International Journal of Pediatric Otorhinolaryngology 79 (2015) 1892–1895

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Mutation spectrum of autosomal recessive non-syndromic hearing loss in central Iran

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ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 25 August 2015
Accepted 27 August 2015
Available online 2 September 2015

Keywords:
ARNSHL
GJB2 gene
GJB6 gene
Central Iran

ABSTRACT

Objective: To identify the spectrum of mutations in *connexin 26* gene and frequency of two deletions in *connexin 30* gene in central Iran.
Methods: After extraction of DNA from 300 blood samples, *connexin 26* gene coding region was amplified using specific primers. PCR products were used for bidirectional sequencing. Multiplex PCR was used for detection of del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) in the *GJB6* gene.
Results: Eighteen different mutations including two novel variants in *GJB2* gene were detected. The *GJB2* mutations were observed in 23.3% of all the subjects. In addition, none of the deaf patients carried the del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) in the *GJB6* gene. The 35delG mutation was the most common mutation, accounting for 32.65% of the mutant alleles.
Conclusion: The present study indicates that mutations in the *GJB2* gene particularly 35delG are important causes for ARNSHL. 60% of the patients were heterozygous carriers. Thus, further investigation is needed to detect the genetic cause of hearing loss in patients with mono allelic mutations in the coding region of *GJB2*.

1. Introduction

There are more than 400 genetic syndromes that have hearing loss as a feature, with up to 1% of human genes estimated to be involved in hearing process and over 130 loci associated with nonsyndromic hearing loss [1,2]. Hearing loss is the most heterogeneous human trait. Both genetic and nongenetic factors cause hearing loss. Hearing impairment occurs in 1 to 3 of 1000 live births given that over 60% of significant congenital and early-onset hearing loss cases are caused by genetic defects [3–5]. An estimated 30% of genetic deafness cases are syndromic and 70% are non-syndromic [6]. Autosomal recessive non-syndromic hearing loss (ARNSHL) is a condition occurring in about 80% of cases and often there is no positive family history for hearing loss [7].

Despite the genetically heterogeneous state of hereditary deafness, mutations in the *GJB2* gene encoding connexin 26 and two deletions in the *GJB6* gene encoding connexin 30, del(GJB6-D13S1830) and del(GJB6-D13S1854), in DFNB1 locus, account for up to 50% of recessive deafness and 15–40% of all deaf individuals in a variety of populations [1,7]. Type and frequency of mutations differ considerably among ethnic groups. In many populations, mutations in connexin 26, a gap-junction protein encoded by the *GJB2* gene with two exons in which exon 1 is untranslated, is identified in a large proportion of ARNSHL [7–11]. In many ethnic groups especially European populations, a single mutation, c.35delG, accounts for the majority of hearing loss caused by *GJB2* mutations [5,6,12,13].

The goal of genetic evaluation for individuals with hearing impairment is to define mutation spectrum of genes associated with hearing loss which may be of vital importance in elucidating the etiology of the disease. Defining the spectrum of mutations in a gene also provides the basis for precise carrier screening, genetic testing, as well as in providing accurate counseling [5,13–15]. Countries like Iran, which have a heterogeneous population and a

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http://dx.doi.org/10.1016/j.ijporl.2015.08.039
0165-5876/

high consanguinity marriage rate, offer a potential valuable source to investigate autosomal recessive disorders including ARNSHL. In the present study we have investigated the spectrum and prevalence of mutations in coding region of *GJB2* (exon 2) and two deletions in the *GJB6*gene using direct sequencing technique.

2. Materials & methods

2.1. Sampling and DNA extraction

The study included 220 patients with moderate to profound ARNSHL and 80 unrelated normal hearing participants from central Iran. The patients and normal participants were referred by the general ear, nose, and throat (ENT) clinic to the medical genetics lab of Alzahra University hospital, Isfahan, Iran. Medical history, physical examination record and informed consent for each subject were obtained before the study. All procedures performed in this study were in accordance with the ethical standards of the Isfahan University of Medical Sciences' research committee. Blood samples were obtained and genomic DNA was extracted from peripheral blood leukocytes by standard salting out procedure.

2.2. Screening of GJB2 by direct PCR-sequencing

To identify GJB2 mutations, the coding region (exon 2, 681 bp) of the *GJB2* gene was PCR amplified using the approach described previously [16]. Following primers were used for amplification of exon 2of the GJB2 gene: F1 (5-GCTTACCCAGACTCAGAGAAG-3) and R1 (5-CTACAG GGGTTT CAA ATGGTTGC-3). In brief, PCR reactions were performed in a 25 μl mixture containing 100–200 ng genomic DNA, 1.5 mM $MgCl_2$, 0.4 mM dNTP, PCR buffer 1× (50 mM KCl, 10 mM Tris–HCl at PH 8.8), 0.4 μM of each primer, 1.25 U AmpliTaq Gold polymerase (Applied Biosystems). Reactions were run under following conditions: initial denaturation at 95 °C for 5 min, 30 cycles at 95 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and final extension cycle at72 °C for 5 min. ABI 3130 sequencer (Applied Biosystems) was used for bidirectional sequencing of purified PCR product.

2.3. Screening of GJB6 deletions

The presence of del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) in GJB6 was investigated by multiplex PCR Using following primers: del(GJB6-D13S1830) breakpoint junction: F1 (5-CACCATGCG-TAGCCTTAACCATTTT-3) and R1 (5-TTTAGGGCATGATTGGGGT-GATTT-3); del(GJB6-D13S1854) breakpoint junction: F2 (5-CAGCGGCTACCCTAGTTGTGGT-3) and R2 (5-TCATAGTGAAGAACTC-GATGCTGTTT-3); GJB6 (exon 1): F3 (5-CATGAAGAGGGCGTACAAGT-TAGAA-3) and R3 (5′-CGTCTTTGGGGGTGTTGCTT-3).

Table 1
Allelic frequency of*GJB2* gene mutations.

Nucleotide change	Protein change	Allelic frequency (%)	Mutation type
35delG		32.65	Recessive
551G > C	R184P	21.42	Recessive
459G > A	V153I	9.18	Polymorphism
35insG	Frameshift	2.04	Recessive
c.127delG	Frameshift	2.04	Recessive
487A > G	M163V	6.12	Unknown
167delT	Frameshift	2	Recessive
35G > T	G12V	2	Recessive
358-360delGAG	delE120	4.08	Recessive
380G > A	R127H	3.06	Polymorphism/recessive
79G > A	V27I	6.12	Polymorphism
445G > A	A144T	1.02	Recessive
88A > C	I30L	3.06	Polymorphism
397T > G	W133G	1.02	Recessive
IVS1-12C > T	–	1.02	Unknown
109G > A	V37I	1.02	Recessive
IVS1-15C > T	–	1.02	Polymorphism
551G > A	R184Q	1.02	Dominant

The mixture used for PCR-reaction and PCR conditions were the same as*GJB2* gene analysis.

3. Results

GJB2 gene variants were identified in 70 out of the 300 (23.3%) participated in this study. Among individuals having mutant allele, forty two were heterozygous (60%) in which 17.1% suffered from hearing loss and 42.9% were normal and twenty eight subjects were homozygous (40%) in which 37.17% were patient and 2.85% were normal. Compound heterozygous mutations were found in four subjects (1.32%). These mutations include M163V/V153I, G12V/35delG and W133G/R184P detected in approximately 0.66%, 0.33% and 0.33% of the patients, respectively. Eighteen different variants in the *GJB2* gene were found in this study, which two of them were novel (Table 1).

Heterozygous mutation p.I30L (c.88A > C) was identified in two unrelated patients; however, further analysis of DNA samples from their family indicated the same mutation in both healthy and affected members (Fig. 1). Homozygous mutation c.127delG was identified in a boy born to consanguineous parents. Further analysis of the extended pedigree members indicated the same mutation in proband's cousin. Normal parents were heterozygous for c.127delG mutation and their affected son was homozygous for this mutation. These results in addition to pedigree information suggest the mode of inheritance is autosomal recessive (Fig. 2). These mutations are not reported in the dbSNP database (http://

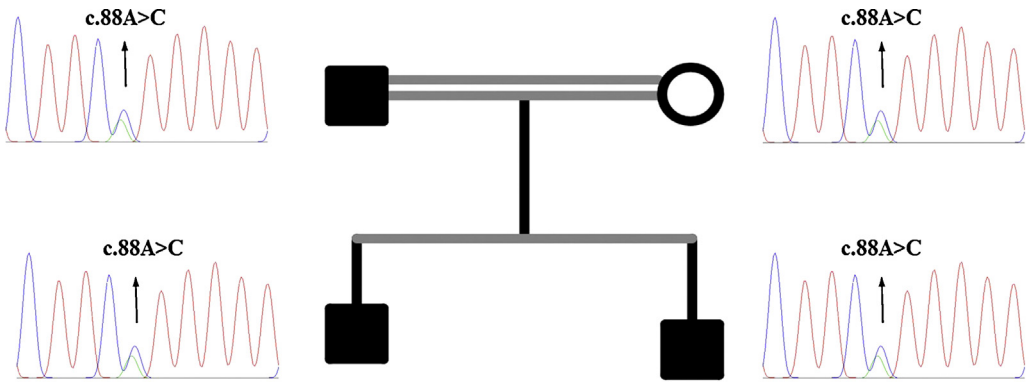

Fig. 1. Sequencing graph and pedigree of a family with c.88A > C (p.I30L) mutation. This pedigree shows two boys with their father affected with ARNSHL. Both affected and unaffected family members were heterozygous for c.88A > C (p.I30L) mutation.