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Study of modifiers factors associated to mitochondrial mutations in individuals with hearing impairment

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ABSTRACT

Hearing impairment is the most prevalent sensorial deficit in the general population. Congenital deafness occurs in about 1 in 1000 live births, of which approximately 50% has hereditary cause in development countries. Non-syndromic deafness can be caused by mutations in both nuclear and mitochondrial genes. Mutations in mtDNA have been associated with aminoglycoside-induced and non-syndromic deafness in many families worldwide. However, the nuclear background influences the phenotypic expression of these pathogenic mutations. Indeed, it has been proposed that nuclear modifier genes modulate the phenotypic manifestation of the mitochondrial A1555G mutation in the *MTRNR1* gene. The both putative nuclear modifiers genes *TRMU* and *MTO1* encoding a highly conserved mitochondrial related to tRNA modification. It has been hypothesizes that human *TRMU* and also *MTO1* nuclear genes may modulate the phenotypic manifestation of deafness-associated mitochondrial mutations. The aim of this work was to elucidate the contribution of mitochondrial mutations, nuclear modifier genes mutations and aminoglycoside exposure in the deafness phenotype. Our findings suggest that the genetic background of individuals may play an important role in the pathogenesis of deafness-associated with mitochondrial mutation and aminoglycoside-induced.

Introduction

In the developed countries, the aminoglycoside antibiotics are mainly used in the treatment of hospitalized patients with aerobic Gram-negative bacterial infections, particularly in patients with chronic infections. [1]. However, in developing countries aminoglycosides are used even for relative minor infections.

These drugs are known to exert their antibacterial effects by direct binding to 16S ribosomal RNA in the 30S subunit of the bacterial ribosome causing premature termination of protein synthesis [2]. The aminoglycosides may become concentrated in the perilymph and endolymph of the inner ear, potentially leading to ototoxicity [3].

In familial cases of ototoxic deafness, the aminoglycoside hypersensitivity is usually maternally transmitted, suggesting mitochondrial genome involvement [4].

A number of distinct mutations in the mitochondrial DNA (mtDNA) have been found to be associated with both syndromic and non-syndromic forms of hearing loss [4,5].

The most commonly reported SNHL associated with mtDNA mutations are A1555G, T1095C, C1494T and C insertion or deletion at position 961 in the rRNA gene [6–9], and the A7445G, 7472insC, T7510C and T1511C in the $tRNA^{Ser(UCN)}$ gene [10–13]. Of these, the homoplasmic A1555G mutation was associated with aminoglycoside-induced and non-syndromic deafness in many families from different ethnic backgrounds [14,15]. In absence of aminoglycosides, the A1555G mutation was responsible for a clinical phenotype that ranges from severe congenital deafness to moderate progressive hearing loss with later onset [16], to completely normal hearing [16,17].

Recently, A827G mutation in the 12S rRNA gene has been identified in a Chinese families with aminoglycoside-induced and SNHL, suggesting that mitochondrial 12S rRNA gene is a hot-spot

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for deafness-associated mutations [18,19]. There are proposed an interaction between nuclear and mitochondrial genes that modulate the phenotypic manifestation of the mitochondrial mutations. The nuclear modifier genes TRMU and MTO1 encode a highly conserved mitochondrial protein related to tRNA modification, developing an important role in the phenotypic expression of SNHL-induced by aminoglycosides associated mitochondrial mutations [20,21]. Therefore, we studied the mutations in mitochondrial genes, genes nuclear modulators and their interactions with the use of aminoglycoside antibiotics and/or ototoxic drugs. We performed a mutational screening of TRMU and MTO1 genes to examine the role of genes in the phenotypic expression mitochondrial mutations.

In the present study, we reported the clinical and genetic findings in Brazilian patients with mitochondrial A827G mutation and aminoglycoside-induced SNHL. We have identified eight cases carrying the A827G mutation and were observed five cases with mutations in the TRMU and MTO1 nuclear genes.

Subjects and methods

Subjects. A total of 85 Brazilian individuals were enrolled. Written informed consent was obtained from all the subjects included in the study or their parents. These subjects were divided in four groups in order to elucidate the etiology of their hearing impairment and the bases of aminoglycoside ototoxicity. *Group A*—25 newborns at risk with aminoglycoside-induced and sensorineural non-syndromic hearing impairment. *Group B*—25 newborns at risk with aminoglycoside-induced and normal hearing. *Group C*—25 individuals with sensorineural non-syndromic deafness and no history of ototoxy medication. *Group D*—10 adults individuals with sensorineural non-syndromic deafness and positive for A1555G mutation in the *12S rRNA* mitochondrial gene and with no aminoglycoside history.

The DNA samples of individuals of Groups A–C were obtained from Maternidade-Escola Assis Chateaubriand (Assis Chateaubriand Maternity-School) from Universidade Federal do Ceará (Federal University of Ceará). The samples of individuals of Group D were collected from Universidade Estadual de Campinas (State University of Campinas).

After obtaining written informed consent, DNA samples were extracted from whole blood by standard phenol–chloroform method. The samples were tested for the presence of mutations in the coding region of *GJB2*, the two deletions affecting *GJB6* [del(*GJB6*-D13S1830), del(*GJB6*-D13S1854)], the A827G, C1494T and A1555G mitochondrial mutations in the *12S rRNA* gene were analyzed. In addition, G28T mutation in the TRMU nuclear gene and mutations in the *MTO1* nuclear gene were analyzed.

Mutation analysis of the DFNB1 locus. The DNA fragments spanning the entire coding region of *GJB2* gene were amplified by PCR and subsequent sequencing analysis were performed. The results were compared with the wild type *GJB2* sequence (GenBank Acession No. GI62999485) to identify the mutations. To detect *GJB6* deletions, a specific PCR assay was used, as described by [22].

Mutational screening of the mitochondrial A827G mutation. Genomic DNA was isolated from whole blood of participants using the standard phenol–chloroform method. The A827G variant was PCR amplified using the oligonucleotides corresponding to the mitochondrial genome at positions 611–1411. PCR conditions were follows as: initial denaturation at 94 °C for 5 min, followed by 32 three-step cycles (94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min), with a final extension at 72 °C for 10 min. The fragments were analyzed by direct sequencing in an ABI PRISM® 3700 DNA analyzer.

Mutational screening of the mitochondrial C1494T mutation. For the detection of the C1494T mutation, the amplified segments were digested with a restriction enzyme HphI as described by Wang et al. (2006) [23]. The digested samples were then analyzed by electrophoresis through 1.5% agarose gel.

Mutational screening of the mitochondrial A1555G mutation. The detection of the A1555G mitochondrial mutation was performed by PCR amplification followed by the digestion with restriction endonuclease BsmAI and according to the manufacturer's recommended digestion condition [24,25].

Mutational analysis of the G28T mutation in the TRMU nuclear gene. The G28T variant was PCR amplified followed by digestion with the restriction enzyme Bsp1286I. The digested products were analyzed on 1.5% agarose gels [21].

Mutation analysis of the MTO1 nuclear gene. The 12 exons of *MTO1* nuclear gene were screening by denaturing high-performance liquid chromatography (DHPLC) followed by direct sequencing in an ABI PRISM® 3700 DNA analyzer.

Results and discussion

The Table 1 below summarizes the mutations found in different genes studied.

Mutational analysis of DFNB1 locus

To examine the role of *GJB2* we also conducted the mutation screening of *GJB2* gene in the 85 subjects. We detected five individuals homozygous for the 35delG mutation in the *GJB2* gene.

Mitochondrial DNA analysis

To further elucidate the molecular basis of hearing loss, we have performed a mutational analysis of A827G, C1494T and A1555G in the *12S rRNA* mitochondrial gene in 85 subjects. No one individual presented the C1494T mitochondrial mutation. The mutation A1555G was not found in group A–C. As mentioned, the group D was previously selected by the presence of A1555G mutation. The A827G mutation was found in all four groups. In group A two patient was detected positive for A287G, one of them has both A827G mitochondrial mutation and G28T mutation in TRMU nuclear gene. In group B two individuals presented the A827G mutation and one of them presented the I392M mutation in the *MTO1* nuclear gene too. In group C five individuals were detected positive for A827G, two of them presented two different alterations in the MTO1 nuclear gene. In group D one subject is positive for A1555G, A827G mutation in the *12S rRNA* and G28T in the *TRMU* gene, as has been described.

Mutation analysis of TRMU nuclear gene

To determinate whether *TRMU* modulates the phenotypic expression of the A1555G or the A827G mutation, we performed a G28T mutation analysis of *TRMU* gene. We detected G28T mutation in three groups. In group A two cases of TRMU mutation were found, one of them has both G28T mutation in *TRMU* nuclear gene and A827G mitochondrial mutation in the *12S rRNA*. In group C one patient was detected positive for G28T mutation. Finally, in group D one individual has three mutations, among them A1555G, A827G in the *12S rRNA* gene and G28T.

Mutation analysis of MTO1 nuclear gene

To determinate whether *MTO1* modulates the phenotypic expression of the A1555G or the A827G mutation, we performed mutation screening of *MTO1* nuclear gene. We detected four different alterations in the *MTO1* gene. We failed to detect mutations in