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PharmGKB Summary: Very Important Pharmacogene Information for MT-RNR1

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Introduction

Mitochondria generate cellular energy in the form of adenosine triphosphate (ATP) through the process of oxidative phosphorylation. The generation of ATP by mitochondria is critical for survival, and conditions that affect mitochondrial function can lead to serious and incurable diseases. Unlike other organelles, mitochondria contain their own genetic system, with mitochondrial DNA (mtDNA) that undergoes replication, transcription and translation [1]. While most proteins involved in oxidative phosphorylation are encoded by nuclear genes, a subset are encoded in the mitochondrial DNA. Genetic variations within one particular mitochondrial gene, *MT-RNR1* (also referred to as *MTRNR1*), have been strongly linked with the development of hearing loss following administration of aminoglycoside antibiotics. This review provides information on the pharmacogenetics of *MT-RNR1*, preceded by a brief synopsis of the mitochondrial genetic system and associated diseases. This very important pharmacogene summary on *MT-RNR1* is available with interactive links to genetic variants and drugs on the PharmGKB website at www.pharmgkb.org/gene/PA31274.

The mitochondrial genetic system

Mitochondria are ellipsoid organelles surrounded by both an outer and inner membrane. The outer membrane, a simple phospholipid bilayer, encases the entire mitochondria, while the inner membrane consists of a series of deep folds known as cristae. The proteins mediating oxidative phosphorylation associate with the inner membrane, with the cristae allowing for a

larger surface area on which oxidative phosphorylation can occur. The inner membrane encloses the matrix, where the mtDNA and other genetic machinery are located [2]. The number of mitochondria within a cell varies depending on the required energy production, with some cells containing hundreds or even thousands of these organelles [2].

The genetic material of mtDNA is circular, double-stranded, and exists in multiple copies within each single mitochondrion. Just as mitochondrial density can vary between cells, the number of mtDNA copies can vary between individual mitochondria. A single cell can harbor thousands of copies of mtDNA within its mitochondria [3]. The mtDNA encodes only 37 genes, with 13 coding for proteins involved in cellular respiration and 22 coding for transfer RNAs (tRNA). The remaining two genes, *MT-RNR1* and *MT-RNR2*, code for ribosomal RNAs (rRNA): *MT-RNR1* for 12S rRNA (small ribosomal subunit) and *MT-RNR2* for 16S rRNA (large ribosomal subunit) [4].

Mitochondrial DNA is present in ova, but not sperm, resulting in strict maternal inheritance: males can inherit diseases encoded in mitochondrial DNA from their mothers, but cannot pass it on to following generations. Females with a condition due to mutations in mitochondrial DNA can pass the condition onto their children [5]. The likelihood of children being affected depends on frequency and distribution of the disease-causing mutation in the offspring. In most individuals, all copies of their mtDNA will be identical at a specific site, a state known as homoplasmy. However, individuals can have a mixture of alleles at one or more positions within the mtDNA, a state known as heteroplasmy [3]. Heteroplasmy can occur due to the presence of multiple mtDNA alleles in the fertilized ovum, or may arise due to the accumulation of somatic mutations over time. The process of oxidative phosphorylation produces free radicals that can damage mtDNA, and the mitochondrial DNA repair system is much less effective than its nuclear counterpart [5–7]. The concept of heteroplasmy is important to consider in the context of mitochondrial disease: individuals with a mitochondrial condition may be heteroplasmic for damaging mutations within the mtDNA, but for symptoms to develop the proportion of mtDNA with the pathogenic genetic variant must reach threshold in the relevant tissue [3]. Though homoplasmy for mutations which have a significant effect on oxidative phosphorylation is possible, affected embryos are unlikely to survive past the early development stages, due to the significant impact on energy production. Thus many pathogenic mutations in the mtDNA are present in the heteroplasmic state [8].

Mitochondrial disease and hearing loss

Since mitochondria play such a critical role in cellular energy production, any form of genetic variation that affects the normal operation of mitochondria can result in a significant, negative impact on health. Hearing loss is a common symptom across many mitochondrial conditions [9, 10], and some of the same variants in the *MT-RNR1* gene that are linked with aminoglycoside antibiotic-induced hearing loss have also been associated with hearing loss unrelated to antibiotic use.

Anatomy of the ear and hearing loss

The process of hearing involves a complex series of steps in which sound waves are transduced into electrical signals that are transmitted via the auditory nerve to the brain for processing. Sound waves enter through the outer ear and travel down the ear canal, striking the tympanic membrane (eardrum) causing it to vibrate. These vibrations are transmitted to the ossicles, small bones in the middle ear. From the ossicles, the vibrations are further transmitted to the cochlea in the inner ear, a fluid-filled, snail-shaped organ. Inside the cochlea are specialized sensory cells known as hair cells, named for the rows of cilia that project from their surface into the cochlear fluid. As the sound waves reach the cochlea and travel through the cochlear fluid, the hair cells transduce the sound waves into the electrical signal sent to the brain. Hair cells in the cochlea do not regenerate, and can be permanently damaged by loud noises, conditions such as high blood pressure and diabetes, or certain drugs, including aminoglycoside antibiotics and some chemotherapeutic agents [11].

Hearing loss can be categorized based on several factors, one of which is the section of the ear that is affected. Conductive hearing loss occurs when sounds cannot travel to the inner ear, such as when the ear canal or eardrum is damaged. Sensorineural hearing loss occurs when the sound cannot be transmitted to the brain, as with injury to the hair cells within the cochlea, the auditory nerve, or the brain itself. Conductive hearing loss can often be treated, either with medication or surgery, but sensorineural hearing loss can rarely be corrected, and is often permanent [12, 13]. In addition to classification by the affected anatomy, hearing loss can also be categorized as unilateral or bilateral, and pre-lingual or post-lingual, depending on when the hearing loss occurs with regard to language acquisition.

Nonsyndromic hearing loss occurs in the absence of additional clinical findings, while syndromic hearing loss is accompanied by other features. Hearing loss severity, measured in decibels (dB HL), can range from mild to profound, as shown in Table 1. Severity levels and dB HL range classifications vary among authorities [14]. Individuals with a diagnosis of “deafness” typically have profound hearing loss [15].

MT-RNR1 and nonsyndromic hearing loss

Nonsyndromic hearing loss can be caused by a variety of environmental and genetic factors [16]. A small percentage of cases of nonsyndromic hearing loss are due to mutations within the *MT-RNR1* gene [17]. One of the specific variants causing mitochondrial hearing loss is the 1555A>G (rs267606617) variant within *MT-RNR1*. This variant exists in numerous different ethnicities worldwide with variable frequency. The 1555G allele is strongly associated with the development of aminoglycoside antibiotic-induced hearing loss. Some evidence suggests that this allele may also cause nonsyndromic hearing loss independent of aminoglycoside use, but the current data are conflicting [18, 19]. However, a number of pedigree studies do report hearing loss in individuals with 1555G and no aminoglycoside exposure on recall [20]. Many studies of 1555A>G and nonsyndromic hearing loss have been conducted by analyzing the matrilineal line within a pedigree of a proband with hearing loss. Because *MT-RNR1* is a mitochondrial gene, matrilineal relatives of a proband with the 1555G allele are also expected to have the 1555G allele. Penetrance of the mutation has varied widely between different cohorts – one family in South Africa had 18 individuals homoplasmic for the 1555G allele across three generations. The nine exposed to

streptomycin all had hearing loss, while none of the non-exposed had any form of hearing loss [21]. In individuals without a reported history of aminoglycoside use in a large Arab-Israeli family and in 19 Spanish pedigrees with confirmed deafness, penetrance was ~65% and ~54%, respectively [22, 23]. A large study of 43 Chinese pedigrees reported an overall penetrance of 15.5% when excluding those with aminoglycoside exposure; within each pedigree, penetrance ranged from 0 to 47.8% [24]. Modifying factors, such as variation within nuclear-encoded genes or mitochondrial haplotypes, may be responsible for this marked variability in penetrance [25]. The methodology of these studies may also have introduced bias, in that many included pedigrees on the basis of a proband with hearing loss rather than the presence of the 1555A>G mutation. The definitive contribution of the 1555A>G mutation to hearing loss in the absence of aminoglycoside exposure has not been well established.

In most studies on hearing loss and the 1555A>G variation, individuals are homoplasmic for the G allele. However, several studies by del Castillo *et al.* and Ballana *et al.* found a correlation between the level of heteroplasmy and the penetrance and severity of hearing loss [26, 27]. The level at which individuals began showing hearing loss varied between studies: del Castillo *et al.* found that those with a mutation load above ~50% had more severe hearing loss, while Ballana *et al.* estimated the level to be 80%. Both studies included less than 20 individuals [26, 27]. Due to the size of the studies, any conclusions about correlation should be drawn lightly. Some exceptions existed in these studies, such as the two individuals within the del Castillo *et al.* study who were homoplasmic for the 1555G allele but asymptomatic, a finding that supports the idea of a modifying factor affecting the development of hearing loss [26].

Other *MT-RNR1* variants associated with nonsyndromic hearing loss, particularly in the presence of aminoglycoside exposure, include 1494C>T (rs267606619) [28] and 1095T>C (rs267606618) [29], though these are less common and have not been studied as extensively as the 1555A>G variant. Within Chinese populations the 1494T allele appears to have an average penetrance of 18% in the absence of aminoglycoside exposure, with the severity of hearing loss ranging from mild to profound. However, the penetrance within individual pedigrees varies greatly, as members of some families with the 1494T allele show no hearing loss [30], while others report an penetrance of nearly 40% [31]. Within one study of 13 Spanish individuals with the 1494T allele who did not take aminoglycosides, the penetrance reached 77% [32]. Again, these studies report pedigrees studied because of a proband with hearing loss, and were later found to have the relevant *MT-RNR1* mutation. No studies have reported individuals who are heteroplasmic for the 1494T allele. Less than ten individuals with the 1095C allele have been found who developed nonsyndromic hearing loss [29, 33, 34]. Heteroplasmy has been seen in these individuals [33, 34], though the correlation between level of heteroplasmy and severity or age-of-onset of hearing loss does not have conclusive evidence.

Pharmacogenetics

Multiple variations within the *MT-RNR1* gene have been associated with the development of sensorineural hearing loss in patients treated with aminoglycoside antibiotics. The

aminoglycosides class of antibiotics includes streptomycin, kanamycin, gentamicin, tobramycin, amikacin, and others. They typically consist of three carbon rings onto which amino groups are attached [35]. Aminoglycosides are highly effective against gram-negative bacteria, such as *Escherichia coli*, *Klebsiella*, and *Salmonella* species, as well as *Mycobacterium tuberculosis* [35]. Aminoglycosides work by binding to 16S rRNA within the small ribosomal subunit of bacteria, specifically at the mRNA decoding site (A site). Binding leads to miscoding or premature termination of protein synthesis [36–38]. While aminoglycosides are the most commonly used antibiotic worldwide, they can cause toxic side effects, particularly ototoxicity and nephrotoxicity [35]. The ototoxicity has been shown to be a function of the concentration of aminoglycosides in the cochlear hair cells, thereby leading to increased exposure relative to other organs [39]. Individuals who carry *MT-RNR1* variations such as 1555A>G (rs267606617) and 1494C>T (rs267606619) are highly susceptible to hearing loss after exposure to aminoglycosides, regardless of dose, length of treatment or serum drug levels. The prevalence of the deafness-associated *MT-RNR1* variants is unclear and varies by population, but is estimated to be about 1–2% [40–44]. Mitochondrial genes have not been assessed in the populations in the NHLBI Exome Sequencing Project (ESP), 1000 Genomes, or Exome Aggregation Consortium (ExAC) populations.

Important variants

MT-RNR1: 1555A>G (rs267606617)

The 1555A>G variation in the *MT-RNR1* gene strongly associates with the development of bilateral, sensorineural, nonsyndromic hearing loss following aminoglycoside antibiotic use: across over 40 studies in either pedigrees or groups of unrelated patients with hearing loss, 100% of those with the *MT-RNR1* 1555G allele who received an aminoglycoside antibiotic developed hearing loss. In the majority of studies, individuals were treated with systemic streptomycin, gentamicin or kanamycin. Many individuals developed severe or profound hearing loss, though some individuals had only mild or moderate hearing loss following aminoglycoside treatment. The time of onset of hearing loss relative to aminoglycoside therapy remains unclear. While some individuals developed severe or profound hearing loss immediately following treatment, others developed hearing loss months later, and still others initially showed mild hearing loss that later progressed to serious hearing loss over a period of years [45, 46]. This variability in hearing loss is important to consider in the context of prospective studies on the effect of the 1555G allele, since consequences attributable to aminoglycoside administration may be difficult to discern when other factors associated with hearing loss risk may also be present over this extended time course. An overview of the studies linking the 1555A>G variant with aminoglycoside-induced hearing loss can be found in Table 2; more detailed descriptions of each study in the table can be found on PharmGKB at www.pharmgkb.org/clinicalAnnotation/1444608367.

While the vast majority of studies all reported that individuals with the 1555G allele develop hearing loss after receiving aminoglycosides, several recent studies have identified cases where patients did not develop hearing loss after aminoglycoside administration. Most of these studies have involved infants, as neonatal sepsis is commonly treated with gentamicin

[42]. Johnson *et al.*, in a prospective study on premature, low-birth-weight infants in the neonatal intensive care unit (NICU) receiving gentamicin treatment, identified three neonates out of 256 who carried the 1555G mutation; only one of the three failed the hearing screening [43]. In a retrospective study, Ealy *et al.* identified two NICU patients out of 703 with the 1555G allele. Neither of these patients showed hearing loss at the time of screening, which occurred at 10 days after gentamicin treatment for one infant and 25 days for the other [42]. Göpel *et al.* found 10 preterm infants out of a cohort of 7056 who carried the 1555G allele and received aminoglycoside treatment, though only three of these infants failed the hearing screening. However, the combination of aminoglycoside treatment and the 1555G allele was still a significant predictor of failed newborn hearing screening, with an odds ratio of approximately 1.3 (low birth weight was the most significant predictor of a failed newborn hearing screening) [47]. Most infants who fail a newborn hearing screen do not have permanent hearing loss [47], therefore this may not be the best metric for gauging the effect of the 1555G allele on aminoglycoside-induced hearing loss. Additionally, both Ealy *et al.* and Göpel *et al.* note that aminoglycoside-related sensorineural hearing loss may not appear until well after the time period in which their hearing screening was performed [42, 47], a caveat that may explain the results seen in the Johnson *et al.* study; as mentioned earlier, previous studies have shown great variability in the length of time between aminoglycoside treatment and development of hearing loss in those with the 1555G allele. Additionally, neonates and infants are dosed differently than older patients due to their immature renal clearance, and this may impact the incidence of adverse effects on hearing in this population [48]. No studies to date have prospectively followed neonatal or older patients exposed to aminoglycosides to determine the precise timing for hearing loss in those at risk. Studies with long-term follow-up periods are therefore critical to better determine the level of penetrance of the 1555G allele.

In a single study of cystic fibrosis patients, Al-Malky *et al.* reported a case of two children with exposure to aminoglycoside treatments and homoplasmic for the 1555G allele. One child developed severe/profound hearing loss and the other retained normal hearing. The child with normal hearing had three courses of intravenous tobramycin per year over a period of three years; the other child with hearing loss had received three courses of intravenous amikacin over a period of one year. There is no evidence to suggest that the use of tobramycin versus other aminoglycosides affects the development of hearing loss [49], though aminoglycosides antibiotics are known to have differing risks for cochleotoxicity versus vestibulotoxicity [50–52]. While many studies report full penetrance of hearing loss when treated with aminoglycosides in the presence of the 1555G allele, cases such as these highlight the importance of larger and more comprehensive studies on the effect of the 1555G allele on aminoglycoside-induced hearing loss, especially given its potential for preemptive genetic screening.

Heteroplasmy of the 1555G allele and its modulation of the incidence of aminoglycoside-induced hearing loss has been studied in a limited number of individuals. Shen *et al.* reported three individuals who received aminoglycosides and were heteroplasmic for the 1555G allele with a mutation load of approximately 50%. All had subclinical hearing loss at high frequencies detected by audiologic assessment [53]. Treatment with aminoglycosides initially impairs high frequency sounds before progressively impairing the ability to hear

lower frequency sounds [54]. del Castillo *et al.* identified one individual with aminoglycoside-induced hearing loss who had a mutation load of ~61%, and Ballana *et al.* and Shen *et al.* identified heteroplasmic subjects with aminoglycoside-induced hearing loss with mutation loads between ~70% and ~98% [27, 53]. To date, no studies describe adverse outcomes in individuals who have heteroplasmy for the 1555G allele at <50%, which may be due to the fact that individuals in these studies were identified in pedigrees collected for a proband who had already developed hearing loss. Additionally, the level of heteroplasmy is assessed in peripheral blood rather than the cochlea, and levels of heteroplasmy can vary between tissues.

MT-RNR1: 1494C>T (rs267606619)

The 1494C>T variant has also shown strong associations with the development of aminoglycoside-induced hearing loss, albeit with fewer studies compared to the 1555A>G variant. Across ten studies, all individuals with the 1494T allele developed hearing loss after receiving an aminoglycoside antibiotic. However, the cohort sizes are smaller as compared to those for the 1555A>G variant, and studies are more ethnically homogenous: eight of the ten studies were conducted in Chinese populations, with the final two studies being conducted in Spanish and North American populations. An overview of the studies linking the 1494T allele with aminoglycoside-induced hearing loss can be found in Table 3; more detailed descriptions of each study in the table can be found on PharmGKB at www.pharmgkb.org/clinicalAnnotation/1444699308.

As with the 1555G allele, hearing loss resulting from aminoglycosides in those with the 1494T allele results in bilateral, sensorineural and nonsyndromic hearing loss. Within the ten studies that linked the allele with hearing loss, the severity ranged from mild to profound. Hearing loss was noted to occur three days after treatment in one study [31] and two weeks to one month after treatment in another [28]. Currently, no studies have described individuals heteroplasmic for the 1494T allele. Only one study identified a patient with the 1494T allele who received aminoglycosides but did not develop hearing loss: Johnson *et al.*, in their prospective study on neonates within the intensive care unit identified one infant who received gentamicin but passed the initial hearing assessment [43]. However, as noted earlier in regard to the 1555A>G variant, studies have shown great variability in the length of time between aminoglycoside treatment and development of hearing loss. This may also apply to the 1494C>T variant, though few studies have provided information about the time between treatment and development of hearing loss. Aminoglycoside-related sensorineural hearing loss may not appear until much later than the time period in which newborn hearing screening is typically performed [42, 47].

Mechanism of action of the 1555A>G and 1494C>T variants

Aminoglycoside antibiotics exert their bacteriocidal action by binding to the 16S rRNA within the small subunit of bacterial ribosomes, specifically the mRNA decoding site, and disrupting protein synthesis [36]. Mitochondria are believed to have evolved from bacteria and mitochondrial ribosomes exhibit stronger similarities to bacterial ribosomes than do nuclear ribosomes. Therefore, even in the absence of additional genetic risk factors, aminoglycosides likely target the small subunit of mitochondrial ribosomes, binding and

inhibiting protein synthesis. Aminoglycosides within hair cells also leads to increased formation of reactive oxygen species (ROS) or free radicals, which may lead to apoptotic cell death [38]. Aminoglycoside antibiotics concentrate within the inner ear, resulting in prolonged exposure of these tissues relative to other regions of the body. This may explain, at least in part, the ototoxicity experienced by many individuals receiving aminoglycosides [38, 55–57].

At the highest doses of aminoglycosides, most individuals develop ototoxicity, regardless of genetic predisposition [55]. However, in those with the 1555A>G or 1494C>T changes, hearing loss can occur with single doses and nontoxic serum levels. Both the 1555A>G and 1494C>T variants are located in a region of nonpairing sequence within the A site of the ribosome where additional amino acids are accepted for incorporation into the elongating protein. The A>G transition at position 1555 within human mitochondrial 12S rRNA results in the abnormal pairing of the new G allele with the C allele at position 1494 within the rRNA secondary structure. Likewise, the transition from C to T (U in rRNA) at position 1494 results in the pairing of the new U allele with the A allele at position 1555. These new pairings make the human 12S rRNA secondary structure more closely resemble the corresponding region of bacterial 16S rRNA, increasing the affinity for aminoglycosides, and may even create a binding pocket for aminoglycoside antibiotics [55, 58, 59]. The binding of aminoglycosides to this region impairs the translation of mitochondrial proteins involved in cellular respiration, leading to a level of energy production insufficient for cellular function. Hair cells are at increased risk due to the increased concentration of aminoglycosides in cochlear fluid and the intrinsic high energy requirements of these hair cells; if energy production levels are too low for the hair cells to function, a deafness phenotype may result [37, 38, 55].

The 1555A>G and 1494C>T variants which render human mitochondrial rRNA more similar to bacterial rRNA may also reduce the accuracy of mRNA decoding. The resultant errors in the amino acid sequences of translated mitochondrial proteins may impact cellular energy production [56]. This potential mechanism underlying the development of hearing loss independent of aminoglycoside use may not be sufficient to produce a significant hearing loss phenotype independently. Additional modifying factors, such as coexisting mutations in nuclear genes, may be required to bring energy production below the minimum threshold necessary for basic metabolic requirements. Indeed, in support of this theory, suggested nuclear modifier genes, such as *TRMU*, *GTPBP3* and *TFB1M*, are associated with tRNA or rRNA modification [37, 60–62].

Other studies have suggested that the 1555G allele results in hypermethylation of the rRNA, leading to aberrant mitochondrial biogenesis and an increased risk for stress-induced hair cell death. Aminoglycosides would act as an additional stressor leading to hearing loss under this proposed mechanism [63, 64]. However, a recent study by O'Sullivan *et al.* described evidence contradicting this hypothesis [65].

MT-RNR1: 1095T>C (rs267606618)

Only a handful of studies link the 1095T>C variant with aminoglycoside-induced hearing loss. However, all the patients in these studies developed hearing loss after receiving the

antibiotics. The severity of hearing loss varied from moderate to profound, and occurred between 7 days [66] and 3 months [67] after treatment. An overview of the studies linking the 1095C allele with aminoglycoside-induced hearing loss can be found in Table 4; more detailed descriptions of each study in the table can be found on PharmGKB at www.pharmgkb.org/clinicalAnnotation/1444699743.

Some heteroplasmy has been reported for the 1095C allele. Thyagarajan *et al.* found an individual who developed hearing loss after aminoglycoside treatment with a C allele mutation load of 98% [33]. Tessa *et al.* found two individuals from a single family who developed aminoglycoside-induced hearing loss and members of the mitochondrial lineage exhibited between 2–45% mosaicism for the risk allele [34].

Yao *et al.* suggested that the 1095T>C variant may not be pathogenic, since, along with ten other mutations, it defines the mitochondrial haplogroup M11 (haplogroups represent major branchpoints within the ancestral mitochondrial lineage and are defined by particular variants within mtDNA) [68]. They also suggest that the absence of the variant in the 364 Chinese controls in a study by Zhao *et al.* [29] is not actually indicative of pathogenicity, since it is not out of range of the expected frequency for this population [68]. Muyderman *et al.* refutes the haplotype argument by noting that their sequencing analysis excludes the possibility that the 1095T>C variant defines the M11 haplogroup; the individual sequenced in their study was placed with haplogroup HV and V [69]. However, very little is understood about the mechanism of hearing loss in those with the 1095T>C variant who receive aminoglycosides. This mutation is located within the P site of the ribosome, where protein translation initiation occurs, and disrupts an evolutionarily conserved base pair within the 12S rRNA [33, 69]. Muyderman *et al.* found that the cells with the 1095T>C variant exposed to aminoglycoside antibiotics had a ten-fold increase in the number of apoptotic cells compared to controls [69], providing *in vitro* evidence for pathogenicity.

Other variants

MT-RNR1: 827A>G (rs28358569)

Multiple studies have identified individuals homoplasmic for the 827A>G variant who have developed non-syndromic hearing loss without aminoglycoside treatment. Penetrance was incomplete, suggesting that modifying factors may be necessary for development of hearing loss [66, 70–72]. Only two studies report individuals with the 827G variant who developed hearing loss after aminoglycoside treatment. Xing *et al.* found three individuals within a 4-generation Chinese family who were homoplasmic for the 827G allele and developed moderate or severe hearing loss two to four days after treatment with streptomycin [70]. Chaig *et al.* identified two members of a 4-generation Argentinean family with 827G homoplasmy who developed severe or profound hearing loss within 30 days after gentamicin treatment [73]. Conrad *et al.* identified a cystic fibrosis patient with the 827G allele who had received over 20 lifetime courses of intravenous tobramycin without developing clinically significant hearing loss. However, no audiometric testing was done on this individual [44]. The 827G allele was shown to be absent in unrelated controls or non-matrilineal relatives in multiple studies [70, 73, 74], though it was present at a frequency of 2.9% (6/211) in normal controls in a study by Zhu *et al.* [71]. As with 1555A>G and 1494C>T, 827A>G is located

within the A site of the human ribosome which accepts amino acids to add to the nascent protein. Both its location within the ribosome and low frequency in controls suggest a possible pathogenic role for the allele.

MT-RNR1: Position 961

A number of different variations at position 961 within *MT-RNR1* have been found in individuals with nonsyndromic hearing loss, both with and without a history of aminoglycoside use. These include 961delT, 961delTinsC(2_7) (a deletion of a thymidine with an insertion of a varying number of cytosines) [75–81], 961 T>G [79, 82], 961T>C (rs3888511) [66] and 961insC [66]. Bacino *et al.* found the 961delT variant in one individual out of 35 with aminoglycoside-induced deafness [75]. Casano *et al.* found five individuals within a family who all developed severe or profound hearing loss within a few months of aminoglycoside treatment and all possessed 961delT [76]. Yoshida *et al.* presented a case study of an individual with the 961delT variant who developed deafness after streptomycin treatment [80].

Despite these studies linking variations at position 961 and hearing loss, its pathogenicity remains controversial. Though some studies have found an absence of the variant in controls [75, 77, 82], others have found a frequency high enough to question its possible pathogenicity. Kobayashi *et al.* found that the 961delT variant had a frequency of 2.1% in patients with hearing loss and 2.2% in the general Japanese population [81]. Elstner *et al.* found the 961delT variant in 3.2% of controls, 961 T>G in 1.6%, and 961 T>C in 1.9% [83]. Multiple other studies have also found unexpectedly high frequencies of the 961delT [78, 84–86], and 961 T>G [77, 86] variants in controls or the general population. Conrad *et al.* identified a cystic fibrosis patient with the 961G allele who had received six lifetime courses of intravenous tobramycin, but had no hearing loss [44]. Position 961 is also in a region that is not evolutionarily conserved, and its pathogenic mechanism of action is unclear at this time [55, 87].

Conclusions

Significant evidence links variants within the *MT-RNR1* gene, particularly 1555A>G and 1494C>T, with aminoglycoside-induced hearing loss. The penetrance of hearing loss in individuals with these alleles who receive aminoglycosides is 100% in almost every study, and mechanistic explanations support the associations. These pathogenic variants within the *MT-RNR1* gene may be good candidates for pre-emptive screening – recent advances in testing allow for rapid, accurate, and inexpensive screening, particularly useful for neonates entering intensive care, for whom sepsis treatment cannot be delayed [85, 88]. Screening could also be applied prior to treatment outside of intensive care, where the benefits of avoiding aminoglycoside treatment may have a greater likelihood of outweighing the risks. Indeed, screening might be of particular benefit in developing countries, where aminoglycosides are frequently used for routine infections due to their effectiveness and low cost. Though aminoglycoside use has declined in industrialized countries, they are still used to treat multiple-drug-resistant bacterial strains, patients with cystic fibrosis, and AIDS patients who develop tuberculosis [35]. Hearing loss at any age leads to a significant

economic burden, but particularly when the onset is prelingual [89]. Despite studies connecting *MT-RNR1* variants with aminoglycoside-induced deafness that stretch back decades, large, prospective, long-term clinical studies analyzing this association have yet to be performed. These studies are critical to fully understand the penetrance of these associations, and whether screening would be effective and economically feasible.

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Conflicts of Interest:

RBA is a stockholder in Personalis Inc. and a paid advisor for Personalis Inc., Pfizer and Karius. TEK is a paid scientific advisor to Rxight Pharmacogenetics.

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Table 1**Levels of hearing loss**

The threshold hearing level in decibels indicates the softest sound range that an individual with that severity of hearing loss can detect. For a typical person with normal hearing, the softest sound that can be heard is 0 decibels [14].

Severity of hearing loss	Threshold Hearing Level (dB HL)
Mild	26 to 40
Moderate	41 to 55
Moderately severe	56 to 70
Severe	71 to 90
Profound	91+

Table 2
Summary of *MT-RNR1* 1555A>G and aminoglycoside antibiotic studies

The number of patients with the 1555G allele who developed hearing loss after receiving aminoglycoside antibiotics is provided, as well as the number of patients with the 1555G allele who did not develop hearing loss after receiving aminoglycoside antibiotics. A blank row indicates zero patients.

Hearing loss	No hearing loss	Ethnicity	Reference
71		Chinese	[24]
48		Chinese	[90]
40		Spanish	[22]
40		Spanish	[91]
21		Mongolian	[92]
20		Japanese	[93]
16		Chinese	[67]
15		Chinese	[94]
15		Chinese	[95]
14		Chinese	[66]
14		Japanese	[96]
13		Chinese	[97]
11	3?1	Chinese	[53]
11		Japanese	[81]
10		Chinese	[98]
10		Polish	[99]
9		Chinese	[100]
9		Japanese	[101]
9	1?2	South African	[21]
7		American (Caucasian, Asian, Hispanic)	[45]
7		Chinese	[102]
6		Polish	[77]
5		Japanese	[103]
5		Japanese	[104]
5		Polish	[105]
4		Chinese	[106]
4		Chinese	[107]
4		Japanese	[46]
4		Spanish	[108]
4		Spanish	[27]
3		Chinese	[109]
3		Chinese	[110]
3	7	Germany	[47]

Hearing loss	No hearing loss	Ethnicity	Reference
2		Greek	[111]
2		Japanese	[112]
2		Japanese	[113]
2		Moroccan Jewish	[114]
2		Spanish	[26]
2		Korean	[115]
2		North American	[44]
1		British (Asian)	[116]
1	2	American	[43]
1		Belgian	[78]
1		Chinese	[117]
1		Israeli	[118]
1		Italian	[119]
1	1	British (Caucasian)	[49]
	2	American	[42]

¹Three patients were described as having “normal” hearing but also as having “mild hearing loss at high frequencies”. The paper does not state whether it considers these patients as having hearing loss or not.

²One patient was treated for tuberculosis and was not deaf, but the authors could not confirm whether she received an aminoglycoside antibiotic.

Table 3
Summary of *MT-RNR1* 1494C>T and aminoglycoside antibiotic studies

The number of patients with the 1494T allele who developed hearing loss after receiving aminoglycoside antibiotics is provided, as well as the number of patients with the 1494T allele who did not develop hearing loss after receiving aminoglycoside antibiotics. A blank row indicates zero patients.

Hearing loss	No hearing loss	Ethnicity	Reference
19		Chinese	[30]
8		Chinese	[31]
6		Chinese	[120]
5		Chinese	[121]
3		Spanish	[32]
3		Chinese	[122]
2		Chinese	[123]
2		Chinese	[28]
2		Chinese	[90]
1		North American	[44]
	1	Unknown	[43]

Table 4
Summary of *MT-RNR1* 1095T>C and aminoglycoside antibiotic studies

The number of patients with the 1095C allele who developed hearing loss after receiving aminoglycoside antibiotics is provided, as well as the number of patients with the 1095C allele who did not develop hearing loss after receiving aminoglycoside antibiotics. A blank row indicates zero patients.

Hearing loss	No hearing loss	Ethnicity	Reference
3		Chinese	[67]
2		Unknown	[34]
1		Chinese	[66]
1		Chinese	[29]
1		Italian	[33]