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FREQUENCY OF M.1555A>G MUTATION IN *MT-RNR1* GENE OF MITOCHONDRIAL DNA AMONG DEAF INDIVIDUALS IN YAKUTIA

ABSTRACT

It has been established that the mutation m.1555A>G in the *MT-RNR1* gene in the homoplasmic state is associated with non-syndromic sensorineural hearing loss caused by the use of aminoglycoside antibiotics in many families of different ethnic origin. Earlier, the m.1555A>G mutation was detected on a small sample of patients (n = 65) in Yakutia with a frequency of 1.54%. In this study, we performed a search of the m.1555A>G mutation among additional sample of 108 hearing impaired individuals from Yakutia (Eastern Siberia, Russia). As a result, we found no mutation in this sample. When combining both samples (n=65 and n=108), the m.1555A>G mutation frequency in Yakutia is – 0.57% (1/173), and among the Yakut patients frequency of this mutation is 0.92% (1/108). The frequency of the m.1555A>G mutation among deaf patients in Yakutia is 0.57%, and is relatively low when compared with the global data.

Keywords: hearing loss, mitochondrial genome, m.1555A>G, *MT-RNR1*, Yakutia.

INTRODUCTION

It has been established that the mutation m.1555A>G in the *MT-RNR1* gene in the homoplasmic state is associated with non-syndromic sensorineural hearing loss caused by the use of aminoglycoside antibiotics in many families of different ethnic origin [3-5, 7, 8, 16]. The action of aminoglycosides is based on binding with the bacterial 16S rRNA of the small subunit of the ribosome, which results in the protein synthesis blocking. When adenine is replaced with guanine in *MT-RNR1* gene at 1555 bp position C-G pairing takes place in the human 12S rRNA site, which leads to a similarity to the A site of bacterial 16S rRNA, which is the target for aminoglycoside drugs [6] (fig. 1). Currently, most of the aminoglycoside drugs are used only for the treatment of severe infections, such as endocarditis, sepsis and tuberculosis [4]. However, in some developing countries, they are still being used as broad-spectrum drugs [9].

Earlier in Yakutia (Eastern Siberia, Russia) the m.1555A>G mutation was detected with a frequency of 1.54% in a small sample of patients (n = 65), 2.08% (n = 48) among Yakut patients, and the frequency of this mutation was 0.83% (n = 120) in the control sample of Yakuts without hearing impairment, [1]. The obtained values of the m.1555A>G mutation frequency indicated the urgency of conducting preventive diagnostics for the presence of this mutation before application of aminoglycoside antibiotics among the indigenous population of Yakutia. It is necessary to screen this mutation on larger sample of patients with hearing impairment to clarify the frequency of m.1555A>G in Yakutia.

Aim of study: To update data on frequency of the m.1555A>G mutation of the mitochondrial *MT-RNR1* gene in a sample of deaf patients in Yakutia in comparison with the world data.

MATERIALS AND METHODS

The sample of 108 hearing impaired individuals (66 female and 42 male) aged between 25 and 63 (mean age 44.7 ± 7.1 years) was selected. Ethnic composition of the sample: Yakuts - 60 patients; Russians - 20; individuals of other and mixed ethnicities - 28. Hearing impairment in the participants in the study was confirmed by an audiological study involving threshold tone audiometry using an audiometer "MAICO ST 20" (Germany) for air conduction at frequencies 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and bone conduction at frequencies 0.25, 0.5, 1.0, 4.0 kHz, step 5.0 DB. The degree of hearing loss was estimated at hearing thresholds of bet-

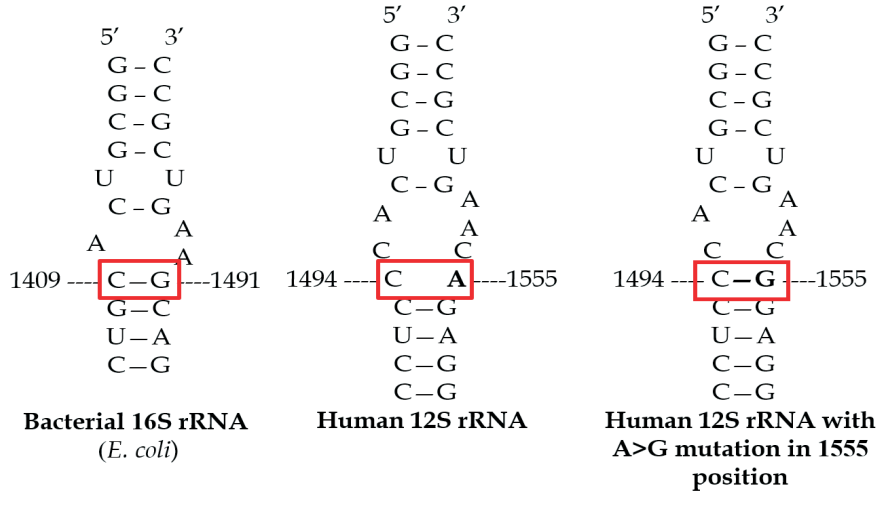

Figure 1. Molecular-genetic principle of aminoglycoside drugs action in case of m.1555A>G mutation. When adenine is replaced with guanine at the1555 bp position in the MT-RNR1 gene C-G pairing takes place in the human 12S rRNA site, which leads to a similarity to the A site of bacterial 16S rRNA, which is the target for aminoglycoside drugs [6].

ter hearing ear in the speech frequency range 0.5, 1.0, 2.0, 4.0 kHz according to the international classification.

The DNA was extracted from blood samples by a standard phenol-chloroform method with with proteinase K. The *MT-RNR1* gene of the mitochondrial genome was amplified by PCR using forward (GCTCAGCCTATATACCGCCATCTTCAGCAA; position 1247-1276) and reverse of oligonucleotide primers (TTTCCAGTACACTTACCATGTTACGACTGG; 1556-1585) flanking the 1555 bp position. The reverse primer is a mismatch primer replacing A with C at the 1557 bp position, thus in case of the m.1555A>G mutation, an artificial *HaeIII* restriction site (5 '... GG ↓ CC ... 3') is formed (fig. 2.C, the nucleotide replaced by mismatch primer is signed with black square). The results of the PCR-RFLP analysis were visualized by electrophoretic separation of the restriction products in a 3% agarose gel stained with ethidium bromide, followed by visualization in a UV-light.

All procedures in this work were conducted with the written informed consent of the participants. The local biomedical ethics committee at the FGBNU «YSC CMP» (Yakutsk, protocol No. 41, November 12, 2015) has approved this research.

RESULTS AND DISCUSSION

Among 108 hearing impaired s, the m.1555A>G mutation of the *MT-RNR1* gene was not detected (0/108). When combining results of previous [1] and the present study in Yakutia, the m.1555A>G mutation frequency is – 0.57% (1/173), and among the Yakut patients frequency of this mutation is 0.92% (1/108).

To compare the obtained data with the literature, we analyzed the global prevalence of the m.1555A>G mutation. For this purpose, data published during the period from 1999 to 2016 were used (total of 42 sources) [the list of sources is available on request]. The analyzed studies included a variety of sample scales (from 33 to 2417). Analysis showed that frequency of the m.1555A>G mutation among patients with hearing impairment in the world varies in wide range (in Australia – 0.27%, America – 0.72%, Africa – 0.97%, Europe – 1.62%, Asia – 4.42% [data available on request]. The world maximum of the occurrence among patients was recorded in Spain - 20% [10], also high incidence of m.1555A>G was registered in Morocco - 3.6% [14], China - 5.1% [2], Indonesia (5.3%) [15] and Japan - 5.4% [12]. Among Russian patients, the m.1555A>G mutation was previously registered only in the sample from St. Petersburg with a frequency of 0.8%, and was not found in the Altai Republic and in the populations of the Volga-Ural region [1]. Thus, the refined frequency of the mutation m.1555A>G in Yakutia (0.57%) corresponded to Saint Petersburg and is relatively low.

We performed additional genealogy and molecular-genetic analysis of previously described family [1], members of which were positive for m.1555A>G mutation. This family presented a variable penetrance of hearing loss. Grandmother of the proband (II:5) is positive for m.155A>G mutation but has normal hearing, possibly due to absence of

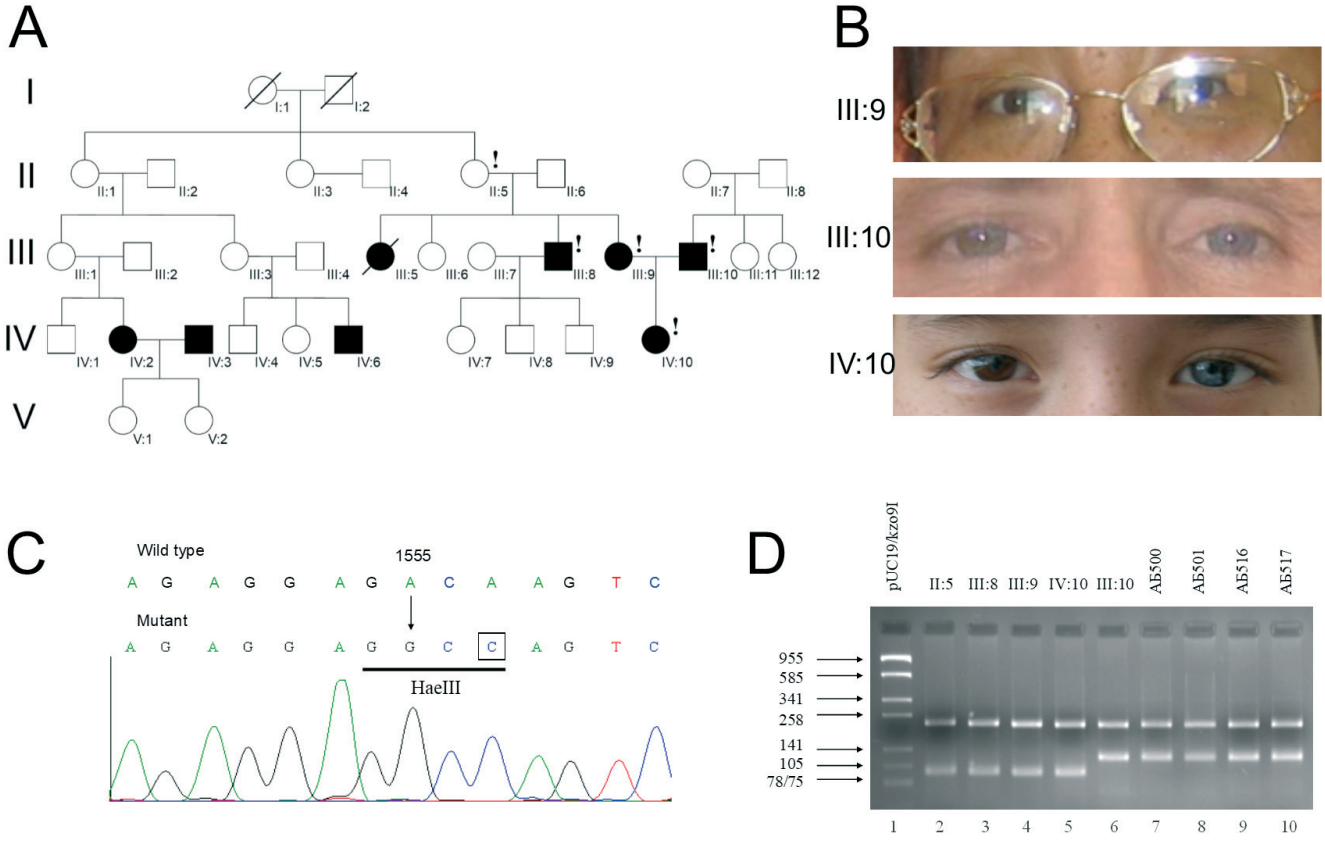

Figure 2. A. Fragment of the family tree of the proband (IV: 10), patients with hearing impairment are identified by black color, the «!» sign indicates the members who were tested for m.1555A>G mutation. B. The picture shows the iris heterochromia in the proband (IV:10) and in the father of the proband (III:10), the mother (III:9) does not have iris heterochromia. C. MT-RNR1 gene nucleotide sequence in proband (IV:10): Wild type - normal sequence, Mutant – sequence with m.1555A>G mutation. The arrow shows the nucleotide position of the mutation, the nucleotide in square is replaced in the structure of the reverse mismatch primer; the HaeIII restriction site is underlined. D. The electrophoregram of the m.1555A>G mutation PCR-RFLP analysis in a 3% agarose gel. In normal state 218 bp and 121 bp bands are observed, in case of mutation bands in 218, 91 and 30 bp are formed. (fragment 30 bp are not visualized); Left to right: lane 1 – molecular weight marker pUC19 / kzo9I; lanes 2 to 5 – samples containing m.1555A>G in the homoplasmic state; lanes 6 to 10 – samples with a normal sequence;

aminoglycosides usage history (fig. 2.A). Moreover, two cousins of the mother of the proband (III:1 and III:3) also have normal hearing and but both had deaf children (fig. 2.A). Perhaps, different penetrance is due to use of the aminoglycoside antibiotics. The m.1555A>G mutation in the homoplasmic state was previously confirmed in the proband (IV:10) and her mother (III:9) [1]. But later it was noticed that in addition to hearing loss, the proband also has heterochromia of the iris, which is also observed in the father of the proband (III:10), but the mother of the proband (III:9) has a normal color of the iris (fig. 2.B). Earlier it was reported about the possible association of m.1555A>G with the Waardenburg syndrome [11, 13], signs of which are deafness, telecant, fused eyebrows, and partial albinism [17]. The study reported a patient with a lighter skin and hair pigmentation than other relatives [13]. In this study we additionally tested three other members of the family: the probands father (III:10), the uncle of the proband (III:8) and the grandmother of the proband (II:5) (fig. 2.D). Among them, m.1555A>G was not detected only in the father of the proband (III:10), whose hearing loss reasons remain unknown and may be due to the Waardenburg syndrome. Thus, in the studied family, the iris heterochromia in the proband (IV:10) is not associated with the m.1555A>G mutation, since the proband inherited the heterochromia from the father (III:10), who did not have this mutation.

CONCLUSIONS

The frequency of aminoglycoside-induced hearing loss caused by the m.1555A>G mutation of the *MT-RNR1* gene among deaf patients in Yakutia is 0.57%, and is relatively low when compared with the global data.

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