ARTICLE

The prevalence of mitochondrial mutations associated with aminoglycoside-induced deafness in ethnic Latvian population: the appraisal of the evidence

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Abstract

Aminoglycosides are potent antibiotics which are used to treat severe gram-negative infections, neonatal sepsis, and multidrug-resistant tuberculosis. Ototoxicity is a well-known side effect of aminoglycosides, and a rapid, profound, and irreversible hearing loss can occur in predisposed individuals. *MT-RNR1* gene encoding the mitochondrial ribosomal 12S subunit is a hot spot for aminoglycoside-induced hearing loss mutations, however, a variability in the nature and frequency of genetic changes in different populations exists. The objective of this study was to analyze *MT-RNR1* gene mutations in a Baltic-speaking Latvian population, and to estimate the prevalence of such genetic changes in the population-specific mitochondrial haplogroups. In the cohort of 191 ethnic non-related Latvians, the presence of two deafness-associated mutations, m.1555A>G and m.827A>G, three potentially pathogenic variations, m.961insC(n), m.961T>G and m.951G>A, and one unknown substitution, m961T>A was detected, and the aggregate frequency of all variants was 7.3%. All genetic changes were detected in samples belonged to the haplogroups H, U, T, and J. The presence of several aminoglycoside ototoxicity-related *MT-RNR1* gene mutations in Baltic-speaking Latvian population indicates the necessity to include ototoxicity-related mutation analysis in the future studies in order to determine the feasibility of DNA screening for patients before administration of aminoglycoside therapy.

Introduction

Hearing ability is one of the key human senses which is important for the satisfactory quality of life. 360 million people worldwide have disabling hearing loss, and 32 million of those are children [1]. The causality of the hearing loss is multifactorial. Hereditary hearing loss can be conductive or sensorineural; it can be a part of a syndrome or a sole clinical symptom [2]. Acquired hearing causes include infectious diseases, chronic ear infections, otitis media, head or ear injury, excessive noise, physically blocked the ear canal, ageing, and use of certain medicines [1]. More than 150 drugs are currently known to be ototoxic; while the ototoxicity of many medicines, such as loop diuretics, macrolide antibiotics, and quinine resolves after treatment discontinuation, the use of platinum derivatives and aminoglycosides is associated with permanent hearing loss (reviewed in Lanvers-Kaminsky and Ciarimboli, 2017) [3].

Despite known nephro side effects and ototoxic side effects, aminoglycosides are still commonly prescribed antibiotics in clinical practice used to treat severe or hospital-acquired gram-negative infections, neonatal sepsis, and multidrug-resistant tuberculosis [4, 5]. Ototoxic side effects occur within days or weeks after systemic application of any amount (including a single dose) of the antibiotic, and could manifest as either cochlea damage with permanent hearing loss or vestibular damage with dizziness, ataxia and/or, nystagmus [6, 7]. Streptomycin and gentamicin are primarily vestibulotoxic, whereas

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amikacin, neomycin, dihydrosterptomycin, and kanamicin are primarily cochleotoxic [8]. The main etiology of the sensory deficit is death of inner ear sensory hair cells selectively triggered by aminoglycosides, and known mechanisms include drug trafficking across endothelial and epithelial barrier layers, sensory cell uptake of these drugs, and disruption of intracellular physiological pathways (reviewed in Jiang et al., 2017) [9].

Patients' individual susceptibility to aminoglycoside-induced hearing loss (AIHL [MIM: 580000] [10]) varies considerably, and several risk factors have been identified including high doses, high plasma concentrations, frequent applications, long treatment periods, renal dysfunction, older age, noise exposure, preexisting hearing impairment, and co-administration of other ototoxic or nephrotoxic drugs [3]. AIHL also reflects environmental-genetic interactions, as it occurs more likely in persons with specific variants in the mitochondrial genome (mtDNA) and is transmitted by maternal inheritance. A mitochondrion is a very essential organelle of a eukaryotic cell that provides cellular energy, and large deletions in the mitochondrial genome and definite mitochondrial point mutations usually lead to syndromic deafness [11]. MtDNA abnormality as a possible basis of the maternal inheritance of the aminoglycoside ototoxicity was firstly noted by Higashi in 1989 [12]. Later, several mutations, such as m.1555A>G, m.1494C>T, m.961_962delTinsC(n), m.1095T>C and m.827A>G were discovered in the mitochondrial ribosomal 12S subunit gene (MT-RNR1) and associated with both AIHL and nonsyndromic hearing loss (NSHL) [13–18].

The pooled prevalence of the AIHL-related MT-RNR1 gene mutations in general global population was shown to be 2%; however, in different populations it seems to be variable [19]. Comprehensive worldwide data are still lacking, making it difficult to estimate the risk of AIHL in general, and to differentiate pathological mtDNA mutations from nonpathogenic polymorphisms. Moreover, variation in the degree of hearing loss in patients with mtDNA mutations indicates the involvement of additional environmental factors or genetic modifiers, many of which still have to be deciphered [20].

In Latvia, aminoglycosides are commonly prescribed as a second-line anti-tuberculosis drugs used for multidrug-resistant tuberculosis treatment [21]. In addition, the use of aminoglycoside antibiotics in the treatment of infections in babies in neonatal intensive care units has been considered as an important risk factor of sensorineural hearing loss among children and, especially, preterm infants [22]. The goal of this study was to cognize the frequency of AIHL-related MT-RNR1 gene mutations in Baltic-speaking ethnic Latvian population, and to estimate the prevalence of such mutations in the population-specific mitochondrial haplogroups (mtDNA hgs).

Materials and methods

Subjects

DNA samples of 191 ethnic non-related Latvians (European population, the Baltic region), i.e., individuals who are considered themselves as Latvians for three generations, were used in this study. Among them, 107 were females (56%), 84 males (44%); mean age was 53 years (16–88 years).

The samples and information were obtained from the Genome Database of the Latvian Population (VIGDB). The project was conducted in accordance with the Helsinki Declaration; the study protocol was approved by the Central Medical Committee of Ethics in Latvia.

mtDNA *MT-RNR1* gene analysis

For the detection of mtDNA *MT-RNR1* gene mutations which were associated with AIHL, a polymerase chain reaction (PCR) amplification of the partial mtDNA sequence and sequencing of the obtained fragment was performed. Briefly, a 1580-bp mtDNA fragment was amplified targeting whole-length *MT-RNR1* gene using following primers: forward 12smtF 5′- CCGCTTCTGG CCACAGCACT -3′ (mtDNA bases 314–333), and reverse 12smtR 5′- CTTTGGCTCTCCTTGCAA –3′ (bases 1894-1877). The PCR was performed in a final volume of 25 µl. PCR mix for one reaction contained 14 ng of DNA template, 0.025 U/µl of Thermo Scientific Dream Taq Hot Start DNA polymerase (Thermo Fisher Scientific, USA), 1× Dream Taq Buffer, 0.2 µM of each primer, 0.2 mM of dNTP mix (Thermo Fisher Scientific, USA).

The PCR was performed under the following conditions: an initial denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 30 s, extension at 72° for 1 min; final extension at 72 °C for 5 min. The PCR products were separated using electrophoresis on a 1.5% agarose gel (TopVision Agarose, Thermo Fisher Scientific, USA) in Tris-acetate EDTA buffer containing 0.2 mg of ethidium bromide/ml and were visualized with transillumination under UV light. Positive PCR products were purified using the ExoI and FastAP enzymes (Thermo Fisher Scientific, USA) and subsequently sequenced on both strands by Sanger method using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). All sequences were analyzed using *Contig Express* software (ContigExpress, USA).

The mtDNA reference sequence which was used for primer design was obtained from the National Center for Biotechnology web site (http://www.ncbi.nlm.nih.gov/GenBank Accession No. NC_012920: *Homo sapiens* mitochondrion, complete genome). The Human Mitochondrial DNA Revised Cambridge Reference Sequence was used for mutation/polymorphism analysis. This sequence was obtained from the MITOMAP database available from https://www.mitomap.org/foswiki/bin/view/MITOMAP/HumanMitoSeq.

mtDNA HV1 and HV2 variation

The analysis of the two hypervariable regions (HV1 and HV2) of the control region of the mtDNA was performed by sequencing. A 609-bp fragment, which contains the HV1 region, was amplified by PCR using primers HVS1-F (5'TACACCAGTCTTGTAAACC) and HVS1-R (5'CCT GAAGTAGGAACCAGATG). The PCR was performed in a final volume of 20 μl. The PCR mix contained (per reaction) 1× Taq Buffer with 20 mM $(NH_4)_2SO_4$, 3.125 mM $MgCl_2$, 100 μM of each dNTPs, 0.25 μM of each primer, 1 U of Taq DNA polymerase (recombinant) and 21 ng of DNA template. The PCR assays were performed under the following conditions: an initial denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 56 °C for 30 s, and elongation at 72 °C for 40 s; and a final elongation step at 72 °C for 5 min.

A 738-bp fragment, which contains the HV2 region, was amplified by PCR using primers HVS2-F (5'TGGGG GTAGCTAAAGTGAACT) and HVS2-R (5'GGTGAT GTGAGCCCGTCTAA). The PCR master mix was identical to that used for HV1 region. The PCR assays were performed under the following conditions: an initial denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 54 °C for 45 s, and elongation at 72 °C for 40 s; and a final elongation step at 72 °C for 5 min.

The PCR products were separated using electrophoresis on a 1.5% agarose gel (TopVision Agarose, Thermo Fisher Scientific, USA) in Tris-acetate EDTA buffer containing 0.2 mg of ethidium bromide/ml and were visualized with transillumination under UV light. Positive PCR products were purified using the ExoI and FastAP enzymes (Thermo Fisher Scientific, USA) and subsequently sequenced on both DNA strands using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations on an ABI Prism 3100 Genetic Analyzer (PerkinElmer, USA).

The PCR reagents were purchased from Thermo Fisher Scientific, USA.

Mitochondrial haplogroups

To investigate the mtDNA hg affiliation, hierarchical PCR–RFLP analysis was performed using the method described elsewhere [23]. The classification of mtDNA hgs was based on their position in the hierarchy of the mitochondrial phylogenetic tree available from www.phylotree.org [24].

Statistical analysis

The statistical analysis was made using GraphPad Software (GraphPad Software Inc., USA) two-tailed Fisher's exact test for categorical data (2 × 2 contingency table was analyzed). Two-tailed *p* value less than or equal to 0.05 was considered significant.

Results

mtDNA *MT-RNR1* gene mutation analysis

PCR amplicons of the desired length were successfully obtained from all DNA samples, and sequence data for all targeted mutations were included in this study. The representative cases are shown in a Supplementary Fig. S1. In total, 7.3% of samples (14/191) had shown *MT-RNR1* gene changes; 10 samples were from females (9.3%) and 4 from males (4.8%) (*P* = 0.2732). All detected mtDNA mutations were homoplasmic and were present in separate DNA samples.

The results have revealed the presence of six different genetic changes in the *MT-RNR1* gene among ethnic Latvians (Table 1). The most frequently observed mutations in this study were m.961insC(n) and m.961T>G; these changes were identified with a frequency of 2.09% (4/191) each (Table 1).

Further, m.827A>G and m.951G>A genetic changes were found in 2 out of the 191 DNA samples each (estimated frequency in our cohort = 1.05%), while both m.1555A>G and m.961T>A mutations were identified in one carrier each with an estimated frequency of 0.52%. Sequence data analysis revealed that m.961delTinsC(n), m.1494C>T and m.1095T>C mutations, which have been previously associated with AIHL, were not present in our cohort (Table 1).

The observed prevalence of m.1555A>G mutation in Latvians (i.e., 0.52) was statistically similar to those found in studies in Finland, Poland, the United States of America (USA), New Zealand and the United Kingdom (UK) (Table 1). Similarly, the frequency of m.827A>G genetic change in Latvians was in agreement to those reported in Poland and the USA (1.05 vs. 0.2 and 0.78, respectively). The absence of rare m.1494C>T and m.1095T>C genetic

Table 1 Prevalence of *MT-RNR1* gene mutations, which have been associated with aminoglycoside-induced hearing loss (AIHL), among ethnic Latvians and general populations around the world

Variation	Compliance with mtDNA haplogroup[a]	Clinical meaning[b]	Prevalence % (No) in general populations								
			Latvia	Finland [34]	Poland [35]	USA, Iowa [32]	USA, Texas [39]	NZ [47]	Argentina [48]	UK [49]	China [33]
m.1555A>G	No match	Pathogenic	0.52 (1/191)	0.12 (1/813)	0.4 (2/500)	0.23 (5/2176)	0.09 (1/1161)	0.48 (1/206)	0 (0/1042)	0.26 (19/7350)	0.27 (103/37573)
m.1494C>T	No match	Pathogenic	0 (0/191)	0 (0/813)	0 (0/500)	0.05 (1/2176)	—	—	—	—	0.02 (9/37573)
m.1095T>C	M11, R1b	Pathogenic	0 (0/191)	0 (0/813)	0 (0/500)	0 (0/2176)	—	—	—	—	—
m.961insC(n)	Several	Likely benign	2.09 (4/191)	—	0.6 (3/500)	—	**0.43**[c] **(5/1161)**	—	—	—	—
m.961delTinsC(n)	No match	Pathogenic	0 (0/191)	—	0.4 (2/500)	0.78 (17/2176)	0.6 (7/1161)	—	—	—	—
m.961T>G	H11a	Likely benign	2.09 (4/191)	—	0.8 (4/500)	—	**0.52**[d] **(6/1161)**	—	—	—	—
m.961T>A	No match	Unknown	0.52 (1/191)	—	0 (0/500)	—	—	—	—	—	—
m.827A>G	Several	Pathogenic	1.05 (2/191)	—	0.2 (1/500)	0.78 (17/2176)	—	—	—	—	—
m.951G>A	Several	Likely benign	1.05 (2/191)	—	0.4 (2/500)	—	—	—	—	—	—

References for the studies are shown

Those results shown in bold are significant when compared to the ethnic Latvians (*p* ≤ 0.05)

USA The United States of America, *NZ* New Zealand, *UK* The United Kingdom

[a]Compliance with mtDNA haplogroup was based on data provided by www.phylotree.org [24]

[b]Clinical meaning of the mutation as described in ClinVar archive available at https://www.ncbi.nlm.nih.gov/clinvar [50]

[c]*p* = 0.0273

[d]*p* = 0.0406

changes in our study cohort was concordant to the results from other countries; previously, the low number of m.1494C>T mutation-positive samples has been reported only in the general USA and Chinese populations. Similarly, m.961delTinsC(n) mutation was absent in the ethnic Latvian population in this study; while this genetic change has been reported in Poland and the USA (frequency 0.4 and 0.78, respectively), the difference was not statistically significant ($P > 0.05$).

The results of this study indicated that both m.961insC(n) and m.961T>G mutations were observed significantly more often in the ethnical Latvians than in the general USA population ($P = 0.0273$ and $P = 0.0406$, respectively) (Table 1). The frequency of both mutations (i.e., 2.09) was also higher in Latvians than those reported in Polish population (0.6 and 0.8, respectively), however, the difference was not statistically significant for this study cohort.

MT-RNR1 gene mutations in mtDNA haplogroups

In order to evaluate the occurrence of *MT-RNR1* mutations within specific maternal hgs, the mtDNA hg composition in the samples has been analyzed. The observed mtDNA hg frequencies found among Latvians are summarized in Table 2. As it was expected, the results showed that the composition of observed mtDNA hgs was similar to that reported earlier in Latvian population, and the vast majority of observed mtDNA variants belong to hgs which are common to most European populations [25]. Meanwhile, *MT-RNR1* mutations were found in four mtDNA hgs: H, U, T, and J; these hgs were also the most frequent in our sample cohort (Table 2).

Hg H, which is the most frequent mtDNA hg in all European populations except the Saami, accounted for 38.74% of the samples in this study. Among hg H samples, three different *MT-RNR1* mutations were found: m.961T>G was detected in four, m.951T>A – in two, and m.961T>A – in one sample. None of these mutations were detected in samples belonged to the other mtDNA hgs (Table 2). All four m.961T>G-positive samples belonged to the subclade H11a of the hg H. Approximately one third (32.46%) of mtDNA variants belonged to the hg U in this study. This observation was similar to the earlier results obtained for the Latvian gene pool and neighboring populations [25]. All four m.961insC(n)–positive samples belonged to the hg U in our study, and the estimated frequency of this mutation was as high as 6.45% for this mtDNA hg. Both hgs T and J were found at a relatively high proportion in our cohort: 7.33% and 9.95%, respectively. The single m.1555A>G mutation–positive sample in our study belonged to the hg J, while both samples with m.827A>G mutation belonged to the hg T. No *MT-RNR1* gene mutations have

been observed in the minor mtDNA hgs, such as V, I, HV, and W.

mtDNA HV1 and HV2 variation

In order to analyze if DNA samples which carried identical mutations were not inherited the same maternal lineage, sequences from the hypervariable region HV1 of the control region of the mtDNA were obtained and analyzed. Based on this analysis, all but two samples have shown different haplogroup confirming that these DNA samples came from unrelated individuals (Table 3).

Further, two DNA samples with identical HV1 region were studied by analysis of the hypervariable region HV2.

Again, an identical haplogroup was observed indicating strong possibility of the same maternal lineage (Table 3). Thus, while the information obtained from the VIGDB did not indicate any known family relationships between these samples, the founder effect could not been ruled out for these samples.

Discussion

Numerous studies have highlighted the mtDNA *MT-RNR1* gene as a hotspot for the late-onset NSHL, and several mutations have been strongly associated with increased susceptibility to AIHL in different populations [3]. In this

Table 2 Estimated frequencies of of *MT-RNR1* gene mutations among mtDNA haplogroups in ethnic Latvians

mtDNA haplogroup	No (%)	No (%) of mtDNA mutations						
		956–960insC	T961G	A1555G	A827G	G951A	T961A	Total
H	74 (38.74)		4 (5.41)			2 (2.7)	1 (1.35)	7 (9.46)
U	62 (32.46)	4 (6.45)						4 (6.45)
T	14 (7.33)				2 (14.3)			2 (14.3)
J	19 (9.95)			1 (5.26)				1 (5.26)
V	5 (2.62)							
I	1 (0.52)							
HV	3 (1.57)							
W	6 (3.14)							
Other	7 (3.67)							
Total	191	4 (2.09)	4 (2.09)	1 (0.52)	2 (1.05)	2 (1.05)	1 (0.52)	14 (7.33)

Table 3 The analysis of the hypervariable regions HV1 and HV2 of the control region of the mtDNA

Sample Nr	MT-RNR1 gene mutation	mtDNA haplogroup	Haplotype[a]	
			HV1	HV2
A5	956–960insC	U	16192-16256-16270-16311-16465	n/a
B5	956–960insC	U	16192-16256-16270-16475	n/a
C7	956–960insC	U	16134-16173-16356-16362	n/a
C6	956–960insC	U	16189-16193-16256-16270-16311	n/a
C4	T961G	H11	16263-16278-16293-16311-16390	n/a
D3	T961G	H11	16224-16278-16311	n/a
E6	T961G	H11	16278-16311	n/a
B8	T961G	H11	16278-16293-16311	n/a
C8	A827G	T	16126-16163-16186-16189-16294	73-152-195-263-311
B7	A827G	T	16126-16163-16186-16189-16294	73-152-195-263-311
B2	T951A	H	16354	n/a
D7	T951A	H	16104-16193-16354	n/a

[a]Numbers indicate the positions of the nucleotide changes

n/a not applicable

study, for the first time, the frequency of mitochondrial *MT-RNR1* gene mutations were studied in the Baltic-speaking ethnic Latvian population. Overall, the genotyping results of our sample cohort were consistent with those previously reported in neighboring European populations and Caucasians. The mutational screening of *MT-RNR1* gene showed the presence of six known and putative AIHL and NSHL-associated mutations, and the aggregate frequency of all variants was as high as 7.3%.

The pathogenic m.1555A>G mutation has been frequently reported among patients with AIHL in white cohort from Poland (5.5%), and Asian populations in China and Japan (up to 13.3% and 33%, respectively) [26–29]. In the Spanish population, the frequency as high as 17% was reported in adult patients affected by NSHL [30]. In general populations, m.1555A>G mutation has been rarely reported, and the frequency did not exceed 0.5%; similar results were obtained in this study for the ethnic Latvians (0.52%).

The m.1494C>T variant has also shown strong associations with the development of AIHL. Across several studies in Chinese, Spanish and North American populations, all individuals with the m.1494C>T allele have developed hearing loss after receiving an aminoglycoside antibiotic (reviewed in ref. [31]). However, this pathogenic variant has been found only rarely in the general populations in China and the USA; the carrier frequency was determined to be 0.024% and 0.05%, respectively [32, 33]. The lack of m.1494C>T mtDNA variant have been noted among Brasilian, Finnish, and Polish patients, normal controls, and/or general populations, thus indicating that it is thought to be absent from some populations [26, 34–36]. In accordance to these observations, the m.1494C>T allele was not detected in any of our cohort's samples. Similarly, m.1095T>C pathogenic variant was not detected in ethnic Latvians either. Overall, m.1095T>C mutation has been very rarely reported in general populations or in normal hearing controls worldwide, however, it was linked with SNHL in patients in China, Russia, and Japan [27, 28, 37, 38]. The absence of m.1095T>C and m.1494C>T variants in our study indicates that either these mutations are very rare or that both changes do not exist in ethnic Latvian population. Similar results were observed in the neighboring Finnish population [34].

A number of different variations at position 961 within *MT-RNR1* gene have been found in individuals with AIHL and NSHL. M961delTinsC(n) mutation, which has been previously reported in studies in Poland and USA (refs. 26, 32, 39), was absent in our sample cohort. In oppose, the putatively pathogenic mutations m.961T>G and m.961insC (n) were the most frequent in our study: the prevalence rate for both mutations separately was as high as 2.09%. The carrier frequency for both mutations in our cohort was significantly higher than the rates reported in the population

of the USA (Table 1). All m.961insC(n)–positive samples belonged to the hg U in our study; on the other hand, this genetic change was detected in the Caucasian hg T, the Native American/Asian hg A, and at least one other mtDNA hg in the USA, indicating that multiple ethnic groups may be at risk for carrying this mutation [39].

There are various opinions concerning m.961T>G allele: it is currently a defining mutation of H11a mitochondrial hg and is often considered as a non-pathogenic polymorphism; however, there are reports about its association with AIHL and SNHL [24, 40–42]. In the previous study, hg H, which is the most frequent mtDNA hg in all European populations except the Saami, accounted for 45% of the mtDNA variants in Latvians, while its subclade hg H11a was detected in 3.3% (10/299) of samples (i.e., 7.5% of all hg H) [25]. In this study, the mtDNA analysis have shown that all four m.961T>G mutation-positive samples in our cohort belonged to the hg H11a, thus providing an additional indirect evidence that m.961T>G is an hg-specific variant.

In oppose to m.961T>G, the compliance of m.961T>A with any of mtDNA hgs has not been shown previously [24]. In our study, this mutation has been detected in one sample which belonged to the hg H. Up to date, m.961T>A variant has been reported only in one NSHL patient in Russia [37]. Thus more studies are required to decipher whether this genetic change is indeed pathogenic or it is normal ethnic-associated mtDNA hg variation existing among certain populations.

M.827A>G has been frequently reported in Chinese patients with NSHL and AIHL, but not in normal hearing controls; the reported frequency rate in Chinese patients was from 3.3% to 4.4% [27, 28]. Controversially, in Brasil, this mutation has been observed in both patients and normal controls with a frequency 13.2% and 8.6%, respectively [43]. In the general population studies, the carrier frequencies for m.827A>G were 0.2% and 0.78% in Poland and USA, respectively [32, 35]. In our study, m.827A>G mutation has been detected in two samples (estimated frequency 1.05%); both samples belonged to the hg T.

Worldwide studies revealed that the prevalence of mtDNA mutations considerably varies between patients and might not necessarily result in a deafness phenotype. Thus, it has been proposed that other genetic variants, including mtDNA hgs, may contribute to the manifestation of AIHL [44]. It was also shown that mtDNA hg B increases the risk for hearing loss among the Eastern Asian pedigrees carrying m.1555A>G mutation [45]. On the other hand, Torroni and colleagues showed that m.1555A>G mutation occurs on mtDNA hgs that are common in all European populations, and concluded that mtDNA backgrounds do not play a significant role in the expression of this mutation [46]. In our study, all detected *MT-RNR1* gene variations occurred

in separate mtDNA hgs. Whether there are some interactions between specific hgs, mtDNA *MT-RNR1* gene mutations and aminoglycoside ototoxicity is an intriguing yet pending puzzle.

In conclusions, the presence of several aminoglycoside ototoxicity-related mtDNA *MT-RNR1* gene mutations in Baltic-speaking Latvian population indicates the necessity to include ototoxicity-related mutation analysis in the future studies in order to determine the feasibility of DNA screening for patients before administration of aminoglycoside therapy. The exact reason and the magnitude for the occurrence of specific *MT-RNR1* mutations in particular mtDNA hgs that was observed in our study remains unclear and urges additional research.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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