RESEARCH NOTE

The c.IVS1+1G>A mutation inthe *GJB2* gene is prevalent and large deletions involving the *GJB6* gene are not present in the Turkish population

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Introduction

Congenital hearing loss is the most common sensorial problem, affecting approximately one in 500 children. Genetic factors are believed to be causative in at least half of these children. Although significant genetic heterogeneity exists as the cause of sensorineural hearing loss, one locus, *DFNB1*, comprising the *GJB2* and *GJB6* genes, is responsible for up to 20–50% of cases with congenital nonsyndromic hearing loss in many populations (Kelsell *et al*. 1997; Kenneson *et al*. 2002). Homozygous or compound heterozygous mutations in *GJB2* are detected in most cases with *DFNB1*-related hearing loss. It was, nevertheless, evident in some studies that up to 50% of deaf subjects with *GJB2* mutations carried only one mutant allele. During the search for an accompanying mutation in these heterozygotes, a large deletion of about 342 kb (later corrected to be 309 kb), which does not include *GJB2* but truncates the neighboring *GJB6*, was discovered (Lerer *et al*. 2001; Del Castillo *et al*. 2002; Pallares-Ruiz *et al*. 2002). This large deletion, referred to as del(*GJB6*-D13S1830), was found to be located in *trans* in about 50% of Spanish individuals with nonsyndromic congenital or prelingual hearing loss with only one heterozygous mutation in *GJB2* (Del Castillo *et al*. 2002). Significant differences in the frequency of this deletion among probands with sensorineural hearing loss have been reported in different populations. It was found to be common in Spain, France, the United Kingdom, Israel and Brazil, less frequent in the United States, Belgium and Australia, and detected in only a few individuals in Germany and the Czech Republic (Gabriel *et al*. 2001; Del Castillo *et al*. 2005; Seeman *et al*. 2005). It was not found in Austria and China (Günther *et al*. 2002; Liu *et al*. 2002). It is remarkable that different frequencies have also been reported in different parts of the same country such as in Italy or in the United States (Del Castillo *et al*. 2005). We and others were not able to demonstrate this deletion in a limited number of probands from Turkey (Uyguner *et al*. 2003; Tekin *et al*. 2005).

Another large deletion encompassing 232 kb, referred to as del(*GJB6*-D13S1854), has recently been discovered at the *DFNB1* locus (Del Castillo *et al*. 2005). This deletion was found in 25.5% of the affected *GJB2* heterozygotes in Spain, 22.2% in the United Kingdom, 6.3% in Brazil, and 1.9% in northern Italy. However, it was absent in France, Belgium, Israel, the Palestinian Authority, the United States and Australia (Del Castillo *et al*. 2005).

In this study we searched for del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) in a large group of Turkish probands with sensorineural hearing loss from different parts of Anatolia.

Materials and methods

We previously screened 371 unrelated probands with congenital or prelingual-onset nonsyndromic sensorineural hearing loss for mutations in *GJB2* (Tekin *et al*. 2005). The results of these studies revealed that deafness could be explained by two sequence alterations in the single coding exon (exon 2) of *GJB2* in 73 (19.7%) probands. A total of 279 probands were negative for *GJB2* mutations and 19 (5.1%) probands were found to have only one pathogenic exon 2 *GJB2* mutation. Our screening strategy was to initially search for c.35delG with a PCR-RFLP protocol (Storm *et al*. 1999). No further tests were performed in homozygotes after this initial screening. The entire exon 2 was later sequenced in heterozygotes. Sequencing of the coding exon either following SSCP or directly was performed in all the remaining probands.

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A total of 295 probands (142 multiplex, 153 simplex) were included in this study. All probands were affected by congenital or prelingual-onset moderate to profound non-syndromic sensorineural hearing loss. Sixteen probands (12 multiplex, 4 simplex) had single exon 2 *GJB2* mutations, whereas 279 probands did not have any pathological *GJB2* alleles. Fifteen heterozygote probands had the c.35delG and one proband had the p.W24X mutations. DNA samples of the remaining three probands, who were found to be heterozygote in the initial study, were not available.

Appropriate consent forms were signed by each participant or parent before the study. The noncoding exon 1 and the promoter region of *GJB2* were sequenced in 16 probands who remained heterozygous after screening of exon 2. Forward 5′-CCG GGA AGC TCT GAG GAC -3′ and reverse 5′-GCA ACC GCT CTG GGT CTC-3′ primers were used for PCR amplification. Betaine (10%; Sigma, USA) was added in the master mix of the PCR reactions to facilitate amplification. PCR products were purified, used in cycle sequencing reactions with Beckman Coulter Cycle Sequencing Kit (Fullerton, USA), and loaded on a Beckman Coulter CEQ 2000XL automated sequencer. Results were visualized using Beckman Coulter CEQ 2000XL software. PCR reactions for del(*GJB6*-D13S1830), del(*GJB6*-D13S1854) and a wild-type *GJB6* allele were carried out by a protocol reported by Del Castillo *et al*. (2005), using primers GJB6-1R 5′-TTT AGG GCA TGA TTG GGG TGA TTT-3′ and BKR1 5′-CAC CAT GCG TAG CCT TAA CCA TTT T-3′ for del(*GJB6*-D13S1830); DelBK1 5′-TCA TAG TGA AGA ACT CGA ATG CTG TTT-3′ and DelBK2 5′-CAG CGG CTA CCC TAG TTG TGG T-3′ for del(*GJB6*-D13S1854); and Cx30Ex1A 5′-CGT CTT TGG GGG TGT TGC TT-3′ and Cx301B 5′-CAT GAA GAG GGC GTA CAA GTT AGA A-3′ to amplify *GJB6* exon 1.

PCR products were analysed by electrophoresis through a 2% agarose gel containing 3 μl ethidium bromide.

Positive control samples were included in each PCR reaction.

Results

Eight of 16 samples, which were heterozygous for an exon 2 mutation, were found to have heterozygous c.IVS1+1G>A (−3172G >A) mutation (figure. 1).

PCR screening for del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) did not show a mutant allele in 279 probands without *GJB2* exon 2 mutations in Turkey (figure 2). Screening for both deletions was also negative in eight probands who remained heterozygous after screening for *GJB2* mutations.

Discussion

Our study demonstrates that both large deletions at the *DFNB1* locus involving *GJB6* are not present in a large representative series from Turkey. Our previous screening for *GJB2* mutations in Turkey revealed that 19 in 371 (5.1%) unrelated probands with sensorineural hearing loss remained heterozygous for a *GJB2* exon 2 mutation. Further screening of these samples in this study shows that eight out of 16 *GJB2* heterozygous samples are indeed compound heterozygous with the c.IVS1+1G>A (−3172G>A) mutation. As a result, the heterozygosity rate of *GJB2* mutations

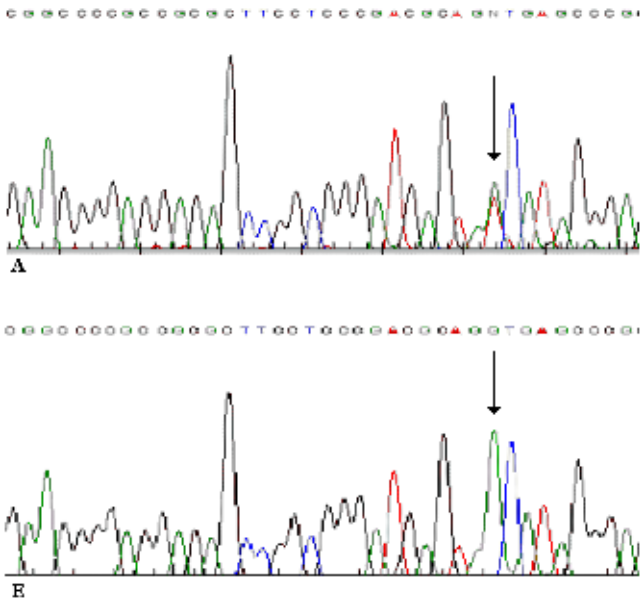

Figure 1. (A) Heterozygous c.IVS1+1G>A (−3172G>A) mutation; (B) wild-type allele. Arrows indicate the mutation point.

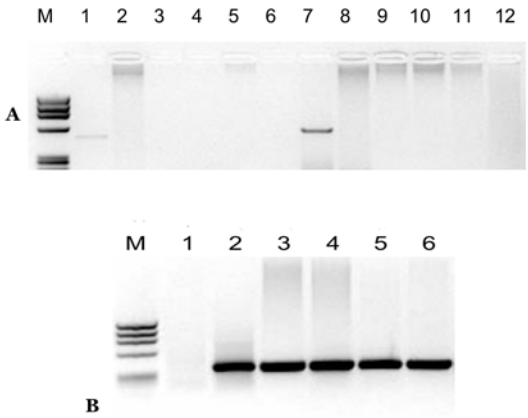

Figure 2. A representative example of screening for the *GJB6* deletions. (A) M: ϕX174 DNA/*Hae*III marker. PCR results for del(*GJB6*-D13S1830) are shown in lanes 1–6; lane 1 is homozygous del(*GJB6*-D13S1830), lanes 2–6 are negative samples. PCR results for del(*GJB6*-D13S1854) are shown in lanes 7–12; lane 7 is heterozygous del(*GJB6*-D13S1854), lanes 8–12 are negative samples. (B) M: ϕX174 DNA/*Hae*III marker. PCR results for amplification of exon 1 of *GJB6* are shown in lanes 1–6; lane 1 is homozygous del(*GJB6*-D13S1830), lane 2 is heterozygous del(*GJB6*-D13S1854).

Table 1. Results of screening for del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) in different populations.

	Del(*GJB6*-D13S1830)		Del(*GJB6*-D13S1854)		
	Heterozygous persons/ *GJB2* heterozygous persons	Positive alleles/ all chromosomes in deaf (*GJB2* is wt)	Heterozygous persons/ *GJB2* heterozygous persons	Positive alleles/ all chromosomes in deaf (*GJB2* is wt)	Reference
Australia	2/29	NA	0/27	NA	Del Castillo *et al.* 2003, 2005
Austria	0/18	0/634	NA	NA	Günther *et al.* 2003
Belgium	2/19	0/202	0/20	NA	Del Castillo *et al.* 2003, 2005
Brasil	2/9	NA	1/16	NA	Del Castillo *et al.* 2003, 2005
Czech	1/13	NA	NA	NA	Seeman *et al.* 2005
Dutch	4/6	0/482	NA	NA	Santos *et al.* 2005
France	23/60	1/416	0/40	NA	Del Castillo *et al.* 2003, 2005
Germany	2/4	0/38	NA	NA	Bolz *et al.* 2003
Iran	Was not found	0/230	NA	NA	Najmabadi *et al.* 2005
Israel	12/27	1/191-2/382	0/11	0/318	Del Castillo *et al.* 2003, 2005
Italy	23/60	1/476	0/40	NA	Del Castillo *et al.* 2003, 2005
Palestine	NA	NA	NA	0/80	Del Castillo *et al.* 2003, 2005
Spain (Barcelona)	5/35	2/472	1/53	NA	Del Castillo *et al.* 2003, 2005
Spain (Madrid)	29/68	4/850	12/47	NA	Del Castillo *et al.* 2003, 2005
Turkey	0/8	0/558	0/8	0/558	This study
United Kingdom	6/19	NA	4/18	NA	Del Castillo *et al.* 2003, 2005
United States/lowa	7/95	NA	0/88	NA	Del Castillo *et al.* 2003, 2005
United States/Virginia	14/88	6/972	0/92	NA	Del Castillo *et al.* 2003, 2005

among the deaf probands in Turkey drops to approximately 2.2%. Although this rate of heterozygosity appears to be still higher than the carrier frequency of c.35delG in the hearing population of Turkey, which has been reported to be 1.8% (Tekin *et al*. 2001), the difference is not statistically significant ($P = 0.654$). Therefore, a common yet-to-be-identified deletion at the *DFNB1* locus appears to be unlikely.

Table 1 shows the results of screening for these two deletions in different populations. It has been suggested that both of these deletions have a common founder although different origins could not be completely ruled out (Del Castillo *et al*. 2003, 2005). Anatolia has been home and transit route from Asia to Europe for many populations since ancient times. Thus, absence of del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) in modern Turkey suggests that both deletions are not as old as c.35delG, for which an ancient founder has been reported in Europe and Anatolia (Van Laer *et al*. 2001; Rothrock *et al*. 2003; Tekin *et al*. 2005). A less possible explanation for the absence of these deletions in Turkey would be random genetic drift.

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