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Mohammad Hamid, Morteza Karimipoor, Morteza Hashemzadeh Chaleshtori, Mohammad Taghi Akbari. A novel 355-357delGAG mutation and frequency of connexin-26 (GJB2) mutations in Iranian patients.. Journal of Genetics, Indian Academy of Sciences, 2009, 88 (3), pp.359-362. 10.1007/s12041-009-0054-6 . pasteur-00750567

HAL Id: pasteur-00750567

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Submitted on 11 Nov 2012

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RESEARCH NOTE

A novel 355–357delGAG mutation and frequency of connexin-26 (*GJB2*) mutations in Iranian patients

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Introduction

The common form of autosomal recessive non-syndromic deafness is caused by the mutation in gap junction beta 2 (*GJB2*) gene (GenBank M86849, OMIM# 121011) which is located at the DFNB1 locus at 13q11. *GJB2* is a small gene about 5500-bp length with two exons, of which only one contains the coding region (Kelley *et al.* 2000). The sequence of the coding region consists of 681 bp, encoding a gap-junction protein with 226 amino acids (Schrijver 2004).

The genetics of hearing loss is highly heterogeneous and more than 100 mutations in connexin 26 (*GJB2*) genes are reported to be responsible for 30%–40% of hereditary hearing loss in deaf subjects (Ballana *et al.* 2001; Schrijver 2004). The most frequent mutation 35delG has been detected in different populations; especially in European countries where it is established to be due to founder effect (Van Laer *et al.* 2001; Rothrock *et al.* 2003).

In this study, we performed mutation screening in 33 families who met clinical criteria of non-syndromic hereditary hearing loss (NSHL) to evaluate the type and frequency of *GJB2* mutations in Iranian population.

Materials and methods

This study was conducted on 33 unrelated families affected by NSHL were referred to our laboratory. All patients had moderate-to-profound sensorineural hearing loss. The hearing loss of patients was confirmed by audiologic testing; air and bone conduction were evaluated in frequencies of 250, 500, 1000, 2000, 4000 and 8000 Hz with intensities up to

120 dB. The autosomal-recessive inheritance of the disease was established by construction of the pedigree. However, the hearing loss patients did not have other clinical features.

After obtaining informed consent from all participants, 5 mL peripheral blood was taken from patients and genomic DNA was extracted by using standard salting out method. Detection of mutations within *GJB2* gene was carried out by DNA sequencing for all samples. The entire coding region of *GJB2* gene (GenBank accession no. M86849) was amplified using the primers: Cx148F2 (5-CCTGTGTTGTGTGYGCATTCGTC-3) and Cx929R3 (5-CTCATCCCTCTCATGCTGTC-3). For PCR amplification at 2 min, initial denaturation at 94°C was followed by three steps including 94°C for 2 min, 59°C for 45 s and 72°C for 2 min in first loop (four cycles) and 94°C for 1 min, 59°C for 30 s and 72°C for 1 min in second loop (25 cycles). Fifty µL of the PCR product was purified using PCR clean up kit (Qiagen, Hilden, Germany), and it was subjected to sequencing by chain termination method on ABI 3730 XL sequencer (Primm, Milan, Italy). The sequencing results were analysed by Chromas 2.13 software (Technelgsim, Queensland, Australia). The 342-kb del(*GJB6*-D13S1830) was also studied by multiplex PCR as previously described (Sadeghi *et al.* 2005).

Results

In this study, we analysed the frequency of *GJB2* mutations in 50 NSHL patients from 33 unrelated Iranian families. The patients were from different parts of the country with different ethnic background. Ten mutations including 35delG, R127H, V27I+E114G, Y155X, M163V, R143W, R32H, R165W, 333–334 delAA and a novel

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Keywords. hearing loss; connexin 26 (Cx26); gap junction beta-2 (*GJB2*); 35delG; Iranian population; human genetics.

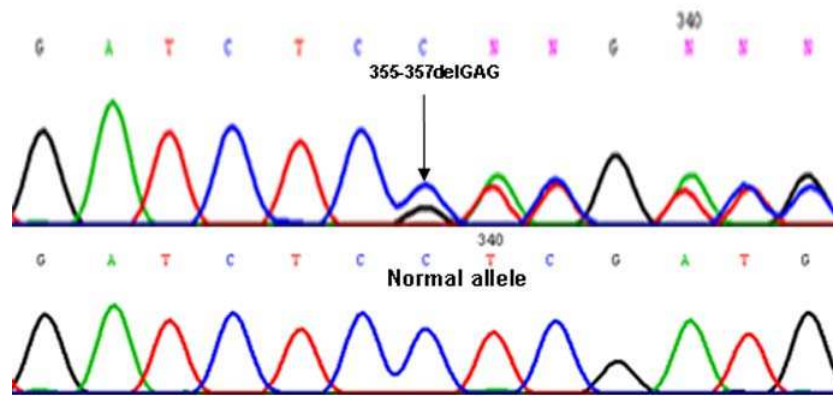


Figure 1. Nucleotide sequence of the novel variant (355–356 delGAG) compared with normal control.

Table 1. Frequency of *GJB2* genotypes detected in NSHHL Iranian individuals.

Name of variants	Deaf individuals no. (%)
Mutation	
M163V/wt	1 (2)
35delG/wt	4 (8)
R127H/wt	1 (2)
V27I+E114G/wt	2 (4)
R32H/35delG	1 (2)
35delG/35delG	2 (4)
R143W/R143W	1 (2)
R165W/wt	1 (2)
333–334delAA/wt	1 (2)
Unknown mutation	
Y155X/wt	1 (2)
Novel mutation	
355–357delGAG/wt	1 (2)
Detected	16 (33.3)
Total	50 (100)
Polymorphism	
F146F +F154F /wt	1 (2)
V153I/wt	3 (6)
S86T/S86T	50 (100)

355–357delGAG (figure 1) were diagnosed in 16 deaf patients (33.3%) (table 1). 35delG, R127H, V27I+E114G, Y155X, M163V, R165W, 333–334delAA and a novel 355–357delGAG were identified in 11 (22.%) deaf patients in heterozygous form. Three (6%) of hearing loss patients had 35delG/35delG and R143W/R143W mutations and one (2%) had (R32H+35delG) mutation in homozygous and compound heterozygous status, respectively (table 2).

Overall, the most common mutation in this cohort was 35delG mutation because nine out of 22 (40.9.%) mutant alleles had this mutation (table 2). In addition, S86T polymorphism was observed in all patients (100%) in homozygous form and V153I and (F154F +F146F) were detected in five families (table 2). This newly found mutation 355–357delGAG has not been reported previously. It causes a deletion at codon 119 and did not alter any amino acids (fig-

ure 1). In this study, 20 normal subjects were used as control group and they did not show any nucleotide variation in coding region of connexin-26 gene except S86T polymorphism, which was observed in all cases.

We also analysed the 342-kb del(*GJB6*-D13S1830) in 10 unrelated patients with non-syndromic hearing loss that had only one *GJB2* mutant allele. We did not detect any deletion among deaf person studied.

Discussion

Mutations in connexin 26 are the most common cause of NSHHL in many populations with different ethnic background. They are responsible for about 50% of autosomal-recessive hearing loss cases (Rabionet *et al.* 2000). In this

Table 2. Frequency of *GJB2* variations detected in NSHHL Iranian individuals

Name of variants	Nucleotide change	Allele no. (%)
Mutation		
M163V	A to G at 487	1 (1)
35delG	Deletion of G at 35	9 (9)
R127H	G to A at 380	1 (1)
V27I	G to A at 79	2 (2)
E114G	A to G at 341	2 (2)
R32H	G to A at 95	1 (1)
R143W	C to T at 427	2 (2)
R165W	C to T at 493	1 (1)
333–334delAA	del of AA at 333–335	1 (1)
Unknown Mutation		
Y155X	T to A at 465	1 (1)
Novel mutation		
355–357delGAG	del of GAG at 355–357	1 (1)
Detected		22 (22)
Total		100 (100)
Polymorphism		
F146F	C to T at 438	1 (1)
F154F	C to T at 462	1 (1)
V153I	G to A at 457	3 (3)
S86T	GC to CG at 257-8	100 (100)

study, 10 different mutations were detected in NSHL patients. Twelve out of 33 families (36.3%) were associated with *GJB2* mutation in heterozygous and homozygous forms. Only four out of 33 families showed the mutations in both alleles (12.1%), whereas eight other families revealed mutation in heterozygous form (24.2%). In our study, the mutation detection rate in connexin 26 was higher than previous studies (Najmabadi *et al.* 2005; Hashemzadeh Chaleshtori 2006) This discrepancy may be due to difference in inclusion criteria for patients.

The contribution of the *GJB2* gene mutations in the present Iranian NSHL families is not much lower than Western populations, 40% in USA (Kelley *et al.* 1998), 49% in Italy and Spain (Estivill *et al.* 1998) and 54% in Russian families (Posukh *et al.* 2005).

This study also shows 35delG is the most common deafness-causing mutation in the Iranian population, which is in agreement with previous reports (Najmabadi *et al.* 2005; Esmaili *et al.* 2007). The allele frequency of 35delG mutation among our cases was 40.9%. Compared with previous reports, it shows lower frequency of 35delG mutation in homozygous forms (Najmabadi *et al.* 2005; Esmaili *et al.* 2007). This result is expected because there are different ethnic groups in our population, each one having its own characteristic allele type. Therefore, every ethnic group should be studied for the existence of mutations in other loci that might be related to deafness. As we see in Japanese, Chinese, Ashkenazi Jews and Slovak Romany populations, 35delG is rare and 235delC, 167delT and W24X were reported as founder effect (Abe *et al.* 2000; Lerer *et al.* 2001; Alvarez *et al.* 2005). Therefore, we will independently need to study each and every ethnic group to identify their founder mutation and private mutations.

We also detected a novel mutation which has not been reported in connexin-deafness homepage (Ballana *et al.* 2001). This mutation (355–357delGAG) is most probably related to deafness condition. It causes a 3-bp deletion at codon 119 in the CL domain. This mutation must be examined for functional effects. The N-terminal domain of connexin 26 associated with transmembrane domain M1 creates a charge complex that acts as a voltage sensor (Verselis *et al.* 1994). Moreover, the extracellular domains E1 and E2 determine the heterotypic compatibility, whereas the cytoplasmic linking domain and C-terminal domain are involved in pH gating of the channel (Kelley *et al.* 1998).

Cx26 and Cx30 are expressed in the same inner-ear structures and connexons made of Cx26 and Cx30 are able to bind together to form heterotypic gap-junction channels (Dahl *et al.* 1996). Therefore, we analysed 342-kb del(*GJB6*-D13S1830) as appropriate candidate in patients with non-syndromic hearing loss that had only one *GJB2* mutant allele. We did not detect any deletion among Iranian patients. Absence of this mutation among studied patients is similar to other studies from Iran and other countries (Gunther *et al.* 2003; Kalay *et al.* 2005; Esmaili *et al.* 2007). This result

supports the view that del(*GJB6*-D13S1830) mutation is associated with only certain populations.

Finally, the high level of heterozygosity detected in our NSHL patients, was also confirmed by another report from Iran (Sadeghi *et al.* 2005). Therefore, we would expect the contributions of other genes related to non-syndromic hearing loss to be important, and this would require further analysis in families without mutations in entire coding region of *GJB2* gene. Consequently, for more characterization of the genetic contribution in these families identification of other responsible loci is required. This will help to detect carriers and patients, and facilitate prevention of deafness in multi-ethnic Iranian population.

Acknowledgements

We thank the staff of Dr Akbari's Medical Genetic Laboratory for their contribution for this study and also our patients for their collaboration.

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Received 19 January 2009, in final revised form 14 July 2009; accepted 16 July 2009

Published on the Web: 8 October 2009