomes studied (6.3%).



**Fig. 1:** Nucleotide sequences of 1 novel variant detected in this study

**Table 1:** Summary of GJB2 variations detected in 79 autosomal recessive non-syndromic and sporadic hearing loss cases in Chehar Mahal va Bakhtiari province

Allele variant	No. of Chromosomes (%)	No. of family	Second variant
<b><u>Mutation</u></b>			
35delG	10	5	35delG
[V27I; E114G]	1	1	-
R127H	1	1	-
<b><u>Polymorphism</u></b>			
V27I	1	1	-
V153I	3	3	-
S86T	158	79	S86T
<b><u>Novel Variant</u></b>			
363delC	4	2	363delC

## Discussion

Apart from genetic and inherited problem, consanguinity is a common pattern of marriage all over the country (11). A consanguineous marriage seems also quite probable when two relatives are disable or suffering from a hereditary disorder. In addition, families with special hereditary disorder prefer to marry each other even they are not relative (12). Our study revealed 72% of consanguinity in deaf families which is relatively high compare to the normal population (11). This high level of consanguineous marriage between deaf families is in the range of that reported elsewhere such as 70% for Gilan and Khorasan provinces (6) and 74% for Tehran and Tabriz provinces (8). The present results revealed a low frequency (7.8% of chromosomes) of GJB2 mutations compare to similar studies reported from north of the country such as Gilan (6, 8) province (27.6% of chromosomes). However, some reports from south of the country like Sistan va Baluchistan (7, 9) province indicated a lower rate of GJB2 mutations frequency (3% of chromosomes).

According to our finding and similar studies in 9 different populations all over the country, we found a gradual decrease in the frequency of the GJB2-related deafness from north (Gilan) to south east (Sistan va Baluchistan) of the country (6-9).

Regarding the mutation types, our results showed 7 different variants from which 3 mutations (35delG, [V27I; E114G], R127H) and 3 polymorphisms (V27I, V153I, S86T) were identified. Only one novel variant including 363delC was detected in the population studied. This novel variant (363delC) was found to be homozygous. This variant results in frame shift and stop codon which are presumed to be pathogenic (5, 13).

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