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A LOCAL FOCUS OF ACCUMULATION OF THE MITOCHONDRIAL FORM OF HEARING LOSS IN EVENO-BYTANTAISKY DISCTRICT OF YAKUTIA

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Previously, the m.1555A>G mutation in the MT-RNR1 gene associated with the mitochondrial form of hearing loss was detected in one patient from Eveno-Bytantaisky district of Yakutia. The aim of this work is to study the mitochondrial form of hearing loss in this region of Yakutia, which probably has a local focus of accumulation of the m.1555A>G mutation in the MT-RNR1 gene. In the work, a clinical-genealogical, clinical-audiological and molecular-genetic examination of 72 residents of Eveno-Bytantaisky district was carried out for the presence of the m.1555A>G mutation in the MT-RNR1 gene of mitochondrial DNA. As a result of molecular genetic analysis, among the examined individuals, the m.1555A>G mutation was found in 6 people. Clinical and genealogical analysis, carried out up to the fifth generation, revealed that these six individuals belong to three families (including 25 deaf people). In the examined individuals with the m.1555A>G mutation of the MT-RNR1 gene, clinical phenotype variability was revealed - from normal hearing to bilateral hearing loss of III degree, with a late debut (onset from 30 to 60 years). The revealed variability is probably due to incomplete penetrance and requires further extensive research aimed at searching for genes that modulate nuclear or mitochondrial genomes.

Keywords: a mitochondrial form of hearing loss, mutation m.1555A>G, MT-RNR1 gene, Eveno-Bytantaisky national dictrict, Yakutia.

Introduction. In the world, congenital deafness is registered on average 1 per 1000 newborns and is one of the most common diseases among children in the world [3]. Both environmental and genetic factors are thought to contribute to the etiology of hearing loss. It is assumed that about half of all cases of congenital or early childhood deafness are due to hereditary causes [4]. For most hereditary diseases associated with the hearing loss, a large number of genes and mutations have been identified that cause their development; shows regional and ethnic differences in the spectrum and frequencies of detected mutations [Hereditary Hearing Loss Homepage: https://hereditaryhearinggloss.org/]. Most inherited forms of hearing loss are transmitted in a number of generations in an autosomal recessive manner, autosomal dominant and X-linked recessive and mitochondrial forms of hearing loss are less common [5].

Although mitochondrial forms of hearing loss are much less common than nuclear genome-related forms of deafness, they were among the first to be described. Thus, in 1993, Prezant et al [6] described a family case of hearing loss associated with m.1555A>G of the *MT-RNR1* gene (OMIM 561000) [6]. At the same time, the family members in whom this substitution was found showed incomplete penetrance - the hearing phenotype varied from profound hearing loss to completely normal hearing. There is a hypothesis that incomplete penetrance during the m.1555A>G substitution in the *MT-RNR1* gene of mitochondrial DNA is probably due to the use of aminoglycoside antibiotics [6-8]. This hypothesis is based on the fact that when adenine is replaced by guanine at position 1555 in the A site of human 12S rRNA, C–G pairing occurs, which leads to similarity with the A site of bacterial 16S rRNA, which is a target for aminoglycoside drugs [7]. Currently, most antibiotics from this series are used only for the treatment of severe infections [9]. However, in many developing countries, they are often used as broad-spectrum drugs [10-12]. In addition, modifier genes most likely located in the nuclear genome can influence the phenotypic manifestation of the mutation [13], it is less likely that the mitochondrial background can have a modulating effect, since this mutation was found on various mtDNA haplotypes [14, 16].

It is now known that the frequency of the m.1555A>G mutation of the *MT-RNR1* gene among patients with hearing impairments in different regions of the world varies widely, on average from 0.27% in Australia to 4.42% in Asia [2] . However, the worldwide maximum occurrence of the m.1555A>G mutation among patients was registered in Spain - 20% [8, 17], and a relatively high mutation frequency was shown in Morocco - 3.6% [18], China - 5, 1% [19], Indonesia - 5.3% [20] and Japan - 5.4% [21]. Among Russian patients, the m.1555A>G mutation of the *MT-RNR1* gene was previously registered only in a sample from St. Petersburg with a frequency of 0.8% (one Russian patient), and in a sample from Yakutia (one patient from the Eveno-Bytantai region), with a frequency of 0.57% and was not found among the examined deaf people from the Republic of Altai and the Volga-Ural region [1]. In another study, among the examined 108 individuals with hearing impairments in Yakutia, the m.1555A>G mutation of the *MT-RNR1* gene was not found (0/108) [2].

Due to the fact that m.1555A>G of the *MT-RNR1* gene most likely has a local distribution, the aim of this study was to study the mitochondrial form of hearing loss in Eveno-Bytantaisky district of Yakutia.

Materials and methods. ***Sample.*** To study cases with hearing loss of unknown etiology, a survey was conducted among residents of the Eveno-Bytantaisky district. The sample consisted of 72 individuals (68 from the village of Batagay-Alyta, 4 from the village of Kustur). Of these, males accounted for 34.7% (n=25), females - 65.2% (n=47). Average age - 44±17.21 years. Ethnic composition of the sample: Evens - 48 people. (66.6%), Yakuts - 22 people. (30.5%), Evenk - 1 person. (1.4%), mixed ethnicity (Even / Yakut) - 1 person. (1.4%).

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Clinical and audiological analysis. Assesment of level of hearing impairment was performed using threshold tone audiometry using a MAICO ST 20 audiometer (Germany) for air conduction at frequencies of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and for bone conduction at frequencies of 0.25, 0.5, 1.0, 4.0 kHz step 5.0 dB. The degree of hearing loss was assessed by the average threshold of hearing in $PTA_{0.5.1.0.2.0.4.0}$ kHz according to the WHO classification: I degree - 26.0-40.0 dB, II degree - 41.0-55.0 dB, III degree - 56.0-70.0 dB, IV degree - 71.0-90.0 dB, deafness -> 91.0 dB.

Clinical and genealogical analysis. To conduct a clinical and genealogical analysis, the collection of anamnestic data was carried out according to the individual card developed by us, which included information about the main ENT diagnosis, the probable cause of hearing loss, age at the onset of hearing loss, the presence or absence of hereditary burden and concomitant diseases. After collecting the necessary information about the proband (a study participant with hearing loss), data about the siblings and parents of the proband, information about relatives on the mother's side and on the father's side, a pedigree was compiled. To confirm the hereditary nature of hearing loss and clarify the type of inheritance, segregation analysis (SF) was performed using the Weinberg proband method [22-24]. The following formulas were used for calculations:

To calculate the probability of registering a trait in families (Fisher's method):

$$\pi = \sum n/\sum r, \quad (1)$$

where: π - probability of registration; n is the number of all probands in all properties; r - the number of affected in all sibstv;

To calculate the expected segregation frequency of a trait in families:

$$SF = \sum r - n/\sum s - n, \quad (2)$$

where: SF - expected segregation frequency, r - number of affected in all sibs, n - number of all probands in all sibs, s - total number of sibs in sibs;

To calculate standard deviation:

$$\sigma = \sqrt{SF\ (1 - SF)/\sum s - n}, \quad (3)$$

where: σ - standard deviation, SF - expected segregation frequency, s - total number of sibs in sibs; n is the number of all probands in all properties;

To test the hypothesis about the type of inheritance:

$$t = SF_0 - SF/\sigma, \quad (4)$$

where: t - Student's t-test, SF_0 - theoretically expected segregation frequency, SF - expected segregation frequency, σ - standard deviation.

Molecular genetic analysis. Genomic DNA was isolated from venous blood using a standard method using phenol-chloroform extraction followed by enzymatic digestion with proteinase K. To detect the m.1555A>G mutation of the *MT-RNR1* gene (mitochondrially encoded 12S RNA; NCBI, Gene ID: 4549; NC_012920.1) the PCR-RFLP method was applied. The sequences of oligonucleotide primers for gene fragment amplification included forward primer F5'GCTCAGCCTATATACCGCCATCTTCAGCAA3', and reverse mismatch primer R5'TTTCCAGTACACTTACCATGTTACGACTGG3'; creating a restriction site for the *Hae*III endonuclease. Visualization of the results of PCR-RFLP analysis was carried out using electrophoretic separation of restriction products in a 3% agarose gel with ethidium bromide in UV light.

Ethical control. The surveys included in the scope of this research work were conducted after the informed written consent of the participants. The research work was approved by the local committee on biomedical ethics at the YSC CMP in 2019 (Yakutsk, protocol No. 7 dated August 27, 2019).

Results and discussion. Clinical genealogical, clinical audiological and molecular genetics analyzes were performed in 72 people for the presence of the m.1555A>G mutation in the *MT-RNR1* gene of mitochondrial DNA. As a result of molecular genetic analysis, the studied mutation was found in 6 out of 72 examined people. Clinical and audiological analysis showed that the hearing loss was clinically significant in 4 people, and in two people the hearing thresholds were within the normal range (Table 1).

As a result of clinical and genealogical analysis, carried out up to the fifth generation, it was found that these six individuals belong to three families, including 25 affected people. Fragments of pedigree families with the m.1555A>G mutation of the *MT-RNR1* gene are shown in Figure.

Since only family members available at the time of the study were tested for the m.1555A>G mutation in the *MT-RNR1* gene, as well as the fact that hereditary forms of hearing loss are characterized by extremely high heterogeneity, it was important to confirm that the identified cases are associated with the mitochondrial form hearing loss. In this regard, a segregation analysis was carried out. For the correctness of the analysis (calculation is carried out only among siblings), out of 25 individuals with hearing loss, 4 people were excluded: family 1, IV-1 was not taken into account, since he was not biologically related (husband IV-2); family 2, I-2 no data on sibs, II:1 half-sibs; family 3, proband II-1 adopted (Figure). Data used for segregation analysis is presented in Table 2.

When establishing the hereditary nature of a pathological trait (deafness/hearing loss) in families, the probability of registration (π) of the trait (probability probability) according to the Fisher method (1) was:

$$\pi = 9/21 = 0.43$$

The resulting probability of registering a trait (π = 0.43) indicates its hereditary nature and corresponds to multiple incomplete registration, where $0 < \pi \leq 1$ [23, 24].

Table 1

Clinical and audiological characteristics of individuals with the m.1555A>G mutation of the *MT-RNR1* gene

Family	Cipher	Sex	Ethnicity	Age	Hearing status	Age of onset of HL	Case of HL
1	III-11	Female	Yakut	56 years	Normal	-	-
	III-25	Female	Even	62 years	Bilateral sensorineural HL (of IV degree (Moderate)	-	-
	IV-8	Female	Yakut	42 years	Bilateral sensorineural HL of III degree (Moderate)	30 years	Hereditary
	V-17	Female	Even	4 years	Normal	-	-
2	II-7	Female	Even	54 years	Bilateral sensorineural HL of III degree (Moderate)	48 years	-
3	II-1	Female	Even	62 years	Bilateral mixed HL of I degree (Mild)	60 years	Age-related

Note: "-" - no answer or data not available.

The segregation frequency (SF) (2) or the expected proportion affected for all sings (3) was:

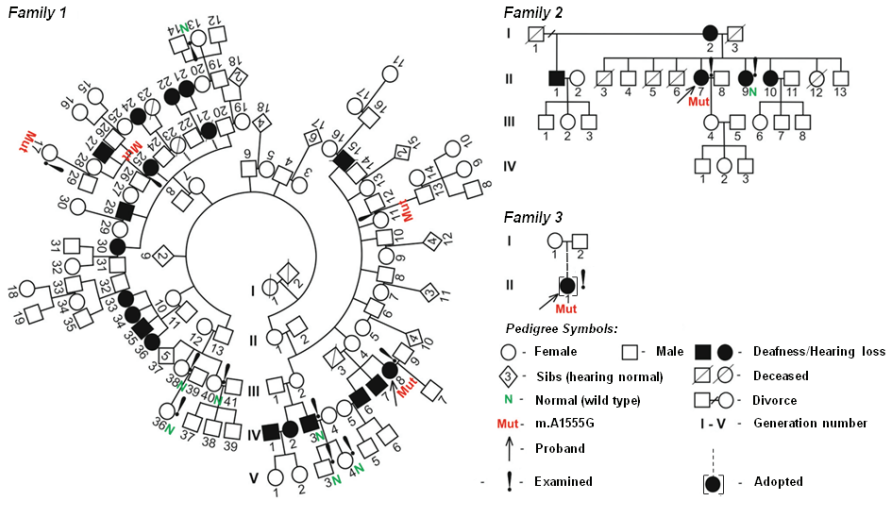
SF = 21 - 9/40 - 9 = 12/31 = 0.39

σ = √0.39 x (1 - 0.39)/40 - 9 = 0.1

The calculated segregation frequency, SF = 0.39, was higher in autosomal recessive inheritance (SF_0 = 0.25), since all probands had hearing parents, except for three nuclear families with one affected parent. Further, the obtained frequency was compared with the theoretically expected one for different types of inheritance (SF_0 = 0.25 - autosomal recessive (AR), SF_0 = 0.50 - autosomal dominant (AD)) using Student's t-test (4). As a result, negative values were obtained (t = 0,25 - √0,39/0,1 = -1,7; t = 0,50 - √0,39/0,1 = -1,4.), which refute these types of inheritance.

In general, given the results of segregation analysis, which did not confirm the autosomal dominant and autosomal recessive types of inheritance, it can be assumed that the most likely type of transmission of the disease in a number of generations is the mitochondrial type of inheritance. The obtained results testify in favor of a local focus of accumulation of hearing impairment in Eveno-Bytantaisky district of Yakutia, associated with the mitochondrial form of hearing loss associated with m.1555A>G of the *MT-RNR1* gene.

Family 1 in family 1, 11 people were examined, according to which a clinical and genealogical analysis was carried out up to the fifth generation, as a result of which it was possible to obtain information about 19 people affected by deafness who belonged to this family (Figure). In this family, testing for the presence of the mutation was carried out in 11 family members (9 nuclear families), of which four people had the m.1555A>G mutation in the mitochondrial DNA *MT-RNR1* gene. Despite the results of segregation analysis that the type of transmission of the disease in a number of generations does not contradict the mitochondrial type of inheritance, in family 1, cases of "slippage" of the pathological trait were identified, which are likely due to incomplete penetrance or incomplete information about the hearing status of members of this family in the first two - three generations (Figure). According to the results of the clinical and audiological analysis, two family members had a clinically significant hearing loss (III:25 and IV:8), and two (III:11 and V:17) had hearing thresholds within the normal range (Table 1). Thus, in this family, the m.1555A>G mutation of the *MT-RNR1* gene was found among two hearing family members. The revealed variability in the manifestation of the phenotype may be due to incomplete penetrance associated with the use of the aminoglycoside group of drugs, which, as is commonly believed, can be the main triggers of this form of hearing loss [6, 7, 11]. However, according to the results of the survey, it turned out that the affected individuals did not associate hearing loss with taking medications, and the onset of signs of hearing loss occurs at a fairly mature age (from 30 to 60 years) (Table 1). In addition, it is known that gene variants of the nuclear or mitochondrial genomes may have a modulating effect, or this may be associated with a different level of heteroplasmy. Thus, in one member of this family with hearing loss (IV-3), the m.1555A>G mutation in the *MT-RNR1* gene of mitochondrial DNA was not identified (Figure).



Pedigrees of families with the m.1555A>G mutation of the *MT-RNR1* gene

Table 1

Segregation analysis in families with deafness cases from the Eveno-Bytantai region

Sibling size	Number of nuclear family/probands (n)	Number of siblings with affected children				Total number of children		
		1	2	3	4	Affected	Non-affected	Total
						(r)	-	(s)
2	3	1	2	-	-	5	1	6
3	2	1	-	1	-	4	2	6
4	1	-	-	-	1	4	-	4
7	1	-	-	-	1	4	3	7
8	1	1	-	-	-	1	7	8
9	1	-	-	1	-	3	6	9
Total	9	3	4	6	8	21	19	40

Family 2 in family 2, two sisters with hearing loss were examined (II:7 and II:9), according to which, in this family there are three more family members (siblings and mother) with signs of hearing loss. As a result of the clinical and genealogical analysis carried out in this family, it was possible to draw a pedigree line up to the fourth generation. It should be noted that as a result of molecular genetic analysis, the m.1555A>G mutation was found only in one of the sisters (II:7), while this mutation was not detected in the other (II:9) (Figure). In this case, as in family 1, where no mutation was identified in one of the affected members of family 1 (IV:3), there may be several explanations for the lack of segregation. One of the most likely reasons may be different levels of mtDNA heteroplasmy [25] in different organs and tissues, which

makes it difficult to detect this mutation from DNA samples isolated from venous blood. Another likely reason may be the lack of maternal biological relationship between the examined individuals. However, given the common pathological phenotype present in both sisters, this variant is less likely.

Family 3 in family 3, only a 62-year-old female proband (II-1) was examined, in which the m.1555A>G mutation of the *MT-RNR1* gene was found (Figure). The patient's hearing loss was characterized as bilateral mixed hearing loss of the first degree (Table 1), and the onset of the disease was at 60 years of age. Since the proband was an adopted child, it was not possible to conduct a clinical and genealogical analysis in this family.

Conclusions

1) As a result of molecular genetic analysis, among the examined 72 individuals from the Eveno-Bytantaisky national region, the m.1555A>G mutation of the *MT-RNR1* gene was found in 6 people. At the same time, hearing loss was clinically significant in 4 people, and in two people, hearing thresholds were within normal limits. The revealed clinical variability of the phenotype in individuals with the m.1555A>G mutation of the *MT-RNR1* gene - from normal hearing to bilateral hearing loss of the III degree, is probably due to incomplete penetrance and requires further extensive research aimed at searching for modulator genes of the nuclear or mitochondrial genomes.

2) As a result of clinical and genealogical analysis, it was found that these six individuals belong to three families, including 25 people affected by deafness, in which hearing loss segregated according to the mitochondrial type of inheritance. Thus, the study revealed a local focus of accumulation of hearing impairment in Eveno-Bytantaisky district of Yakutia, associated with the mitochondrial form of hearing loss associated with m.1555A>G of the *MT-RNR1* gene. The revealed absence of the m.1555A>G mutation of the *MT-RNR1* gene in two deaf people from two different nuclear families, in which the mitochondrial type of inheritance was traced, may indicate a different level of mtDNA heteroplasmy.

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