

RESEARCH NOTE

GJB2 mutations in Baluchi population

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Introduction

Hearing impairment is the most common sensory disorder world-wide and affects one in 1000 newborns (Cohen and Gorlin 1995). Over 100 loci have been associated with the nonsyndromic hearing loss, with the majority of the cases (~80%) having autosomal recessive pattern of inheritance (Cryns *et al.* 2004). To date, up to 60 loci for autosomal recessive nonsyndromic deafness have been identified (<http://web01.ua.ac.bc/hhh/>), indicating it as an extremely heterogeneous disorder. DFNB1 was the first locus incriminated in autosomal recessive deafness; two genes have been associated with this locus: *GJB2* and *GJB6* encoding gap-junction proteins connexin 26 and connexin 30, respectively. Despite this heterogeneity, up to 50% of prelingual recessive nonsyndromic deafness can be attributed to mutations in *GJB2* (Denoyelle *et al.* 1997; Green *et al.* 1999). More than 90 different mutations of the *GJB2* gene have been reported. Many are 'private' mutations, having observed in only one or few pedigrees, although very common alleles have also been identified in several populations including the 35delG allele in Caucasians, 167delT allele in Ashkenazi Jews, 235delC allele in east Asian population, and R143W mutation in Ghana (Nance 2003). Although a broad spectrum of recessive deafness mutation in the *GJB2* gene is known, one particular mutation, the 35delG is very frequent in populations of European origin (Gasparini *et al.* 2000). Recently, a deletion Δ (*GJB6*-D13S1830), truncating the *GJB6* gene was shown to be the accompanying mutation in 50% of deaf persons heterozygous for only one *GJB2* mutant allele in a cohort of Spanish patients.

Because population-specific differences are common, in this study we sought to determine the prevalence and spectrum of *GJB2* mutation in the deaf Baluchi and Sistani population in Sistan and Baluchistan province in southeast of Iran. Sistan and Baluchistan province is bound to the north by Khorasan province and Afghanistan, to the east by Afghanistan and Pakistan, to the south by the sea of Oman and to the west by Kerman province. Consanguinity and assortative mating are very common in these populations.

Materials and methods

One proband from each family was examined by our clinicians, using audiologic testing confirming existence of hearing loss, and syndromic cases were excluded during clinical examinations. Genetic counselling was performed based on which a pedigree was drawn for each family. A consent approved by ethical committee of the University of Social Welfare and Rehabilitation Sciences was signed by all participating patients. From each patient 10 ml of blood sample was taken and DNA extraction was performed according to standard protocols (Miller *et al.* 1988). All procedures were approved by the Institutional Review Board of the Social Welfare and Rehabilitation Sciences University, Tehran, Iran, and the University of Iowa, USA.

GJB2 mutation screening began with an allele-specific polymerase chain reaction (ASPCR) assay to screen all subjects for the 35delG mutation using previously described primers (Scott *et al.* 1998). Samples negative or heterozygous for 35delG mutation, were screened for other *GJB2*

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mutations by denaturing high performance liquid chromatography (DHPLC) analysis followed by directed sequencing if elution profiles were not normal. After exon 1 screening for cases which had heterozygous mutations in exon 2, all individuals from whom only one deafness-causing allele variant was identified were screened for the $\Delta(GJB6-D13S1830)$ and $\Delta(GJB6-D13S1854)$ mutations using PCR primers that amplified the breakpoint junction of this deletion (del Castillo *et al.* 2002, 2005).

Results

In this study, DNA samples of 100-unrelated patients with an autosomal recessive nonsyndromic deafness (ARNSD) from endogamous and inbred populations of two different ethnic groups (45 Baluchis and 55 Sistani) from Sistan and Baluchistan province in southeast of Iran were screened for *GJB2* mutations. *GJB2* deafness causing allele variants were diagnosed in 10 chromosomes among Baluchi patients (11%). These mutations were W24X and R127H. In four families, deaf persons were homozygous for W24X mutation. Table 1 shows the genotypes found in this population.

Table 1. Genotypes found in Sistani and Baluchi populations.

Sistani genotypes	Baluchi genotypes
R127H/R127H	R127H/wt (2 families)
167delT/167delT	W24X/W24X (4 families)
W24X/W24X	
M93I/wt	
K122I/wt	

Also *GJB2* mutations were found in eight chromosomes among Sistani subjects (7.2%). Interestingly, we found M39I mutation in one family and K122I in another. These two mutations have not been reported previously in Iranian population. Table 1 shows the genotypes found in Sistani population. In addition to mutations that are mentioned, three polymorphisms were found in study subjects, V153I was detected in both groups, whereas V27I and E114G were found only in Sistani probands. Taken together *GJB2*-related deafness was diagnosed in seven families (7%) of the study participants and frequency of *GJB2* mutant alleles in two population studied was 18% (table 2). $\Delta(GJB6-D13S1830)$ was not detected in patients screened for this mutation.

Discussion

Mutations in *GJB2* are the most common cause of hereditary congenital hearing loss in many countries (Zelante *et al.* 1997; Kelley *et al.* 1998; Morell *et al.* 1998; Scott *et al.* 1998; del Castillo *et al.* 2002), accounting for about half of all moderate-to-profound deafness in many world populations (Sobe *et al.* 2000). One mutation, the 35delG allele

Table 2. Frequency of *GJB2* deafness-causing alleles among the study populations.

Mutation	No. of alleles in Baluchis	No. of alleles in Sistanis	Total no. of alleles
W24X	8	2	10
R127H	2	2	4
167delT	-	2	2
M93I	-	1	1
K122I	-	1	1
Total	10	8	18

variant, is the most common in population of northern European ancestry. A very high frequency of the 35delG mutation in Spanish, Italian and Israeli ARNSD patients is also found (Morell *et al.* 1998). This suggests that this ancient deletion mutation has spread in Europe and Middle East. Surprisingly, the 35delG mutation which is the most common *GJB2* mutation in white population was not found in the Japanese, Chinese and Taiwanese population, and a different mutation, the 235delC, predominates in these east Asian groups (Kudo *et al.* 2000). Although no studies have been carried out in Afghanistan, Brown *et al.* (1996) studied 27-Pakistani families segregating for ARNSD and found only one family linked to *GJB2*. On the other hand, a study of 215-Indian families with ARNSD found that W24X mutation has high frequency in this group (Ram Shankar *et al.* 2003). These data suggest that there is an ethnic bias in the contribution that *GJB2* makes to the ARNSD genetic load. The Iranian population is composed of many different ethnic groups, so it is important to generate ethnic-specific data. In this study, *GJB2*-related deafness among participating patients was 7%, as against 15% reported for the rest of Iran (Najmabadi *et al.* 2005). We identified two different deafness-causing mutations in exon 2 of *GJB2*, W24X and R127H in Baluchi population. In our study, W24X has the highest frequency among *GJB2* mutations detected in Baluchi population (80%). This mutation has also been found to be the most observed in India. (Ram Shankar *et al.* 2003).

We did not find the 35delG mutation, which is very common in European and Ashkenazi Jewish population (Estivill *et al.* 1998; Scott *et al.* 1998; Roux *et al.* 2004). It is also the most common *GJB2* deafness-causing allele in the rest of Iran (Najmabadi and Cucci 2002). However, the Baluchi population is ethnically distinct from the rest of Iran. We can conclude that: (i) W24X is a common allele among the *GJB2* alleles in southeast Iran, (ii) W24X may be a common mutation in Pakistani population, warranting further investigation, (iii) this mutation has spread in east Asia, like 35delG in Europe, and east of Iran (Baluchi group) is the end point of this distribution, and finally, (iv) further studies are needed to find the other genes that have a causal role in ARNSD in these populations. These data are compatible with other data about ethnic biases in *GJB2* mutations in different world populations. These type of biases sometimes may be impor-

tant and they reflect the limited admixture of some ethnicities and must be considered in government health care policy issues.

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