Gentamicin Therapy and *MT-RNR1* Genotype

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Introduction

Gentamicin is an aminoglycoside antibiotic that is active against gram-negative bacteria. It is administered by injection to treat serious infections caused by susceptible strains of the following microorganisms: *Pseudomonas aeruginosa*, *Proteus* species, *Escherichia coli*, *Klebsiella-Enterobacter-Serratia* species, *Citrobacter* species and *Staphylococcus* species (1). Gentamicin may also be used topically to treat ophthalmic and dermatological infections.

There are reports that a single injection of gentamicin may cause hearing loss in individuals who have a variant in the mitochondrial gene *MT-RNR1*, known as m.1555A>G. Hearing loss is bilateral, usually moderate to profound, and irreversible. Importantly, this occurs in genetically susceptible individuals even in cases where drug levels remain within the therapeutic range. Note that this effect is distinct from "dose-dependent ototoxicity" (damage to the inner ear), which can affect any individual, typically occurring after 5-7 days of aminoglycoside therapy (2).

Currently, the FDA-approved drug label for gentamicin does not include a statement about m.1555A>G. However, an American College of Medical Genetics and Genomics (ACMG) guideline includes the following recommendation: "Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics" (3, 4).

Drug: Gentamicin

Aminoglycosides such as gentamicin are among the earliest formulations of antibiotics (5). They act by inhibiting protein synthesis in susceptible microorganisms through

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ribosome binding and are effective against most Gram-positive and -negative aerobic bacteria but are inactive against anaerobes. They may be used in combination with another antibiotic, such as a beta-lactam antibiotic or a cephalosporin, to increase coverage (1).

Six aminoglycoside drugs currently are approved for use by the FDA: amikacin, gentamicin, neomycin, paromomycin, streptomycin, and tobramycin. The ending of these drug names, -mycin or -micin, reflects from which genus of bacteria the aminoglycoside was derived, respectively *Streptomyces* or *Micromonospora* (6).

Aminoglycosides exert antibacterial effects by binding to bacterial ribosomes and inhibiting bacterial protein synthesis. They bind to the 30s ribosomal subunit, which interferes with the decoding site—this is where the ribosome has to accurately select tRNA in accordance with the appropriate mRNA codon. Errors here lead to inappropriate translation of the mRNA codons so that incorrect amino acids are inserted into the polypeptide chain, which can disrupt elongation of the peptide chain (7, 8).

Like all aminoglycosides, gentamicin is poorly absorbed from the gut so is not administered orally. It is either given by injection, with regular blood tests to monitor drug levels, or given topically in the form of drops, cream, or ointment, to treat infections of the eye or skin.

The toxicity of aminoglycosides, along with the discovery of equally potent but less toxic antibiotics, has meant that the use of aminoglycoside injections is reserved for serious infections that are proven, or strongly suspected, to be caused by susceptible microorganisms. They are most commonly used in the treatment of neonatal septicemia, especially in premature babies. They are used as surgical prophylaxis in patients who are allergic to penicillin and for febrile neutropenia, septic shock, and drug-resistant tuberculosis (5).

The main toxicities of aminoglycoside injections are kidney damage (nephrotoxicity) and damage to the inner ear (ototoxicity) (9). Nephrotoxicity primarily involves the proximal tubules and is generally reversible (10). In contrast, aminoglycoside-induced otoxocity is usually irreversible. Damage may occur to the cochlea, resulting in sensorineural hearing loss, and/or to the vestibular system, causing problems with balance, vertigo, ataxia, nausea, and vomiting. Gentamicin is considered to be more toxic to the vestibular system, and for this reason is used for vestibular ablation to treat Ménière's disease. Amikacin and neomycin are examples of aminoglycosides that are more toxic to the cochlea (9, 11).

Rarely, neuromuscular blockade can occur after aminoglycoside therapy. The boxed warning on the FDA-approved drug label recommends that aminoglycosides "be used with caution in patients with neuromuscular disorders, such as myasthenia gravis or parkinsonism, because they may aggravate muscle weakness (1)"; whereas the British National Formulary states that aminoglycosides should not be given to patients with myasthenia gravis (12).

Gene: *MT-RNR1*

Mitochondria are the principal source of energy in most cells—they use oxygen and sugars and fats to create energy in the form of ATP, in a process known as oxidative phosphorylation. Mitochondria have their own genome, which is small and circular, resembling the bacterial prokaryotes from which they evolved. The genome is passed down from mother to child (maternal inheritance) and contains 37 genes, one of which is the *MT-RNR1* gene (mitochondrially encoded 12S RNA).

The *MT-RNR1* gene may have variants associated with both aminoglycoside-induced and nonsyndromic hearing loss (see Nomenclature). The rRNA encoded by *MT-RNR1* is found only within mitochondria, and it is essential in the synthesis of proteins that carry out oxidative phosphorylation.

Consistent with their bacterial origin, mitochondrial rRNA resembles bacterial rRNA more closely than human rRNA found in the cell cytoplasm. However, at the highly conserved decoding region in the 12S RNA gene, the sequence in humans is different to the corresponding site in bacterial ribosomes. Thus, aminoglycosides that bind to bacterial ribosomes do not normally bind to human ribosomes (7).

However, sequence variants in the ribosomal decoding region make mitochondrial RNA more similar to bacterial rRNA, thereby facilitating the binding of aminoglycosides. Although the mechanism is unclear, aminoglycosides damage the sensory hair cells in the cochlea that mediate hearing, which may be mediated by the generation of free radicals (13-15).

The most common *MT-RNR1* variant is a single nucleotide substitution of a guanine at position 1555 for an adenine (m.1555A>G). Individuals with this variant are exquisitely sensitive to aminoglycoside-induced hearing loss, which is moderate to profound, bilateral, irreversible, and may have a rapid onset. This presentation occurs in the setting of receiving standard doses of aminoglycosides with monitoring to ensure the drug levels are within therapeutic range. Even a single dose can be sufficient to cause ototoxicity (2, 16).

The m.1555A>G variant is nearly always homoplasmic (present in all mitochondria), and the penetrance of hearing loss after exposure to aminoglycosides is high (16). Susceptible individuals who are not exposed to aminoglycosides may nonetheless develop hearing loss, referred to as "non-syndromic mitochondrial hearing loss." The course of hearing loss may be affected by the presence of additional genetic factors as well as environmental factors, such as exposure to loud noise. However, normal hearing is usually preserved until at least 44 years of age (2). In cases with m.1555A>G heteroplasmy (variant is present in some but not all mitochondria), the proportion of variant mitochondria generally correlates with the degree of hearing loss (17).

The prevalence of the m.1555A>G variant varies among different populations but frequency data are limited. In the US, the population prevalence is estimated to be 0.09%,

and in the UK, 0.20% (4, 18, 19). In hearing impaired populations, the prevalence is much greater, but the estimates vary widely based on study differences such as the age of onset of hearing loss and whether there has been exposure to aminoglycosides. Estimates include a prevalence of 3.5% among the hearing impaired population in Japan (20), 5% among deaf individuals in Indonesia (21), and 6% of individuals with post-lingual hearing loss from the UK and Southern Italy (22). Additionally, a prevalence of 15% has been reported in "ethnically diverse patients in the United States with hearing loss after aminoglycoside exposure" (23), and in 15-20% of individuals from Spain with hearing loss (24).

Interestingly, although m.1555A>G is present in mitochondria in all tissues of affected individuals, it appears that only the cochlea of the inner ear—but not the vestibular system—is extremely susceptible to aminoglycosides. In contrast, "dose-dependent ototoxicity" can occur after aminoglycoside therapy in any individual, including those with wild-type *MT-RNR1*, and the toxicity involves both the cochlea and vestibular system (see above) (9).

Several studies have highlighted the complex issues regarding genetic testing for m.1555A>G, with the aim of preventing avoidable hearing loss in carriers by administering an alternative antibiotic whenever possible. These issues include the costs and benefits of universal screening, for example, as part of the newborn screening program, given that the prevalence of m.1555A>G is thought to be 1 in 385 Caucasians (2, 25, 26), versus limiting genetic testing to a case-by-case basis (e.g., patients with tuberculosis, children with leukemia, individuals with cystic fibrosis, and surgical patients allergic to beta-lactam antibiotics)(4).

Genetic screening may provide more benefit in countries where aminoglycosides are more commonly used, or when their use becomes more widespread because of growing resistance to other antibiotics (27). In the US, aminoglycoside use is most common in the neonatal intensive care unit where acute, life-threatening situations may dictate that aminoglycosides are given before the results of genetic testing are available (28). One potential alternative would be to screen all pregnant women, because mitochondrial variants are maternally inherited and m.1555A>G is almost always homoplasmic (4).

A report from the World Health Organization's Essential Medicines and Pharmaceutical Policies comments that "pre-treatment screening is an important consideration to prevent aminoglycoside related hearing loss but given cost and access issues, asking about a maternal family history of deafness may be more practical" (29). In countries where the use of aminoglycosides is more common, a quarter of people with aminoglycoside-induced hearing loss have maternal relatives who also have drug-related hearing loss (30, 31).

Genetic Testing

As with all mitochondrial variants, variants of the *MT-RNR1* gene are either maternally inherited, or occur sporadically.

Genetic testing is available for the *MT-RNR1* gene. Targeted mutation panels vary among testing laboratories but laboratories typically include m.1555A>G at a minimum.

MT-RNR1 variants are associated with both extreme idiosyncratic aminoglycoside hypersensitivity, resulting in post-exposure deafness, and nonsyndromic mitochondrial hearing loss, which tends to develop gradually over time. While the presence of an *MT-RNR1* variant indicates a predisposition to aminoglycoside hypersensitivity, the test results do not predict the age of onset or severity of nonsyndromic mitochondrial hearing loss (16).

Therapeutic Recommendations based on Genotype

Excerpt from the American College of Medical Genetics and Genomics (ACMG) Guideline for the Clinical Evaluation and Etiologic Diagnosis of Hearing Loss:

For individuals lacking physical findings suggestive of a known syndrome and having medical and birth histories that do not suggest an environmental cause of hearing loss, a tiered diagnostic approach should be implemented.

Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing should be ordered.

Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
m.1555A>G	A1555G MTRNR1 1555A-G	NA	NA (encodes ribosomal RNA)	rs267606617

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Tests in GTR by Gene

MT-RNR1 gene