International Journal of Pediatric Otorhinolaryngology 74 (2010) 494–498

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

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

Five new OTOF gene mutations and auditory neuropathy

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ARTICLE INFO

Article history:
Received 3 December 2009
Received in revised form 1 February 2010
Accepted 2 February 2010
Available online 7 March 2010

Keywords:
Auditory neuropathy
Children
Cochlear implantation
Congenital deafness
DFNB9
Otoferlin

ABSTRACT

Objective: Purpose of this paper is to analyse OTOF gene in a series of subjects affected by auditory neuropathy.
Methods: Four children showing mild to profound prelingual deafness, confirmed by the absence of a clear and detectable responses at auditory brainstem responses (ABR), associated with the presence of bilateral OAE, were enrolled in the study.
Results and Conclusions: Genetic analysis identified five new mutations (a nonsense, a small and a large deletion and two splicing site mutations), and one missense mutation (F1795C) previously described. These results further confirm the role of OTOF gene in auditory neuropathy. In the absence of a context of neurological syndrome, the combination of absent ABR and positive OAE responses should lead to an auditory neuropathy diagnosis and to a mutational screening in OTOF.

1. Introduction

Prelingual deafness affects about 1 in 1000 newborns; for the 60% of these subject's deafness has a genetic cause and in the 70% it is not associated to other clinical features (non-syndromic). Non-syndromic hearing loss has a recessive inheritance in the 80% of the cases and until now 77 different loci have been described (DFNB) (see Hereditary Hearing Loss Homepage at http://web-h01.ua.ac.be/hhh/). DFNB9 locus was described in families of Lebanese origin showing a congenital, profound and fully penetrant sensorineural deafness [1]. Three years later, OTOF was discovered as the gene defective in DFNB9 deafness [2], which is associated with auditory neuropathy (AN), a particular form of hearing loss, characterized by elevating threshold on pure tone audiogram, no acustic reflex, very poor speech discrimination, abnormal or absent ABR responses but normal otoacustic emissions (OAEs) [3]. The prevalence of AN is still not yet defined: data ranging from 1.78% [4] to 14.58% [5] of the impaired children. A recent study suggests that auditory neuropathy/dys-synchrony accounts for approximately 7% of permanent hearing loss in children [6].

Auditory neuropathy could be congenital or late onset; sometimes it has been associated with neonatal hyperbilirubinemia, respiratory distress syndrome, mitochondrial disorders, and peripheral neuropathies [7–11]. The absence of a clear ABR response with the preservation of OAEs suggests that the defect is caused by a primary lesion located in inner hair cells (IHC), in the auditory nerve or in the intervening synapse, or in neuronal populations of the auditory pathway [7,12,13].

DFNB9 seems to belong to a subclass of isolated AN in which the synaptic transmission, IHCs-auditory neurons mainly, would be impaired. Therefore molecular diagnosis of OTOF mutations in patient can lead to a differential diagnosis and prognosis.

OTOF gene encodes for otoferlin, a member of the ferlin family, coding for membrane anchored cytosolic proteins that contain six C2 domains, four of which are expected to bind Ca^{2+} [14]. Otoferlin is expressed in cochlea and vestibule, in cochlear and vestibular nuclei, hippocampus, cerebellum and testis. In adult cochlea, otoferlin is expressed only in IHC, at the basolateral region, where the afferent synaptic contacts are located, suggesting that this molecule has a role in controlling neurotrasmitter release [15]. Otoferlin seems to be implied in the late step of synaptic vesicle exocytosis, probably acting as the major Ca^{2+} sensor triggering membrane fusion at IHC ribbon synapse: KO mice have structurally normal synapses between the hair cell and the afferent fibre, but lack calcium-triggered dumping of the synaptic vesicle contents [15]. OTOF gene contains 48 exons, coding for short and long isoforms of the protein. Long isoforms are constituted of six C2 motifs and of a transmembrane c-terminal domain and are exclusively expressed in the cochlea. Short isoforms, coded by exons 20–48, present only the last three C2 domains [16].

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doi:10.1016/j.ijporl.2010.02.004

Until now 41 OTOF mutations have been worldwide described [17–23].

Here, we report the data obtained analysing a series of four Italian patients affected by hearing loss and AN for the presence of mutations in otoferlin gene.

2. Materials and methods

2.1. Patients

Four children showing mild to profound prelingual deafness, in the absence of a clear and detectable response at the ABR, associated with the presence of bilateral OAEs were included in the study. The degree of hearing loss and the status of the auditory pathway were determined by standard pure tone audiometry, auditory brainstem responses (ABR) and electrocochleography (EcochG). The outer hair cells (OHCs) function was assessed using evoked otoacoustic emission tests (TEOAE and DPOAE). The etiological work-up consisted of a CT scan of the petrous part of the temporal bones, a cerebral MRI and a genetic mutation screening test. Informed consent was obtained in order to have a peripheral blood sample from the subjects, and DNA was isolated from blood leukocytes according to standard protocols.

Samples used in the present study were attending the Medical Genetics Service of the Institute of Child Health IRCCS Burlo Garofolo for diagnostic purposes related to their hearing loss problem. Each subject (and/or the parents) was properly informed about the examination and then asked to sign a consent form prepared according to Italian national rules and laws, in particular: (i) Art.10 and 22 of the Italian law 196/03 on privacy and following updates published on the Gazzetta Ufficiale n.65 of 19 March 2007, (ii) national authorization n.2-2004 to conduct research activities on genetics, and (iii) government authorization to referral research centers (an IRCCS in our National Health Care System) to perform genetic tests 02-2007.

2.2. Genetic analysis

Forty-eight different primer pairs were designed in order to amplify the 48 coding exons of the OTOF gene, including the splice sites [The numbering schemes used are those of otoferlin (GenBank accession number NM_194248.1)]. All amplicons were screened using WAVE 4500 denaturing high-performance liquid chromatography system (transgenomics) according to supplier protocols. DHPLC data analysis was based on a subjective comparison of sample and reference chromatograms. PCR products showing an abnormal chromatographic profile were analysed by direct sequencing on an ABI PRISM 3130 Genetic Analyzer (Perkin Elmer). Mutated alleles were tested on 200 normal chromosomes. In one patient, the possible presence of a large deletion involving otoferlin gene was assessed by SNPs genotyping using the Human1M-Duo BeadChip Infinium whole genome genotyping assay on Illumina Beadstation 500, according to supplier method. This assay interrogates more than 1.1 million evenly distributed loci per sample. CNV data analysis was done using PennCNV, a hidden Markov model (HMM) based approach for kilobase-resolution detection of CNVs from Illumina high-density SNP genotyping data. This algorithm incorporates multiple sources of information, including total signal intensity and allelic intensity ratio at each SNP marker, the distance between neighboring SNPs, the allele frequency of SNPs, and the pedigree information were available [24].

3. Results

Five new alleles were identified (Table 1). All these genetic variations, checked in 200 normal chromosomes, were not detected in healthy subjects.

3.1. Case 1

The first mutation identified was an C > A nucleotide change at position 2316, which replaces a conserved cystine residue at position 722 leading to a stop codon (C722X).

C722X is located in exon 21; as the short isoforms are transcribed from exon 20, this mutation will affect both short and long isoforms. This residue is located in the region between C2C and C2D domains, and causes the loss of the last three C2 domains (C2D-F) and of the membrane-anchoring domain, producing a truncation of 1225 aa. This mutation was firstly assessed in homozygous state, but the analysis of the healthy parents shows that it was present only in the mother (hemizygous): the Human1M-Duo BeadChip, shows the presence of a CNV region defined by snps rs_2272070, located in intron 18 of OTOF gene, and rs_7423300, located in C2orf39 (Fig. 1). As the assay has no snps in the area between the CNV and the nearest heterozygous snps (rs_4491689 and rs_13004993), we can presume that this deletion spans in the genomic region defined by snps rs_4491689 and rs_13004993. This data demonstrated that the patient has inherited the C772X pathogenic allele from the mother and a deletion spanning at least 52.8 kb utmost 61.6 kb, completely disrupting OTOF gene, from the father. This mutation causes the loss of the last three C2 domains, leading to a protein with domains C2A–C; therefore these two mutations detected in the patient seem to have a similar effect on the protein.

The patient is the first daughter of healthy unrelated parents with family history negative for significant genetic conditions or birth defects. At 13 months, an ABR performed, revealed an hearing threshold of 40 dB HL bilaterally. Brain MRI examination was normal. At age 3, OAEs were bilaterally present but there was no V wave detectable at the ABR at the maximum intensity of stimulation. At the EcochG the compound action potential was present till 45 dB HL bilaterally. Temporal bone CT scans as well as at the MRI scans were both normal. A right CI (cochlear implant) was performed when she was 3 and 10 months. The child showed poor perceptive skills, only detection of sounds and words using hearing aids. After surgery, the hearing perceptive abilities improved slowly, achieving the identification level after 24 months of CI use.

Table 1
Mutations detected in OTOF gene (NM_194248.1) in our 4 cases.

Location	DNA level	Homo/heterozygosity	Protein level	Case	Ref.
Intron 9	IVS9-2T > A	Homozygosity		4	This work
Intron 17	IVS17-2C > T	Heterozygosity		2	This work
Intron 18	Deletion 52.8–61.6 kb	Heterozygosity		1	This work
Exon 21	2316C > A	Heterozygosity	C722X	1	This work
Exon 30	3704del16 bp	Heterozygosity	T1264X	2	This work
Exon 44	5384T > G	Homozygosity	F1795C	3	[25]