Braz J Otorhinolaryngol.
2013;79(1):95-9.

DOI: 10.5935/1808-8694.20130016

ORIGINAL ARTICLE

BJORL.org

A study of GJB2 and delGJB6-D13S1830 mutations in Brazilian non-syndromic deaf children from the Amazon region

Luciana Santos Serrão de Castro[1], Anderson Nonato do Rosario Marinho[2], Elzemar Martins Ribeiro Rodrigues[1], Giorgio Christie Tavares Marques[3], Tarcísio André Amorim de Carvalho[4], Luiz Carlos Santana da Silva[5], Sidney Emanuel Batista dos Santos[6]

Keywords:
connexins;
deafness;
genetic counseling.

Abstract

Hearing impairment affects about 1 in 1000 newborns. Mutations in the connexin 26 (GJB2) gene rank among the most frequent causes of non-syndromic deafness in different populations, while delGJB6-D13S1830 mutation located in the DFNB30 locus is known to cause sensorineural hearing loss. Despite the many studies on the involvement of GJB2 mutations in hearing impairment in different populations, there is little information on genetic deafness in Brazil, especially in the Amazon region.

Objective: To determine the prevalence of GJB2 mutations and delGJB6-D13S1830 in 77 sporadic non-syndromic deaf patients.

Method: The coding region of the GJB2 gene was sequenced and polymerase chain reaction was performed to detect the delGJB6-D13S1830 mutation.

Results: Mutant allele 35delG was found in 9% of the patients (7/77). Mutations M34T and V95M were detected in two distinct heterozygous patients. Non-pathogenic mutation V27I was detected in 28.6% of the patients (22/77). None of the deaf patients carried the delGJB6-D13S1830 mutation.

Conclusion: Mutant alleles on gene GJB2 were observed in 40% (31/77) of the subjects in the sample. Pathogenic variants were detected in only 12% (9/77) of the individuals. More studies are required to elucidate the genetic causes of hearing loss in miscegenated populations.

[1] PhD in Genetics and Molecular Biology (Researcher).
[2] PhD in Genetics and Molecular Biology (Researcher).
[3] Degree in Biomedicine.
[4] MSc in Genetics and Molecular Biology (Researcher).
[5] PhD (Associate Professor I - Institute of Biological Sciences - Federal University of Pará).
[6] PhD (Associate Professor III - Institute of Biological Sciences - Federal University of Pará).
Institute of Biological Sciences - Federal University of Pará.
Send correspondence to: Luciana Santos Serrão de Castro. Tv. 14 de Abril, nº 1235, apto 601. Belém - PA. Brazil. CEP: 66060-460.
Paper submitted to the BJORL-SGP (Publishing Management System - Brazilian Journal of Otorhinolaryngology) on October 16, 2012;
and accepted on November 2, 2012. cod. 10524.

INTRODUCTION

Hearing impairment affects about 1 in 1000 newborns[1]. Deafness can be due to either genetic or environmental causes or a combination of both. In developed countries, about 60% of all cases are related to genetic origin[2]. It has been estimated that 30% of genetic deafness cases are syndromic and 70% are non-syndromic[3]. In Brazil, the frequency of non-syndromic congenital deafness is approximately four in 1000 births, 16% of which are of genetic etiology[4]. Within the hereditary non-syndromic hearing loss category, autosomal recessive forms (DFNB) make up about 75-80% of all cases, autosomal dominant forms (DFNA) about 20%, X-linked forms (DFN) 2-5%, and mitochondrial forms about 1%[5]. To date, more than 120 different loci have been estimated to be involved in deafness and 70 genes have been identified and characterized[6].

The *GJB2* locus has been reported to be the major cause of autosomal recessive non-syndromic sensorineural deafness (ARNSD)[3,7,8]. *GJB2* gene mutations have been associated to 50% of autosomal recessive non-syndromic deafness in many populations[9,10]. More than 101 different mutations in connexin 26 gene are known to be associated with hearing impairment[11]. The prevalence of some *GJB2* mutations differs considerably among ethnic groups. The 35delG mutation is the most common variant in European populations[12,13]. The carrier frequency of 35delG ranges between 0.97% and 2.24% in the southeastern region of Brazil[14,15]. In São Paulo state, the 35delG mutation was the most frequent (12.4%)[16]; it was found in 23% of the family cases, and 6.2% of the simplex cases. The 235delC mutation is predominant in Asian populations as a whole[17-21], whereas the 167delT mutation is also frequent in Ashkenazi Jewish population[22-24].

It is known that *GJB6* mutations are a common cause of deafness[25]. A deletion of 243Kb named del*GJB6*-D13S1830 mutation in *GJB6* gene is the second most frequent genetic cause of non-syndromic prelingual hearing impairment in the Spanish population[26]. It has also been described among Ashkenazi Jews[27] and in French non-syndromic hearing loss patients[28].

Some studies have reported the genetic frequency of *GJB2* mutations and del*GJB6*-D13S1830 mutation related with non-syndromic deafness in Brazilian populations[12,16,29-31]. North Brazilian populations are composed of a highly interethnic admixture with an added European gene contribution[32] and no previous data are available for the allelic variants in *GJB2* or the frequency of the del*GJB6*-D13S1830 mutation in the Amazon region. In order to establish the prevalence of *GJB2* mutations among deafness patients in this region, we investigated 77 simplex cases of prelingual non-syndromic hearing impairment. Additionally, we investigated the prevalence of the del*GJB6*-D13S1830 deletion.

METHOD

This study was approved by the Research Ethics Committee of João de Barros Barreto University Hospital (protocol n. 2241/05).

Subjects and clinical evaluation

The study was conducted on seventy-seven children with prelingual non-syndromic hearing loss. All seventy-seven probands were unrelated and simplex cases of deafness. Samples were obtained from a School for the Deaf in Belém, Pará, Brazil. Syndromic patients were not included in this study. All the seventy-seven children were severe-to-profound prelingual hearing impairment cases. For each child, the complete medical history and questionnaires were administered to ensure that hearing loss was not the result of environmental causes: maternofetal infection, perinatal complications, meningitis, mumps, prenatal, prolonged use of antibiotics/drug ototoxicity and acoustic trauma. All children underwent an otoscopy, audiovestibular tests and available audiometry, and a general examination including systematic examination of syndromic form was evaluated. Written informed consent was obtained from all the patients' parents.

Molecular Analysis

DNA was extracted from EDTA anticoagulated whole blood using the phenol-chloroform method and precipitation with ethanol. To identify GJB2 mutations, a DNA fragment containing the entire coding region was amplified using the primer pair in Polymerase Chain Reaction (PCR) and two additional internal primers were used in the DNA sequencing of Cx26: GJB2-1F (5´-GTGTTGTGTGCATTCGTCTTTTC-3´) forward primer for PCR and sequencing; GJB2-2R (5´-CCTCATCCCTCTCATGCTGTCTA-3´) reverse primer for PCR and sequencing; GJB2-4F (5´-GGAAGTTCATCAAGGGGGAGATA-3´) primer for internal forward sequencing, and GJB2-3R (5´-ACCTTCTGGGTTTTGATCTCC TC-3') primer for internal reverse sequencing.

PCR conditions were for 35 cycles with denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. In all cases of *GJB2* gene analysis, bi-directional DNA sequencing was performed and in some cases internal primers were used to confirm the result. They were sequenced on an *ABI Prism Big-Dye Terminator Cycle Sequencing Kit™ (Applied Biosystems)* and electrophoresed on an *ABI Prism 377 DNA Sequencer (Applied Biosystems)*. PCR amplification of delGJB6-D13S1830 mutation was performed by using primers and conditions previously described[26].