Review

Mitochondrial DNA Mutations Associated with Aminoglycoside Ototoxicity

GUAN Min-Xin
Division of Human Genetics and Center for Hearing and Deafness Research, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio 45229, USA
Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, USA

Abstract The mitochondrial 12S rRNA has been shown to be the hot spot for mutations associated with both aminoglycoside-induced and non-syndromic hearing loss. Of all the mutations, the homoplasmic A1555G and C1494T mutations at a highly conserved decoding region in the 12S rRNA have been associated with aminoglycoside-induced and non-syndromic hearing loss in many families worldwide. The A1555G or C1494T mutation is expected to form novel 1494C-G1555 or 1494U-A1555 base-pair at the highly conserved A-site of 12S rRNA. These transitions make the secondary structure of this RNA more closely resemble the corresponding region of bacterial 16S rRNA. Thus, the new UA or G-C pair in 12S rRNA created by the C1494T or A1555G transition facilitates the binding of aminoglycosides, thereby accounting for the fact that the exposure to aminoglycosides can induce or worsen hearing loss in individuals carrying these mutations. Furthermore, the growth defect and impairment of mitochondrial translation were observed in cell lines carrying the A1555G or C1494T mutation in the presence of high concentration of aminoglycosides. In addition, nuclear modifier genes and mitochondrial haplotypes modulate the phenotypic manifestation of the A1555G and C1494T mutations. These observations provide the direct genetic and biochemical evidences that the A1555G or C1494T mutation is a pathogenic mtDNA mutation associated with aminoglycoside-induced and nonsyndromic hearing loss. Therefore, these data have been providing valuable information and technology to predict which individuals are at risk for ototoxicity, to improve the safety of aminoglycoside antibiotic therapy, and eventually to decrease the incidence of deafness.

Hearing loss affects one in every 1, 000 newborns. Hearing loss can be classified as genetic or non-genetic, prelingual or postlingual, and syndromic or nonsyndromic. About 50% of hearing loss cases have a genetic etiology or predisposition with autosomal dominant, autosomal recessive, X-linked or maternal patterns of inheritance. These have rapidly been defined at molecular levels. Hearing loss can result from a mutation in a single gene or from a combination of mutations in different genes. Hearing loss can also be caused by environmental factors, including perinatal infection, acoustic or cerebral trauma affecting the cochlea or ototoxic drugs such as aminoglycoside antibiotics. Additionally, deafness can result from the interaction of hereditary and environmental factors.

Aminoglycoside antibiotics such as gentamicin, streptomycin, kanamycin and tobramycin, are clinically important drugs. These antibiotics are composed of amino sugars linked to a 2-deoxystreptamine ring (Figure 1, ring II). The conserved elements among aminoglycosides are rings I and II, and particularly the amino groups at positions 1 and 3 within ring II. These elements are essential for binding to the decoding site of 16S ribosomal RNA (rRNA). The 2-deoxystreptamine ring is substituted most commonly at positions 4 and 5, as in the neomycin class, or at positions 4 and 6, as in the kanamycin and gentamicin class (Figure 1). They are particularly active against aerobic, gram-negative bacteria and act synergistically against certain
gram-positive organisms. In the developed countries, these drugs are mainly used in the treatment of hospitalized patients with aerobic gram-negative bacterial infections, particularly in patients with chronic infections such as cystic fibrosis or tuberculosis \[9, 10\]. However, in developing countries, aminoglycosides are more routinely used, even for relative minor infections. These drugs are highly polar cations, which are not easy to be metabolized \[11\].

Glomerular filtration rapidly clears aminoglycosides from the majority of tissues and organs \[11, 12\]. However, these drugs may become concentrated in renal tubular cells, and the perilymph and endolymph of the inner ear \[13, 14\]. The use of these drugs can frequently lead to toxicity, which involves the renal, auditory and vestibular systems \[9, 10, 15\]. The renal impairment is usually reversible, but the auditory and vestibular ototoxicity is usually irreversible. Although all of aminoglycosides are capable of affecting cochlear and vestibular functions, some (streptomycin and gentamicin) produce predominately vestibular damage, while others (neomycin and kanamycin) cause mainly cochlear damage. Tobramycin affects both equally \[10\].

Nearly four million courses of aminoglycosides are administrated annually in the United States \[16\] and it is estimated that at least 2-5% of patients treated with these antibiotics develop clinically significant hearing loss \[17-19\]. The problem of ototoxic side effects is more acute in developing countries, where highly effective and low cost drugs such as aminoglycosides are often prescribed without adequate monitoring. The use of these antibiotics has been widespread in China and the administration of various aminoglycosides was believed to be responsible for 22% of all deaf-mutes in one Shanghai district alone \[20\]. Of these, 28% had relatives with aminoglycoside ototoxicity \[20\]. Recently, we showed that 48% of 128 Chinese pediatric deaf subjects could be the result of aminoglycoside treatment \[21\]. The type and doses of aminoglycoside medication, the length of treatment, and age of drug administration also contribute to the severity of hearing impairments in some subjects. At the highest doses of the drugs, most individuals exhibit toxicity. By contrast, some patients developed aminoglycoside-induced hearing loss after treatment with conventional doses - even a single dose - over a short period. These cases of aminoglycoside ototoxicity may have a genetic etiology or predisposition with autosomal dominant, autosomal recessive, X-linked or mitochondrial patterns of inheritance. In particular, susceptibility to aminoglycoside-induced hearing loss is maternally inherited in humans in a significant proportion of cases \[22-24\]. Hu et al. described 36 Chinese families with maternally transmitted predisposition to aminoglycoside ototoxicity \[20\], while Higashi reported that 26 of 28 Japanese families with streptomycin-induced deafness had maternally inherited transmission \[25\].

These drugs are known to exert their antibacterial effects by directly binding to 16S rRNA in the 30S subunit of the bacterial ribosome, causing mistranslation or premature termination of protein synthesis \[26-27\]. In particular, the aminoacyl-tRNA-binding site (A-site) of small rRNA has been shown to be the primary target site for aminoglycoside antibiotics \[28-30\]. As the mitochondria are evolved from bacteria, mitochondrial ribo-
somes share more similarities to bacterial ribosomes than do cytosolic ribosomes \cite{23, 31}. Therefore, it is suggested that one of the primary targeting sites for the aminoglycoside antibiotics is the small ribosomal RNA of mitochondrial ribosome \cite{23}. In fact, in familial cases of ototoxic deafness, the aminoglycoside hypersensitivity is often maternally transmitted, suggesting that mutation(s) in mitochondrial DNA (mtDNA), particularly in 12S rRNA gene, could be the molecular basis for this susceptibility \cite{22, 23}. Thus, it has been proposed that the exposure to aminoglycosides leads to an impairment of mitochondrial translation in susceptible subjects by interacting with the binding sites of mitochondrial 12S rRNA \cite{24, 31, 32}.

**Identification of mitochondrial 12S rRNA mutations associated with aminoglycoside ototoxicity.**

Prezant et al. first investigated the molecular basis of this disorder by mutational analyses of the mitochondrial genome of three Chinese families with maternally transmitted aminoglycoside ototoxicity and a large Arab-Israeli family with maternally inherited nonsyndromic deafness \cite{32}. As a result, the A-to-G transition at position 1555(A1555G) in the 12S rRNA gene was identified in matrilineal relatives of all three Chinese families and the Arab-Israeli family but not in 278 control subjects \cite{32}. The A1555G mutation is located at the A-site of the small ribosomal subunit, which is highly conserved from bacteria to mammals \cite{31-34}. The homologous region of 16S rRNA in *Escherichia coli* is an essential part of the decoding site of the ribosome \cite{34, 35} and is crucial for subunit association either by RNA-protein or RNA-RNA interaction \cite{36}. This region is also an important locus of action for aminoglycosides \cite{37, 38}. In particular, the wild type *E. coli* exhibits sensitivity to aminoglycosides, because drugs including paromomycin, neomycin interact with the C1409-G1491 base-pairing of A-site of bacterial 16S rRNA \cite{37, 38}. Mutations, which disrupted the 1409-1491 base pair of *E. coli* 16S rRNA or *Saccharomyces cerevisiae* mitochondrial 15S rRNA or *Tetrahymena thermophila* 17SrRNA, confer aminoglycoside resistance \cite{39-41}. In humans, the nucleotide at position 1555 in the mitochondrial 12S rRNA (equivalent to position 1491 in the *E. coli* 16S rRNA) in wild-type cells is A, which, when mutated to a G, as in the Arab-Israeli family, paired with the C at position 1494 (Figure 2). This transition makes the secondary structure of this rRNA more closely resemble the corresponding region of *E. coli* 16S rRNA (Figure 2). Thus, this new G-C pair in 12S rRNA is also expected to create a binding site for aminoglycosides, which facilitates the binding of these drugs \cite{44}.

Subsequently, the A1555G mutation has been found in many families worldwide, such as Asian, Caucasian or African \cite{21, 31, 45-60}. Aminoglycoside-induced deafness associated with the A1555G mutation has also been reported in many sporadic individuals \cite{21, 31, 47, 55}. This mutation is often present in homoplasmic form in the affected or at-risk individuals, while matrilineal relatives of eight Spanish families carry the heteroplasmic form \cite{28, 30}. The dosage and the age at the time of aminoglycoside administration seem to be associated with the severity of hearing loss in some individuals carrying the A1555G mutation. In particular, we showed that nine matrilineal relatives with aminoglycoside ototoxicity in a Chinese family exhibited varying severity and audiometric configuration of hearing impairment, although these subjects share some common features: bilateral and sensorineural hearing impairment \cite{60}. This discrepancy likely reflects the dosage and the age at the time of aminoglycoside administration, and different nuclear backgrounds in those individuals.

In the absence of exposure to aminoglycosides, the A1555G mutation also produces a nonsyndromic hearing loss in many families. However, symptoms of progressive matrilineal hearing loss, premature graying, depigmented patches, and digital anomalies in a large Filipino-American family were also associated with the A1555G mutation in the 12S rRNA gene in both affected and unaffected maternal relatives \cite{54}, while maternally inherited cardiomyopathy and hearing loss was associated with the A1555G mutation \cite{61}. Matrilineal relatives of intra-families or inter-families carrying the A1555G mutation exhibited variable penetrance and expressivity including severity and age-of-onset in hearing impairment, ranging from profound congenital deafness, to severe and moderate progressive hearing loss of later onset, to completely normal hearing \cite{32, 48, 53}. These data indicate that the A1555G mutation is a primary factor underlying the development of deafness and that other modifier factors play a role in the phenotypic expression of the hearing loss associated with the A1555G mutation \cite{62-67}.

To identify additional mutations causing drug susceptibility in the 12S rRNA gene, a systematic and extended mutation screening of the mitochondrial 12S rRNA gene has been initiated in two hearing-impaired Chinese populations \cite{21, 55, 66}. As a result, a homoplasmic C-to-T transition at position 1494(C1494T) in the 12S rRNA gene has been identified in a large Chinese family with maternally transmitted
aminoglycoside-induced and non-syndromic deafness \(^{68}\). In the absence of aminoglycosides, some matrilineal relatives in this family exhibited late-onset/progressive deafness, with a wide range of severity and age at onset. Notably, the average age of the onset of deafness has changed from fifty-five years (generation II) to ten (generation IV). Clinical data show that the administration of aminoglycosides can induce or worsen deafness in matrilineal relatives. The age at the time of drug administration appears to be correlated with the severity of hearing loss experienced by affected individuals. As shown in Figure 2, the C1494T mutation is expected to form a novel U1494-1555A base pair, which is in the same position as the C1494-1555G pair created by the deafness-associated A1555G mutation, at the highly conserved A site of 12S rRNA \(^{32, 44, 68}\). This alteration in the tertiary structure of 12S rRNA is also expected to create a binding site for aminoglycosides, thereby causing the sensitivity to these drugs \(^{32, 44, 68}\).

Furthermore, sequence analysis of the complete mitochondrial genomes in four Chinese subjects with aminoglycoside-induced and nonsyndromic hearing impairment identified homoplasmic T1095C mutation in 12S rRNA \(^{21, 69}\). This mutation was absent in 364 Chinese controls \(^{68}\). This mutation was identified in an Italian
family with auditory neuropathy, aminoglycoside-induced hearing loss and Parkinsonism\(^{70}\), another Italian family with maternally inherited hearing loss \(^{71}\) and a Chinese subject with auditory neuropathy \(^{64}\). In fact, the occurrence of the T1095C mutation in these genetically unrelated subjects affected by hearing impairment strongly indicates that this mutation is involved in the pathogenesis of hearing impairment, including aminoglycoside ototoxicity. This T-to-C transition disrupted an evolutionarily conserved base-pair at stem loop of the helix 25 of 12S rRNA \(^{34}\). This nucleotide is also located at the P-site of ribosome, suggesting an important role in the initiation of mitochondrial protein synthesis \(^{70}\). The alteration of the tertiary or quaternary structure of this RNA by the T1095C mutation may lead to impairing mitochondrial protein synthesis, thereby causing the mitochondrial dysfunction associated with hearing impairment.

The mutations at position 961 occur in genetically unrelated families and sporadic subjects affected by aminoglycoside-induced and nonsyndromic hearing loss. These include ET961Cn mutation in Caucasian and Asian subjects \(^{53-55}\), 961-Cinsertion in Caucasian and Asian subjects \(^{21, 53, 68, 75, 76}\), T961G mutation in Caucasian subjects \(^{77}\) and T961C mutation in Chinese subjects \(^{21}\). These observations imply that this mutation is involved in the pathogenesis of this disorder. The 961 mutation localizes at the C-cluster of the region between loop 21 and 22 of 12S rRNA \(^{34}\). This region is not very evolutionarily conserved and its function is not well defined, specifically for its interaction with aminoglycosides in bacterial homologues. It is possible that the alteration of the tertiary or quaternary structure of this RNA by the 961 mutation may indirectly affect the binding of aminoglycosides. Alternatively, this alternation may result in a mitochondrially translational defect.

**Molecular pathogenesis of mitochondrial 12S rRNA mutations associated with aminoglycoside ototoxicity.**

The A1555G and C1494T mutations, as shown in Figure 2, created a highly conserved 1494-1555 G-C or G•U base-pairing at the A site of mitochondrial ribosome where the codon-anticodon recognition occurs \(^{28, 29, 34}\). This corresponding base-pair of *E. coli* 16S rRNA is adjacent to the A-site tRNA-binding bases including A1408, A1492 and A1493 \(^{37, 72, 73}\). Thus, the A1555G or C1494T mutation may result in a local conformational change in the A-site of mitochondrial 12S rRNA, thereby affecting the efficiency and accuracy of codon-anticodon interaction. As a result, these mutations lead to impairment of mitochondrial translation. In fact, ~28% and 50% average decrease, despite a considerable variability, in the rate of mitochondrial protein labeling, were observed in lymphoblastoid cell lines derived from 9 asymptomatic and 10 symptomatic individuals of the Arab-Israeli family, respectively \(^{62}\). Under a constant nuclear background, ~35% and ~37% average reduction in the rate of mitochondrial protein labeling was observed in cybrid cell lines derived from asymptomatic individuals or from symptomatic individuals from this family \(^{63}\). Similarly, cybrid cell lines derived from asymptomatic members, and cybrid cell lines derived from asymptomatic members of the Chinese family carrying the C1494T mutation exhibited ~38% and 43% decrease in the rate of mitochondrial protein labeling, respectively, compared with cybrids derived from Chinese control individuals \(^{78}\). Thus, the A1555G or C1494T mutation indeed produces the primary mitochondrial translational defect with a decrease of ~30-40% mitochondrial protein synthesis rate. However, ~50% decrease in the rate of mitochondrial protein synthesis responsible for a significant reduction in oxygen consumption is likely below the threshold level to produce the deafness phenotype associated with the A1555G or C1494T mutation \(^{78}\). The incomplete penetrance of hearing loss and the mild biochemical defects clearly indicated that the A1555G or C1494T mutation is the primary factor for the hearing impairment but itself is not sufficient to produce a clinical phenotype. Therefore, other modifier factors, such as nuclear modifier genes, mitochondrial haplogroups and aminoglycosides, modulate the phenotypic manifestation of the A1555G or C1494T mutation.

Nuclear modifier genes have been proposed to modulate the phenotypic manifestation of the A1555G or C1494T mutation. Extensive genome-wide linkage studies in Arab-Israeli and Spanish-Italian families revealed that the phenotypic expression of the A1555G mutation is influenced by a complex inheritance of multiple nuclear-encoded modifier genes \(^{79}\). Despite the statistical support for the linkages of several putative modifier loci, including one locus localized to chromosome 8p23.1 \(^{80, 81}\), none of mutations in these modifier genes have been identified. Thus, it is very difficult to identify such nuclear modifier genes by using conventional genetic approaches such as genome-wide linkage analysis. Recently, we have proposed an interesting model for nuclear-mtDNA interaction for the phenotypic manifestation of the A1555G or C1494T mutation. The mutant alleles of yeast *S. cerevisiae* *MTO1* or *MSS1* or *MTO2*, encoding mitochondrial proteins, manifest a respiratory-deficient phenotype on-
ly when coupled with the paromomycin-resistance mitochondrial 15S rRNA C1409G mutation (P₈ₑ₅₄ or P₉) corresponding to sites for human deafness-associated 12S rRNA A1491G and C1409T mutations [67, 82, 83]. These strongly indicate that Mss1p or Mto1p or Mtop2 affects the phenotypic expression of the C1409G mutation by functionally interacting with the region of the C1409G mutation in the15S rRNA. In E.coli, the products of mnmE (homolog of MSS1) [84], gidA (homolog of MTO1) [85] and trmU [86] have been shown to be involved in the biosynthesis of the hypermodified nucleoside 5-methyl-aminomethyl-2-thio-uridine (mmn’s t34) [87]. This modified nucleotide, found in the wobble position of several bacterial tRNAs specific for glutamate, lysine, arginine, and glutamine, has a pivotal role in the structure and function of tRNAs, including structural stabilization, aminoaclonylation and codon-recognition at the decoding site of small ribosomal RNA [85, 87]. Recently, we demonstrated that isolated human MTO1 or GTPBP3 (homolog of MTO1) or TRMU (homolog of MTO2) cDNA can complement the respiratory deficient phenotype of yeast mto1, mss1 or mto2 cells carrying the phenotype of yeast mto1, mss1 or mto2 cells carrying the 15S rRNA C1409G mutation, suggesting that the functions of those proteins are evolutionarily conserved [66, 67]. Thus, mutations in MTO1, MTO1/S1/TPBP3 and MTO2/TRMU likely act as a modifier factor leading to a failure in mitochondrial tRNA metabolism, thereby worsening defects in mitochondrial protein synthesis in cells carrying the A1555G or C1494T mutation. Of these, genotyping analysis of TRMU in 613 subjects of an Arab-Israeli kindred and 210 Italian/Spanish families carrying the A1555G or C1494T mutation revealed a missense mutation(G28T) altering an invariant amino-acid residue(A10S) in the evolutionarily conserved N-terminal region of Trmu protein [88]. Interestingly, all eighteen Arab-Israeli or Italian-Spanish matrilineal relatives carrying both the TRMU A10S and 12S rRNA A1555G mutations exhibited congenital profound deafness. Functional analysis showed that this mutation did not affect importing of Trmu precursors into mitochondria. However, the homozygous A10S mutation leads to a marked failure in mitochondrial tRNA metabolisms, specifically reducing the steady-state levels of mitochondrial tRNAs. As a consequence, these defects contribute to the impairment of mitochondrial protein synthesis. Resultant biochemical defects aggravate the mitochondrial dysfunction associated with the A1555G mutation, exceeding the threshold for expressing the deafness phenotype. These findings indicate that the mutated TRMU, acting as a modifier factor, modulates the phenotypic manifestation of the deafness-associated 12S rRNA mutations.

Furthermore, mtDNA variants/haplotypes have been implicated to influence the penetrance and expressivity of hearing loss associated with primary mutations. The second LHON-associated mtDNA mutations at positions 4216 and 13708 were implicated to increase the penetrance of the deafness-associated A7445G mutation in the precursor of tRNASer(UCN) gene in the New Zealand family than the Scottish pedigree [89-93]. The ND1 T3308C and tRNAAlaT5655C mutations likely contribute to the higher penetrance of deafness in an African pedigree than Japanese and French families carrying the tRNASer(UCN) T7511C mutation [94-98]. Furthermore, the G7444A mutation in the COI gene /precursor of tRNASer(UCN) gene was implicated to influence the penetrance and expressivity of the A1555G mutation [99] and the primary LHON-associated mtDNA mutations [100]. On the H strand of mtDNA, this mutation results in a read-through of the stop codon AGA of the COI message, thereby adding three amino acids(Lys-Gln-Lys) to the C-terminal of the polypeptide. Thus, the mutated polypeptide may retain a partial function. Alternatively, the G7444A mutation is adjacent to the site of 3’ end endonucleolytic processing of L-strand RNA precursor, spanning tRNA-Ser(UCN) and ND6 mRNA [60, 90, 101]. Thus, the G7444A mutation, similar to the A7445G mutation, may also cause a defect in the processing of the L-strand RNA precursor, thus causing mitochondrial dysfunctions.

Aminoglycoside is the predominating modifier factor for hearing loss associated with 12S rRNA mutations. In fact, the new U-A or G-C pair in 12S rRNA created by the C1494T or A1555G transition makes the decoding site of the secondary structure of this rRNA more closely resemble the corresponding region of E. coli 16S rRNA [7, 14, 24]. Thus, these mutations facilitate the binding of aminoglycosides [36, 40]. These drugs, which are concentrated in the perilymph and endolymph of the inner ear [5, 6], may impair mitochondrial translation in cochlear cells in susceptible subjects carrying the A1555G or C1494T mutation. As a consequence, the exposure to aminoglycosides leads to tissue specific defects in those cells, thereby inducing or worsening hearing loss in individuals carrying these mutations. In fact, the significant defects growth rate in the presence of high concentration of paromomycin or neomycin were observed in lymphoblastoid or cybrid cell lines or derived from either symptomatic or asymptomatic individuals carrying the A1555G or C1494T mutation,
as compared with the average rate in control cells\textsuperscript{54, 56, 60, 70}. The reduced growth rate of mutant lymphoblastoid or cybrid cells carrying the A1555G or C1494T mutation in media containing paromomycin is most probably due to a worsening of mitochondrial translation and subsequent respiration defect\textsuperscript{56, 70}. However, no mutant proteins were detected in lymphoblastoid or cybrid cells carrying the A1555G mutation\textsuperscript{37, 38, 56}. Thus, aminoglycosides, acting as modifier factors, lead to an additional 30% decrease in the rate of mitochondrial protein synthesis in cells carrying the A1555G or C1494T mutation, reducing the overall mitochondrial translation rate down to and below the minimal level required for normal cell function, thus inducing the deafness phenotype\textsuperscript{54, 70}.

**Prevalence of ototoxic mitochondrial 12S rRNA mutations.**

In the familial cases of aminoglycoside otoxicity, the susceptible mutations in the 12S rRNA gene account for a significant portion\textsuperscript{21, 32, 49-57, 60, 68, 69}. In the sporadic populations with aminoglycoside otoxicity, the A1555G mutation appears to be more frequently than other mutations such as A1494T and T1095C mutations. The incidence of the A1555G mutation varies in hearing-impaired populations from different ethnic origins. In certain populations, the A1555G mutation appears to be more frequent, while in other populations, this mutation exhibited very low incidence. The A1555G mutation accounts for 33% of a Japanese outpatient population with a history of exposure to aminoglycosides\textsuperscript{102}, while 13% of Chinese pediatric subjects with aminoglycoside otoxicity carry the A1555G mutation\textsuperscript{21}. In two different cohorts of sporadic Chinese subjects with aminoglycoside otoxicity, 5-6% of sporadic patients carry the A1555G mutation\textsuperscript{31, 73, 74}. In two different Caucasian populations, 17% and 17.7% of cases in the United States and Spain with aminoglycoside otoxicity carry the A1555G mutation, respectively\textsuperscript{48, 105}. However, the incidence of A1555G mutation in nonsyndromic hearing loss is much lower than in those with aminoglycoside otoxicity. In other Caucasian clinical populations with nonsyndromic hearing loss, the frequency of the A1555G mutation varies: 2.4% in a Danish cohort\textsuperscript{104}, 1.8% and 0.7% in Hungarian and Germany populations\textsuperscript{105}, 2.5% in a UK cohort\textsuperscript{106}, 1.8% in Turkish pediatric population\textsuperscript{107} and 0.6% in 164 Caucasian American pediatric subjects\textsuperscript{108}. However, in Asian nonsyndromic hearing-impaired populations, the incidence of the A1555G mutation appears to be higher than in Caucasians: 2.9% in Chinese\textsuperscript{21}, 3% in Japanese\textsuperscript{108} and 5.3% in Indonesian\textsuperscript{109}.

**Potential pathophysiological mechanisms of maternally transmitted aminoglycoside otoxicity.**

Based on these genetic and biochemical evidences, we propose the following mechanism for maternally transmitted aminoglycoside otoxicity, as shown in Figure 3. We hypothesize that the site of susceptibility to aminoglycosides is in the mitochondrial ribosomes, specifically that carrying these A1555G or C1494T mutation. Aminoglycosides enter and then accumulate in the cochlear and vestibular mitochondria. Then, these drugs worsen defects in mitochondrial protein synthesis caused by the mitochondrial 12S rRNA A1555G or C1494T mutation. These mitochondrial translational defects result in a decline in ATP production in the cochlear and vestibular cells. At same time, these defects in oxidative phosphorylation would increase the production of reactive oxygen species (ROS), thereby damaging mitochondrial and cellular proteins, lipids and nuclear acids. The hair cells and cochlear neurons may be preferentially involved because they are somehow exquisitely sensitive to subtle imbalance in cellular redox state or increased level of free radicals. Consequently, the mitochondrial permeability transition pore opens and activates apoptosis. This would lead to the dysfunction or death of cochlear and vestibular cells, thereby producing the phenotype of hearing impairment.

**References**

Figure 3. Schematic representation of pathways leading to hearing loss associated with mitochondrial 12S rRNA mutations.
370: 659-662.
44 Hamasaki K, Rando RR. Specific binding of aminoglycosides to a human rRNA construct based on a DNA polymorphism, which causes aminoglycoside-induced deafness. Biochemistry 36: 12323-12328.


