ORIGINAL RESEARCH

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ANALYSIS OF PATHOGENIC VARIANTS OF THE MTDNA *MT-TS1* GENE IN PATIENTS WITH HEARING LOSS IN BURYATIA

Pathogenic variants of mitochondrial tRNA genes remain poorly understood in the context of the study of the etiology of hearing loss, despite the fact that they are one of the important causes of syndromic and non-syndromic hearing impairment as well as aminoglycoside-induced hearing loss. In this work, we searched for pathogenic variants of the mtDNA *MT-TS1* gene in patients with hearing impairments in Buryatia. One rare variant m.7445A>C was found in the *MT-TS1* gene out of five investigated variants in one patient (1/165; 0.6%). This variant is localized in the coding region of two genes the *MT-CO1* (H-chain) and *MT-TS1* (L-chain), but on different mtDNA chains. However, a pathogenetic role for the m.7445A>C substitution has been shown for the *MT-TS1* gene, but not for the *MT-CO1* gene. In databases, the m.7445A>C variant is associated with non-syndromic hearing loss, including those caused by aminoglycoside antibiotics. A comparative genotype-phenotypic analysis of our case and four cases with m.7445A>C of the *MT-TS1* gene described earlier in the literature showed that hearing loss in all cases is not congenital, but at the same time varies in severity with low penetrance. The results obtained indicate the involvement of other modulating factors in the clinical manifestation of hearing impairment associated with this variant. Thus, further study of rare variants of *MT-TS1* gene will contribute to our understanding of the pathogenetic mechanisms of mitochondrial forms of hearing loss.

Keywords: non-syndromic hearing loss, mtDNA, *MT-TS1*, m.7445A>C, Buryatia.

Introduction. Hearing impairment is one of the most common sensory pathologies. The worldwide prevalence of congenital hearing loss and deafness is estimated as 1 per 1000 newborns [6]. It is known that up to 50% of congenital deafness has an inherited etiology [18; 4]. Currently, more than 120 genes are known to be associated with hearing impairment [18; 4; 7]. About 75% of all cases of non-syndromic hearing loss are autosomal recessive forms, 10-15% – autosomal dominant and 1-2% – X-linked recessive and mitochondrial forms [18; 4; 15]. Despite the low contribution of pathogenic variants of the mitochondrial genome, they have wide clinical variability and are associated with various forms of hearing loss [2; 15], including hearing loss induced by aminoglycoside antibiotics [5; 16; 14; 22; 15].

The *MT-TS1* gene encodes the mitochondrial serine tRNA (UCN), which is involved in mitochondrial protein biosynthesis processes [11]. Currently, according to ClinVar database, 34 variants have been described in the *MT-TS1* gene, 12 of which are annotated as pathogenic and likely pathogenic https://www.ncbi.nlm.nih.gov/clinvar/?term=MT-TS1 [gene]). Among them, four variants are associated with non-syndromic deafness (OMIM:500008), MERF syndrome (OMIM:545000) and MELAS (OMIM:540000), mitochondrial cytochrome c oxidase deficiency with neurologic features (OMIM:590080.0003), palmoplantar keratoderma and deafness (OMIM:590080.0002), as well as exercise intolerance with muscle pain and lactic acidemia (OMIM:590080.0008). Most of the pathogenic variants of the *MT-TS1* gene (7 out of 12) are associated with hearing loss: m.7443A>G (rs397507452), m.7445A>T (rs199474818), m.7445A>C (rs199474818), m.7445A>G (rs199474818), m.7471dup (rs111033319), m.7510T>C (rs199474820) and m.7512T>C (rs199474817).

In the DNA diagnosis of hereditary hearing loss, pathogenic mitochondrial DNA variants are included in the research protocols in a limited number and in most cases search for only one mitochondrial variant m.1555A>G of the *MT-RNR1* gene. In Russia, analysis of the clinically relevant nuclear genes (*GJB2, SLC26A4*, and *STRC*), followed by a whole exome sequencing, is most commonly performed for DNA diagnosis of hereditary hearing loss [20]. Previously, mitochondrial variants of the *MT-RNR1* and *MT-TS1* genes were studied only in one research with a multi-ethnic sample of patients (n=410) with non-syndromic sensorineural hearing loss (Russians, Tatars, Bashkirs, Yakuts, Altaians, and Kazakhs) [1]. However, the pathogenetic role of the variants found in the *MT-RNR1* and *MT-TS1* genes, except for m.1555A>G of the *MT-RNR1* gene, remained undefined. In this regard, the aim of this study was to investigate pathogenic variants of the *MT-TS1* gene in patients with hearing impairment in the Republic of Buryatia.

Materials and methods. *Study samples.* A total of 165 hearing-impaired people were included in the study, with an average age 48.73 years old in the Republic of Buryatia. Nationality of the studied were represented in approximately equal proportions by Buryats (47.9%) and Russians (46%), the remaining 6.1% were Mongols, Nanai, Evenks, Chuvashes, Uzbeks and Germans.

Clinical and audiological analysis. Hearing impairment was determined by threshold tone audiometry using a MAICO ST 20 audiometer (Germany) by air conduction at frequencies 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and by bone conduc-

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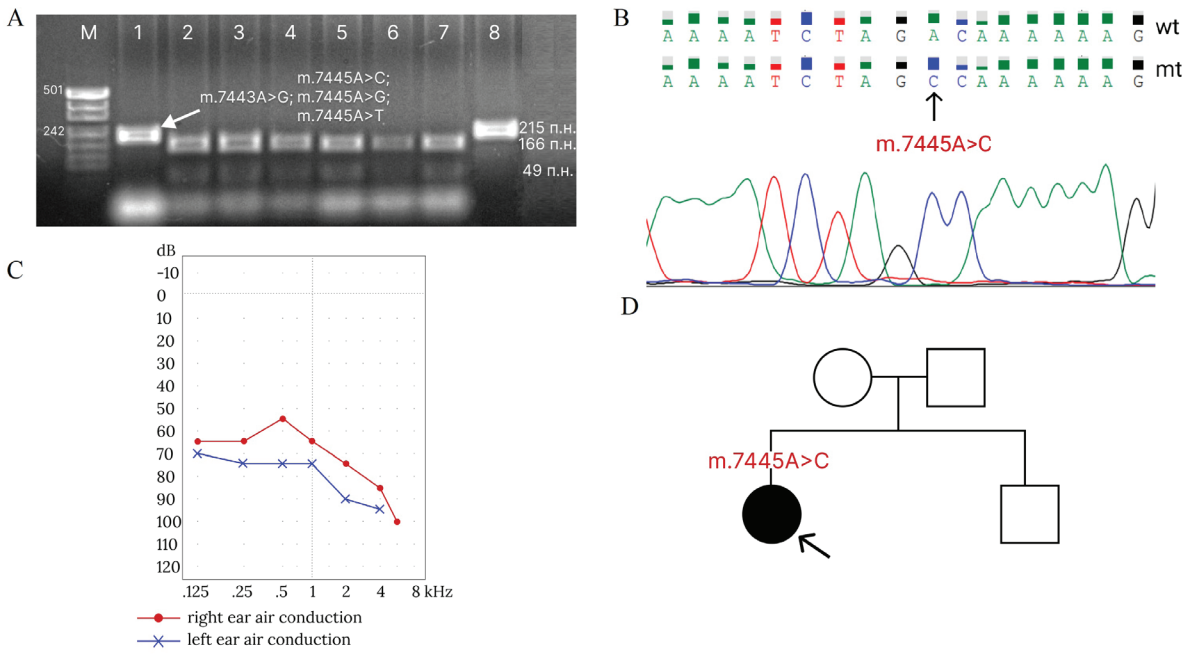

Fig. 1. Identification of the m.7445A>C variant in the *MT-TS1* gene in a patient with non-syndromic hearing loss.
Note. A - electrophoregram of RFLP analysis: M - molecular weight marker, 1 - sample with substitution in positions m.7443 A and m.7445A, 2-7 - samples without replacement, 8 - control sample not treated with XbaI enzyme; B - chromatogram section of *MT-TS1* gene sequence with m.7445A>C variant.; C - audiogram of proband; D - fragment of pedigree of patient with m.7445A>C in *MT-TS1* gene, proband marked by arrow.

tion and at frequencies 0.25, 0.5, 1.0, 4.0 kHz in 5.0 dB steps. Severity of hearing loss was defined by the average hearing threshold at 0.5;1.0;2.0;4.0 kHz, according to the WHO classification: mild - 26-40 dB, moderate – 41-70, severe – 71-90, profound - >90 dB.

Molecular genetic analysis. Genomic DNA samples were isolated from venous blood, using the phenol-chloroform method. The search for m.7443A>G (rs397507452), m.7445A>G (rs199474818), m.7445A>C (rs199474818) and m.7445A>T (rs199474818) variants was performed by PCR-RFLP using *Xba*I restriction endonuclease [10] (Fig. 1, A). Based on the results of PCR-RFLP analysis, Sanger sequencing was performed for samples lacking the *Xba*I restriction site at positions m.7443 and m.7445 (Fig. 1, B). Restriction endonuclease *Hinf*I was used to identify the m.7510T>C variant (rs199474820) by PCR-RFLP [19].

Ethical control. Surveys for this research were performed after informed written consent of the participants. This study was approved by the Local Committee on Biomedical Ethics at YSC CMP in 2019 (Yakutsk, Minutes no. 7 of 27 August 2019).

Results and discussion. In this study, among 165 patients with hearing loss in the Republic of Buryatia 5 of 7 known pathogenic variants of *MT-TS1* gene associated with non-syndromic hearing loss, including those induced by aminoglycoside antibiotics, were studied: m.7443A>G, m.7445A>C, m.7445A>G, m.7445A>T and m.7510T>C. The other two variants of the *MT-TS1* gene are not included in this study because the m.7512T>C variant is associated with MERF (OMIM:545000) and MELAS (OMIM:540000) syndromes, and the m.7471dup variant is associated with cytochrome c oxidase deficiency and neurologic features (OMIM:590080.0003). Only the m.7445A>C variant was found in one patient in the study sample, with an incidence of 0.6% (1/165). In the world, m.7445A>C occurs with a rather low frequency – in China from 0.04% (1/2651) [13] to 0.11% (1/887) [12], in Mongolia 0.42% (2/480) [8], in Russia 0.19% (1/520) [1].

The patient with the identified variant m.7445A>C was diagnosed with bilateral severe sensorineural hearing loss (Figure 1, C). The patient was 51 years old at the time of the study. From the anamnesis, it is known that the age of onset of hearing loss was 3 years after antibiotic treatment. Patient studied at a school for the deaf and hard of hearing (Ulan-Ude), does not wear hearing aids in daily life, and use speech and sign language in communication. Genealogical analysis of the patient with variant m.7445A>C showed the absence of other affected family members, which can be interpreted as a low penetrance of this variant (Fig. 1, D).

The pathogenic variant m.7445A>C in the *MT-TS1* gene is associated with non-syndromic mild hearing loss (OMIM:500008), including that caused by aminoglycosides (OMIM:580000) [14; 8; 17; 22]. Other substitutions at position m.7445, adenine (A) for guanine (G) and thymine (T), have also been described as pathogenic variants [3; 21; 9; 14; 17; 22]. Position m.7445A (Figure 2, A) is localized in the coding region of two genes: at the 3′-end of the *MT-TS1* gene (L-chain mtDNA) encoding the serine tRNA precursor (UCN) and at the 3′-end of the *MT-CO1* gene (H-chain mtDNA) encoding cytochrome c oxidase subunit 1 [21]. On the L-chain, position m.7445 is part of the 3′-endonuclease (3′-RNase Z) processing site, so replacing adenine with cytosine (A>C) leads to a failure in the serine tRNA precursor (UCN) processing (Figure 2, B) [21; 14; 17]. The exact pathogenic mechanism has been described for the m.7445A>G variant [21 and 9]. However, a similar failure of serine tRNA precursor (UCN) processing can occur with other nucleotide substitutions at the m.7445A position [14]. On the H-chain, in the *MT-CO1* gene, an adenine to cytosine substitution (A>C) results in the loss of the stop codon with the addition of three amino acids to the C-terminal of the polypeptide (Fig. 2, B), which is not thought to result in a functionally significant change in the protein [21; 14; 22].

The phenotypic effect of mitochon-

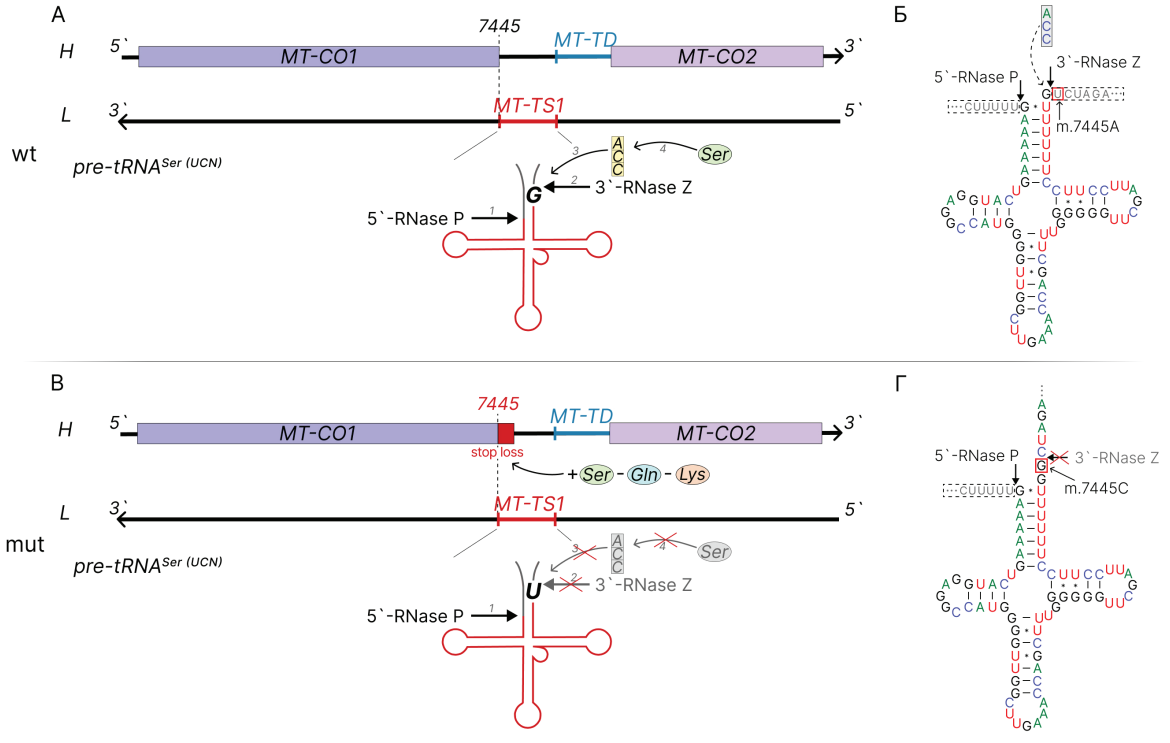

Fig. 2. Effects of adenine (A) to cytosine (C) substitution at position m.7445 for *MT-TS1* gene (L-chain) and *MT-CO1* gene (H-chain).
Note. A - Position of MT-CO1 (H-chain) and *MT-TS1* (L-chain) genes in normal, B - serine pre-tRNASer(UCN) processing and serine pre-tRNA structure in normal; C - Loss of stop codon in MT-CO1(H) gene at m.7445 substitution A>C and disruption of serine pre-tRNA processing; D - Structure of serine pre-tRNA when m.7445A>C is replaced. Figure adapted from [Guan et al., 1998 and Levinger et al., 2004].

Phenotypes of patients with the pathogenic variant m.7445A>C in the *MT-TS1* gene

Level of hearing impairment	Audiological curve configuration	Age at the time of the study (in years)	Age of manifestation (in years)	The cause of hearing loss	History of the use of aminoglycosides	Heteroplasmy/Homoplasmy	Penetrance	Frequency	Ethnicity	Literature
severe	NA	students	1-4	Due to disease	no	homoplasmy	NA	0.42% (2/480)	mongols (Mongolia)	[8]
mild	ravine	17	10	NA	no	homoplasmy	low	0.04% (1/2651) - 0.11% (1/887)	NA (China. Zhejiang Province)	[12-14]
severe	flat	19	1	NA	no	homoplasmy	low	NA	NA (China. Xingjiang Province)	[17]
moderate	NA	NA	NA	NA	NA	NA	high	0.19% (1/520)	Kazakh (Russia. Altai Republic)	[1]
severe	slope	51	3	Due to disease	yes	homoplasmy	low	0.6% (1/165)	Buryat (Russia. Republic of Buryatia)	Present study

drial variants on auditory function is highly heterogeneous and depends on the proportion of mutant and normal mtDNA copies in certain tissues, on the energy dependence of tissues, as well as on the role of modulators, in particular aminoglycosides [16]. Therefore, we performed a comparative analysis of the phenotypes of our case and four cases with m.7445A>C previously described in the literature (Table 1). In total, clinical data from five patients were included in the analysis [8; 14; 17; 1]. In most studies, the pathogenic variant m.7445A>C is found in the homoplasmy. The level of hearing impairment of patients ranged from mild to profound hearing loss with an age of manifestation ranging from 1 to 10 years. Only one patient in the present study had a history of antibiotic use. Hearing impairment due to the disease, however, has also been reported in patients from Mongolia, while no information about the disease or the use of medication has been described in other papers (Table 1). In general, for most families with m.7445A>C, the authors observed a low penetrance. According to some researchers, the biochemical defects associated with the m.7445A>C variant alone probably do not cause hearing impairment, which may be reflected in low penetrance. In this case, modifiers, in particular – antibiotics with ototoxic effects [14], associated with damage of hair cells as well as non-sensory cells modulating their function, leading to nerve damage and affecting auditory perception may play a significant role.

Conclusions. The m.7445A>C pathogenic variant in the *MT-TS1* gene was detected at a frequency of 0.6% in deaf patients from the Republic of Buryatia, which generally corresponds to the relatively low frequency of this variant in previously studied Asian regions (0.04% to 0.42%). Analysis of phenotypes of patients with this variant from different regions showed that hearing loss is not congenital in all identified cases, but varies in severity. For the majority of cases associated with m.7445A>C of the *MT-TS1* gene, low penetrance was shown, indicating the likely involvement of other modulating factors in the clinical manifestation of hearing loss. Thus, further study of rare *MT-TS1* gene variants will contribute to our understanding of the pathogenic mechanisms of mitochondrial forms of hearing loss.

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