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Mitoribosome dysfunction in sensorineural hearing loss: genetic mechanisms and clinical implication

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ABSTRACT

Mitochondrial translation depends on coordinated contributions from the mitochondrial and nuclear genomes and is essential for oxidative phosphorylation. Disruption of this system can cause isolated sensorineural hearing loss or auditory impairment within multisystem disease. This review examines how mitochondrial rRNA variants, nuclear-encoded mitoribosomal protein defects, and environmental exposures converge on mitochondrial translation to impair hearing. The mitochondrial 12S rRNA 1555A > G and 1494C > T mutations alter decoding-site architecture and increase susceptibility to aminoglycoside ototoxicity, whereas pathogenic variants in nuclear mitoribosomal protein genes can compromise subunit assembly, stability, or abundance. Nuclear modifiers, particularly TRMU, and secondary mitochondrial tRNA variants illustrate how RNA modification and tRNA function influence the consequences of a primary rRNA defect. We propose mitochondrial translational reserve as a testable framework for incomplete penetrance and cochlear vulnerability, while recognizing that its quantitative relationship to hearing outcomes remains unresolved. Current clinical applications include molecular diagnosis, genotype-informed aminoglycoside prescribing, cascade testing of maternal relatives, and individualized hearing rehabilitation. Patient-derived auditory models and genetic rescue studies provide opportunities to clarify disease mechanisms and evaluate interventions, although modifier correction and pharmacological otoprotection remain experimental. Integrating molecular mechanisms with clinical assessment can strengthen prevention of avoidable ototoxicity and guide care for mitochondrial hearing loss.

KEYWORDS

mitochondrial ribosome, mitochondrial translation, 12S rRNA, nuclear modifiers, aminoglycoside ototoxicity, sensorineural hearing loss.

1 Introduction

Sensorineural hearing loss results from pathogenic variants in nuclear or mitochondrial genes, environmental insults such as noise and ototoxic drugs, and age-related degeneration of the auditory system (Morton and Nance, 2006; Cunningham and Tucci, 2017; Petit et al., 2023). Cochlear hair cells and auditory neurons depend on mitochondrial metabolism to sustain ion homeostasis, calcium regulation, synaptic transmission, and cellular maintenance. Impaired mitochondrial translation can compromise these functions, causing isolated hearing loss or auditory impairment within a multisystem disorder (Fischel-Ghodsian, 2003; Guan, 2011). The mitochondrial translation machinery synthesizes the 13 mitochondrial DNA (mtDNA)-encoded polypeptides that form essential subunits of oxidative phosphorylation (OXPHOS) complexes I, III, IV, and V. Its function requires coordinated contributions from the mitochondrial and nuclear genomes. mtDNA encodes 13 mRNAs for these polypeptides, two ribosomal RNAs, and 22 tRNAs, whereas nuclear genes encode mitoribosomal proteins, assembly factors, RNA-processing and modification enzymes, aminoacyl-tRNA synthetases, and translation initiation, elongation, termination, and recycling factors (Andrews et al., 1999; Ott et al., 2016). At the center of this machinery is the human 55S mitoribosome, comprising a 28S small subunit containing mitochondrial 12S rRNA and approximately 30 nuclear-encoded proteins, and a 39S large subunit containing mitochondrial 16S rRNA, approximately 50 nuclear-encoded proteins, and a structural mitochondrial tRNA (Brown et al., 2014; Amunts et al., 2015; Greber et al., 2015; Rorbach et al., 2016). This dual-genome organization creates complementary routes to mitochondrial translation dysfunction. The mitochondrial 12S rRNA 1555A>G and 1494C>T mutations alter the decoding region and predispose

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carriers to maternally inherited hearing loss and aminoglycoside ototoxicity (Prezant et al., 1993; Zhao et al., 2004; Lu et al., 2010a). Biallelic pathogenic variants in nuclear mitoribosomal protein genes can impair subunit assembly or stability and cause multisystem mitochondrial disease, sometimes including hearing loss (Menezes et al., 2015; Lake et al., 2017; Gardeitchik et al., 2018; Pulman et al., 2019) (Table 1). This review compares these mechanisms, evaluates genetic and environmental modifiers, and considers mitochondrial translational reserve as a testable framework for understanding incomplete penetrance and auditory vulnerability.

2 Mitochondrial 12S rRNA mutations and decoding defects

The m.1555A>G and m.1494C>T mutations, identified in many families with maternally inherited aminoglycoside-induced and nonsyndromic hearing loss worldwide, affect the conserved decoding region of mitochondrial 12S rRNA. In the conventional secondary-structure model, the m.1555A>G mutation converts the C1494–A1555 mismatch into a C1494–G1555 base pair, whereas the m.1494C>T mutation generates a U1494–A1555 base pair at the corresponding position in the mature rRNA. Both substitutions increase the structural resemblance of the mitochondrial decoding site to its bacterial counterpart, providing a basis for enhanced aminoglycoside susceptibility (Prezant et al., 1993; Zhao et al., 2004) (Fig. 1). Aminoglycosides, including gentamicin, interact with the bacterial 16S rRNA decoding center and disrupt the codon–anticodon discrimination (Carter et al., 2000). The evolutionary conservation of this region helps explain their unintended effects on mitochondrial translation. Consistent with this mechanism, hybrid-ribosome experiments incorporating mitochondrial decoding-site sequences demonstrated a direct contribution of this region to antibiotic susceptibility (Hobbie et al., 2008). Qian and Guan (2009) investigated the interactions of six aminoglycosides —paromomycin, neomycin, gentamicin, kanamycin, tobramycin, and streptomycin —with synthetic RNA analogues representing the wild-type and m.1555A>G mutation-containing mitochondrial decoding regions. Dimethyl sulfate probing revealed mutation-dependent changes in nucleotide accessibility and antibiotic-dependent protection. Within the mutant analogue, G1555 showed marked protection with neomycin, gentamicin, and kanamycin across the tested concentrations, whereas protection with paromomycin, tobramycin, and streptomycin was more concentration dependent (Qian and Guan, 2009). These findings indicate that the m.1555A>G or m.1494T>C mutation alters the local conformation of the mitochondrial decoding region and its interactions with aminoglycosides. Cellular studies connect these decoding-site alterations to impaired mitochondrial function. Lymphoblastoid cells carrying the m.1555A>G mutation exhibited reduced mitochondrial protein synthesis and respiration, with more pronounced abnormalities in cells from affected than unaffected matrilineal relatives (Guan et al., 1996). Aminoglycoside exposure further aggravated translational dysfunction in cells carrying the m.1555A>G mutation (Guan et al., 2000). Similarly, transmitochondrial cybrids carrying the m.1494C>T mutation showed reduced mitochondrial protein synthesis and respiratory capacity, together with increased growth inhibition following paromomycin exposure (Zhao et al., 2005). More recent work has linked the m.1555A>G mutation to broader disturbances in mitochondrial and cellular homeostasis (He et al., 2025). These studies support a mechanism in which pathogenic 12S rRNA mutations impair mitochondrial translation and sensitize the decoding machinery to aminoglycosides.

3 Nuclear mitoribosomal protein defects and subunit assembly

Nuclear-encoded mitoribosomal proteins link nuclear gene expression to the assembly and function of mtDNA-encoded rRNAs. Following synthesis in the cytosol and import into mitochondria, these proteins establish protein–protein and protein–rRNA interactions required to generate functional ribosomal subunits. Quantitative pulse-labeling studies demonstrated ordered protein incorporation during mitoribosome biogenesis (Bogenhagen et al., 2018). Subsequent biochemical purification, quantitative mass spectrometry, and mathematical modeling identified protein-only assembly modules that associate with their cognate rRNA components in a coordinated manner (Lavdovskaia et al., 2024). Pathogenic variants may therefore impair protein folding or stability, disrupt assembly interfaces, or prevent maturation of an rRNA-containing intermediate. The resulting loss of functional subunits reduces the capacity for mitochondrial protein synthesis and compromises OXPHOS biogenesis. These mechanisms differ from the predominantly decoding-associated effects of pathogenic 12S rRNA mutations, although assembly and decoding defects are not mutually exclusive. Unlike maternally transmitted mitochondrial rRNA disorders, the nuclear disorders discussed here generally follow autosomal recessive inheritance and frequently affect multiple organs including cochlea.

Table 1 Comparison of mitochondrial 12S rRNA variants and nuclear mitoribosomal protein disorders

Genetic lesion	Inheritance	Clinical presentation	Principal molecular mechanism	
12S rRNA 1555A > G / 1494C > T mutation	Maternal; often homoplasmic; incomplete penetrance	Nonsyndromic or aminoglycoside-triggered sensorineural hearing loss	12S rRNA decoding-center remodeling; antibiotic susceptibility; partial translation defect	Prezant et al., 1993; Zhao et al., 2005
MRPS2	Autosomal recessive (biallelic)	Hearing loss, hypoglycaemia, multiple OXPHOS deficiencies	Reduced small-subunit abundance / impaired translation	Gardeitchik et al., 2018
MRPS7	Autosomal recessive (biallelic)	Congenital deafness; lactic acidaemia; hepatic/renal dysfunction	Small-subunit and translation impairment	Menezes et al. 2015
MRPS28	Autosomal recessive (biallelic)	Growth restriction, craniofacial dysmorphism, multisystem involvement; variable auditory findings	Mitoribosome biogenesis / small-subunit dysfunction [27]	Pulman et al., 2019
MRPS34	Autosomal recessive (biallelic)	Leigh syndrome and severe neurological disease; hearing loss not obligatory	Small-subunit instability and defective mitochondrial translation	Lake et al., 2017
MRPL49	Autosomal recessive (biallelic)	sensorineural hearing loss, ovarian insufficiency neurological disease	Large-subunit instability, defective assembly, and impaired ribosome function	Thomas et al., 2025

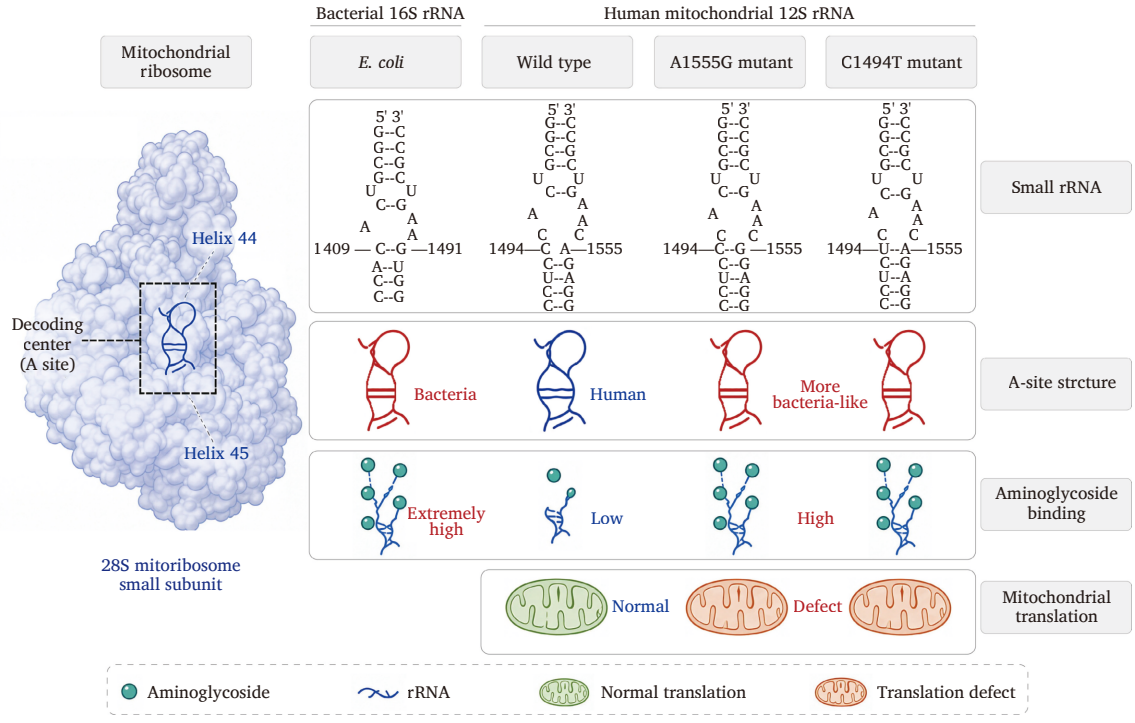


Figure 1 Structural basis of aminoglycoside susceptibility associated with mitochondrial 12S rRNA variants. Left, schematic localization of the decoding center (A site) within the human 28S mitochondrial ribosomal small subunit, showing helices 44 and 45. Right, comparison of the decoding-region secondary structures of *Escherichia coli* 16S rRNA and wild-type, m.1555A>G, and m.1494C>T human mitochondrial 12S rRNA. The wild-type mitochondrial C1494–A1555 mismatch is replaced by a C1494–G1555 base pair in m.1555A>G mutation or a U1494–A1555 base pair in m.1494C>T mutation. Both substitutions increase the resemblance of the mitochondrial decoding site to its bacterial counterpart, enhancing susceptibility to aminoglycoside interactions and associated mitochondrial translation defects. Turquoise circles represent aminoglycosides; green and orange mitochondrial symbols indicate preserved and impaired translation, respectively. Binding and functional effects are illustrated schematically and do not represent quantitative measurements.

MRPS2-associated disease illustrates how disruption of a single structural component can impair small-subunit maturation. Gardeitchik et al. (2018) identified biallelic variants in two unrelated individuals with sensorineural hearing impairment, mild developmental delay, hypoglycemia, and combined OXPHOS deficiency. Patient fibroblasts showed reduced abundance of MRPS2 and other small-subunit proteins, abnormal assembly profiles, and a generalized mitochondrial translation defect. Complexome profiling supported a role for MRPS2 in a relatively late assembly step, suggesting that its deficiency can prevent completion of the small subunit despite persistence of an earlier protein-containing intermediate. Reintroduction of wild-type MRPS2 restored mitochondrial translation and OXPHOS assembly, directly connecting the protein defect to impaired respiratory-complex biogenesis.

MRPS7-associated disease demonstrates the dependence of mitochondrial rRNA stability on a nuclear-encoded binding partner. Menezes et al. (2015) identified a homozygous p.Met184Val substitution in siblings with congenital sensorineural deafness, lactic acidemia, and variable progressive hepatic and renal dysfunction. MRPS7 binds 12S rRNA and contributes to small-subunit assembly. Patient fibroblasts exhibited reduced 12S rRNA abundance and respiratory-chain abnormalities, whereas expression of wild-type MRPS7 restored 12S rRNA levels and improved complex I and IV activities. These findings support a mechanism in which defective MRPS7 compromises the accumulation or maintenance of an rRNA-containing small subunit, thereby limiting mitochondrial translation. Reduced steady-state rRNA abundance is consistent with impaired stabilization during assembly, although it does not alone establish the rate or pathway of rRNA degradation.

MRPS28 and MRPS34 disorders further demonstrate how small-subunit instability propagates into translation failure. Pathogenic MRPS28 variants were identified in an individual with intrauterine growth restriction, craniofacial dysmorphism, severe developmental abnormalities, and multisystem involvement. Patient fibroblasts showed defective small-subunit assembly and mitochondrial translation; wild-type MRPS28 expression restored assembly, protein synthesis, and OXPHOS biogenesis (Pulman et al., 2019). In MRPS34-associated Leigh or Leigh-like syndrome, reduced MRPS34 abundance was accompanied by depletion of other small-subunit proteins and reduced assembled monosomes (Lake et al., 2017). Thus, failure to maintain a competent small subunit can also restrict its association with the large subunit, reducing the pool of functional 55S mitoribosomes. The predominantly neurological presentation of MRPS34 disease also emphasizes that defective mitochondrial translation does not invariably produce a hearing-dominant phenotype.

Large-subunit disease provides an important mechanistic qualification. Biallelic MRPL49 variants cause phenotypes ranging from sensorineural hearing loss with ovarian insufficiency to severe neurological disease, including leukodystrophy (Thomas et al., 2025). Patient-cell analyses identified reduced mitoribosomal subunit abundance and OXPHOS abnormalities without evidence of altered assembly profiles. Consequently, reduced ribosome abundance, defective assembly, and impaired ribosome function should be distinguished experimentally rather than inferred interchangeably from a mitoribosomal gene diagnosis.

These studies support several routes from a nuclear variant to mitochondrial translation dysfunction: destabilization of an individual protein, disruption of an assembly module, reduced rRNA accumulation, and depletion of functional ribosomes. Systematic

knockout studies further show that different mitochondria-specific small-subunit proteins produce distinct assembly defects with variable effects on protein synthesis (Hilander et al., 2024). Mechanistic classification therefore requires complementary measurements. Protein and rRNA abundance assess component availability; sucrose-gradient analysis and complexome profiling resolve subunits and assembly intermediates; mitochondrial translation assays measure synthetic output; and wild-type complementation tests whether restoring the affected protein reverses the defect. Applying these approaches together can distinguish defective ribosome production from dysfunction of assembled particles and identify where nuclear and mitochondrial components become functionally uncoupled.

4 Nuclear modifiers of RNA metabolism determine whether a ribosomal defect crosses the disease threshold

Nuclear-encoded RNA-modifying enzymes can influence the biochemical consequences of 12S rRNA mutations by altering the tRNAs and rRNAs required for mitochondrial translation. Among these enzymes, TRMU provides particularly strong mechanistic evidence for a nuclear modifier of mitochondrial hearing loss. TRMU catalyzes 2-thiolation of wobble uridine at position 34 in mitochondrial $tRNA^{Lys}$, $tRNA^{Glu}$, and $tRNA^{Gln}$, supporting efficient codon recognition and protein synthesis (Guan et al., 2006; Wang et al., 2010; Zhang et al., 2018). The *TRMU* c.28G>T (p.Ala10Ser; A10S) variant was identified as a modifier of deafness-associated 12S rRNA mutations (Guan et al., 2006). Subsequent biochemical studies showed that A10S reduces TRMU protein stability and tRNA thiolation and exacerbates mitochondrial dysfunction in cells carrying the m.1555A>G mutation (Meng et al., 2017). These findings support a mechanism in which defective tRNA modification compounds the decoding defect of the mutant ribosome, further impairing mitochondrial translation and respiratory-chain function. Patient-derived induced pluripotent stem cells (iPSCs) extend this mechanism to an auditory differentiation model. Cells carrying both m.1555A>G and *TRMU* c.28G>T mutations exhibited more pronounced abnormalities during differentiation into otic epithelial progenitors and hair-cell-like cells than cells carrying the m.1555A>G mutation alone. CRISPR/Cas9 correction of the *TRMU* allele improved cellular morphology and electrophysiological function while retaining the mitochondrial variant (Chen and Guan, 2022). This rescue supports a causal contribution of the nuclear modifier to the auditory cellular phenotype.

Other mitochondrial RNA-modifying enzymes, including MTO1, GTPBP3, and TFB1M, have been investigated as candidate modifiers. MTO1 and GTPBP3 participate in the formation of taurine-containing modifications at mitochondrial tRNA wobble uridines, providing a mechanistic basis through which altered tRNA decoding could aggravate m.1555A>G-associated translation defects (Li and Guan, 2002; Li et al., 2002). Family-based linkage and linkage-disequilibrium analyses implicated *MTO1* and *GTPBP3* in the variable expression of hearing loss associated with m.1555A>G mutation (Bykhovskaya et al., 2004b). However, pathway involvement and genetic association do not establish that a particular allele modifies clinical penetrance; variant-specific functional evidence and independent replication remain necessary. TFB1M catalyzes dimethylation of conserved adenosines in mitochondrial 12S rRNA and was also proposed as a modifier of m.1555A>G-associated hearing loss (Bykhovskaya et al., 2004a). Its proposed contribution through pathological rRNA hypermethylation remains uncertain. O'Sullivan et al. (2015) found methylation of all detectable transcripts at the examined site in primary samples from both carriers and controls, challenging increased methylation as a general explanation for disease expression. Thus, although mitochondrial RNA modification provides a plausible interface between nuclear background and rRNA-associated deafness, the strength of modifier evidence differs among genes, with *TRMU* supported by biochemical studies and genetic rescue in patient-derived auditory cells.

5 mtDNA background adds a second layer of mitochondrial genetic modification

Pathogenic mitochondrial 12S rRNA variants are inherited within mitochondrial genomes containing linked sequence variation that may modify their biochemical effects. Analysis of various pedigrees carrying the m.1555A>G mutation have implicated mitochondrial haplotypes and secondary variants in the variable penetrance of associated hearing loss, supporting a role for inherited mitochondrial variation in phenotypic expression (Torroni et al., 1999; Yuan et al., 2005; Young et al., 2006; Ding et al., 2009; Lu et al., 2010b). In particular, the haplogroup B was associated with an increased risk of hearing loss among East Asian pedigrees carrying the m.1555A>G mutation (Ying et al., 2015). These findings suggest that the surrounding mtDNA background contributes to phenotypic expression, although associations may be confounded by ancestry, nuclear genetic background, aminoglycoside exposure, and ascertainment. Comprehensive mtDNA analysis can therefore identify candidate modifiers, but haplogroup membership alone is not a validated predictor of individual hearing outcomes.

Secondary mitochondrial tRNA variants provide more specific mechanistic explanations for modification of the auditory phenotype. The mt-$tRNA^{Thr}$ 15927G>A mutation affects conventional tRNA position 42, disrupting a conserved base pair in the anticodon stem. Studies of four Chinese families implicated this variant in altered tRNA metabolism and increased phenotypic expression of m.1555A>G-associated hearing loss (Wang et al., 2008). Meng et al. (2018) provided complementary functional evidence for the mt-$tRNA^{Ile}$ 4317A>G mutation, which affects position 59 in the T-loop and perturbs tRNA conformation, stability, and aminoacylation. Lymphoblastoid cells carrying both m.4317A>G and m.1555A>G mutations exhibited greater impairment of mitochondrial translation and respiration, reduced mitochondrial ATP production and membrane potential, and increased reactive oxygen species compared with cells carrying the m.1555A>G mutation alone. These findings support a model in which reduced availability or function of aminoacylated tRNAs compounds the translational defect imposed by the mutant ribosome. Nevertheless, patient-derived cell lines retain additional genetic differences, limiting attribution of the combined phenotype to the secondary variant alone.

Together with the *TRMU* findings, these studies identify tRNA modification, structural integrity, and aminoacylation as interacting determinants of mitochondrial translation in 12S rRNA-associated deafness. The evidence should nevertheless be interpreted according to experimental strength: pedigree associations identify candidate modifiers, biochemical assays establish functional consequences, and genetic correction or complementation more directly tests allele contribution. Because mitochondrial variants are inherited together, conservation and maternal cosegregation alone cannot establish a modifier effect. Independent replication

and comparisons that control nuclear and mitochondrial genetic backgrounds are required before secondary variants can support individualized prediction of hearing-loss risk.

6 Incomplete penetrance and the translational reserve model

Families carrying the m.1555A>G or m.1494C>T mutation exhibit substantial variation in penetrance, age at onset, and severity of hearing loss (Prezant et al., 1993; Estivill et al., 1998; Zhao et al., 2004; Chen et al., 2007). Several Chinese pedigrees show particularly low penetrance, with hearing loss confined to a small proportion of maternal relatives carrying the m.1555A>G or m.1494C>T mutation (Dai et al., 2006; Chen et al., 2007; Lu et al., 2010b). Such families may appear to contain sporadic cases despite maternal transmission; an isolated affected individual does not necessarily indicate a *de novo* mutation. Similarly, a population-based British cohort found comparable hearing thresholds in m.1555A>G carriers and noncarriers at 44–45 years, although aminoglycoside exposure histories were unavailable (Rahman et al., 2012). These observations demonstrate that homoplasmic carriers can retain hearing and that mutant load alone cannot explain clinical expression.

Transmitochondrial cybrid studies help distinguish the biochemical effects of the primary rRNA mutations from those of the nuclear background. Mitochondria derived from symptomatic and asymptomatic maternal relatives carrying the m.1555A>G mutation produced similar biochemical abnormalities after transfer into a nearly constant nuclear background, despite differences between the original lymphoblastoid cell lines (Guan et al., 2001). Comparable findings were reported for cybrids bearing the m.1494T>C mutation (Zhao et al., 2005). In these models, mitochondrial protein synthesis was reduced by approximately 35–37% for m.1555A>G mutation and 38–43% for m.1494C>T mutation, indicating substantial residual translational capacity. These measurements describe protein-synthesis output under specific experimental conditions and should not be interpreted as uniform effects across tissues or direct measurements of decoding fidelity. We propose mitochondrial translational reserve as a framework for understanding these findings: the capacity of mitochondrial protein synthesis, together with compensatory responses, to maintain sufficient OXPHOS under genetic or environmental stress (Fig. 2). A primary 12S rRNA variant reduces this reserve, while nuclear modifiers, secondary mtDNA variants, and aminoglycoside exposure may impose additional burdens. Auditory function may remain preserved when residual capacity meets cellular requirements, whereas hearing loss may develop when cumulative impairment exceeds the functional tolerance of susceptible cochlear cells. This model provides a testable explanation for incomplete penetrance, but translational reserve has not been established as a clinical biomarker. An approximately 50% reduction in mitochondrial translation was previously proposed as a level associated with deafness, based on selected cellular observations (Guan et al., 1996; Zhao et al., 2005). This estimate should be regarded as a model-dependent interpretation rather than a universal pathogenic threshold. The relationship between translation and respiratory function depends on metabolic demand, protein turnover, and compensatory capacity; measurements in lymphoblastoid cells or osteosarcoma-derived cybrids cannot define the corresponding threshold in the cochlea. Hair cells, supporting cells, strial cells, and auditory neurons have distinct physiological requirements that may confer different tolerances to mitochondrial

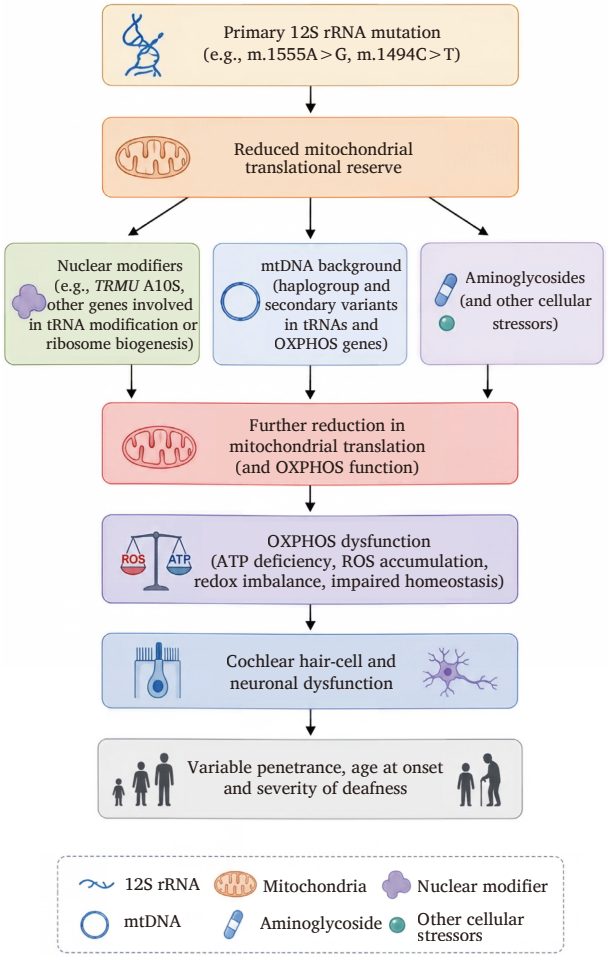


Figure 2 Genetic and environmental determinants of mitochondrial translational reserve. Primary m.1555A>G or m.1494C>T mutations establish a partially compromised translational state. Nuclear modifiers, mtDNA background and secondary mtDNA variants can further reduce translational capacity, whereas aminoglycoside exposure adds an environmental stress. When cumulative impairment remains within compensatory capacity, hearing is preserved; when translational reserve falls below a tissue-specific threshold, OXPHOS dysfunction, ATP deficiency and redox imbalance can impair cochlear cells.

dysfunction. Sustained energetic demand, redox sensitivity, limited hair-cell regeneration, and cochlear aminoglycoside accumulation may together contribute to auditory vulnerability. Although nuclear mitoribosomal protein disorders also impair mitochondrial translation, their assembly defects and multisystem manifestations represent distinct disease mechanisms that cannot be arranged along a single severity continuum with 12S rRNA-associated hearing loss (Fig. 3).

7 Aminoglycoside exposure and downstream cochlear injury

Aminoglycosides are potent environmental modifiers of 12S rRNA-associated hearing loss because they target the decoding machinery already compromised by pathogenic 12S rRNA mutations. Their ototoxic effects also depend on access to and persistence within the cochlea. Systemically administered aminoglycosides can cross the blood–endolymph barrier and enter sensory hair cells through their apical surfaces (Li and Steyger, 2011), with mechanotransduction channels providing an important entry route (Alharazneh et al., 2011). Cochlear accumulation and reten-

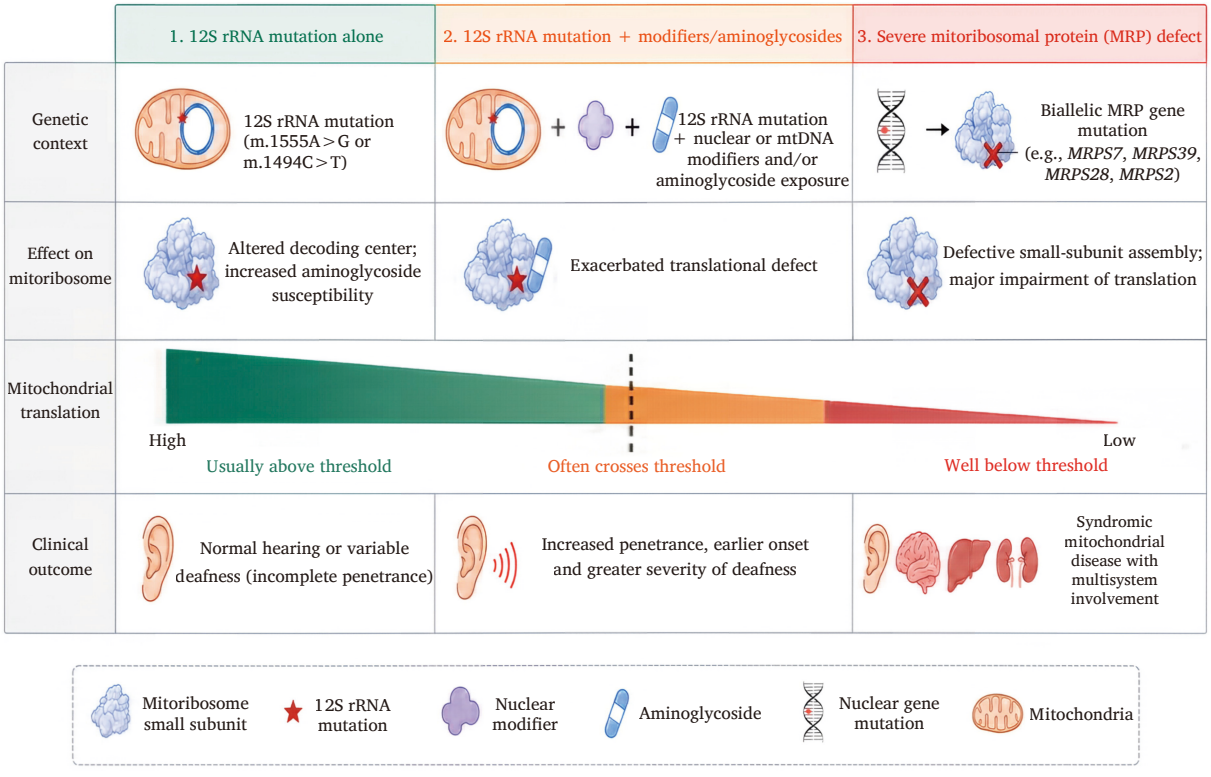


Figure 3 A comparative model of mitochondrial genetic architectures underlying sensorineural deafness. Left, primary 12S rRNA mutations cause partial, incompletely penetrant translational impairment. Middle, genetic and environmental modifiers further reduce translational reserve and increase penetrance and severity. Right, biallelic mitoribosomal protein defects can cause severe assembly or stability defects and multisystem mitochondrial disease. The model emphasizes a continuum defined by the magnitude and context of mitochondrial translational impairment.

tion may therefore increase local exposure and contribute to selective auditory injury. Following cellular entry, oxidative stress and regulated cell-death pathways may amplify mitochondrial injury. Huo et al. (2024) reported that a palladium single-atom nanozyme attenuated neomycin-induced ototoxicity, accompanied by suppression of ferroptosis-associated changes and improved mitochondrial function. These findings implicate iron-dependent lipid peroxidation as a potential contributor to hair-cell injury and an experimental target for otoprotection. Mitochondrial quality control provides another potential link between aminoglycoside exposure and hair-cell survival, although the effects of mitophagy vary among experimental settings. Zhang et al. (2023) observed impaired PINK1–Parkin-mediated mitochondrial clearance after neomycin exposure and protection following enhancement of mitophagy. Conversely, reducing components of RIPOR2-associated autophagy, including PINK1 or Parkin, protected against aminoglycoside injury in another mouse study (Li et al., 2022a). These divergent findings suggest that the consequences of mitochondrial turnover depend on exposure intensity, timing, and the cellular pathways engaged. Mechanistic studies should therefore measure mitophagic flux, mitochondrial degradation, and respiratory function alongside genetic perturbation, because changes in marker abundance alone cannot establish effective organelle clearance. Whether these pathways specifically amplify the translation defects caused by pathogenic 12S rRNA variants requires direct investigation in genetically defined auditory models.

8 Clinical applications in otologic practice

8.1 Molecular diagnosis and clinical assessment

Distinguishing mitochondrial 12S rRNA-associated susceptibility from nuclear mitoribosomal disease has practical implications for diagnosis and management. Clinical evaluation should integrate the audiologic phenotype, age at onset, a three-generation pedigree, aminoglycoside exposure, and associated neurological, metabolic, hepatic, or renal abnormalities. Maternal clustering or hearing loss following aminoglycoside treatment should raise suspicion of 12S rRNA-associated disease, although incomplete penetrance can obscure maternal inheritance. A negative family history therefore does not exclude a mitochondrial cause. Genetic testing should be guided by the clinical findings and accompanied by counseling about potential results and their implications, consistent with the ACMG practice resource (Li et al., 2022b). Testing strategies should distinguish rapid pharmacogenetic assessment from comprehensive etiologic investigation. Targeted testing for m.1555A>G and m.1494C>T mutations can inform aminoglycoside prescribing, but a negative result does not exclude other clinically relevant 12S rRNA variants or other genetic causes of hearing loss. When broader investigation is warranted, testing should encompass nuclear and mitochondrial hearing-loss genes, using clinical or whole-exome sequencing complemented by dedicated mtDNA analysis to ensure adequate mitochondrial genome coverage (Petit et al., 2023). Hearing loss accompanied by developmental delay, hypoglycemia, lactic acidemia, or organ dysfunction should prompt consideration of mitoribosomal disorders, including those associated with *MRPS2* and *MRPS7*, and coordinated assessment by otology, clinical genetics, and mitochondrial medicine services. Variants of uncertain significance and unvalidated modifier alleles should not be regarded as definitive molecular diagnoses (Menezes et al., 2015; Gardeitchik et al., 2018; Li et al., 2022b).

8.2 Pharmacogenetic prevention of aminoglycoside ototoxicity

Prevention of aminoglycoside-induced hearing loss is the most immediately actionable clinical application of 12S rRNA testing. The Clinical Pharmacogenetics Implementation Consortium (CPIC) identifies the m.1555A>G, m.1494C>T, and m.1095T>C as variants associated with increased susceptibility and recommends avoiding aminoglycosides unless the severity of infection and lack of safe, effective alternatives outweigh the risk of permanent hearing loss. When aminoglycosides are necessary, treatment should involve infectious disease specialists and use the shortest effective course, therapeutic drug monitoring, renal function assessment, and audiovestibular monitoring during and after therapy. Serum concentrations within the therapeutic range do not eliminate genetically mediated ototoxicity. Similarly, a negative genetic test does not exclude conventional aminoglycoside ototoxicity or remove the need for standard precautions (McDermott et al., 2022b). Rapid genotyping offers a practical route to incorporating this information into prescribing. In the PALOH study, point-of-care testing for m.1555A>G mutation generated results in approximately 26 minutes and enabled three variant-positive newborns to receive alternative antibiotics without increasing the mean time to antibiotic administration in the evaluated pathway (McDermott et al., 2022a). These findings demonstrate clinical feasibility, although long-term hearing-loss prevention was not directly measured. Implementation requires validated assays, clear procedures for failed or unavailable results, appropriate antibiotic alternatives, and integration with urgent infection care. Results should be documented in accessible clinical records and communicated to patients, families, and future prescribers to support lifelong prescribing precautions. The 2026 CERSI-PGx guideline further supports pre-emptive testing when aminoglycoside use is foreseeable, while emphasizing that urgent treatment should not be delayed when genetic results are unavailable (McDermott et al., 2026).

8.3 Family counseling and hearing rehabilitation

Identification of a pathogenic 12S rRNA mutation has implications for maternal relatives, including those with normal hearing. Genetic counseling should explain maternal inheritance, incomplete penetrance, and the distinction between increased susceptibility to aminoglycoside ototoxicity and the likelihood of hearing loss without drug exposure. Cascade testing can identify relatives who may benefit from prescribing precautions. For autosomal recessive mitoribosomal protein disorders, counseling should establish the causal variants, their allelic phase, and parental carrier status to inform recurrence-risk assessment. Mitochondrial haplogroups and candidate modifier alleles do not currently provide validated estimates of individual hearing-loss risk, and mitochondrial translational reserve remains a research concept (Li et al., 2022b; McDermott et al., 2022b). Molecular diagnosis should be integrated with timely audiologic assessment and hearing rehabilitation. Baseline evaluation and follow-up should reflect age, hearing phenotype, symptoms, and exposure history, with monitoring during and after aminoglycoside treatment in susceptible individuals. Patients with established hearing loss should receive individualized communication support, appropriate amplification, and cochlear implant assessment when standard candidacy criteria are met. Treatment planning should consider speech perception, auditory-neural involvement, developmental needs, and systemic manifestations. Although genetic findings can clarify etiology and guide preventive care, 12S rRNA or MRPS genotype alone has not been established as a predictor of cochlear implant outcomes. Experimental mitochondrial therapies therefore require evaluation alongside established hearing care (Cunningham and Tucci, 2017; Li et al., 2022b).

9 Outstanding questions and therapeutic opportunities

Several priorities emerge for translating this framework into improved prevention and treatment of mitochondrial hearing loss. Candidate modifiers require independent replication and allele-specific functional validation to distinguish causal effects from linked genetic variation. Comparisons among cochlear hair cells, supporting cells, strial cells, and auditory neurons are also needed to determine how differences in mitochondrial translation, OXPHOS, redox regulation, and proteostasis influence vulnerability. A central challenge is to define mitochondrial translational reserve experimentally and establish whether it predicts auditory dysfunction. Mitochondrial ribosome profiling can characterize ribosome occupancy and translational behavior, but should be combined with direct measurements of protein synthesis, respiration, and responses to controlled stress, because occupancy alone does not establish productive translation (Rooijers et al., 2013). Patient-derived hair-cell-like models provide a platform for these investigations, although cellular maturity and reproducibility require careful assessment (Chen and Guan, 2023). Experimental therapies may act at several stages of aminoglycoside injury. Strategies that reduce drug uptake must preserve mechanotransduction and antimicrobial efficacy, whereas interventions targeting mitophagy or ferroptosis require evidence that their effects are protective in the relevant genetic and exposure context. Correction of the TRMU modifier allele provides proof of principle that a nuclear contribution to an mtDNA-associated cellular phenotype can be reversed (Chen and Guan, 2022). Translation to treatment will require effective cochlear delivery, sustained benefit, and intervention before irreversible auditory-cell loss. Gene supplementation or variant-specific correction may also be considered for nuclear mitoribosomal protein disorders, although multisystem involvement may require delivery beyond the cochlea. Cochlear gene therapy offers a delivery precedent for hereditary hearing loss. In a 2024 single-arm study, bilateral AAV1-hOTOF administration was associated with improved hearing in five children with biallelic OTOF-associated DFNB9 (Wang et al., 2024). These findings support the feasibility of molecular intervention in the human cochlea. However, 12S rRNA-associated hearing loss and nuclear mitoribosomal disorders involve different molecular defects and potentially broader cellular targets; therapeutic efficacy must therefore be established independently for each condition. Mitochondrial replacement addresses a separate objective: reducing maternal transmission of pathogenic mtDNA through reproductive intervention. Clinical pronuclear-transfer studies have reported live births with reduced transmission of maternal pathogenic variants (Hyslop et al., 2025). Its potential use requires assessment of disease risk, reproductive alternatives, residual mtDNA carryover, long-term outcomes, and applicable regulations. Therapeutic development should therefore define distinct goals and outcome measures for preventing aminoglycoside injury, preserving auditory cells, correcting molecular defects, and reducing transmission.

10 Conclusions

Mitoribosome-associated hearing loss arises from interactions

among the mitochondrial genome, nuclear genes, and environmental exposures. Pathogenic mitochondrial 12S rRNA mutations alter decoding and increase aminoglycoside susceptibility, whereas nuclear-encoded mitoribosomal protein defects can disrupt ribosome assembly or stability and cause multisystem disease with variable auditory involvement. Findings involving TRMU and secondary mitochondrial tRNA variants demonstrate how RNA modification and tRNA function can influence the consequences of a primary rRNA defect. Mitochondrial translational reserve provides a testable framework for incomplete penetrance and cochlear vulnerability, although its quantitative relationship to hearing outcomes remains to be established. Current clinical priorities include etiologic diagnosis, genotype-informed aminoglycoside prescribing, family counseling, and individualized hearing rehabilitation. Integrating genetic evidence with functional studies and patient-derived auditory models should improve variant interpretation and support the development of interventions to preserve mitochondrial function and hearing.

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Data Availability

All data that support the findings of this review are contained in the cited literature.

Ethical Approval

Not applicable.

Conflicts of Interest

The authors declare no competing interests.

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