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Original Article

Auditory Phenotype of a Novel Missense Variant in the *CEACAM16* Gene in a Large Russian Family With Autosomal Dominant Nonsyndromic Hearing Loss

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BACKGROUND: Autosomal dominant hearing loss is represented by a large number of genetically determined forms. Over 50 genes associated with dominant nonsyndromic hearing impairments were described. Pathogenic variants in the *CEACAM16* gene lead to the development of DFNA4B hearing loss. Currently, 8 pathogenic variants in this gene have been described. The objective of this study was to study the audiological and molecular genetic characteristics of a large family with *CEACAM16*-associated autosomal dominant nonsyndromic hearing loss.

METHODS: A detailed anamnesis was collected, and a comprehensive audiological examination was performed for 21 family members. Genetic testing was performed, including whole-genome sequencing for the proband's son and Sanger sequence analysis for the proband and for all available family members.

RESULTS: In a large Russian family, including 5 generations, an autosomal dominant type of slowly progressing nonsyndromic late-onset hearing loss was observed. Eleven family members suffer from hearing impairment, which starts with tinnitus and threshold increase at high frequencies, since the age of 5-20 years. Hearing loss slowly progresses with age in each person and is similar to age-related hearing loss. We have detected the novel likely pathogenic variant c.419C>T (p.(Thr140Ile)) in exon 3 of the *CEACAM16* gene, which segregates with late-onset nonsyndromic hearing loss in this family.

CONCLUSION: The clinical data obtained in the examined family correspond with the phenotype in previously described cases. In general, the study widened the mutation spectrum of the gene, allowing to carry out medical genetic counseling and to answer the questions about the hearing impairment prognosis for future generations.

KEYWORDS: Age-related hearing loss, autosomal dominant nonsyndromic hearing loss, CEACAM16, DFNA4B, late-onset hearing loss

INTRODUCTION

Autosomal dominant hearing loss is represented by a large number of genetically determined forms. As of date, there are over 50 described genes associated with dominant nonsyndromic hearing impairments, and many more are still to be discovered.[1]

The *CEACAM16* gene was first discovered in 1995 in a case of nonsyndromic autosomal dominant sensorineural hearing loss (ADSNHL) linked to the DFNA4B locus on chromosome 19q12-q13.4 (OMIM:614614).[2,3,4,5] *CEACAM16* encodes a cellular adhesion

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molecule, which represents a secreted glycoprotein similar to cancer embryonic antigen. This protein is found in mammals on the stereocilia tips of the outer hair cells (OHC), in interdental cells, in Deiters' cells, and in the tectorial membrane (TM).[6] It has been shown that it interacts with α-tectorin, one of the TM proteins.[7,8] Immunofluorescent staining and other methods prove *CEACAM16* to be a secreted protein.[2] Considering such a specific localization, it has been suggested that the protein might play a certain role in sustaining TM integrity.[9] The *CEACAM16* protein is concentrated in the attachment points of the stereocilia and TM; therefore, it is presumed to provide adhesion between stereocilia and TM and mechanical enhancement. *CEACAM16* is the third important non-collagen TM protein, and it belongs to glycoproteins like α-tectorin (TECTA) and β-tectorin (TECTB). Previous studies have shown that the products of the TECTA and TECTB genes form the TM matrix consisting of collagen fibers. In the absence of the *CEACAM16* protein, the β-tectorin levels are decreased, and the striated matrix does not develop in a proper manner. The Hensen's stripe, which is usually clearly observed in wild-type mice (wt/wt), is also absent. The *CEACAM16* protein supposedly stabilizes the interaction between α- and β-tectorin and forms structures that have an influence on the TM's physical properties. In *CEACAM16* mice (–/–), the TM is stretched, while in wild-type mice, it is contracted and detached from the OHC.[6]

Genetic modification of the main TM proteins leads to an alteration of hearing thresholds and frequency selectivity, which can only be explained by the changes in TM's mechanical properties.[10] OHC motility (changing their length due to potential alterations) provides the enhancement of the main membrane movement. The absent feedback from OHC leads to hearing impairment by approximately 50-60 dB.[7,11]

Studies on mice with absent *CEACAM16* gene showed that animals aged 6 and 12 months, compared to animals at the age of 1 month, have progressive impairment of TM matrix structure, which is more profound in the apical part of the cochlea responsible for lower frequencies. At the age of 6-7 months, the distortion product otoacoustic emissions (DPOAE) are reduced in contrast to younger animals. In mice aged 12 months, DPOAE are not registered, and the visual detection threshold of auditory brainstem responses elicited by broad-band clicks corresponds to 40 dB nHL.[8] Recent studies have shown that the TM mechanical properties are decreased in adult mice without functioning *CEACAM16* protein in comparison with the control cohort of wild-type mice. However, TM properties in young mice without this protein and wild-type mice were similar. Thus, this experiment showed that the alteration of audiological phenotype corresponds with the changes in mechanical and wave properties of the TM, which are provided by the Ceacam16 protein.[12] This demonstrates the role of TM in the development of progressive late-onset hearing loss, which was previously associated with changes in sensory receptor cells only.

MAIN POINTS

- Likely pathogenic variant c.419C>T (p.(Thr140Ile), NM_001039213.4) of the *CEACAM16* gene was described for the first time.
- The novel variant leads to slowly progressive late-onset autosomal dominant nonsyndromic hearing loss.
- The hearing impairment manifests in the age of 5-20 years with tinnitus and thresholds increase at high frequencies. It should be differentiated with age-related hearing loss if identified late.
- The study has widened the mutation spectrum of the *CEACAM16* gene.

As of date, there are published reports on only eight pathogenic variants in the *CEACAM16* gene. Audiological data are presented in a small number of studies. Five dominant and 3 recessive variants were detected in families with progressive hearing loss with the first- and second-decade onset.[2,7,13,14,21,22]

The goal of the current study was to examine the audiological and molecular genetic characteristics of a large family with *CEACAM16*-associated autosomal dominant nonsyndromic hearing loss.

MATERIALS AND METHODS

Subjects

Audiological and genetic examination was carried out for a large family with bilateral ADSNHL in 5 generations similar to age-related hearing loss (Figure 1). Comprehensive anamnesis was collected during personal consultation to exclude external factors. We obtained information on 41 family members, including 11 people with hearing impairments (I-3, II-3, III-2, III-5, III-7, IV-4, IV-5, IV-8, IV-9, IV-12, and V-7), 1 child with manifestations represented by tinnitus at the age of 8 years (V-9), and 29 relatives with normal hearing. At the moment of the initial examination, 7 children were less than 10 years old and had normal hearing (V-6, V-10, V-11, V-14, and V-15) (Table 1). In the family of the proband's mother III-5, her sister III-2 and brother III-7 had hearing impairments, and 4 brothers, III-1, III-3, III-6, and III-8, had normal hearing. All descendants of the healthy relatives had normal hearing. The proband's aunt III-2 participated only in the audiological examination in 2010 and passed away in 2015. The uncle III-7 did not take part in the audiological examination; his clinical data have not been preserved. Informed consent to participate in the study was obtained from each subject. The study was approved by the local ethical committee of Russian Medical Academy of Continuous Professional Education (Approval No: 4.3.19, Date: March 12, 2019).

Audiological Examination

A comprehensive audiological examination in accordance with age was carried out for 21 family members. It included visual examination, pure-tone audiometry (PTA) (AC-40, Interacoustics AS, Denmark), and tympanometry (AZ-26, Interacoustics AS, Denmark). The proband was observed for 10 years. The previous audiometry information of participants, if available, was obtained from their personal archives. Audiograms of the core family (proband, her mother, siblings, and children) performed at different ages are presented in Figure 2. The audiometry results for relatives residing in other regions who could not participate in our examination personally were obtained through their regional audiologist.

During PTA, the air and bone conduction thresholds at 0.25, 0.5, 1, 2, 4, and 8 kHz were determined. The severity of hearing loss was estimated as average air conduction thresholds at frequencies of 0.5, 1,

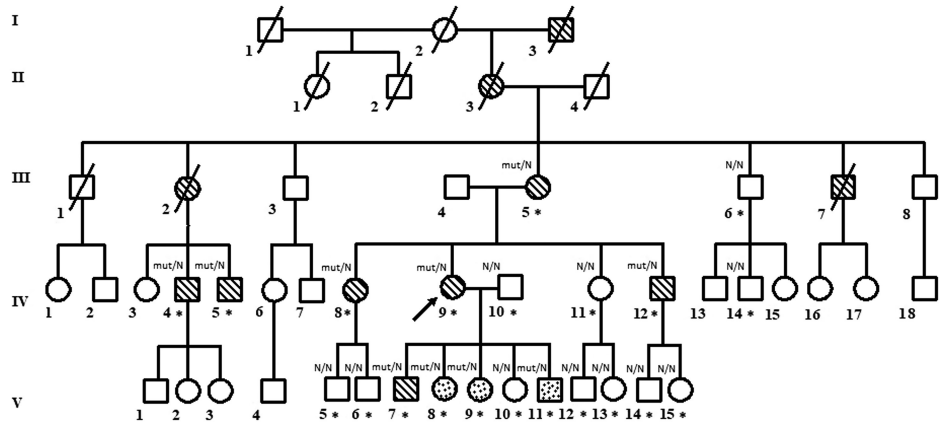

Figure 1. Family tree of a large Russian family with late-onset ADSNHL carrying a missense mutation in the *CEACAM16* gene. Squares and circles represent females and males; shaded: family members with hearing impairment; white: healthy. Subjects younger than the onset age with an intangible hearing status are marked with minute shading and a question mark, and deceased family members are marked with a slash. The arrow points at the proband (IV:9). Twenty-one family members included in the linkage analysis are marked with an asterisk.

Table 1. Clinical Data and Genotypic Characteristics of all Examined Family Members

n	Subjects	Gender	Year of Birth-Death	Age of the First Audiogram	Age at Last Test	Severity[1]	Hearing Aids	Tinnitus[2]	Alleles[3]
1	**III-2**	F	1950-2020	25y	62y	Profound	Since 50	+	–
2	IV-4*	M	1977	21y	45y	Profound	Since 40	+	C/T
3	IV-5*	M	1979	18y	43y	Profound	refused	+	C/T
4	V-2	F	2001	—	21y	Normal		–	–
5	**III-3**	M	1952	—	70y	Normal		–	–
6	**III-5***	F	1954	23y	68y	Severe	Since 55	+	C/T
7	IV-8*	F	1977	17y	45y	Moderate to severe	Since 30	+	C/T
8	V-5*	M	1997	—	25y	Normal		–	C/C
9	V-6*	M	2017	—	5y	intangibility		–	C/C
10	IV-9*	F	1978	5y	44y	Moderate	Since 31	+	C/T
11	IV-10*	M	1974	—	48y	Normal		–	C/C
12	V-7*	M	2004	10y	18y	Mild	Do not need	+	C/T
13	V-8*	F	2006	8y	16y	Normal/Mild[4]	Do not need	+	C/T
14	V-9*	F	2009	9y	13y	Normal/Mild[4]	Do not need	+	C/T
15	V-10*	F	2013	6y	9y	Intangibility	-	–	C/C
16	V-11*	M	2015	7y	7y	intangibility	Do not need	–	C/C
17	IV-11*	M	1985	—	47y	Normal		–	C/C
18	V-12*	M	2011	—	11y	Normal		–	C/C
19	V-13*	F	2012	—	17y	Normal		–	C/C
20	IV-12*	M	1987	19y	35y	Moderate	Since 21	+	C/T
21	V-14*	M	2011	7y	11y	intangibility		–	C/C
22	V-15*	F	2017	—	5y	intangibility		–	C/C
23	**III-6***	M	1956	—	66y	Normal		–	C/C
24	IV-14*	M	1986	—	36y	Normal		–	C/C
25	**III-7**	M	1960-2015	?	50y	Moderate	refused	+	–

F, female; M, male; +, present; –, not present; ND, no data; y, years of age.*Blood sample obtained.

[1]According to criteria in published literature.[15,16]

[2]Hearing loss initially manifests as tinnitus.

[3]Alleles refer to *CEACAM16* c.505, at position 45207410 (hg19).

[4]For 5 children (V-8, V-9, V-10, V-11, and V-14), the age is less than 10 years before our last audiological investigation and genetic analysis.

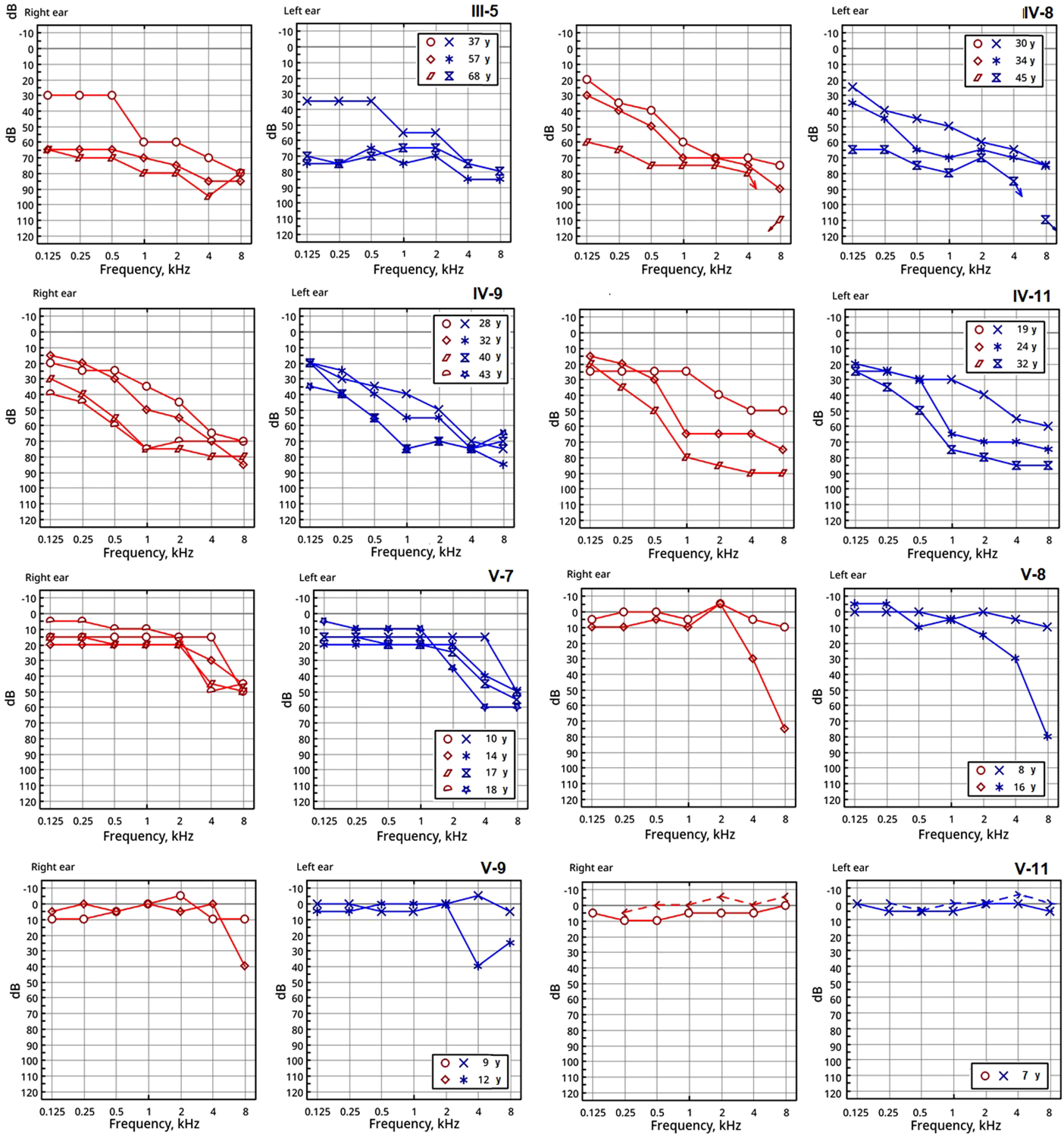

Figure 2. Air conduction audiograms of the core family (proband, her mother, siblings, and children) performed at different ages.

2, and 4 kHz in the better hearing ear and defined as normal (<25 dB HL), mild (26-40 dB HL), moderate (41-55 dB HL), moderate to severe (56-70 dB HL), severe (71-90 dB HL), and profound HL (>90 dB HL).[15,16] Computerized tomography (CT) of the temporal bone and vestibular examination were carried out for the proband; no pathology was detected.

Genetic Investigation

Samples of blood were collected from the proband and her relatives, and standard methods were used to extract genomic DNA. Upon routine diagnostic testing following the previously described protocol,[17] no pathogenic variants were detected in the *GJB2* gene.

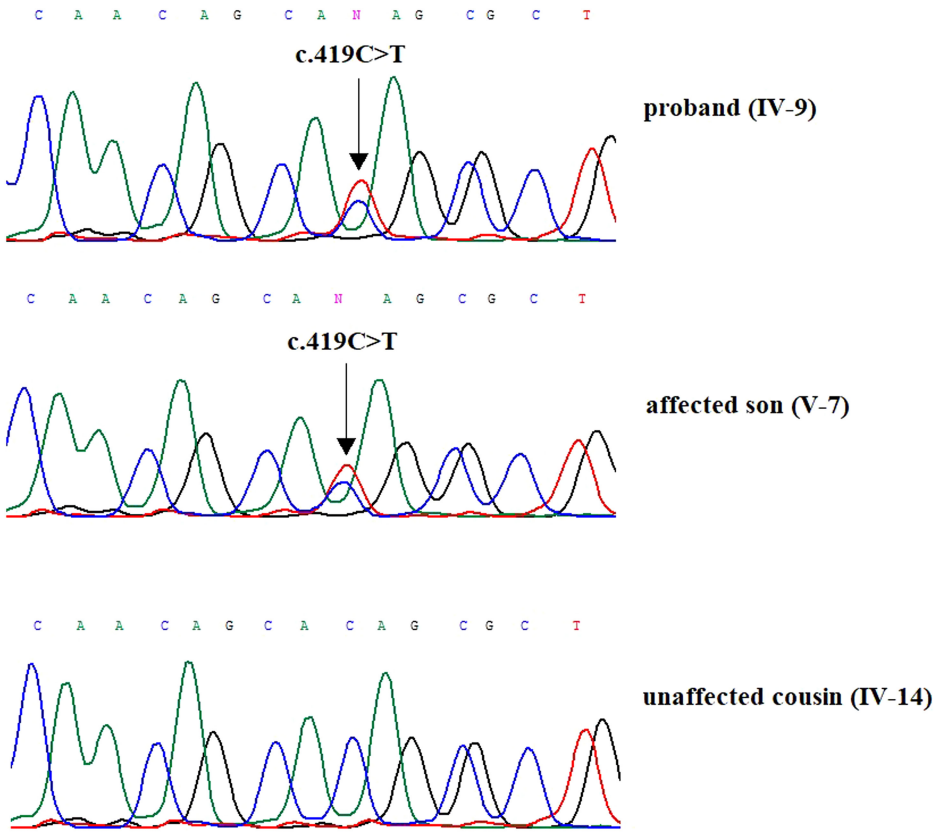

Figure 3. Genetic testing of the family with a variant in the *CEACAM16* gene. Sanger traces demonstrating the c.419C>T heterozygous missense *CEACAM16* mutation in the proband and the proband's son.

To further analyze the affected proband's son, whole-genome sequencing was conducted. The samples were prepared with the MGIEasy stLFR Library Prep Kit (MGI Tech Co., Ltd., China). Sequencing was carried out using paired-end reading (2 × 100 bp) on a DNBSEQ-T7 instrument (MGI Tech Co., Ltd., China). The obtained data underwent processing using NGSData software (http://ngs-data.ru/). The average coverage achieved for this particular sample was ×32.5. Visualization of the sequenced fragments was accomplished using Integrative Genomics Viewer software (© 2013-2018 Broad Institute and the Regents of the University of California, USA).

To filter the variants, frequency-based criteria were applied: variants with a frequency of less than 1% in gnomAD and those affecting coding regions, including missense, nonsense, coding indels, and splice sites. The clinical significance of the identified variants was evaluated in accordance with the guidelines for the interpretation of massive parallel sequencing (MPS) data.[18]

Validation of the genome variant in the *CEACAM16* gene of the proband's son, as well as its presence in the proband and other relatives, was performed through amplification and Sanger sequencing. Amplification was performed using PCR with Taq polymerase on a Veriti Dx Thermal Cycler (Thermo Fisher Scientific, Waltham, Mass, USA). The amplification algorithm consisted of the following steps: initial denaturation at 95°C for 5 minutes, followed by 32 cycles of denaturation at 94°C for 45 seconds, annealing at 62°C for 45 seconds, extension at 72°C for 45 seconds, and a final extension step at 72°C for 5 minutes, with a holding temperature of 4°C. Sanger sequencing was performed using an ABIPrism 3100xl Genetic Analyzer (Applied Biosystems, Foster City, Calif, USA), following the manufacturer's instructions. Analysis of the sequencing results was conducted using Chromas software (Technelysium Pty Ltd., South Brisbane, Australia). Custom primers were utilized (based on the NM_001039213.4 reference sequence) to amplify the fragment encompassing the candidate variants: *CEACAM16*_4F: ACCTGCCTCC TAAAACCATTCT, *CEACAM16*_4R: CATGAGGTTTGGACACTGGGTA.

RESULTS

Whole-genome sequencing was conducted for the affected proband's son (V-7), resulting in the detection of a novel heterozygous variant, c.419C>T (p.(Thr140Ile), NM_001039213.4), in exon 4 of the *CEACAM16* gene (Figure 3). This variant was not identified in the Genome Aggregation Database (gnomAD v.2.1.1) or in the exomes of 2000 Russian patients. Various prediction programs, such as PROVEAN and SIFT, suggested this variant to be disease causing, while others like FATHMM and DEOGENE2 predicted it not to be disease causing. Previously described pathogenic variants, c.418A>C/p.(Thr140Pro) and c.418A>G/p.(Thr140Ala), were reported in the 140-protein codon of the *CEACAM16* gene.[7,19]

Sanger sequencing confirmed the presence of this variant in the proband (IV-9), 4 of the proband's children (V-7, V-8, V-9, and V-11), and other affected relatives (III-5, IV-4, IV-5, IV-8, and IV-12) (Figure 2). An asterisk was used to mark all 21 family members included in the linkage analysis. This variant was absent in the adult unaffected relatives of the proband (III-6, IV-10, IV-11, and IV-14) and the children of the proband's sisters (V-5, V-6, V-12, V13, V-14, and V-15). Thus, the

variant's segregation with the disease in the family was confirmed. The Lod score was determined to be 3.2 (Lod 3.2 at $\theta = 0.00$).[20]

Based on the guidelines for interpretation of MPS data (criteria PS4, PM2, and PM5),[18] the variant was classified as likely pathogenic.

The PTA examination was repeated for all proband's children just after receiving these results. Children V-8 and V-11 do not complain of hearing impairment and were considered healthy by their mother; 1 child V-9 complained of tinnitus.

The proband IV-9, a female born in 1978, attended for consultation in 2010 at the age of 32 years to explore the etiology of hearing loss. Repeated cases of late-onset, slowly progressing bilateral age-related hearing loss were traceable in her family. The patient was concerned about the future hearing condition of her 3 children and wanted to determine the risk of the disease for future children in the family. Considering the family tree, the inheritance type of the hearing loss was undoubtedly autosomal dominant. At that moment, in the 5 generations of the family, 10 relatives had hearing impairments, including the grandmother, great-grandfather, mother, sister, and brother of the proband (Figure 1). Her older sister IV-8 and younger brother IV-12 suffered from hearing impairments; however, her younger sister IV-11 had normal hearing. Their mother III-5 suffered from severe bilateral sensorineural hearing loss at the age of 57 years. The preserved results of audiometry carried out at the age of 38 years with a downward-sloping profile showed hearing thresholds corresponding to moderate bilateral sensorineural hearing loss.

According to the proband, the hearing impairment in her family usually manifests at the age of 10 years. The family members assumed that if there was no hearing loss at 10 years, there was no need to worry about hearing later in life. The proband states that her hearing has been impaired since the age of 6 years, when she first noticed tinnitus as mild, high-pitched ringing in both ears. At the moment of examination in 2010, the proband's children (V-7, V-8, and V-9) were younger than 10 years. The oldest son V-7 was examined at the age of 10 years and found to have a threshold increase at 8 kHz and tinnitus. The late examination age of 18 showed a gradual, high-frequency hearing loss. His sister V-8 had normal hearing at the age of 8 years; until the age of 15 years, she did not have any complaints of hearing loss and refused examination. Her mother was sure that her daughter was healthy. The last examination detected elevated thresholds at high frequencies at the age of 16. The girl admitted having high-pitched ringing in the ears for a long time. Her 12-year-old sister V-9 had elevated thresholds at high frequencies as well, although a previous examination at 9 demonstrated normal thresholds, but she complained of tinnitus. The proband's little son, V-11, aged 7 years, has normal hearing.

The 10-year hearing dynamics of the oldest family members are presented in Figure 2. As of date, the proband has 5 children. She suspected manifestations of hearing loss in one of the daughters, V-9, seeing that the girl complained of tinnitus, but PTA at the age of 10 years was normal. The youngest son, V-11, was examined at the age of 7 years, and no hearing pathology was detected. According to the genetic analysis results, all of the proband's children except V-10 inherited the mutant allele from their mother.

Thus, the history of hearing impairment in the family corresponds with ADSNHL, with the onset at 10 years or later. Most family members had high-pitched ringing in their ears prior to the development of hearing loss. All affected family members had similar phenotypes characterized by bilateral sensorineural progressive hearing loss, with the onset in the second half of the first decade of life. It is worth noting that the visits to an audiologist were so late because the patients believed their hearing to be sufficient for life and did not have any trouble with education or communication until the age of approximately 20 years. Audiological examination showed that the hearing loss in this family manifested in the first or second decade of life and was accompanied by an increase in thresholds at high frequencies. Later on, every 5 or 10 years, the thresholds gradually increased at medium and then at low frequencies up to moderate-to-severe hearing loss. A CT of temporal bones and vestibular examination did not detect any structural alterations or signs of vestibular dysfunction or ear malformation. Characteristic clinical and audiological data are presented in Table 1 and Figure 2.

It is worth noting that the proband's mother III-5 did not lose hearing significantly during the last 10 years (from 58 to 68 years of age); however, during the previous 20 years (from 37 to 57 years), her hearing thresholds increased drastically at all frequencies. She did not wear a hearing aid until the age of 55, seeing that her occupation did not require communication, and she presumed her hearing to be sufficient for everyday life. Her children IV-8, IV-9, and IV-12 had a significant increase in hearing thresholds between the ages of 30 and 40 years. Her daughters were fitted with hearing aids after 30 years of age, and her son after 20 years of age (Table 1).

DISCUSSION

In our study, we identified a previously unreported likely pathogenic variant c.419C>T p.(Thr140Ile) (NM_001039213.4) in exon 4 of the *CEACAM16* gene. This variant segregates with late-onset nonsyndromic progressive hearing loss in a large Russian family with ADSNHL. Notably, the variant affects the same codon as a previously described variant reported by Zheng et al in 2011 and Zhang et al in 2020.[7,19] However, the nucleotide change in our variant results in a different amino acid substitution. In a separate study, Zheng et al (2011) identified a novel heterozygous missense variant, c.418A>C p.(Thr140Pro), in the *CEACAM16* gene in a family from the United States with postlingual bilateral sensorineural moderate hearing loss.[7] Pure-tone auditory testing revealed that hearing-impaired family members have sensorineural, postlingual, bilateral, moderate hearing loss commencing during adolescence and progressing to ~50 dB in adulthood. The authors also conducted experiments where they introduced the corresponding human nucleotide variant T140P in the mouse *CEACAM16* at position 142 (T142P), showing that the proline change at position 142 affected glycosylation. Similarly, in another study, a family from China with high-frequency ADSNHL was found to have a heterozygous missense variant, c.418A>G p.(Thr140Ala), in the *CEACAM16* gene. This variant segregated with age-related hearing loss, which manifested in affected family members after approximately 20 years of age.[19]

All previously published studies characterize *CEACAM16* as a protein interacting with α-tectorin and report pathogenic variants in the coding gene leading to ADSNHL in the DFNA4 locus. Published studies describe 5 families with different pathogenic variants of this gene,

leading to a similar clinical picture of hearing loss with late onset, slowly progressing with age. However, 2 studies demonstrate 3 pathogenic variants with presumed autosomal recessive inheritance types.

Wang[2] in 2015 detected a missense variant c.505G>A (p.(G169R)) in a heterozygous state in exon 3 of the *CEACAM16* gene due to a combined strategy based on linkage analysis and next-generation sequencing in a large pedigree from China spanning 5 generations. The variant segregated with ADSNHL in the family and was not detected in a control cohort of 200 non-related Asian people. This was the second report of variants in this gene in the case of ADSNHL.[2] The study of this protein's secretion in a HEK293T cell culture showed that the secretion effectiveness of the mutant protein was significantly lower than the wild-type protein, which indicated the pathogenicity of this genetic variant. Wang et al (2015) wrote that the majority of the affected family members had a high-frequency tinnitus at the onset of hearing loss. The affected subjects in our family had very similar phenotypes as described in their work. Hearing loss was late-onset bilateral, postlingual, sensorineural, and progressive. The higher frequencies were initially decreased in the first or second decade and progressed to profound hearing loss involving all frequencies.[2]

One more pathogenic variant, c.1094T>G (p.(Leu365Arg), also being the first *de novo* variant of the *CEACAM16* gene, was presented in a study by Hofrichter M.A. in 2015.[21] This variant affects a highly conservative amino acid in a highly conservative domain of the *CEACAM16* protein. The authors gave a detailed description of the proband whose hearing loss was detected at the age of 11 and corresponds exactly with the description of manifestation and severity in the case of the DFNA4B phenotype. His hearing loss has not progressed in the short span of 1-year, and no episodes of tinnitus have been reported. His audiogram exhibits a stable, flat threshold throughout all frequencies except 0.125 kHz in the 40-60 dB range. He was the only one affected child in the family and has no other relatives with hearing impairments.

Another recent study in a Chinese family consisting of 4 generations with late-onset progressive hearing loss has identified a novel missense c.763A>G; (p.(Arg255Gly)) variant in the *CEACAM16* gene. Experiments *in vitro* showed that this variant results in increased secretion of the mutant *CEACAM16* protein, potentially affecting its function. These results widen the *CEACAM16* variant spectrum and contribute to understanding the function and significance of *CEACAM16* in the case of a disease. They presented the audiograms of 4 affected subjects, which showed bilateral sensorineural hearing loss. Audiometry of probands with bilateral hearing loss detected at 8 years showed a down-sloping configuration. Their 7-year-old brother had no noticeable hearing loss, but the audiogram revealed high-frequency hearing loss. Their father and uncle presented with bilateral moderately severe sensorineural hearing loss, which had started around the age of 10 and got progressively worse with age.[22]

Previously, researchers described 2 more *CEACAM16* variants, which affect splicing and cause autosomal recessive nonsyndromic hearing loss. Both variants were described in Iranian families with consanguineous marriages. Booth (2018) identified 2 variants in the *CEACAM16* gene: c.37G>T and c.662-1G>C, which lead to splicing alterations. Both variants were present in a homozygous state and segregated with hearing loss in each family. Splicing studies using the minigene approach showed that the c.37G>T variant leads to total skipping of exon 2 and loss of the AUG start site. The c.662-1G>C variant activates a cryptic splice site within exon 5, leading to a mRNA frameshift.[13]

Dias et al[14] reported a novel loss-of-function variant c.436C>T (p.(Arg146Ter)) in the *CEACAM16* gene segregating with late-onset progressive autosomal recessive hearing loss. This variant is assumed to decrease the size of the wild-type protein. It is thus presumed that loss-of-function *CEACAM16* variants are able to cause autosomal recessive hearing loss.

The current study presents audiological data of patients from a large Russian family with ADSNHL in which a previously non-described likely pathogenic variant c.419C>T (p.(Thr140Ile), NM_001039213.4) in exon 4 of the *CEACAM16* gene segregates with late-onset nonsyndromic hearing loss. The clinical data obtained in the examined family correspond with the phenotype in previously described cases. In general, the study widened the mutation spectrum of the gene and the audiological phenotype, allowing to carry out medical genetic counseling and to answer the questions about the hearing impairment prognosis for future generations.

Ethics Committee Approval: This study was approved by Ethics committee of Russian Medical Academy of Continuous Professional Education (Approval No: 4.3.19, Date: March 12, 2019).

Informed Consent: Informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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