International Journal of Pediatric Otorhinolaryngology 78 (2014) 1057–1060

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

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

Particular distribution of the *GJB2*/*GJB6* gene mutations in Mexican population with hearing impairment

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ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 30 March 2014
Accepted 1 April 2014
Available online 12 April 2014

Keywords:
GJB2 gene
GJB6 gene
Hypoacusia
Gene mutations
Connexins

ABSTRACT

Background: Hereditary sensorineural hearing loss (SNHL) is a genetically heterogeneous disorder worldwide. Mutations in the *GJB2* gene are a frequent cause of hereditary SNHL. There is a prevalence of certain mutations in various populations which suggests that specific mutations may be influenced by ethnic background.
Objective: To analyze the prevalence of *GJB2*, *GJB6* mutations in several geographic areas of Mexico in patients with hereditary SNHL.
Materials and methods: One hundred and forty Mexican unrelated propositi with prelingual SNHL were included in the study. All patients had three previous generations born in Mexico and belonged to no specific ethnic group. Analyses of the *GJB2* and *GJB6* genes and mt.1555A < G were performed in all subjects.
Results: Twenty-three homozygous mutations, 57 heterozygous mutations, 1 double heterozygous (*GJB2*/*GJB6*) and 59 wild-type genotypes in the *GJB2* gene were observed. Three patients had the homozygous c.del35 mutation whereas 26 patients were heterozygous for this gene defect. Only one patient with the *GJB6* gene deletion was present (it includes the double heterozygous *GJB2*/*GJB6*). The mt.1555A > G mutation was not detected.
Conclusion: We found a great variety of mutations depending on the analyzed region in patients with SNHL; 57.86% of patients had affection in one or two alleles in *GJB2* or *GJB6* genes whereas 42.14% were wild-type. In some cases, allele distribution depended on region. Molecular studies of more genes involved in hereditary non-syndromic SNHL are required to completely confirm the molecular basis of hearing loss in Mexican population.

1. Introduction

Hereditary sensorineural hearing loss (SNHL) is a genetically heterogeneous disorder that presents an incidence of 1 in 1000 children [1]. Non-syndromic deafness accounts for 60–70% of inherited hearing impairment cases (http://hereditaryhearingloss.org/main.aspx?c). Several mutations in various genes have been associated with recessive SNHL impairment. Mutations in the *GJB2* gene are an important cause of hereditary SNHL, they are responsible for as much as 50% of such cases in several populations [2–5]. More than 150 mutations, including polymorphisms, have been described in the *GJB2* gene (http://davinci.crg.es/deafness). Individuals that are homozygous for the *GJB2* gene mutations manifest a wide spectrum of clinical data that ranges from moderate to profound SNHL; this suggests the participation of epigenetic and environmental factors in the phenotypic expression [6]. Analysis of the *GJB2* gene indicates that some proportion of patients has only one mutation in the *GJB2* gene with the participation of other genes in the recessive effect [7–9]. Mutations in the *GJB2* and *GJB6* genes, both in the DFNB1 locus on chromosome 13, are the principal cause of SNHL in Europe and America; in some cases autosomal dominant deafness is due to heterozygous mutations in *GJB2* gene (DFNA3A) [10–13].

GJB2 gene encodes the gap junction beta-2 protein connexin 26. Hexamers of connexins join within the plasmatic membrane and

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http://dx.doi.org/10.1016/j.ijporl.2014.04.002
0165-5876/

form a connexon [14]. Connexons form gap junctions between adjacent cells, they are important intercellular communication channels [15]. When connexons are composed of identical subunits they are called homomeric but when they are composed of divergent subunitis they are heteromeric. In the same sense, gap junctions are homotypic (two identical connexons) or heterotypic (connexons with differing connexin isotypes) [16]. Connexins, present in epithelial and connective tissues of the cochlea, are primordial for normal auditory function [17]. The *GJB2* gene mutations lead to abnormal Cx26 expression and subsequently to hearing impairment with a variable degree of affection [18]. There is a prevalence of certain mutations in various populations which suggests that specific mutations may be influenced by ethnic background and vary among populations. The aim of the present study was to analyze a sample of 140 Mexican patients in several geographic areas to identify the prevalence of the *GJB2/GJB6* gene mutations in hereditary SNHL.

2. Materials and methods

A total of 140 Mexican non-related probands with prelingual SNHL and ancestry of three generations born in Mexico were included; they belonged to no specific ethnic group. This cohort consisted of 76 males and 64 females ranging in age from one month to 43-years-old. Twelve cases were familial and 128 were sporadic. Three families were endogamic. In familial cases, siblings were products of normal hearing parents. Autosomal recessive inheritance was possible in all familial cases but in some cases X-linked or autosomal dominant inheritance could not be discarded. In the sporadic cases, X-linked or autosomal dominant inheritance could not be ruled out. Samples were obtained from west, northwest, east, northeast and center of México (Table 1). Protocol was approved by the Ethics Committee of the General Hospital of Mexico. Informed consent was obtained from all subjects or their parents prior to obtain the blood samples. Parents or patients were interviewed with regard to age at onset, family history, mother's pregnancy, infections, head or brain injury, and the use of aminoglycoside antibiotics. Careful medical examinations revealed SNHL with no other clinical data. Thyroid parameters were performed in all patients. All subjects showed bilateral SNHL impairment on audiograms; all cases with syndromic findings or evidence of environmental factors were excluded. Hearing loss of patients was evaluated with audiometric tests. None of the patients showed any sign of vestibular dysfunction. Hearing status of all parents was investigated to discard any alteration, when possible, molecular analysis was performed in parents and possible carriers. DNA genomic was obtained through conventional methods from the 140 unrelated probands, members of the family (when possible), and from 100 ethnicity matched controls with normal hearing. CT scan of temporal bones was performed in affected homozygous to discard Pendred syndrome or any anomaly.

3. Mutational analysis

Analyses of the *GJB2* and *GJB6* genes and mt.1555A < G mutation were conducted as described previously [14,19]. The coding exon and flanking intronic regions of *GJB2* and *GJB6* genes and mtDNA were amplified by PCR. All the PCR products were directly sequenced. DNA was sequenced on an ABI 3730XL automated sequencer (Applied Biosystems, Inc., Foster City, CA). The *GJB2/GJB6* genes were sequenced for all cases and in 100 normal controls. DNA sequence variations were identified by comparison of subject DNA sequence to *GJB2/GJB6* reference sequences: Genbank Accession Numbers M86849, U43932, and/or XM_007169. Numbering of *GJB2/GJB6* nucleotides starts with the A of the ATG start codon in exon 2 as position number +1.

4. Results

Among the 140 unrelated probands included in this study, all of them had congenital prelingual SNHL. Sequence analysis of the *GJB2* gene indicated that only three (2.14%, west and northeast) out of 140 non-related Mexican patients carried the homozygous c.35delG (p.L10fsX13 or p.G12fsX13) mutation whereas 26 cases (18.57%, all regions) were heterozygous c.35delG. Only one Mexican patient carried the Del*GJB6*-D13S1830 heterozygous mutation (Northeast). Fifty-nine patients (42.14%) present the wild-type genotype. The rest of mutations are described in Table 1. Heterozygous mutations were present in a similar ratio in all regions whereas homozygous predominate in northwest and east

Table 1
Frequency of *GJB2*/*GJB6* gene mutations in different geographic areas of Mexico.

	West	Northwest	East	Northeast	Center	Total
c.35delG/wt	7	3	3	6	10	29
p.V84M/wt	4	7	4		5	20
p.G12D/wt				1		1
p.V63A/wt					1	1
p.S19R/wt					2	2
p.F31I/wt		1			3	4
Total heterozygous	11	11	7	7	21	57
c.35delG/c.35delG	2			1		3
Total homozygous	2			1		3
p.V84M/p.R32S	1		1		1	3
p.V84M/p.F31I		5	7			12
p.V84W/p.F31I			1			1
p.F31I/p.R32S					2	2
p.R32S/p.S19R					1	1
p.M34T/p.E47X				1		1
Total compound heterozygous	1	5	9	1	4	20
DelGJB6-D13S1830/c.35delG				1		1
Total double heterozygous				1		1
Total	14	16	16	10	25	81
wt/wt/benign	11	1	1	12	34	59
Total number of subjects						140