Journal of Human Genetics (2019) 64:257–260
https://doi.org/10.1038/s10038-018-0546-4

BRIEF COMMUNICATION

Further evidence for loss-of-function mutations in the *CEACAM16* gene causing nonsyndromic autosomal recessive hearing loss in humans

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Received: 8 October 2018 / Revised: 13 November 2018 / Accepted: 14 November 2018 / Published online: 4 December 2018
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Abstract

Mutations in the *CEACAM6* gene were first described as causing autosomal dominant nonsyndromic hearing loss, but two splice-altering variants have been recently described as causing autosomal recessive nonsyndromic hearing loss. We describe the novel and extremely rare loss-of-function variant c.436 C > T/p.(Arg146Ter) in the *CEACAM16* gene segregating with post-lingual progressive autosomal recessive hearing loss. This variant is predicted to significantly reduce the size of the wild type protein. Our results give additional support that loss-of-function variants in *CEACAM16* cause autosomal recessive hearing loss in humans.

Introduction

Hearing loss is the most frequent sensory disability, with a wide array of etiologies, reflecting the number of cellular types and proteins necessary to maintain the process of sound perception. Nonsyndromic hearing loss corresponds to 70% of hereditary hearing loss and may be transmitted according to any genetic pattern of inheritance, with a broad spectrum of genes and phenotypes involved [1]. Mutations in more than 150 genes (http://hereditaryhearingloss.org) have been related to hereditary hearing loss. The organ of Corti (OC), located in the cochlea, is the main organ of hearing in humans. It is a neuroepithelium composed of two types of hair cells and accessory structures such as the tectorial membrane (TM) [2], an extracellular matrix that spirals along the length of the OC and is composed of collagen fibrils and glycoproteins, such as α-tectorin (TECTA), β-tectorin (TECTB), and the carcinoembryoinic antigen-related cell adhesion molecule 16 (CEACAM16 #OMIM 614614) [2, 3]. The CEACAM family is a group of Ig-related glycoproteins with diverse functions [4]. Though it is unknown how the proteins of the TM are assembled within the lumen of the inner ear [4], their relevance in the hearing process is unquestionable, due to the large number of pathogenic variants in genes encoding TM proteins that lead to hearing loss [5, 6].

The DFNA4, an autosomal dominant nonsyndromic hearing loss (ADNSHL) locus, maps on chromosome 19q13.31 and is subdivided into DFNA4A and DFNA4B according to the mutated genes (*MYH14* gene and *CEACAM16* gene, respectively [7–10]).

CEACAM16 is well conserved among mice, rats, and humans [11].

In 2006, we described a Brazilian family in which oculocutaneous albinism and hearing loss were segregating [12]. Hearing loss was caused by the homozygous mutation c.35delG/p.(Gly12Valfs) in the *GJB2* gene and albinism was explained by the homozygous mutation c.1121delT/p.(Phe374Serfs) in the *MATP* gene. However, hearing loss in a second sibship from the same pedigree remained unexplained. In this study, we reanalyzed this sibship with three affected siblings presenting sensorineural, post-lingual, and progressive hearing loss, with age of onset in the second

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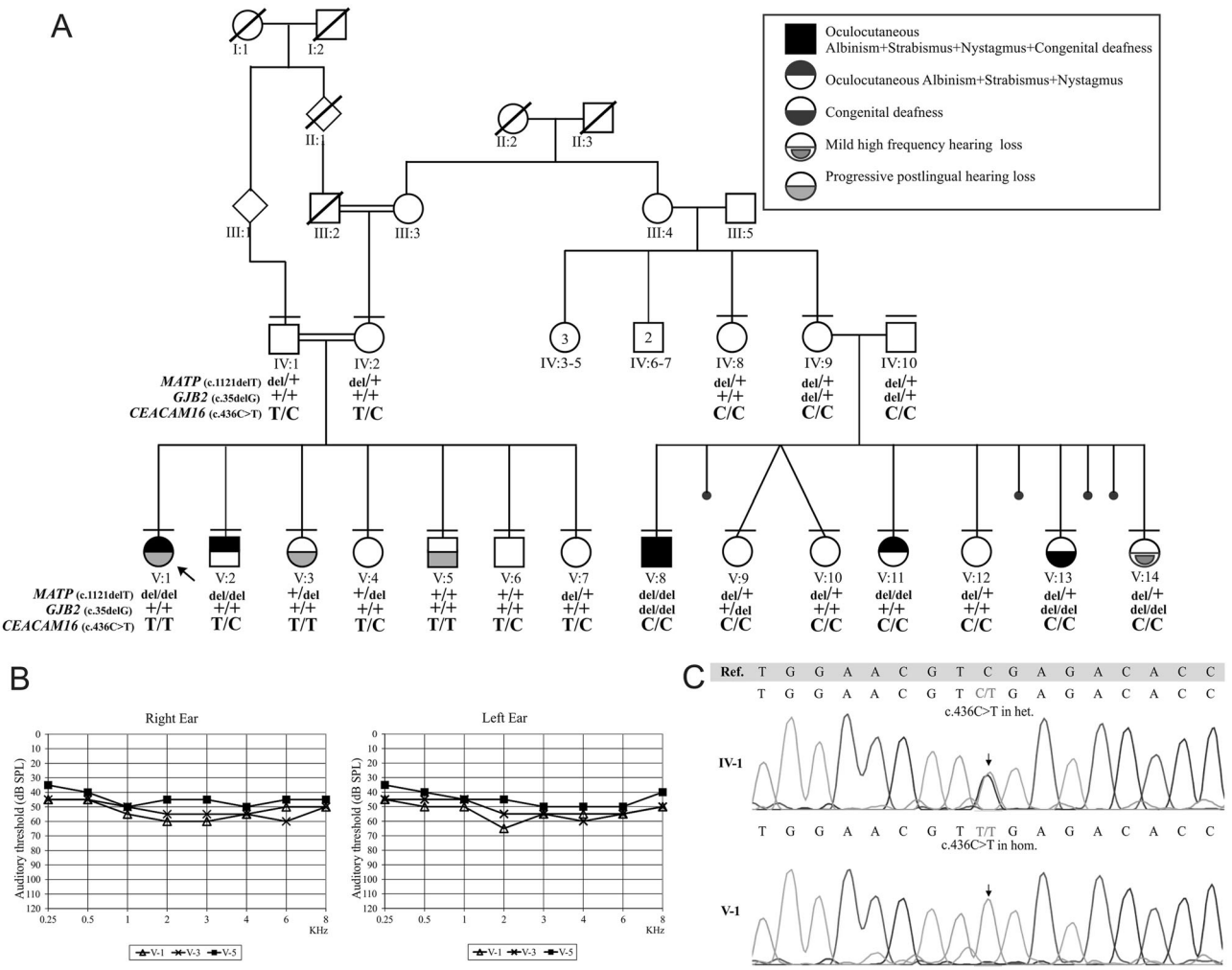

Fig. 1 **a** Pedigree of the family showing the segregation of the deafness and albinism related variants. Examined individuals are indicated with a bar above their symbol. **b** Audiograms of three affected homozygotes for c.436 C > T. **c** Chromatograms of the *CEACAM16* gene (NM_001039213.3) showing the c.436 C > T/p.(Arg146Ter) variant in heterozygous state (IV-1 sample) and in homozygous state (V-1 sample)

decade of life, and four hearing siblings, all children of a normal-hearing consanguineous couple (Fig. 1a). All individuals underwent evaluation of hearing threshold levels: pure tone audiometry, both air (frequencies range: 250–8000 Hz) and bone conduction (frequencies range: 500–4000 Hz) (Fig. 1b).

After obtaining informed consent from family members, blood samples were collected. This study was approved by CONEP, the Brazilian National Committee on Ethics in Research.

Next-generation Targeted Sequencing of 100 genes related to hearing loss was performed in the proband's sample, using SureSelect QXT Target Enrichment System Kit for Illumina Multiplexed Sequencing protocol (Agilent, Santa Clara, USA) and Illumina MiSeq System (Illumina, San Diego, USA). Data analysis was performed according to bioinformatic procedures, as described [13]. Variants were first filtered according to frequency (<1% in: 1000 Genomes Project database; The National Heart, Lung, and Blood Institute Exome Sequencing Project Exome Variant Server and ExAC). The remaining variants were filtered based on pathogenicity prediction (SIFT, PolyPhen2, Mutation Taster) and on phenotypic description (Clinvar, HGMD, DeafnessVD). The homozygous substitution c.436 C > T/p.(Arg146Ter) was found as the sole likely pathogenic variant in the proband. This variant is extremely rare; it was only described in Genome Aggregation Database (gnomAD), where it was found in heterozygous state in one individual (minor allele frequency of 0.000004128). It generates a premature stop codon at position 146 in exon 4 (*CEACAM1*6 has seven exons). It is expected that the resulting RNAm would be targeted to nonsense-mediated-decay (NMD). In case it escapes from degradation, translation would result in a severely truncated polypeptide, in

comparison to the wildtype protein, which is composed of 425 amino acids.

Sanger sequencing revealed co-segregation of the variant with HL. It was present in homozygous state in all three affected siblings and in heterozygous state in both hearing parents and in the four normal-hearing siblings (Fig. 1a, c). According to ACMG criteria for classification of variants [14], the variant is considered pathogenic (PVS1 + PM2 + PP1).

Gain-of-function mutations in the *CEACAM16* gene results in ADNHSL [8–10]. A recent study revealed that two splice-altering variants in the *CEACAM16* gene were responsible for ARNSHL in two Iranian families [15]. One of these (c.662-1 G > C) resides in the donor site of exon 5 of *CEACAM16* that leads to two abnormal splicing events, resulting in loss of the first nucleotides of exon 5 (c.662_764del) and leading to a premature stop codon (p. Phe221Cysfs*16) that alters the Ig-like C2-type 2 domain. The variant described herein generates a premature stop codon at position 146, significantly reducing the size of the protein. Once the interruption of protein synthesis in exon 5 was reported as resulting in hearing loss [15], we hypothesize that the interruption of the protein in exon 4, which has a Ig-like V-type domain, leads to a similar phenotype. Indeed, the phenotype in our patients is similar to that described [15], with all affected individuals presenting postlingual progressive hearing loss, initially affecting high frequencies, with age of onset in second decade of life. Targeted deletion of Ceacam16 in mice results in alteration of the tectorial membrane and also causes progressive hearing impairment [11]. The variant here described may have similar effects, once it is a well conserved protein among mice and humans.

ADNSHL and ARNHSL caused by mutations in the same gene is a common phenomenon in genetics of deafness, but generally the resulting phenotypes vary among different mutations and patterns of inheritance. This was not observed in *CEACAM16* until now. Our results, in combination with the first report of variants of *CEACAM16* gene causing ARNSHL [15], add *CEACAM16* gene to the list of genes that are related to different modes of transmission of hearing loss. In this particular case, the resulting phenotype seems to be very similar in gain-of-function variants, related to dominant inheritance, and loss-of-functions variants, related to recessive transmission.

In conclusion, we report on a novel homozygous nonsense variant segregating with progressive ARNSHL, giving further support that loss-of-function variants in the *CEACAM16* are implicated in progressive ARNSHL.

Acknowledgements The authors are indebted to Humberto Cezar Marcolino for technical assistance. We thank Dr. Alfredo Tabith Junior and all DERDIC staff for clinical assistance. This work was supported by FAPESP - CEPID Human Genome Research Center 2013/08028-1, Coordinator: Mayana Zatz). We thank all family members for participation in the study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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