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WFS1 and non-syndromic low-frequency sensorineural hearing loss: A novel mutation in a Portuguese case

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ABSTRACT

Low-frequency sensorineural hearing loss (LFSNHL) is an unusual type of HL in which frequencies at 2000 Hz and below are predominantly affected. Most of the families with LFSNHL carry missense mutations in *WFS1* gene, coding for wolframin.

A Portuguese patient aged 49, reporting HL since her third decade of life, and also referring tinnitus, was shown to display bilateral moderate LFSNHL after audiological evaluation.

Molecular analysis led to the identification of a novel mutation, c.511G>A (p.Asp171Asn), found in heterozygosity in the exon 5 of the *WFS1* gene, and changing the aspartic acid at position 171 to an asparagine, in the extracellular N-terminus domain of the wolframin protein. This novel mutation wasn't present either in 200 control chromosomes analyzed or in the hearing proband's half-brother, and it had not been reported in 1000 Genomes, Exome Variant Server, HGMD or dbSNP databases. No mutations were found in *GJB2* and *GJB6* genes.

Multi-alignment of 27 wolframin sequences from mammalian species, against the human wolframin sequence in ConSurf, indicated a conservation score corresponding to 7 in a 1–9 color scale where 9 is conserved and 1 is variable. In addition, the mutation p.Asp171Asn was predicted to be damaging and possibly damaging by SIFT and Polyphen-2, respectively.

The auditory phenotype of this patient could thus be due to the novel mutation p.Asp171Asn. Further functional characterization might enable to elucidate in which way the change in the residue 171, as other changes introduced by LFSNHL-associated mutations previously described, leads to this type of HL.

1. Introduction

Low-frequency SNHL (LFSNHL) is an unusual form of HL, often associated with tinnitus, in which frequencies at 2000 Hz and below are predominantly affected. Due to maintenance of high-frequency hearing, LFSNHL patients retain excellent understanding of speech. So, many of them are not aware of their HL until presbycusis or noise exposure may cause high-frequency hearing loss later in their life (Bespalova et al., 2001). DFNA1, DFNA6/14/38 and DFNA54 are the *loci* so far reported as being associated with LFSNHL (Fujikawa et al., 2009).

Most of the families with LFSNHL carry mutations in *WFS1* gene (DFNA6/14/38) that maps to chromosome 4p16 and has a coding transcript of 2673 bp. *WFS1* gene has 8 exons, of which the last seven are coding (Gürtler et al., 2004; Minami et al., 2012). The product of *WFS1* is wolframin, a membrane glycoprotein that is located primarily in the endoplasmic reticulum (ER). Its expression in the human cochlea remains unknown (Gürtler et al., 2004). However, its location in the ER suggests a possible role for wolframin in ion homeostasis retained by the canalicular reticulum, a specialized form of ER (Minami et al., 2012). Functional studies suggest that the autosomal dominant pattern of LFSNHL is due to reduced amount of wolframin (Gürtler et al., 2004).

Here we report the identification of a novel mutation, p.Asp171Asn, in the N-terminus of wolframin, which might be the cause of the LFSNHL phenotype presented by the affected individual.

2. Methods

A Portuguese non-syndromic HL female patient, reporting loss of hearing since her third decade of life was audiologically evaluated and referred for genetic analysis when she was 49 years old. Her hearing status was recently re-examined at the same ORL Service in order to evaluate the degree and progression of her HL. At both examinations, the hearing levels were determined by pure-tone audiometry with a diagnostic audiometer in a sound proof room across a range of frequencies

Abbreviations: Asp, Aspartic acid; Asn, Asparagine; BLAST, Basic Local Alignment Search Tool; ER, Endoplasmic reticulum; Glu, Glutamic acid; HGMD, Human Gene Mutation Database; HL, Hearing loss; LFSNHL, Low-frequency sensorineural hearing loss.

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between 125 and 8000 Hz. A complete clinical history was taken to exclude etiologies for HL as infection, acoustic trauma or ototoxic drugs.

The proband's half-brother on the mother's side, reportedly presenting normal hearing, was the only family member available for genetic study, as the proband's parents and closer family members were already dead or living abroad at the time of the study. No other HL history was known in the family, excepting one uncle from the mother's side, also deceased, reported to be hearing impaired since he was very young. Due to the scarcity of family data, it was difficult to establish if this is a familial or a sporadic case.

Blood samples were collected after written informed consents were signed. Total genomic DNA was extracted from peripheral blood using the JetQuick Blood and Cell Culture Kit (GENOMED), according to the manufacturer's instructions.

Molecular study of the proband included screening of *GJB2* and *GJB6* genes, and exons 4, 5, 6 and 8 of the *WFS1* gene, those where most pathogenic mutations have been found (Lesperance et al., 2003).

Automated sequencing was performed for the coding exon of *GJB2* gene using primers 2AF and 2BR described previously (Matos et al., 2010). The most common *GJB6* deletions were also screened by multiplex-PCR, using the method described by del Castillo and colleagues (del Castillo et al., 2005). The exons 4, 5 and 6 of *WFS1* gene were sequenced using the following primers, respectively: ex4F (5′-CGG AGA ATC TGG AGG CTG AC-3′), ex4R (5′-CAA CCC TCC AGA GGC TGT TC-3′), ex5F (5′-ACA AGG CCT TTG ACC ACA TC-3′), ex5R (5′-GTG CCC AGG GTG AAT CCT C-3′), ex6F (5′-CTG TTA ATC CAC CCT GTC CC-3′) and ex6R (5′-GAG TCG CAC AGG AAG GAG AG-3′). The exon 8 was amplified in two PCR reactions (designed in this study by 8a and 8b) due to its large size. The primers used were: ex8aF (5′-TTC CCA CGT ACC ATC TTT CC-3′), ex8aR (5′-GGG CAA AGA GGA AGA GGA AG-3′), ex8bF (5′-GTG AGC TCT CCG TGG TCA TC-3′) and ex8bR (5′-CCT CAT GGC AAC ATG CAC-3′). The proband's half-brother, as well as 100 Portuguese normal-hearing controls were screened for *WFS1* exon 5.

PCR product of *WFS1* exon 6 was purified with Zymoclean™ Gel DNA Recovery Kit (Zymoresearch) following the manufacturer's instructions. All the remaining PCR products were purified using JETQUICK PCR Product Purification Spin Kit (GENOMED). The electrophoretograms from bidirectional sequencing were evaluated by visual inspection and pairwise alignment to reference sequences using NCBI's BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1997).

The 1000 Genomes, Exome Variant Server, Human Gene Mutation Database (HGMD) and dbSNP databases were searched for the p.Asp171Asn mutation.

Twenty-seven wolframin sequences from mammalian species were multi-aligned against the human wolframin sequence (supplementary information) in ConSurf (Ashkenazy et al., 2010). Conservation scores were calculated using the Bayesian method (default).

The possible pathogenic effects of the p.Asp171Asn mutation were predicted using Provean (Protein Variation Effect Analyzer) (Choi et al., 2012), which also provided results from SIFT (Kumar et al., 2009), and Polyphen-2 (v2.2.2r398) (Adzhubei et al., 2010) prediction algorithms. The protein accession of the template sequence in Provean was ENSP00000226760, and in Polyphen-2 was O76024.

3. Results

3.1. Audiometric data

The patient here analyzed, aged 49 and reporting loss of hearing since her third decade of life, was shown to display bilateral moderate LFSNHL after audiological evaluation (Fig. 1A).

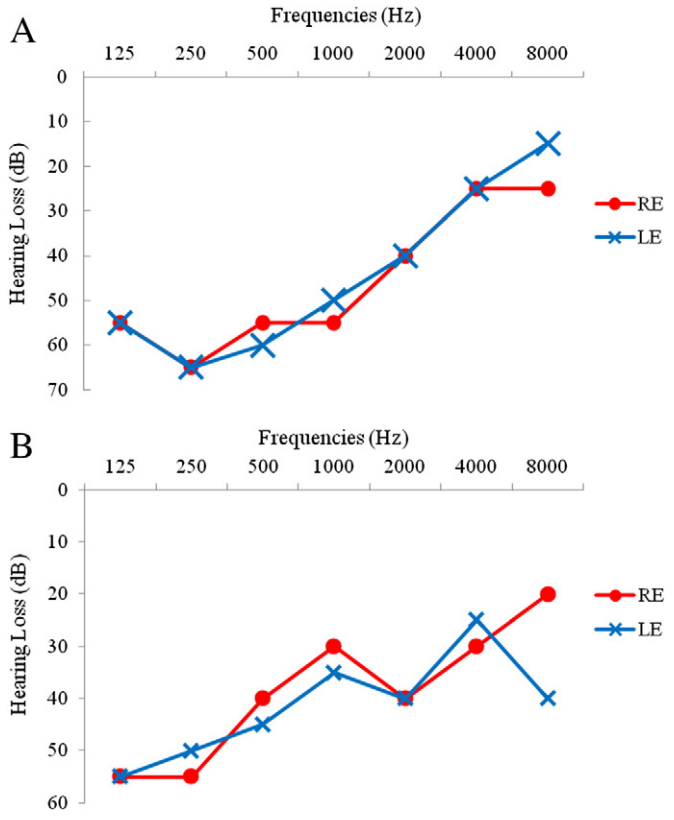

Fig. 1. Pure-tone audiogram of the proband. RE and LE mean the right ear and left ear respectively. 1A — aged 49; 1B — aged 62.

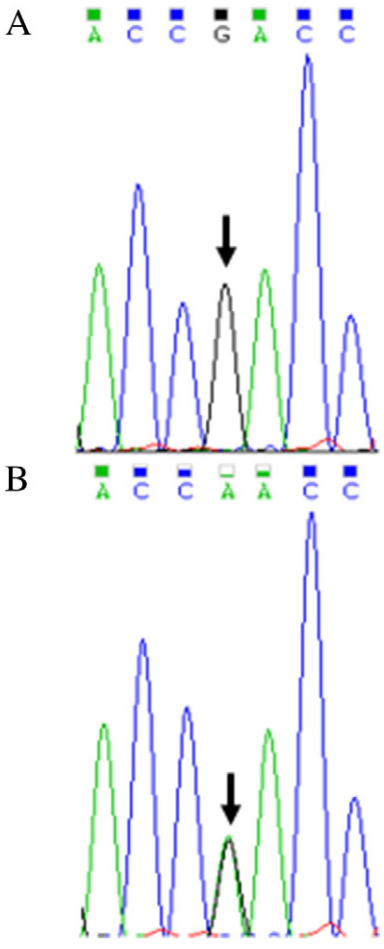

Fig. 2. Electrophoretogram showing: A — wild-type; B — c.511G>A (p.Asp171Asn) mutation in heterozygosity.