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GJB2 mutations in Turkish patients with ARNSHL: prevalence and two novel mutations

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Abstract

Mutations in the connexin 26 gene (*GJB2*) cause a significant proportion of prelingual non-syndromic autosomal recessive deafness in all populations studied so far. To determine the percentage of hearing loss attributed to *GJB2* in northeast Turkey, 93 unrelated patients with autosomal recessive non-syndromic hearing loss (ARNSHL) were screened. Seven different mutations were found in 29 of the patients with severe to profound hearing loss. The 35delG mutation was the most common mutation, accounting for 76% of all mutant *GJB2* alleles. Four already described mutations, W24X, 310del14, delE120 and R184P and two novel mutations, Q80K and P173S, were identified. The allelic Δ(*GJB6*-D13S1830), which can cause hearing loss in combination with *GJB2* mutations, was not present in our patients. Our results are comparable to those reported in other regions in Turkey and indicate that *GJB2* mutations account for about 30% of Turkish patients with ARNSHL. Besides 35delG, W24X and delE120 occur more than once in the Turkish ARNSHL population with a frequency of about 5%.

Keywords: Hearing loss; Connexin 26; *GJB2*; *GJB6*; 35delG

1. Introduction

The incidence of bilateral congenital hearing loss exceeding 35 dB is estimated to be 1.4 in 1000 (Mason and Herrmann, 1998). Approximately half of these cases are hereditary (Marazita et al., 1993). Of the hearing-loss disorders attributable to genetic causes, approximately 70% are classified as non-syndromic and 30% as syndromic (Gorlin et al., 1995). Non-syndromic hearing impairment can be further subdivided by the mode of inheritance. The majority of the non-syndromic cases, about 77%, shows autosomal recessive inheritance, 22% of the cases are autosomal dominant, 1% are X-linked, and <1% are due to mitochondrial mutations (Morton, 1991). In the past decade, remarkable progress has been made in the identification of the molecular basis of hearing loss. To date, 98 loci have been reported for non-syndromic hearing loss. Thirty-seven of the genes from these loci have been identified and characterized (http://www.uia.ac.be/dnalab/hhh, October, 2004). Among these genes, mutations in *GJB2* account for about 50% of all autosomal recessive non-syndromic

Abbreviations: ARNSHL, autosomal recessive non-syndromic hearing loss; C-ARMS, competitive amplification refractory mutation system; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism; ACPTA, air conduction pure-tone average

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hearing loss (ARNSHL) in Caucasians (Rabionet et al., 2000a). *GJB2* encodes connexin 26 (Cx26), a transmembrane protein that oligomerizes with five other connexin molecules to form a homo or heteromeric connexon. Connexons in adjoining cells fuse through disulfide bonding to form gap junctions, which allow molecules to pass from cell to cell (Bruzzone et al., 1996). Connexin 26 is highly expressed in epithelial supporting cells of the mammalian cochlea and is believed to play a key role in the cycling of potassium from the hair cells to the endolymph (Kikuchi et al., 1995; Kelsell et al., 1997; Chang et al., 2003).

To date, more than 100 different deafness-causing *GJB2* mutations have been described (http://www.crg.es/deafness, November, 2004). In the Caucasian population a single founder mutation, 35delG, accounts for the majority of *GJB2* mediated hearing loss (Denoyelle et al., 1997; Gasparini et al., 2000; Sobe et al., 2000; Rothrock et al., 2003). In the Ashkenazi Jewish population, the east Asian population, and the African population the 167delT mutation, the 235delC mutation and R143W mutations, respectively, are predominant (Lerer et al., 2000; Sobe et al., 2000; Kudo et al., 2000, 2001; Wang et al., 2002; Brobby et al., 1998; Hamelmann et al., 2001). *GJB2* mutations can also cause hearing loss in combination with the Δ(*GJB6*-D13S1830) mutation (del Castillo et al., 2003). *GJB6* expresses connexin 30 (Cx30), which can be assembled with connexin 26 in the same gap junction plaques (Ahmad et al., 2003). Although most connexin 26 mutations cause ARNSHL (DFNB1), a few mutations have been identified in dominantly inherited hearing loss (DFNA3) as well (Morle et al., 2000; Hamelmann et al., 2001; Loffler et al., 2001). In addition to non-syndromic hearing loss, some *GJB2* mutations are described for Vohwinkel's syndrome (Maestrini et al., 1999), palmoplantar keratoderma (Kelsell et al., 2000; Uyguner et al., 2002) keratitis–ichthyosis–deafness syndrome (Richard et al., 2002), and erythrokeratodermias (Richard et al., 2000).

According to audiometric examinations there is a correlation between specific *GJB2* mutations and the phenotype in non-syndromic deafness. *GJB2* mutations, which completely inactivate the connexin 26 generally cause severe to profound hearing loss (Cryns et al., 2004). However, it is demonstrated that certain combinations of mutations including the combination of 35delG with the missense mutations L90P, V37I, or the splice-site mutation IVS1 + 1G > A, and the V37I/V37I genotype are associated with significantly less severe hearing impairment as compared to the 35delG homozygous genotype (Cryns et al., 2004).

The aim of this study is to investigate the nature and prevalence of *GJB2* mutations in Turkish patients with ARNSHL.

2. Patients and methods

2.1. Patients

Unrelated children, from boarding schools for the hearing disabled in the provinces of Rize, Trabzon, and Ordu, in Turkey were used as the study population. Students having one or more relatives with hearing impairment in the immediate or extended family were selected. Pedigree analysis and clinical examination revealed 93 cases with prelingual ARNSHL. All the families were informed about the study and consent was obtained from parents. The local ethics committee approved this study. Genomic DNA was extracted from peripheral blood lymphocytes according to standard protocols (Miller et al., 1988).

2.2. Detection of 35delG with C-ARMS

To identify 35delG mutant and normal *GJB2* (NM_004004.3) alleles in a single polymerase chain reaction (PCR) with competitive amplification refractory mutation system (C-ARMS), we designed a wild-type allele specific primer 5′-GGA GTG TTT GTT CAC ACC CCC C-3′ and 35delG specific primer 5′-A GTG TTT GTT CAC ACC CCC A-3′. As a reverse primer 5′-CAT TCG TCT TTT CCA GAG CA-3′ was used (Zelante et al., 1997).

PCR of C-ARMS were performed in a final volume of 25 μl containing 200 ng genomic DNA, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% (v/v) Triton X-100, 1.2 mM $MgCl_2$, 160 μM of each dNTPs, 5 pmol from each primer, and 0.5 U of Taq polymerase (Promega, Leiden, The Netherlands) in a Genius thermal cycler (Techne, Cambridge, England). First, the samples were denatured at 94 °C for 7 min. Subsequently, 35 cycles of denaturation were performed at 94 °C for 35 s, annealing at 64 °C for 3 min, and elongation at 72 °C for 35 s, followed by a final extension step of 72 °C for 5 min. The complete PCR mix was added to 5 μl loading buffer, 0.1% (w/v) bromophenol blue, 0.1% (w/v) xylene cyanol, 50% (w/v) sucrose, and 50 mM Na_2EDTA (pH 8.0). Fifteen microliters of the PCR products were electrophoresed in a 10% native polyacrylamide gel at 350 V and 29 mA for 5 h. DNA fragments were visualized using silver staining.

2.3. SSCP and sequencing analysis

The complete coding region of the *GJB2* gene was screened with single strand conformation polymorphism (SSCP) and sequenced using primers described previously (Zelante et al., 1997). PCR products, which showed altered mobility as compared to normal controls, were excised from the gel and purified with the PCR Preps DNA Purification System (Promega, Leiden,