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Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for the use of aminoglycosides based on *MT-RNR1* genotype

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CONFLICTS OF INTEREST

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Abstract (250 word limit)

Aminoglycosides are widely used antibiotics with notable side effects such as nephrotoxicity, vestibulotoxicity and sensorineural hearing loss (cochleotoxicity). *MT-RNR1* is a gene that encodes the 12s rRNA subunit and is the mitochondrial homologue of the prokaryotic 16s rRNA. Some *MT-RNR1* variants (i.e., m.1095T>C; m.1494C>T; m.1555A>G) more closely resemble the bacterial 16s rRNA subunit and result in increased risk of aminoglycoside-induced hearing loss. Use of aminoglycosides should be avoided in individuals with an *MT-RNR1* variant associated with an increased risk of aminoglycoside-induced hearing loss unless the high risk of permanent hearing loss is outweighed by the severity of infection and safe or effective alternative therapies are not available. We summarize evidence from the literature supporting this association and provide therapeutic recommendations for the use of aminoglycosides based on *MT-RNR1* genotype (updates at https://cpicpgx.org/guidelines/ and www.pharmgkb.org).

INTRODUCTION

The purpose of this Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline is to provide information to allow the interpretation of selected *MT-RNR1* genotype results to guide health care providers on the proper use of aminoglycosides. Detailed guidelines for use of these agents, diagnostic testing, as well as analyses of cost effectiveness, are beyond the scope of this document. CPIC guidelines are periodically updated at www.cpicpgx.org.

FOCUSED LITERATURE REVIEW

A systematic literature review focused on *MT-RNR1* genotypes and aminoglycoside use was conducted (details in Supplement).

DRUGS: AMINOGLYCOSIDES

Background

Aminoglycosides are a large class of antibiotics which are widely used clinically around the world for the treatment of infection. Their safety profile is well understood, they have proven efficacy and can be used in combination with other antibiotics. Streptomycin was the first aminoglycoside antibiotic, isolated in 1943. Since then, the class has grown significantly to include many natural and semi-synthetic agents. They are typically administered by intravenous or intramuscular injection for treatment of serious Gram-negative bacterial infections or as synergistic treatment for serious Gram-positive bacterial infections, and topically for other purposes. Therapeutic dose monitoring is required because pharmacokinetics vary between individuals and high levels are associated with greater toxicity.

Aminoglycoside antibiotics confer their bactericidal effect through the inhibition of protein synthesis by binding to the 16s ribosomal RNA (rRNA) subunit of the bacterial 30S ribosome (1). The 30S ribosome is responsible for mRNA translation within the prokaryotic cell. The 16s component recognizes and binds the Shine-Dalgarno sequence, which ensures the ribosome and the mRNA align effectively allowing protein synthesis to commence (2). If the 16s rRNA subunit is bound to an

aminoglycoside, this will severely interrupt normal protein synthesis and result in mistranslation by inducing codon misreading, causing error prone protein synthesis (3).

In addition to nephrotoxicity, sensorineural hearing loss (cochleototoxicity) and vestibulotoxicity are well-recognized ototoxic side-effects of aminoglycoside antibiotics. These side effects are typically dose-dependent and are observed in patients who receive high doses of aminoglycosides for a protracted period. However, certain individuals appear to have a predisposition towards aminoglycoside-induced hearing loss (AIHL), with reports of single doses causing profound bilateral sensorineural hearing loss (4). Unlike many actionable pharmacogenetic traits that affect the metabolism of a drug and thus drug exposure, the variants in the gene under consideration here predispose individuals to a severe AIHL after exposure to current standard recommended doses of aminoglycosides. This evidence review will focus on that relationship.

GENE: *MT-RNR1*

Background

The human mitochondrial genome contains 37 genes; 13 encode components of the mitochondrial respiratory chain, while the other 24 encode a mature RNA product. Twenty-two of these mature RNA products are mitochondrial tRNA molecules, one is a 16s ribosomal RNA (rRNA) subunit, and one is a 12s rRNA subunit. These subunits are necessary for the translation of messenger RNAs (mRNAs) into mitochondrial proteins (5).

The 12s rRNA subunit is encoded by the *MT-RNR1* gene and is the mitochondrial homologue of the prokaryotic 16s rRNA subunit. Early family studies identified that the predisposition towards AIHL appeared to be inherited down the maternal lineage, in an extra-nuclear (mitochondrial) inheritance pattern. In 1993, mitochondrial sequencing of four families with AIHL identified the m.1555A>G variant in affected individuals in each family. This variant is in a highly conserved region of the 12s rRNA subunit, which has two single stranded regions separated by two stem-loops. In the bacterial homologue, this region is where mRNAs are decoded, and it is where aminoglycosides bind to confer their therapeutic, bactericidal effect. Variants in *MT-RNR1* which pre-dispose to AIHL appear to cause the 12s rRNA subunit to more closely resemble the bacterial 16s rRNA subunit, thus allowing aminoglycosides to bind more readily (6-8). Other than m.1555A>G, additional variants with

sufficient evidence to support a drug-variant interaction are m.1095T>C and m.1494C>T. As such, this guideline concentrates on providing prescribing guidance in the context of these three variants. The ***MT-RNR1*** **allele functionality table (9, 10)** provides allele frequencies based on biogeographical groups.

In addition to m.1555A>G, many additional *MT-RNR1* variants have been proposed as being associated with AIHL (***MT-RNR1*** **allele functionality table (9, 10)**). However, many of these variants are only described in single studies and therefore there is insufficient evidence to support their risk for AIHL. The lack of inclusion of a variant within this guideline should not be interpreted as it being not associated with AIHL; rather, there is insufficient evidence to determine its associated risk with AIHL at this time.

Genetic Test Interpretation

Whereas most nuclear genes exist in diplotype, with one copy found on each autosome, there is a copy of each mitochondrial gene within each mitochondrion. Most cells contain approximately 100 mitochondria, but those cells with high energy requirements can have up to 1000. Unlike with many pharmacogenetic guidelines, there is no metabolizer status assignment based on the patient's diplotype. Rather, the presence of a pathogenic MT-RNR1 variant in an individual can be interpreted as conferring susceptibility to AIHL.

The assignment of a pathogenic variant in *MT-RNR1* is based on multiple lines of evidence, including functional data, *in vitro* data, case-control data, and population allele frequency (***MT-RNR1*** **allele functionality and frequency tables** (9)). It should be noted that one of the limitations inherent in commercially available genotyping tests is that very rare variants will not generally be included or interrogated by these tests.

This CPIC recommendation assumes that genetic testing has been performed and that an *MT-RNR1* variant has been detected, irrespective of the methodology of testing. The identification of a *MT-RNR1* variant that is known to predispose to AIHL is sufficient to assign a phenotype status, but the absence of a variant does not indicate no risk of AIHL.

Available Genetic Test Options

Molecular genetic testing of *MT-RNR1* is available from numerous clinical testing laboratories (see Genetic Testing Registry (GTR®): https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=4549[geneid].

Incidental Findings

In addition to predisposing to AIHL, there is weak evidence that the m.1555A>G variant is also associated with non-aminoglycoside-related sensorineural hearing loss (11). However, this relationship is not clear, and a population-based cohort study found that hearing in individuals with the m.1555A>G, identified from a birth cohort study, was not significantly different from those without the variant at the age of 45 years (12). As such, there is insufficient evidence to support additional hearing assessments in individuals with *MT-RNR1* variants that predispose to AIHL if they have not been exposed to an aminoglycoside. There are no other known health-related associations that would require disclosure to individuals before *MT-RNR1* genetic testing.

Due to the mitochondrial inheritance pattern of *MT-RNR1*, the identification of a clinically relevant *MT-RNR1* variant in an individual will be of relevance to any of their maternal relatives (i.e. mother, siblings, mother's siblings and maternal grandmother) and to all of the children of a female identified to carry the variant. This should be communicated to the patient when a clinically relevant genotype is identified and the advice to avoid aminoglycosides should be cascaded to the relevant individuals within the family. Advice from a clinical genetics service can be sought to support the cascading of information within the family.

Other Considerations

MT-RNR1 variants were historically described as exclusively homoplasmic variants, meaning the same variant was seen in every mitochondrion. However, this likely represented an ascertainment bias due to the choice of genotyping technology. Increasingly sensitive molecular approaches, which allow quantification, more readily facilitate the detection of *MT-RNR1* heteroplasmy, the presence of more than one type of mitochondrial genome within a cell. There are now several reports of variable levels of *MT-RNR1* heteroplasmy for the m.1555A>G variant (13-15). The reasonable clinical question raised by the phenomenon of heteroplasmy is whether there is a threshold at which the administration of an aminoglycoside becomes acceptable. Based on the available literature, at present there is not

sufficient evidence to define a level of heteroplasmy where aminoglycoside administration becomes safe, especially as the mutational load may differ from tissue to tissue and be dependent upon the genotyping technique utilized. As such, we have not tailored this guideline based on the level of heteroplasmy. Rather, we recommend that if a relevant *MT-RNR1* variant is detected, the guidance should be followed as set out for a homoplasmic variant.

Linking Genetic Variability to Variability in Drug-related Phenotypes

Potentially pathogenic *MT-RNR1* variants are present at a relatively low frequency in the population (***MT-RNR1* allele functionality and frequency tables** (9)). As such, many of the studies which investigate the relationship between *MT-RNR1* variants and AIHL have methodological flaws. A large number of clinical studies have been published proposing an association between the *MT-RNR1* variants and AIHL (**Table S1**) (16-20). The majority of these are small retrospective cohort studies, where authors have identified groups with a high prevalence of hearing loss and have subsequently performed genotyping of *MT-RNR1*. Prescribing data detailing aminoglycoside administration is then used to draw conclusions about the relationship between the variant and AIHL. When reviewing the literature, we were conscious that such sampling bias can lead to claims for causality that do not meet the necessary evidence level (21). Establishing a causal and temporal relationship between *MT-RNR1* variants and AIHL is challenging. Furthermore, interpretation guidance for mitochondrial variants does not consider the impact of a phenotype in the context of a drug exposure (22). Despite these issues, there was sufficient and consistent evidence to assign actionability to the m.1555A>G and m.1494C>T variants with high levels of evidence and the m.1095T>C variant with a moderate level of evidence (Table 1 & S1). The m.827A>G variant, despite weak evidence proposing an association with AIHL, has too high a population frequency in certain biogeographic groups to be classified as associated with AIHL ***MT-RNR1* allele functionality and frequency tables** (9)). We therefore classify m.827A>G as conferring no additional (normal) risk for AIHL at this time.

Therapeutic Recommendations

The critical pharmacogenetics recommendation for a person with an *MT-RNR1* variant which predisposes to AIHL is that aminoglycoside antibiotics are relatively contraindicated, meaning that

aminoglycosides should be avoided unless the increased risk of hearing loss is outweighed by the severity of infection and lack of safe or effective alternative therapies (**Table 2**). There is insufficient evidence to suggest that the adverse drug reaction may be more profound with some members of the aminoglycoside class than others. As such, this guidance covers all aminoglycoside antibiotics irrespective of class. We provide a strong recommendation that carriers of *MT-RNR1* variants that predispose to AIHL should avoid aminoglycosides unless the increased risk of permanent hearing loss is outweighed by the risk of infection without safe or effective alternative therapies (see section *Considerations for aminoglycoside use in patients at increased risk of AIHL*). If no effective alternative to an aminoglycoside is thought to be available, we advise use for the shortest possible time, consultation with an infectious disease expert for alternative approaches, therapeutic drug monitoring and frequent assessment for hearing loss, both during and after therapy, in consultation with an audiovestibular physician.

An individual with no detectable *MT-RNR1* variant or carrying *MT-RNR1* variants not considered to be predisposing to AIHL (normal risk), including the m.827A>G variant, should still be considered at risk of AIHL. In addition to *MT-RNR1*, AIHL is often associated with other risk factors such as prematurity, renal impairment, severe inflammatory response syndrome, prolonged therapy regimens, and supratherapeutic plasma concentrations (23, 24). As such, irrespective of the presence of an *MT-RNR1* variant which predisposes to AIHL, precautions such as renal monitoring, therapeutic drug monitoring, and utilizing the lowest effective dose should be applied. Finally, if an individual with an actionable MT-RNR1variant has previously received aminoglycosides and not developed AIHL, this does not preclude them from developing AIHL with subsequent doses.

Considerations for aminoglycoside use in patients at increased risk of AIHL. For the purposes of this guideline, appropriateness for use of aminoglycoside antibiotics can be considered for three scenarios: First, an equally or more effective agent is available for the condition; second, there is reason to believe that an aminoglycoside might lead to superior outcomes, but evidence is poor, the effect-size is small, or the outcome is not clinically meaningful; and third, there is good evidence for significantly superior efficacy of an aminoglycoside-containing treatment regimen for a clinically meaningful outcome.

Examples of the first scenario include treatment of bloodstream or urinary tract infection susceptible to other antibiotics, and empiric use for treatment of fever or suspected infection in a patient without risk factors for broadly resistant gram-negative infection (25-29). In these circumstances, an aminoglycoside is typically being chosen for reasons of cost, antimicrobial stewardship, or personal/institutional preference. The panel recommends *against* use of an aminoglycoside in this scenario for individuals at increased risk of AIHL due to the presence of an *MT-RNR1* variant.

Examples of the second scenario include initial adjunctive empiric therapy for severe sepsis in adults and neonates (30), serious infections in patients at high risk of broadly resistant gram-negative infection, treatment of severe tularemia, plague and brucellosis, and as a component of therapy for enterococcal or streptococcal endocarditis (31-36). In this scenario, the panel recommends that clinicians seek expert opinion about alternative options and use their best judgement to determine the appropriateness of aminoglycoside use, including consideration of patient preferences and co-morbidities, and accounting for the risk of AIHL. If used, the patient or patient's family should be informed of the increased risk of AIHL and the aminoglycoside should be used for the shortest possible period with appropriate precautions as detailed below.

Examples of the third scenario include combination therapy for extensively drug resistant (XDR) tuberculosis where other effective agents are unavailable, treatment of gram-negative organisms resistant to all available alternative therapies, and treatment of serious infections caused by *Mycobacterium abscessus* or other highly resistant *Mycobacteria spp*. (33, 37, 38). In this scenario, the panel recommends seeking expert opinion about alternative options, and use of an aminoglycoside for the shortest possible period with appropriate precautions as detailed below.

In all cases, an aminoglycoside used in patients at increased risk of AIHL due to the presence of an *MT-RNR1* variant should be administered for the shortest possible period, under expert supervision, with therapeutic drug and ototoxicity monitoring, and with clinical audiovestibular assessment performed during and after treatment. Irrespective of whether an individual carries a pathogenic MT-RNR1 variant, all patients who receive aminoglycoside antibiotics, especially those prescribed prolonged courses, should be monitored for ototoxicity in line with existing local and international guidelines (39, 40).

As detailed below, administration of an aminoglycoside by a route other than intravenous or intramuscular may reduce, but not eliminate, the risk of AIHL. So, although there is insufficient evidence to determine the impact of this strategy, it might be considered in a clinically appropriate setting if aminoglycoside use is unavoidable in an individual at increased risk. For example, in a case of mycobacterial lung infection with no acceptable alternative therapy, administration of the drug by the inhaled route might be preferred if it is expected to be efficacious. If any hearing loss or vestibular dysfunction is identified, appropriate rehabilitation should be undertaken. Expert infectious diseases and audiologic opinion is recommended in all cases as new information about alternative approaches may be available.

Alternative routes of aminoglycoside administration. Aminoglycosides are highly bioavailable by intramuscular, intravenous and intraperitoneal administration, so these guidelines apply similarly to each of these routes (41, 42). However, bioavailability of aminoglycosides by enteral, inhaled, topical, intrathecal or intraventricular routes, or by bladder irrigation is lower and it is unknown whether administration by any of these other routes would routinely cause ototoxicity in patients at increased risk of AIHL.

Inhaled administration of aminoglycosides is associated with significant systemic exposure (~10 – 20%) and vestibulocochlear toxicity has been described, albeit usually in the presence of renal dysfunction, so avoidance may be considered in patients with increased risk *MT-RNR1* variants (43-49). Similarly, systemic exposure and ototoxicity have been reported following use of orthopedic cement containing aminoglycosides, and avoidance of use may be considered in patients with increased risk *MT-RNR1* variants (50). There are also reports of hearing loss after intrathecal or intraventricular administration of gentamicin or streptomycin, but the potential role of *MT-RNR1* variants in the risk of this type of AIHL is unknown (51). In contrast to these, enteral and topical administration of aminoglycosides typically has absolute bioavailability <1% so is unlikely to cause AIHL, even in patients at increased risk (52). Absorption of aminoglycosides from the gastrointestinal tract may be elevated in the presence of severe epithelial damage, such as in patients with severe gastrointestinal graft vs. host disease or large areas of deep skin burns, so caution is advised in that that setting (53-55). As in all patients, topical otic use of an aminoglycoside is relatively contraindicated in the presence of a perforated tympanic membrane because of the high risk of

ototoxicity, but is unlikely to lead to AIHL in other circumstances and is not known to be associated with *MT-RNR1* variants (56-58). Minimal systemic absorption of aminoglycosides has been documented from intravesicular administration as bladder washouts for treatment or prevention of urinary tract infections, but few data are available (59, 60).

Pediatrics. These recommendations are not age-based and should apply to any individual with a *MT-RNR1* AIHL-associated genotype where administration of an aminoglycoside is being considered. As hearing, listening and spoken language skills continue to develop in infants and children the impact of aminoglycoside administration in children with an actionable MT-RNR1 variant is likely to be greater than in the adult population (61).

Recommendations for Incidental Findings

Not applicable

Other Considerations

Implementation of this guideline. The guideline supplement contains resources that can be used within electronic health records (EHRs) to assist clinicians in applying genetic information to patient care for the purpose of drug therapy optimization (see *Resources to incorporate pharmacogenetics into an electronic health record with clinical decision support* sections of supplement).

POTENTIAL BENEFITS AND RISKS FOR THE PATIENT

As aminoglycosides are commonly used worldwide, *MT-RNR1* genotype-guided antibiotic prescribing has the potential to reduce the occurrence rates of AIHL. This guidance emphasizes that aminoglycosides should be avoided unless the increased risk of permanent hearing loss is outweighed by the severity of infection and lack of safe or effective alternative therapies. In the rare cases where aminoglycosides are the only antibiotic of choice, it is highly likely that the risk of inadequately treated infection would outweigh the increased risk of ototoxicity. Where *MT-RNR1* genotyping is to be integrated into clinical care, efforts should be made to design clinical pathways which consider the changes required if a *MT-RNR1* variant which predisposes to AIHL is identified.

CAVEATS: APPROPRIATE USE AND/OR POTENTIAL MISUSE OF GENETIC TESTS

Rare *MT-RNR1* and uncertain risk variants are typically not included in common genotyping tests and patients are therefore assigned the "reference" or “wild-type” allele by default. Thus, an assigned “reference” allele may in rare cases harbor an increased risk variant. Similar to all diagnostic tests, genetic tests are one of several pieces of clinical information that should be considered before initiating drug therapy.

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DISCLAIMER

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines reflect expert consensus based on clinical evidence and peer-reviewed literature available at the time they are written and are intended only to assist clinicians in decision-making, as well as to identify questions for further research. New evidence may have emerged since the time a guideline was submitted for publication. Guidelines are updated periodically on https://cpicpgx.org/guidelines/ and it is the responsibility of the guideline user to consult this website for updates. Guidelines are limited in scope and are not applicable to interventions or diseases not specifically identified. Guidelines do not account for all individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It remains the responsibility of the health care provider to determine the best course of treatment for the patient. Adherence to any guideline is voluntary, with the ultimate determination regarding its application to be solely made by the clinician and the patient. CPIC assumes no responsibility for any injury to persons or damage to property related to any use of CPIC's guidelines, or for any errors or omissions.

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Supplementary Files

1. CPIC MTRNR1_aminoglycosides supplemental

TABLE 1. ASSIGNMENT OF MT-RNR1 PHENOTYPE BASED ON GENOTYPE

Likely phenotype	**Genotypes**	**Example genotypes**
MT-RNR1 increased risk of aminoglycoside-induced hearing loss	Individuals with a *MT-RNR1* variant associated with an increased risk of aminoglycoside-induced hearing loss	m.1095T>C m.1494C>T m.1555A>G
MT-RNR1 normal risk of aminoglycoside-induced hearing loss	Individuals with no detectable *MT-RNR1* increased risk variant or a *MT-RNR1* variant associated with normal risk of aminoglycoside-induced hearing loss	m.827A>G
MT-RNR1 uncertain risk of aminoglycoside-induced hearing loss	Individuals with a *MT-RNR1* variant associated with an uncertain risk of aminoglycoside-induced hearing loss	m.663A>G m.669T>C m.747A>G m.786G>A m.807A>G m.807A>C m.839A>G m.896A>G m.930A>G m.951G>A m.960C>del

		m.961T>G m.961T>del m.961T>del+Cn m.988G>A m.1189T>C m.1243T>C m.1520T>C m.1537C>T m.1556C>T

TABLE 2. RECOMMENDED THERAPEUTIC USE OF AMINOGLYCOSIDES IN RELATION TO MT-RNR1 PHENOTYPE IN CHILDREN AND ADULTS

Phenotype	**Implications for phenotypic measures**	**Therapeutic recommendations**	**Classification of recommendations[a]**	**Considerations**
MT-RNR1 increased risk of aminoglycoside-induced hearing loss	Very high risk of developing hearing loss if administered an aminoglycoside antibiotic	Avoid aminoglycoside antibiotics unless the high risk of permanent hearing loss is outweighed by the severity of infection and lack of safe or effective alternative therapies.	Strong	If no effective alternative to an aminoglycoside antibiotic is available, evaluate for hearing loss frequently during therapy and ensure that all appropriate precautions are utilized (e.g., lowest possible dose and duration, utilization of therapeutic drug monitoring, hydration, renal function monitoring).

MT-RNR1 normal risk of aminoglycoside-induced hearing loss	Normal risk of developing hearing loss if administered an aminoglycoside antibiotic.	Use aminoglycoside antibiotics at standard doses for the shortest feasible course with therapeutic dose monitoring. Evaluate regularly for hearing loss in line with local guidance.	Strong	Individuals without *MT-RNR1* aminoglycoside-induced hearing loss increased risk variants are still at risk of aminoglycoside-associated hearing loss, especially with high drug levels or prolonged courses.
MT-RNR1 uncertain risk of aminoglycoside-induced hearing loss	Weak or no evidence for an increased risk of *MT-RNR1*-associated hearing loss if administered an aminoglycoside antibiotic.	Use aminoglycoside antibiotics at standard doses for the shortest feasible course with therapeutic drug monitoring. Evaluate regularly for hearing loss in line with local guidance.	Optional	Individuals without *MT-RNR1* aminoglycoside-induced hearing loss increased risk variants are still at risk of aminoglycoside-associated hearing

				loss, especially with high drug levels or prolonged courses.

[a]Rating scheme described in the Supplemental Material online.