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ORIGINAL ARTICLE

Molecular approach of auditory neuropathy☆

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KEYWORDS
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Abstract
Introduction: Mutations in the otoferlin gene are responsible for auditory neuropathy.
Objective: To investigate the prevalence of mutations in the mutations in the otoferlin gene in patients with and without auditory neuropathy.
Methods: This original cross-sectional case study evaluated 16 index cases with auditory neuropathy, 13 patients with sensorineural hearing loss, and 20 normal-hearing subjects. DNA was extracted from peripheral blood leukocytes, and the mutations in the otoferlin gene sites were amplified by polymerase chain reaction/restriction fragment length polymorphism.
Results: The 16 index cases included nine (56%) females and seven (44%) males. The 13 deaf patients comprised seven (54%) males and six (46%) females. Among the 20 normal-hearing subjects, 13 (65%) were males and seven were (35%) females. Thirteen (81%) index cases had wild-type genotype (AA) and three (19%) had the heterozygous AG genotype for IVS8-2A-G (intron 8) mutation. The 5473C-G (exon 44) mutation was found in a heterozygous state (CG) in seven (44%) index cases and nine (56%) had the wild-type allele (CC). Of these mutants, two (25%) were compound heterozygotes for the mutations found in intron 8 and exon 44. All patients with sensorineural hearing loss and normal-hearing individuals did not have mutations (100%).
Conclusion: There are differences at the molecular level in patients with and without auditory neuropathy.

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PALAVRAS-CHAVE
Surdez;
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Abordagem molecular da neuropatia auditiva

Resumo
Introdução: Mutações no gene da otoferlina (OTOF) são responsáveis pela neuropatia auditiva.
Objetivo: Investigar a prevalência de mutações no gene OTOF em pacientes com e sem neuropatia auditiva.
Método: Estudo de casos em corte transversal sendo avaliados 16 casos índice com neuropatia auditiva, 13 pacientes com deficiência auditiva sensorioneural (DASN) e 20 indivíduos ouvintes. DNA foi extraído de leucócitos do sangue periférico e regiões do gene OTOF foram analisadas pela técnica PCR-RFLP.
Resultados: Dos 16 casos índice, 9 (56%) são do gênero feminino e 7 (44%) do masculino. Dos 13 pacientes com DASN, 7 (54%) são masculinos e 6 (46%) femininos. Dos 20 ouvintes, 13 (65%) são masculinos e 7 (35%) femininos. Treze (81%) casos índice apresentam o genótipo selvagem (AA) e 3 (19%) o genótipo heterozigoto AG para a mutação IVS8-2A-G (intron 8). A mutação 5473C-G (exon 44) foi encontrada em heterozigose (CG) em 7 (44%) dos casos índice e 9 (56%) apresentam o genótipo selvagem (CC). Destes mutantes, dois (25%) são heterozigotos compostos para as mutações encontradas no intron 8 e exon 44. Os pacientes com DASN e os ouvintes não apresentam mutações (100%).
Conclusão: Existem diferenças, ao nível molecular, em pacientes com e sem neuropatia auditiva.

Introduction

After the mutations in GJB2 and GJB6 gene, which account for approximately 80% of autosomal-recessive nonsyndromic hearing impairment (ARNSHI), mutations in the otoferlin gene (OTOF) are the most frequent genetic cause of deafness in children. This type of hearing loss (HL) is more complicated, considering that at an early stage it may present as auditory neuropathy (AN) that is not detected by neonatal screening based on otoacoustic emission testing.[1]

Auditory neuropathy is a unique type of HL in which tympanograms are normal, the acoustic reflex and the brainstem auditory evoked potentials (BAEP) are absent or severely altered, and the outer hair cell function is normal, as shown by the presence of otoacoustic emissions (OAEs). Patients with this disorder can show varied degrees of HL, in addition to severe speech comprehension impairment, which is disproportional to the degree of hearing loss.[1]

In 2003, the term ''autosomal-recessive nonsyndromic auditory neuropathy'' was defined, due not only to the audiological characteristics, but also to genetic discoveries related to mutations in the OTOF gene, which were associated with this type of hearing loss.[2] The OTOF gene, located on chromosome 2 (2p23-p22), contains 48 exons and encodes the protein otoferlin, which is expressed in cochlear inner hair cells and type I vestibular hair cells.[3] Since then, molecular studies have been published that allowed the identification of mutations in the OTOF gene and associated them to the autosomal-recessive nonsyndromic AN.[2–12]

Therefore, this study aimed to investigate the prevalence of mutations in the OTOF gene: 2416T-A (Y730X) in exon 18,[3] IVS8-2-A-G in intron 8/exon 9,[6] 2485C-T (Q829X) in exon 22,[5] 5473C-G (Pro1825Ala) in exon 44,[5] and 3032T-C (Leu1011Pro) in exon 26,[9] in patients with AN and in patients with nonsyndromic HL.

Methods

This cross-sectional study was carried out from April 1, 2010 until July 30, 2011; of 1230 cases of nonsyndromic sensorineural hearing loss (NSHL) treated at the Hearing Loss Outpatient Clinic of the institution, 16 patients were selected (NPT group−neuropathy), of both genders, with AN. These were deemed index cases, according to the following inclusion criteria: normal otoscopy, diagnosis of NSHL by pure tone audiometry, presence of otoacoustic emissions and/or cochlear microphonic in BAEP; absence of BAEP waves or severe alteration in their morphology; normal MRI; absence of risk factors (maternal−fetal infections, meningitis, ototoxic drugs) or peripheral neuropathies. Exclusion criteria included a diagnosis of conductive or mixed hearing loss, the presence of risk factors or peripheral neuropathies, and an age >65 years.

A total of 13 patients of both genders (NSHL group) were also selected, who had received a diagnosis of NSHL and whose audiological tests did not match the clinical picture of AN and had no mutations in the connexin 26 and GJB6 genes.

For the Control group (CG group), 20 individuals of both genders were selected, with no hearing complaints, with normal otoscopy and who were unrelated to the patients in the NPT and NSHL groups.

The index cases, patients, and controls were of the same ethnicity, age $\leq$65 years, with no consanguinity, and from the same geographical area.