DELTA (GJB6-D13S1830) IS NOT A COMMON CAUSE OF NONSYNDROMIC HEARING LOSS IN THE IRANIAN POPULATION

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Background: Mutations in the gene that encodes the gap-junction protein connexin 26 (GJB2) at the DFNB1 locus on chromosome 13q12 are the major cause of autosomal recessive nonsyndromic sensorineural deafness (ARNSD) in many different populations. A fraction of patients with GJB2 mutations have only one mutant allele, and in some familial cases with linkage to the DFNB1 locus, no mutations in GJB2 are reported. Recently, a large deletion involving the GJB6 gene encoding connexin 30, which is also located at the DFNB1 locus (GJB6-D13S1830), has been reported to cause ARNSD in homozygotes for this mutation and in compound heterozygotes-carrying deafness-causing allele variants of GJB2 on the opposite allele. To date, different papers have been published reporting the presence or absence of this deletion in various populations.

Methods: Three hundred eighty-five probands segregating presumed autosomal recessive nonsyndromic deafness were screened for GJB2 mutations using an allele-specific polymerase chain reaction (PCR) assay to detect 35delG mutation. Direct sequencing was performed following DHPLC analysis of all patients except 35delG homozygotes. Screening for $\Delta$(GJB6-D13S1830) was completed using PCR primers that amplified the breakpoint junction of this deletion in all patients heterozygous for only one GJB2 mutation and 116 probands with normal GJB2 alleles.

Results: GJB2-related deafness was diagnosed in 70 probands (18.2%). Sixteen patients were found to carry only one GJB2 mutant allele. Additionally, we found three novel GJB2 allele variants. $\Delta$(GJB6-D13S1830) was not detected in the subjects screened for this mutation.

Conclusion: Our finding indicates that $\Delta$(GJB6-D13S1830) is not a common cause of deafness in Iran and suggests that this mutation is not widespread in the world.

Keywords: Delta(GJB6-D13S1830) • Iranian • nonsyndromic hearing loss

Introduction

Hearing impairment affects 1 in 1000 newborns and in about half of these babies, the loss has a genetic basis.1 Autosomal recessive nonsyndromic sensorineural deafness (ARNSD) is the most common form of severe inherited hearing impairment.2 To date, at least 39 loci for ARNSD have been identified,3–6 indicating that it is an extremely heterogeneous disorder. These loci are referred to as DFNB loci.

Mutations in the gene that encodes the gap-junction protein connexin 26 (GJB2) (MIM#121011) at the DFNB1 locus on chromosome 13q12 are the most common cause of ARNSD in many different populations (MIM # 220290).7–13 A second gap-junction gene, GJB6, also localizes to the DFNB1 interval. Interestingly, the encoded protein connexin 30 is
expressed in the same inner-ear structures as connexin 26 and both connexins are functionally related.\textsuperscript{14}

The importance of GJB6 to normal hearing has been confirmed by the identification of a large deletion involving the first two exons and a part of third exon of GJB6 (MIM# 604418) and a large region of the upstream sequence delta(GJB6-D13S1830) in persons with ARNSD.\textsuperscript{15} Homozygotes for this deletion and compound heterozygotes carrying \(\Delta\) (GJB6-D13S1830) and a deafness-causing allele variant of GJB2 have severe-to-profound congenital deafness. Although, there is a previous study reporting the types of GJB2 mutations in the Iranian deaf population,\textsuperscript{16} no data are available on the prevalence of \(\Delta\) (GJB6-D13S1830) in this heterogeneous population. In this study, we assessed the importance of \(\Delta\) (GJB6-D13S1830) in the Iranian population.

**Patients and Methods**

Three hundred eighty-five patients from 385 families were studied. To be included in this study,
families had to meet the following criteria: 1) hearing loss confirmed by audiologic testing; 2) hearing loss in the absence of other clinical features; 3) a pedigree structure consistent with autosomal recessive inheritance; 4) both parents with normal hearing; and 5) two or more affected family members. Pure-tone audiometry was conducted in a sound chamber. Air and bone conduction were evaluated at the frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz with intensities up to 120 dB. A ten mL blood sample was taken from each patient and DNA extraction was performed according to standard protocol. All procedures were approved by the Human Research Institutional Review Boards of the Social Welfare and Rehabilitation Sciences University, Iran University of Medical Sciences, Tehran, Iran, and the University of Iowa, Iowa City, Iowa, USA.

Genetic testing began with an allele-specific polymerase chain reaction (ASPCR) assay to screen patients for the 35delG mutation (the most frequent deafness-causing allele variant in people of northern European ancestry) using previously described primers.17 Homozygotes for the 35delG mutation were not tested further.

In patients heterozygous for the 35delG mutation, DHPLC analysis of the coding sequence of GJB2 (exon 2) was completed and complemented by direct sequencing if elution profiles were not normal. In samples with no abnormal elution profile, the noncoding exon of GJB2 (exon 1) was sequenced.

In cases in which the 35delG mutation was not detected by the ASPCR, DHPLC was also completed. If abnormal elution profiles were observed, the sample was sequenced. The noncoding exon of GJB2 (exon 1) was sequenced if a single coding sequence mutation was detected.18

After completion of GJB2 mutation screening, we screened for the Δ(GJB6-D13S1830) mutation using GJB6-1R, GJB6del, BKRdel and BKR-1 primers in all persons in whom only one deafness-causing allele variant was identified and 116 probands chosen randomly among subjects with wild type alleles. GJB6-1R and GJB6del primers generate a 325 bp fragment which indicates that the centromeric region of the gene is intact. BKRdel and BKR-1 primers generate a 160 bp fragment which indicates that the telomeric region of the gene is intact. GJB6-1R and BKR-1 primers generate a 460 bp fragment by amplification of the fused region which is made through the deletion (Figures 1A, 1B, and 2).15

Results

Homozygosity or compound heterozygosity for GJB2 deafness-causing allele variants was detected in 70 families (18.2%). A novel GJB2 allele variant (K112N) was diagnosed in compound
heterozygosity with the 35delG mutant allele in one patient. We also found 16 patients heterozygous for known GJB2 mutations (5 with delE120, 6 with 35delG, 2 with R127H, 1 with E129K, and 1 with M93I) as well as two carriers of novel GJB2 allele variants (329delA and I69I). None of the patients screened for $\Delta$(GJB6-D13S1830) was shown to carry this deletion (Table 1).

### Discussion

Although mutations in GJB2 have been shown to be the major cause of ARNSD in many populations, two other connexin genes also have been demonstrated to cause recessive non-syndromic deafness: GJA1 mutations are reported to cause ARNSD in African Americans and a large deletion involving GJB6 called $\Delta$(GJB6-D13S1830) is a common cause of deafness in southern European populations. In fact, $\Delta$(GJB6-D13S1830) has been identified as the second most frequent mutation causing prelingual deafness in the Spanish population.

The presence of $\Delta$(GJB6-D13S1830) has been reported in trans in 4 of 6 patients carrying single GJB2 deafness-causing allele variants and in the homozygous state in one deaf person in France. Likewise, compound heterozygosity for this deletion and common GJB2 mutations have been diagnosed in 7 patients from 4 unrelated Jewish Ashkenazi families with ARNSD and also in 2 Danish deaf persons. Recently, del Castillo et al presented an overview of the deletion in several countries. In addition to the deaf in Spain, UK, France, and Belgium, this mutation has been detected in the deaf in Brazil, the United States, and Australia. The Brazilian subjects were of Portuguese descent, consistent with the high prevalence of this mutation in southern Europe, and both the United States and Australia have a mixed population that includes many people originating from countries in western Europe.

Interestingly, $\Delta$(GJB6-D13S1830) has not been detected in the Chinese deaf population or the Turkish deaf population. In addition, Günther et al screened 393 persons with presumed ARNSD most of whom were of Austrian, Turkish, Serbian, or Bosnian origin, and none carried the deletion. Similar to these studies, screening for $\Delta$(GJB6-D13S1830) was negative in our population (Table 2). These data indicate that $\Delta$(GJB6-D13S1830) is not widespread and suggest a possible founder effect for this deletion in western Europe.

### Acknowledgments

We would like to thank our patients and their families for their collaboration in this study. We are also most grateful to Mrs. M. Shahmoradgoli for her tremendous help in laboratory assistance and Mr. K. Bahadori for his great help in information provision.

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