The Journal of International Advanced Otology

J Int Adv Otol 2023; 19(5): 414–419 • DOI: 10.5152/iao.2023.231107

Review

Research Advances on Deafness Genes Associated with Mitochondrial tRNA–37 Modifications

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Cite this article as: Bian P, Chai J, Xu B. Research advances on deafness genes associated with mitochondrial tRNA-37 modifications. *J Int Adv Otol*. 2023;19(5):414-419.

As the most common cause of speech disorders, the etiological study of deafness is important for the diagnosis and treatment of deafness. The mitochondrial genome has gradually become a hotspot for deafness genetic research. Mitochondria are the core organelles of energy and material metabolism in eukaryotic cells. Human mitochondria contain 20 amino acids, except for $tRNA^{Leu}$ and $tRNA^{Ser}$, which have 2 iso-receptors, the other 18 amino acids correspond to unique tRNAs one by one, so mutations in any one tRNA may lead to protein translation defects in mitochondria and thus affect their oxidative phosphorylation process resulting in the corresponding disease phenotype. Mitochondrial tRNAs are extensively modified with base modifications that contribute to the correct folding of tRNAs and maintain their stability. Defective mitochondrial tRNA modifications are closely associated with the development of mitochondrial diseases. The in-depth study found that modification defects of mammalian mitochondrial tRNAs are associated with deafness, especially the nucleotide modification defect of mt-tRNA-37. This article reviews the research on mitochondrial tRNAs, nucleotide modification structure of mitochondrial tRNA-37, and nuclear genes related to modification defects to provide new ideas for the etiological study of deafness.

KEYWORDS: Mitochondria, mitochondrial tRNA, tRNA modification, *TRMT5*, *TRIT1*, *CDK5RAP1*, deafness gene

INTRODUCTION

Deafness is a common cause of speech impairment and an essential factor affecting the population quality. The latest data published by the World Health Organization shows that the number of people with hearing loss worldwide is about 466 million, accounting for 5.5% of the total population (http://www.who.int/en/). In China, the results of the second national sample survey of people with disabilities show that 27.8 million people have a hearing impairment, which is the highest among all kinds of disability diseases and is growing at a rate of 20 000-30 000 per year, while more than 80 000 children are diagnosed with delayed deafness. Therefore, exploring the causes of deafness and reducing the incidence of deafness is a social problem that needs to be solved urgently.

The leading causes of deafness include genetic and environmental factors, with genetic factors accounting for at least 50%-60%.[1-4] Genetic deafness is inherited in various ways, including autosomal dominant and incomplete dominant (DFNA), autosomal recessive (DFNB), X-linked (DFNX), and mitochondrial maternal inheritance.[5] The study of mitochondrial inherited deafness in humans started in the 1960s, and Tsuiki et al reported a number of mitochondrial inherited deafness families successively. In China, Professor Bu Xingkuan reported for the first time a large family line of aminoglycoside drug-related deafness and verified its maternal inheritance pattern. The mitochondrial genome has gradually become a hotspot for deafness genetic research, and Professor Minxin Guan has discovered that mutations in the *mtDNA12SrRNA* gene are the molecular mechanism of aminoglycoside ototoxicity-induced hearing loss.[6-8] The role of mt-tRNA in the development of various diseases has been gradually revealed along with the excavation of the mitochondrial genome. Mt-tRNA mutations leading to defects in the respiratory chain complex make the mitochondrial oxidative phosphorylation system produce insufficient energy, resulting in different clinical phenotypes. The inner ear is often affected by mt-tRNA mutations because of its high-energy-consuming characteristics.[9] It has also been found that mt-tRNA mutations play an important role in the occurrence and development of deafness, especially the mutations affecting tRNA-37 modification.[10,11] In this article, we briefly introduce mitochondria and mitochondrial genome, and summarize the deafness genes associated with defective mitochondrial tRNA (mt-tRNA)-37 modifications to further reveal future research directions.

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Received: February 23, 2023 • **Accepted:** June 08, 2023 • **Publication Date:** September 29, 2023
Available online at www.advancedotology.org

Mitochondria and Mitochondrial Genome

Mitochondria are important organelles in eukaryotic cells and consist of 4 parts: the outer mitochondrial membrane (OMM), intermembrane space of mitochondria (IMS) between the inner and outer membranes, the inner mitochondrial membrane (IMM), and the mitochondrial matrix located in the innermost layer. Most enzymes required for cellular oxidative phosphorylation are located in the mitochondrial matrix. In contrast, the oxidative respiratory chain (ORC) is found in the inner mitochondrial membrane, a reaction system consisting of a series of hydrogen and electron transporters arranged in a specific order. These transport systems can be divided into 5 complexes: NADH (complex I), succinate oxidoreductase (complex II), cytochrome c oxidoreductase (complex III), cytochrome c reductase (complex IV), and ATP synthase (complex V). The electrons obtained from the oxidation of glucose and other substances are mainly transferred through the ORC, most of which are generated in the reaction of NADH (complex I). Once the mobile electron carrier cytochrome c is reduced, it ferries single electrons from Complex III to Complex IV;[12] some electrons also enter from succinate oxidoreductase (complex II) and are transferred to Complex IV through Complex III. The energy generated by electron transfer is used to generate proton gradients in the inner mitochondrial membrane; protons finally pass through ATP synthase, synthesizing ATP and sustaining the cell's life activities.

Mitochondria are dynamic intracellular organelles with their own DNA (mitochondrial DNA, mtDNA). Mitochondrial DNA, consisting of 16 569 base pairs in humans, is a circular double-stranded genome. Mitochondrial DNA encodes and expresses proteins associated with oxidative phosphorylation. Mitochondria provide more than 90% of the body's energy through oxidative phosphorylation. In addition to synthesizing energy to provide cells with life-sustaining activities, mitochondria play important roles in processes including cell differentiation, cellular messaging and apoptosis, and possess the ability to regulate cell growth and cell cycle.

Mitochondrial DNA mutations are one of the important causes of deafness. Since the mtDNA1555A>G mutation was identified to be associated with deafness, mutations in mtDNA have become a hot region for deafness gene research. In addition to mutations in mtDNA12SrRNA, several tRNA mutation sites associated with deafness have been identified. Mutations in mtDNA contribute to deafness mainly by affecting mitochondrial oxidative respiratory function. Mutations in mtDNA can be classified into primary and secondary mutations depending on their role in pathogenesis. Some mutations in mtDNA can directly cause deafness and are referred to as primary mutations. Other mutations that do not cause deafness alone but have a synergistic effect on the primary mutation are called secondary mutations. Mitochondrial DNA mutations cause both syndromic and non-syndromic deafness. Human mtDNA encodes 2 rRNAs, 22 tRNAs, and 13 subunits involved in oxidative phosphorylation that are essential for mitochondrial protein synthesis.[13,14] And defects in mitochondrial protein synthesis are strongly associated with both non-syndromic and syndromic deafness. Therefore, we speculate that any of the rRNA and tRNA mutations that cause defects in mitochondrial protein synthesis may cause deafness. For example, the common 1555A>G and 1494C>T mutations on *mtDNA12SrRNA* cause altered mitochondrial protein synthesis that specifically binds to aminoglycoside antibiotics and interferes with the normal oxidative phosphorylation process, leading to drug deafness. Previous studies have shown that tRNA mutations associated with mitochondrial diseases with a genetic background also lead to impaired translation of mitochondrial proteins, and although the exact mechanism by which impaired translation of mitochondrial proteins leads to disease symptoms is unclear, it at least provides a new direction for the mechanistic study of defective cellular oxidative phosphorylation.

MAIN POINTS

- Comprehensive analysis of the oxidative phosphorylation function of mitochondria, the effect of tRNA mutations on the function of mitochondrial proteins, and the modification of mt-tRNA by mitochondrial proteins encoded by nuclear genes, this article summarizes all the currently reported mt-tRNA-modified genes that may be associated with deafness.
- The possible deafness-causing mechanism of mt-tRNA-modifier genes is that mutations in the genes lead to impaired synthesis of different tRNA-modifying enzymes, resulting in defective mt-tRNA modification, which in turn affects the oxidative phosphorylation process of mitochondria and ultimately the inner ear function leading to hearing loss.
- This review provides new ideas for the study of deafness genes to contribute to the investigation of the causes of deafness and to reduce the incidence of deafness.

Mitochondrial tRNA

Mitochondrial tRNA genes account for only 8% of the entire mitochondrial genome. However, the frequency of disease-causing mutations in mt-tRNAs is significantly higher than that in mitochondrial messenger RNAs (mt-mRNAs), about 8.5 times the mutation rate of mt-mRNAs. It can be basically concluded that mt-tRNA mutations are the main cause of mtDNA diseases.[15] The mt-tRNA molecule consists of 75-95 nucleotides, and its classical secondary structure is clover-shaped (including D-arm, anticodon arm, T-arm, extra arm, and amino acid receptor arm), which is an important mediator involved in the protein translation process and is mainly responsible for loading the amino acids bound to the ribosome to participate in the synthesis of new peptide chains. Accurate recognition between the anticodon on tRNA and the codon on mRNA is an important factor in maintaining high fidelity of the protein translation process.[16] Human mitochondria contain 20 amino acids, except for $tRNA^{Leu}$ and $tRNA^{Ser}$, which have two iso-receptors (tRNAs carrying the same amino acid but with different structures are called iso-receptor tRNAs); each of the other 18 amino acids corresponds to a unique tRNA, so mutations in any of the tRNAs may lead to protein translation defects in mitochondria and thus affect their oxidative phosphorylation processes resulting in the corresponding disease phenotypes. A variety of tRNA mutations (m.7551A>G, m.12201T>C, m.4263A>G, etc.)[6,17] have been reported to be associated with deafness, and their main pathogenic mechanism is to affect mt-tRNA modification, which leads to impaired mitochondrial protein translation, resulting in impaired mitochondrial oxidative phosphorylation capacity causing inner ear hypoxia and eventually dysfunction.

Mitochondrial tRNA-Modifying Genes

Post-transcriptional modifications play multiple roles in RNA structure and function and have been associated with various biological

processes and physiological events. Approximately 150 RNA modifications have been identified, of which approximately 80% occur in tRNAs[18] (https://www.genesilico.pl/modomics/). Studies[19,20] have shown that mt-tRNAs must undergo a large number of modifications to ensure the accuracy of protein translation, and, in particular, modifications of the anticodon region swing site play an important role in the correct mRNA-tRNA interaction. Some scholars conducted in vivo experiments and found that unmodified tRNAs form various secondary structures without function.[21] In addition, mt-tRNA modification is evolutionarily conserved,[22] which also suggests that it plays an important role in normal mitochondrial oxidative phosphorylation.

The modifications of mt-tRNAs are complex and diverse. To date, 8 human mt-tRNAs with post-transcriptional modifications have been analyzed and 18 chemical modifications involving 137 positions have been identified in human mt-tRNA.[23] A wide variety of RNA modifications exist in the anticodon region of tRNAs, which make important contributions to the accuracy of protein synthesis at various steps of translation, including aminoacylation, decoding, and translocation. The modification located at the mt-tRNA-37 position was found to be closely associated with the stability of codon–anticodon interactions at the first position of the anticodon (swing position) and at the ribosomal A site. These modifications enhance codon–anticodon pairing by enhancing base stacking interactions, thus helping to maintain the correct reading frame during decoding to ensure proteome integrity. Mt-tRNA-37 is a highly conserved site, usually a purine base, and is often highly modified by up to 70%. Modification of this site helps to ensure accurate decoding of mRNA and maintenance of the reading frame.[18,24] The modifications of the mt-tRNA-37 that are now well studied are m^1G37, t^6A37, i^6A37, and ms^2i^6A37 site modifications.[25-27] Some studies have found alterations in any of these modifications in some deaf patients.

m^1G37 Modification Gene

Most of the mt-tRNA-37 modifications are methylation modifications and are formed mainly through tRNA methylation transferase (TrmD) catalysis. What is relatively well studied is the 1-methylguanosine adjacent to the anticodon region 37 sites (m^1G).[28] When A, C, and U are used instead of G, respectively, tRNA cannot be modified by methylation after transcription, which shows that m^1G37 site is the target site of tRNA methylation modification. The TrmD 5 is mainly involved in methylation modification of the mt-tRNA m^1G37 and is associated with compound oxidative phosphorylation defect 26 (COXPD26) (OMIM: 616539). Almost all tRNAs are modified at this site.[29,30]

Defective tRNA modification can be caused by both mtDNA mutations and mutations in the nuclear genes encoding mitochondrial proteins. The *TRMT5* (14q23.1) contains 5 exons and is the gene encoding a mitochondrial protein that primarily encodes tRNA methyltransferase 5, which is a homologous protein with methyltransferase family I, both of which can cause lactic acidosis and respiratory chain complex defects in individuals. Studies have shown that tRNA-(N^1G37) methylation transferase (TRMT5) is extremely sensitive to tRNA-protein tertiary structure alterations.[31,32] The activity of tRNA-(N^1G37) methyltransferase is absent when the protein structure is altered by tRNA mutations. We, therefore, hypothesized that either tRNA mutations causing the inactivation of tRNA-(N^1G37) methyltransferase or mutations in the *TRMT5* gene causing the impaired synthesis of tRNA-(N^1G37) methyltransferase would result in defective modification of the m^1G37. In yeast and bacteria, m^1G37 modification has been shown to play a key role in preventing ribosome shift errors, and defective m^1G37 modification often causes mitochondrial dysfunction.[32] In bacteria, modification of the m^1G37 is accomplished by the involvement of a TrmD encoded by the *TrmD*.[33-35] In *Saccharomyces cerevisiae*, modification of the m^1G37 modification is catalyzed by Trm5p.[30,36,37] In humans, the *TRMT5* is involved in the modification of the m^1G37 by encoding a TrmD.[35,38]

The current phenotypic reports on *TRMT5* mutations are divided into 2 main categories, those that manifest in children with developmental delay and hypertrophic cardiomyopathy and congenital demyelinating sensorimotor neuropathy and those that manifest in adults with lifelong motor intolerance and muscle atrophy.[14,17,30] Partial pathogenic mutations in the *TRMT5* gene have been demonstrated in yeast models, mainly through overexpression in the cDNA of affected individual cells.[22,39,40] Minxin Guan's[40,41] team successively found that mt-$tRNA^{Asp}$ m.7551A>G mutation caused mitochondrial dysfunction associated with deafness; m.7551A>G mutation produced m^1G37 modification of mt-$tRNA^{Asp}$; and $tRNA^{Ile}$ m.4295A>G associated with deafness introduced *TRMT5*-catalyzed m^1G37 modification. The major defect of this mutation affects the stability and aminoacylation of mutant mt-$tRNA^{Asp}$ and mt-$tRNA^{Ile}$, resulting in mitochondrial translation impairment, respiratory impairment, decreased membrane potential, reduced ATP production, increased reactive oxygen species production, and pleiotropic effects that promote autophagy. This deafness manifests as altered nucleotide modifications of mt-tRNA. And the finding provides new insights into the pathophysiology of maternal deafness.[17,40]

t^6A37 Modifier Gene

Nucleotides at position 37 (A or G) of mammalian mt-tRNA contain a complex array of modifications, including N6-carbamoylthreonyl adenosine (t^6A), N6-prenyladenosine (i^6A), 2-(methylthio)-N6-pr enyladenosine (ms^2i^6A).[18] The t^6A37 and its derivatives are universally conserved modifications of mt-tRNA and are responsible for reading codons beginning with adenine (A) (ANN codons). The giant side chain of t^6A37 extends the anticodon loop of mt-tRNA, enhancing the stacking effect with U36-A1 base pairs and stabilizing the codon–anticodon interaction at the ribosomal A site, thus helping to maintain the efficiency and accuracy of translation.[42,43] The t^6A37 modification plays a crucial role in all stages of protein synthesis, including codon recognition, maintenance of reading frame, efficient aminoacylation, and translocation.

YRDC and *OSGEPL1*, homologs of the yeast and human N(6)-threo nylcarbamoyltransferases Sua5 and Qri7, respectively. In human mitochondria, t^6A37 is present in 5 tRNAs.[44,45] During the biosynthesis of t^6A37, Thr residues react non-enzymatically with carbon dioxide or HCO_3^- to generate a carbamate intermediate. *YRDC* activates this intermediate under ATP-supplied conditions and forms threonylcarbamoyl-AMP (TC-AMP). In mitochondria, *OSGEPL1* catalyzes the same reaction and transfers the threonine carbamoyl group of TC-AMP to adenine at position 37 of tRNA, resulting in the formation of t^6A37. It was found that t^6A37 was lowly expressed in mt-tRNAs of *OSGEPL1* knockdown cells, all of which exhibited respiratory defects and reduced mitochondrial translation.[44] Knockdown cells of *OSGEPL1* were found to have low expression of t^6A37 in mt-tRNAs

and exhibited respiratory defects and reduced mitochondrial translation. Existing studies confirm that mutations in genes associated with t^6A37 modification are associated with various human diseases and that c.974G>A mutations in the *OSGEPL1* gene are associated with neurodegeneration and renal tubular lesions. Low expression of t^6A37 was found in mt-$tRNA^{Ile}$ carrying the m.4295A>G mutation, which was isolated from the cells of a deaf patient with cardiomyopathy.[46] This suggests that the lack of t^6A37 has pathological consequences, and it is speculated that the defective t^6A37 modification may be associated with the development of deafness, the exact mechanism of which still needs to be confirmed by numerous studies.

i^6A37 Modifier Gene

The subset of mt-tRNAs undergoes extensive modifications in strict order. N6-isopentenyl adenosine (i^6A) is an evolutionarily conserved modification found at position A37, adjacent to the anticodon. This base was found to enhance stacking on Watson–Crick base pairs and stabilize codon recognition.[47,48] The deletion of the i^6A37 modification leads to mitochondrial dysfunction in the fission yeast model and in mice, respectively. This suggests that the i^6A37 modification plays an important role in mitochondrial function by regulating protein translation in mitochondria.

In yeast, modification of the i^6A37 is accomplished with the involvement of the i^6A modifying enzyme encoded by the *Mod5*,[50] and homologous tRNA isopentenyl transferases (IPTase) have been identified in bacteria (MIAA), yeast (MOD5, TIT1), *Ascaris lumbricoides* (GRO-1), and mammals (TRIT1). In eukaryotes, the heteropentenylation of cytoplasmic and mt-tRNAs is mediated by the product of the same gene, *TRIT1*.[47,49] The *TRIT1* gene is located on chromosome 1p34 and encodes a IPTase that catalyzes the transfer of the isopentenyl group from dimethylallyl pyrophosphate to adenine at position 37 of the tRNA. This post-transcriptional modification of tRNA is essential for protein folding, stability, and maintenance of the correct reading frame during translation. In addition, as a result of this modification, tRNA quadruples the activity of its codons while stabilizing otherwise weak adjacent codon base pairs and increasing the efficiency of decoding the corresponding codons of the genetic code.[49,50]

In mammals, IPTase (TRIT1) catalyzes the i^6A modification of 2 cytoplasmic tRNAs ($tRNA^{Ser\ (AGA)}$ and $tRNA^{Sec}$) and 4 mt-tRNAs (mt-$tRNA^{Trp}$, mt-$tRNA^{Tyr}$, mt-$tRNA^{Ser(UCN)}$, and mt-$tRNA^{Phe}$).[50] Mutations in the *TRIT1* gene have been found to cause autosomal recessive mitochondrial disorders. Fifteen patients with disseminated *TRIT1* mutations causing different clinical manifestations have been reported, and a different and more severe phenotype was found in a child with a truncated *TRIT1* mutation than in other patients, including intrauterine growth retardation, neonatal microcephaly, fibromuscular dysplasia, sensorineural hearing loss, and visual loss.[51] However, the link between these neurodevelopmental symptoms and abnormal tRNA modifications is unclear.

ms^2i^6A37 Modifier Gene

The 2-methylthio-N6-isopentenyl modification of adenosine (ms^2i^6A) is an evolutionarily conserved modification found in mammalian and bacterial mt-tRNA s that are widely present in mt-tRNAs, where the modification of adenosine located at position 37 of mt-tRNA is more clearly studied. Structural analysis of the ms^2i^6A37 modification shows that the 2-methylthio group can directly interact with the first nucleotide of the codon, stabilize codon-anticodon binding and maintain the reading frame, optimize mitochondrial translation and OXPHOS activity, and maintain translation fidelity.[52] Defective ms^2i^6A modification was found to accelerate myopathy and cardiac dysfunction in mice, and reduced levels of ms^2i^6A modification were also found in cells from patients with mitochondrial disease, suggesting that this modification is involved in regulating mammalian mitochondrial translation and energy metabolism.[53,54]

The i^6A37 modification of mt-tRNAs in mammals is further translated into ms^2i^6A37 and is mediated by CDK5 regulatory subunit-associated protein 1 (CDK5RAP1), a radical SAMase homologous to the bacterial MiaB protein, which introduces methylthio groups into N6-isopentenyl adenosine in mt-tRNAs. Mammalian CDK5RAP1 catalyzes the ms^2 modification of mt-$tRNA^{Phe}$, mt-$tRNA^{Trp}$, mt-$tRNA^{Tyr}$, and mt-$tRNA^{Ser\ (UCN)}$, modifying i^6A37 to 2-(methylthio)-N6-isopentenyl A37 (ms^2i^6A37).[50,55]

Recent studies have found that defective mitochondrial translation defects may contribute to early hearing loss by inducing cellular senescence and inner ear cochlear dysfunction. The number of hair cells, IHCs (Inner hair cells), and SGCs (Sprial gangion cells) decreased earlier in CDK5Rap1-KO mice than in normal mice, and the number of senescent cells in the cochlea was significantly higher in CDK5Rap1-KO mice than in CNT mice. This suggests that the accumulation of dysfunctional mitochondria may be associated with the progression of age-related hearing loss AHL, which provides valuable insight into the link between tRNA modification-induced mitochondrial dysfunction and hearing loss, but the exact mechanism remains unclear.[56]

CONCLUSION

By combining the oxidative phosphorylation productivity of mitochondria, the effect of tRNA mutations on mitochondrial protein function, and the modification of mt-tRNA by mitochondrial proteins encoded by nuclear genes, we can speculate that these genes affecting mt-tRNA modification may have some relevance to deafness. Their deafness-causing mechanism may be due to different gene mutations that lead to different tRNA-modifying enzyme synthesis disorders, resulting in defective mt-tRNA modification, which in turn affects its oxidative phosphorylation process and eventually affects inner ear function. The mechanism of deafness may be due to mutations in different genes that lead to impaired synthesis of different tRNA modifying enzymes, resulting in defective mt-tRNA modification, which in turn leads to defective translation of mitochondrial proteins and thus affects their oxidative phosphorylation process, resulting in impaired energy production and ultimately inner ear function. In future studies, we can conduct gene cytology and gene knockout experiments to investigate the functions of these genes and to clarify the correlation between these genes and deafness, in order to identify new deafness-causing genes and supplement the human deafness gene database and to contribute to the investigation of the causes of deafness and to reduce the incidence of deafness.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – P.B., B.X.; Design – P.B., B.X.; Supervision – PB., B.X.; Funding – P.B., B.X.; Materials – J.C.; Data Collection and/or Processing –

J.C.; Analysis and/or Interpretation – P.B., J.C.; Literature Search – J.C.; Writing – P.B., J.C.; Critical Review – B.X.

Declaration of Interests: The authors have no conflict of interest to declare.

Funding: This work was supported by grants from the National Natural Science Foundation of China (Panpan Bian, grant number 32160149), (Baicheng Xu, grant number 31960132); the Health Industry Planning Project of Gansu Province (Panpan Bian, grant number 20JR10RA747), (Panpan Bian, grant number 21JR7RA429); and the Technological Innovation Program of Lanzhou University Second Hospital(Panpan Bian, grant number CY2019-QN18).

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