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A RARE VARIANT OF c.7636C>T p.(Gln2546*) OF THE *MYO15A* GENE IN TWO PATIENTS FROM BURYATIA WITH SENSORINEURAL DEAFNESS

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In the world the role of pathogenic variants of the *MYO15A* gene in the etiology of hearing loss has not been sufficiently studied, since the large size of the gene (66 exons, 71 kb) suggests the search for pathogenic variants using NGS technologies, which are not yet sufficiently used in routine practice. In this regard, it is relevant to study the role of pathogenic variants of the *MYO15A* gene in the etiology of non-syndromic forms of hearing impairments. The purpose of this work is to describe the rare pathogenic variant c.7636C>T p.(Gln2546*) in the *MYO15A* gene, found in a homozygous state in two siblings with prelingual profound sensorineural hearing loss from a Buryat family. Previously, this variant was found only in one patient in a compound-heterozygous state with another nonsense variant in the *MYO15A* gene in Brazil and described as pathogenic. Detection of variant c.7636C>T p.(Gln2546*) in a homozygous state in Buryat siblings may indicate either a rare case of endogamous marriage or a wider distribution of this variant in the Lake Baikal region.

Keywords: autosomal recessive deafness (DFNB3), *MYO15A* gene, variant c.7636C>T p.(Gln2546*), Republic of Buryatia.

In Russia, according to the results of audiological screening of newborns in 2013, the diagnosis of hearing loss was confirmed in 3 of 1000 newborns, of which deafness was detected in 0.6 cases per 1000 newborns [1]. Half of all cases of hearing impairment are believed to have a hereditary etiology, and most of them (70%) are non-syndromic. Currently, more than 120 genes are associated with the nonsyndromic form of hearing loss, of which about 70 genes [https://hereditaryhearingloss.org/recessive-genes the link is active at the time of 27.01.2023] are associated with autosomal recessive forms [8]. One of the first genes to be associated with autosomal recessive hearing disorders were *GJB2* (DFNB1A, OMIM #121011), *MYO7A* (DFNB2, OMIM #276903) and *MYO15A* (DFNB3, OMIM #602666). However, currently, of these three genes, the most well-studied are the *GJB2* gene (2 exons) in non-syndromic forms [7] and the *MYO7A* gene (56 exons) in Usher syndrome [6]. Since the *MYO15A* gene is associated with a non-syndromic form of hearing loss (a less specific phenotype than Usher syndrome) and has a fairly large size (66 exons), its role in the etiology of hearing loss in the world has been studied to a lesser extent. Currently, most pathogenic variants in the *MYO15A* gene have been detected using NGS technologies. The *MYO15A* gene is localized on the 7th chromosome (17p11.2) and encodes an unconventional myosin 15A consisting of 3530 amino acid residues [8]. Myosin 15A is expressed at the tips of the stereocilia of snail hair cells [2] and is necessary for their elongation, as well as for the delivery of molecules to the tips of the stereocilia [17].

In Russia, among 226 *GJB2*-negative patients with hearing impairments, two causative variants c.6046+1G>A (donor splicing site) and c.8910del p.(Val2971fs*63) were found in one patient in the *MYO15A* gene in a compound heterozygous state [15]. The mutational contribution of the *MYO15A* gene among Russian patients was less than 1% [15]. However, recently two more causative variants of c.3576G>A p.(Trp1192Ter) and c.5192T>C p.(Phe1731Ser) were found in one patient from North Ossetia in the *MYO15A* gene [12].

Thus, the molecular genetic search for causative variants of the *MYO15A* gene in cohorts of patients with non-syndromic forms of hearing loss and deafness is relevant, which will contribute to the development of our understanding of the role of this gene in the etiology of hearing loss. In this regard, the purpose of this work is to describe the variant c.7636C>T p.(Gln2546*) in the *MYO15A* gene, found in a homozygous state in two siblings from a Buryat family.

Materials and methods. *Sample of the study.* Two different-sex siblings with hearing impairments from the same Buryat family were studied, their ages at the time of the study were 69 and 65 years. The surveys provided in the framework of this research were conducted after obtaining informed written consent from the participants. This study was approved by the local Committee on Biomedical Ethics at Yakut Scientific Center of Complex Medical Problems in 2019 (Yakutsk, Protocol No. 7 of August 27, 2019).

Clinical and audiological analysis. The study of hearing was carried out using threshold tonal audiometry with the audiometer "AA222" ("Interacoustics", Denmark) for air and bone conduction at frequencies of 0,25, 0,5, 1,0, 2,0, 4,0, 8,0 kHz. The degree of hearing loss was as-

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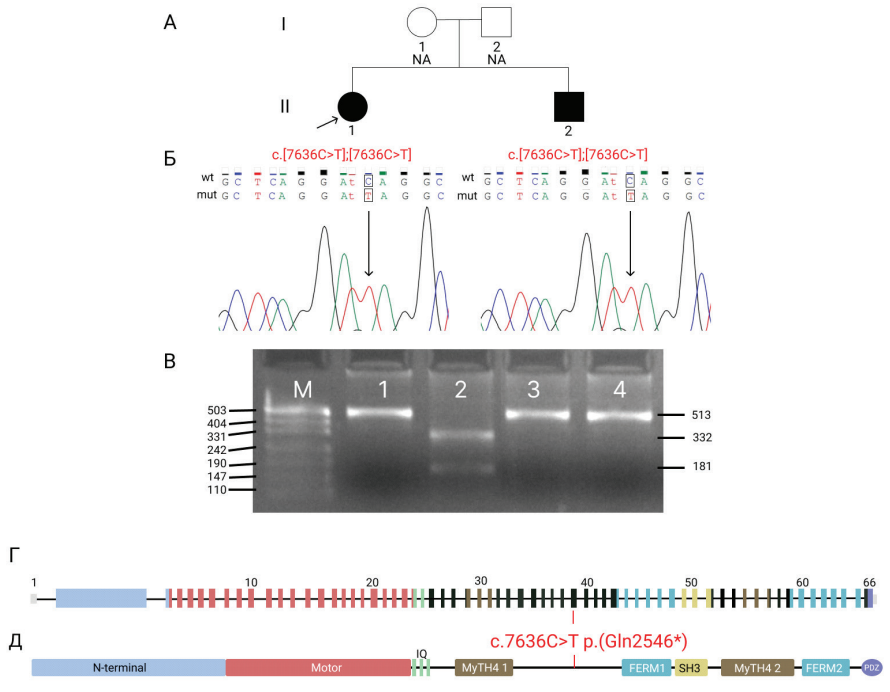
sessed by the average threshold of audibility in RDH0.5; 1.0;2.0;4.0 kHz according to the classification adopted by WHO: I degree - 26-40 dB, II degree - 41-55, III degree - 56-70, IV degree - 71-90, deafness - >90 dB.

Molecular genetic analysis. Genomic DNA was isolated from the peripheral blood leukocytes using the phenol-chloroform method. Massively parallel sequencing (MPS) was performed in one *GJB2*-negative patient with a burdened hereditary history (proband was a 69–year-old woman). MPS of genomic DNA sites corresponding to exons and splicing sites of genes associated with hereditary hearing loss (158 genes) was carried out on a HiSeq 1500, Illumina sequencer with HiSeq Rapid SBS Kit v2 reagents. The median exome coverage is 110x. Sequencing data processing was performed using an automated algorithm that included the alignment of readings to the reference sequence of the human genome (GRCh37/hg19).

Search for pathogenic variant c.7636C>T p.(Gln2546*) in the 39 exon of the *MYO15A* gene was performed in brother proband (sibs, 65 years old) using PCR-RFLP analysis. To amplify fragments in the 39 exon of the *MYO15A* gene (513 bp), primers (F) 5'-CCTGTTCTCCACAGAAACCCC-3' and (R) 5'- AACCCAGTAAGCTGGTGGGC – 3', selected using the Primer BLAST program, were used. *AclW* I endonuclease with GGATC(N)4↑ restriction site was used for restriction. Verification of the results of PCR-RFLP analyses was carried out by Sanger sequencing.

Results and discussion. Massively parallel sequencing was performed in one patient with an identified burdened hereditary history (hearing parents, deaf sibs) who did not have causative variants in the *GJB2* gene. As a result, the nonsense variant c.7636C>T p.(Gln2546*) (rs765936685) was revealed in the 39 exon of the *MYO15A* gene in the homozygous state, previously known as pathogenic (fig.). This variant leads to the replacement of the amino acid glutamine with a stop codon at the 2546th amino acid position, which leads to premature termination of the translation of the polypeptide chain of myosin 15A. The presence of this variant was verified using PCR-RFLP (fig.) followed by Sanger sequencing (fig.). This variant was also found in the homozygous state of the proband's brother (fig.).

Option c.7636C>T p.(Gln2546*) in the Deafness Variation Database (DVD) is presented as pathogenic (https://deafnessvariationdatabase.org/gene/MYO15A 17:18054586:C>T). A total of 12666 variants are annotated in this database, of which 375 were pathogenic/probably pathogenic [18]. In the ClinVar and gnomAD databases, variant c.7636C>T p.(Gln2546*) was not present at the time of the study, probably because of the lack of a phenotypic description.



Identification of the pathogenic variant c.7636C>T p.(Gln2546*) of the MYO15A gene in the Buryat family and the structure of the MYO15A gene: A – a fragment of the Buryat family pedigree; B – chromatograms of the results of sequencing the 39 exon of the MYO15A gene with variant c.7636C>T p.(Gln2546*) in the homozygous state in the proband (indicated by arrow, II-1) and sibs (II-2). NA – genotype has not been clarified; C – electrophoregrams of PCR-RFLP analysis results: M – molecular weight marker pUC 19/Msp I, 1 – sample not treated with AclW I endonuclease (513 bp), 2 – control sample without variant c.7636C>T (preserved restriction site for AclW I – 332 bp and 181 bp), genotype - c.[wt];[wt], 3 and 4 – samples with variant c.7636C>T in homozygous state in proband and sibs (restriction site for AclW I – 513 bp is lost); D – structural organization of the MYO15A gene: 66 exons are represented in the form of rectangles; E – the location of the domains of the myosin 15A protein [13]

In this paper, the variant c.7636C>T p.(Gln2546*) in the *MYO15A* gene was identified in the homozygous state for the first time. Previously, this variant was detected only in one patient with bilateral hearing impairment in a compound heterozygous state with variant c.9319G>T p.(Glu3107*) in Brazil [16]. However, the description of the nature and degree and severity of hearing loss were not described in this study [16]. In our case, audiological analysis of hearing thresholds in the proband and her sibs revealed profound sensorineural hearing loss (bilateral deafness). The onset of hearing impairment is most likely either in early childhood or is congenital. Both siblings studied at the school of the hard of hearing and deaf, and in everyday life they use only sign language. Violations in other organs and systems were not observed.

Previously, the pathogenic variant c.2674A>T p.(Ile892Phe) of the *MYO15A* gene (DFNB3, 600316) in a homozygous state with a high frequency was detected on the island of Bali (Indonesia), where 2.2% of the population had severe/profound hearing loss [3, 5]. However, subsequent studies on the spectrum and frequency of causative variants in the *MYO15A* gene among hearing impaired patients in different regions of the world have shown that in the Middle East, there are also a greater number of variants in the homozygous state [10]. In contrary, in Europe, among patients with hearing impairments, there is a predominance of compound heterozygous variants of the *MYO15A* gene [10]. The authors suggest that the accumulation of homozygous variants in the Middle East is influenced by the customs of consanguineous marriages [10]. In addition, it is known that in

this region, the founder effect is present in the distribution of individual variants of the *MYO15A* gene (c.5807_5813delCCCGTGGG and c.9995_10002dupgccggcc in Turkey, c.1171_1177dupGCCATCT in Oman) [14, 4]. While in Europe, cases of the founder effect on variants of the *MYO15A* gene have not yet been described [11]. In our case, in Buryatia, we found a variant c.7636C>T p.(Gln2546*) of the *MYO15A* gene in a homozygous state, which may indicate either a rare case of endogamous marriage, or a wider distribution of this variant in the Lake Baikal region. Further studies are required to assess the contribution of this variant in etiology of the hearing loss in the Republic of Buryatia.

Conclusion. In this paper, a rare pathogenic variant c.7636C>T p.(Gln2546*) was first detected in a homozygous state in two patients with congenital profound hearing loss. The detection of this variant in a homozygous state in two siblings from a Buryat family may indicate either a rare case of endogamous marriage, or a wider spread of this variant in the Lake Baikal region which requires further research to assess the contribution of this variant to the etiology of hearing impairment in the Republic of Buryatia.

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