

Review

Pathogenic mutations of nuclear genes associated with mitochondrial disorders

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Mitochondrial disorders are clinical phenotypes associated with mitochondrial dysfunction, which can be caused by mutations in mitochondrial DNA (mtDNA) or nuclear genes. In this review, we summarized the pathogenic mutations of nuclear genes associated with mitochondrial disorders. These nuclear genes encode, components of mitochondrial translational machinery and structural subunits and assembly factors of the oxidative phosphorylation, that complex. The molecular mechanisms, that nuclear modifier genes modulate the phenotypic expression of mtDNA mutations, are discussed in detail.

Keywords mitochondria; mitochondrial disorder; nuclear modifier gene; pathogenic mutation

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Introduction

Mitochondrial disorders are clinical phenotypes associated with mitochondrial dysfunction, in particular, abnormalities of oxidative phosphorylation (OXPHOS), which can be caused by mutations in mitochondrial DNA (mtDNA) or nuclear genes. When combining the results of the epidemiology data on childhood and adult mitochondrial diseases, the minimum prevalence is at least 1 in 5000 and could be much higher [1].

The concept of mitochondrial disorders was introduced in 1962, when Luft *et al.* [2] described a young Swedish woman with severe hypermetabolism of non-thyroid origin with a defect in the maintenance of mitochondrial respiratory control. The molecular era of mitochondrial diseases began in 1988, with the description of the first pathogenic mutations in mtDNA, large-scale single deletions in patients with 'mitochondrial myopathies' [3] and G11778A point mutation in the gene encoding subunit 4 of complex I in multiple

families with Leber's hereditary optic neuropathy [4]. Up to August 2008, more than 320 point mutations and hundreds of rearrangements of mtDNA were reported (<http://www.mitomap.org>), indicating that mtDNA may contribute to the pathogenesis of a number of common disorders such as neuromuscular disorders, neurodegenerative diseases, cardiomyopathy, diabetes, and various cancers. In contrast, the identification of nuclear genes responsible for mitochondrial disorders has proceeded at a much slower pace. In 1995, Bourgeron *et al.* [5] identified a homozygous mutation in the succinate dehydrogenase A (*SDHA*) gene. They claimed that this was the first report of a nuclear gene mutation causing a mitochondrial respiratory chain deficiency in humans. Recently, much attention has been paid to investigate the nuclear gene defects that were associated with mitochondrial syndromes.

The studies on mitochondrial disorders in China have been carrying out for a period of time. Yang Fuyu *et al.* reported that a local Keshan disease is one kind of mitochondrial cardiomyopathy, which is associated with malnutrition, particularly lack of selenium. Since 2005, Chinese MiT/International mitochondrial conferences have been organized three times by Institute of Zoology of the Chinese Academy of Science, Wenzhou Medical College, and Tianjin University of Sport. Their topics are 'Mitochondria determine cell life and death', 'Mitochondria and health', and 'From bioenergetics to cell biology and medicine', respectively. The program of mitochondria and diseases got an independent discipline number (C060503) in the Guide of National Science Foundation of China in 2008 [6].

In this review, we summarized the pathogenic expression of nuclear gene mutations in mitochondrial disorders. These nuclear genes encode some mitochondrial-imported proteins, including components of OXPHOS complexes and factors associated with replication, transcription, assembly, function, and turnover. How to

modulate the phenotypic expression of mtDNA mutations by nuclear modifier genes in molecular level is also discussed in detail.

Pathogenic Mutations of Nuclear Genes Encoding OXPHOS Complex Structural Subunits

Mitochondrial proteome is estimated to consist of approximately 1500 gene products. Mitochondrial genome encodes only 13 essential polypeptides of OXPHOS, whereas all other structural subunits and assembly factors are nuclear-encoded and imported into mitochondria. The OXPHOS system is composed of five complexes, four of which, complexes I–IV, cooperate to generate a proton gradient across the mitochondrial inner membrane. Complex V generates the universal energy ATP coupling with proton flow [7]. In the past few years, a number of pathogenic mutations in OXPHOS-related nuclear genes have been identified.

Mutations of nuclear-encoded structural subunits were summarized in **Table 1**.

Complex I (NADH-ubiquinone reductase) catalyzes the first step in the mitochondrial respiratory chain, in which transfer of electrons from NADH to ubiquinone (co-enzyme Q) is accompanied by the translocation of protons across the inner mitochondrial membrane. Complex I is composed of at least 46 subunits, in which seven are encoded by mtDNA and the others by nuclear DNA, whose deficiency is the most common cause of the mitochondrial disease. Nuclear-encoded subunits are termed NADH dehydrogenase ubiquinone (NDU), followed by a description of function/location (FS-iron-sulfur protein region, FV-flavoprotein region, FA-subcomplex α , FB-subcomplex β , FC-undefined subcomplex). Complex I deficiency causes a wide range of clinical disorders, ranging from neurological disorders, such as Leigh's syndrome (LS), to cardiomyopathy, liver failure, or myopathy [8,9]. Pathogenic mutations have now been described in 12 of the nuclear-encoded structural subunits (*NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS4*, *NDUFS6*, *NDUFS7*,

Table 1 Pathogenic mutations of nuclear genes encoding OXPHOS complex structural subunits

Gene	Chromosome	Clinical phenotype	Inheritance	Reference
Complex I				
<i>NDUFS1</i>	2q33–q34	LS	AR	[10]
<i>NDUFS2</i>	1q23	Encephalopathy and cardiomyopathy	AR	[11]
<i>NDUFS3</i>	11p11.11	LS	AR	[12]
<i>NDUFS4</i>	5q11.1	LS	AR	[13]
<i>NDUFS6</i>	5p15.33	Lethal infantile mitochondrial disease	AR	[14]
<i>NDUFS7</i>	19p13.3	LS	AR	[15]
<i>NDUFS8</i>	11q13	LS	AR	[16,17]
<i>NDUFV1</i>	11q13	LS	AR	[15]
<i>NDUFV2</i>	18p11	Cardiomyopathy, hypotonia, encephalopathy	AR	[18]
<i>NDUFA1</i>	Xq24	Encephalomyopathy	X-linked	[19]
<i>NDUFA2</i>	5q31.2	LS	AR	[20]
<i>NDUFA11</i>	19p13.3	Cardioencephalomyopathy, LIMD	AR	[21]
Complex II				
<i>SDH-A</i>	5p15	LS	AR	[5]
<i>SDH-B</i>	1p36.1–p35	Pheochromocytoma and paraganglioma	AD	[22]
<i>SDH-C</i>	1q21	Paraganglioma type 3	AD	[23]
<i>SDH-D</i>	11q23	Paraganglioma type 1 and pheochromocytoma	AD	[24]
Complex III				
<i>UQCRB</i>	8q22	Hypoglycemia, lactic acidosis	AR	[25]
<i>UQCRCQ</i>	5q31.1	Severe psychomotor retardation and extrapyramidal signs	AR	[26]
Complex IV				
<i>COX6B1</i>	19q13.1	Infantile encephalomyopathy	AR	[27]

LS, Leigh syndrome; AR, autosomal-recessive; AD, autosomal-dominant.

NDUFS8, *NDUFV1*, *NDUFV2*, *NDUFA1*, *NDUFA2*, and *NDUFA11*) [10–21] of complex I. Mutations of seven of these nuclear genes (*NDUFS1*, *NDUFS3*, *NDUFS4*, *NDUFS7*, *NDUFS8*, *NDUFV1*, and *NDUFA2*) [10,12,13,15,16,20] result in Leigh or Leigh-like syndromes, whereas mutations of the *NDUFS2* [11], *NDUFS6* [14], *NDUFV2* [18], *NDUFA1* [19], and *NDUFA11* [21] genes are associated with hypertrophic cardiomyopathy and encephalomyopathy. *NDUFS4* and *NDUFS6* are both located in the iron–sulfur fraction of complex I, whose mutations can either prevent complete assembly or destabilize the peripheral arm; the other seven nuclear-encoded subunits (*NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS7*, *NDUFS8*, *NDUFV1*, and *NDUFV2*) [15–18] are constitutive of the core of complex I, considered to be essential for the catalysis of electron transfer from NADH to ubiquinone, and for the generation of the proton motive force. Recently, Fernandez-Moreira *et al.* [19] identified a hemizygous mutation in the X-linked gene *NDUFA1*, resulting in the assembly/stability abnormalities in the mitochondrial respiratory complexes. Hoefs *et al.* [20] identified a homozygous G-to-A transition in intron 2 of the *NDUFA2* gene, resulting in the skipping of exon 2 and generation of a prematurely truncated protein. Further studies showed that the *NDUFA2* mutation resulted in the disturbed assembly and stability of complex I and decreased complex I activity. A splice-site mutation in the *NDUFA11* gene is predicted to abolish the first transmembrane domain of the gene product, thereby destabilizing the enzymatic complex [21].

Complex II (succinate-cytochrome *c* reductase) is an FAD-dependent enzyme at a cross-point between OXPHOS and Krebs-cycle pathways. It comprised four protein subunits encoded by nuclear genes (*SDHA*, *B*, *C*, and *D*). The homozygous *SDHA* (flavoprotein subunit) mutations are associated with LS [5], whereas heterozygous mutations in *SDHB* [22] (iron–sulfur subunit) and in both *SDHC* [23] and *SDHD* [24] (integral membrane-protein subunits) are associated with paraganglioma.

Complex III (ubiquinol cytochrome *c* reductase) catalyzes electron transfer from succinate and nicotinamide adenine dinucleotide-linked dehydrogenases to cytochrome *c*. It is made up of 11 subunits, of which all but one (cytochrome *b*) are encoded by nuclear DNA. Haut *et al.* [25] reported a deletion in *UQCRB*, encoding the human ubiquinone-cytochrome *c* reductase binding protein of complex III (QP-C subunit), in a consanguineous family with hypoglycemia and lactic acidosis. Barel *et al.* [26] identified a single missense (Ser45Phe) mutation in *UQCRQ*, encoding a ubiquinone-binding

protein of low molecular mass, from a large consanguineous Israeli Bedouin kindred with an autosomal-recessive syndrome comprising severe psychomotor retardation and extrapyramidal signs.

Complex IV (cytochrome *c* oxidase, COX) is the terminal complex of the electron transport chain, which transfers electrons from cytochrome *c* to molecular oxygen and contributes to the proton motive force used in the generation of ATP. Complex IV is composed of 13 subunits, in which the three largest ones are encoded by mtDNA, whereas the remaining subunits are encoded by nuclear genes. The mtDNA-encoded subunits function during electron transfer, and the nuclear-encoded subunits may be involved in the regulation and assembly of the complex. Massa *et al.* [27] first reported a disease-associated nuclear gene mutation in *COX6B1*, which encodes cytochrome *c* oxidase subunit Vib polypeptide 1 (ubiquitous), resulting in severe infantile encephalomyopathy.

Complex V (ATP synthase or ATPase) couples proton flow from the inter-membrane space back to the matrix by the conversion of ADP and inorganic phosphate to ATP. ATP synthase comprises an integral membrane component *F*₀ and a peripheral moiety *F*₁. It comprised at least 14 nuclear-encoded subunits and two mtDNA-encoded subunits. Up to now, mutations of nuclear-encoded structural subunits were sought for, but never been found in complex V-defective patients.

Pathogenic Mutations of Nuclear Genes Encoding OXPHOS Complex Assembly Factors

Although many mutations in nuclear-encoded structural subunits have been identified, they account for only a minority of the OXPHOS complex deficiency cases. The fact suggests that the molecular cause of the disease should be found in other factors involved in the catalytic regulation, assembly, or maintenance of the complex. Only in recent years, a number of assembly factors have been validated, as summarized in **Table 2**.

The role of such factors in complex I biogenesis includes involvement in subunit maturation (e.g. folding/co-factor attachment), chaperoning intermediate assemblies, subunit synthesis, and turnover. *BI7.2 L* (*NDUFAF2*) encodes complex I assembly factor 2, which was shown to associate with complex I subunits ND1, *NDUFS1*, *NDUFS2*, *NDUFS7*, and *NDUFS4* in normal mitochondria. It has been attributed to play a direct role in complex I assembly. Ogilvie *et al.* [28]

Table 2 Pathogenic mutations of nuclear genes encoding OXPHOS complex assembly factors

Gene	Chromosome	Clinical phenotype	Inheritance	Reference
Complex I				
<i>NDUFAF2 (B17.2L)</i>	5q12.1	Early-onset progressive encephalopathy	AR	[28]
<i>NDUFAF1 (CIA30)</i>	15q13.3	Cardioencephalomyopathy	AR	[29]
<i>C6orf66 (HRPAP20)</i>	6q16.1	Encephalomyopathy	AR	[30]
<i>C20orf7</i>	20p12.1	Lethal neonatal mitochondrial disease	AR	[31]
Complex III				
<i>BCSIL</i>	2q33	Encephalopathy, hepatic failure, and tubulopathy, LS, GRACILE syndrome	AR	[32,33]
Complex IV				
<i>SURF1</i>	9q34	LS	AR	[34,35]
<i>SCO1</i>	17p13–p12	Neonatal hepatic failure and encephalopathy	AR	[36]
<i>SCO2</i>	22q13	Neonatal cardioencephalomyopathy	AR	[37]
<i>COX10</i>	17p12–p11.2	Neonatal tubulopathy and encephalopathy, LS, cardiomyopathy	AR	[38]
<i>COX15</i>	10q24	Early-onset hypertrophic cardiomyopathy, LS	AR	[39,40]
<i>LRPPRC</i>	2p21–p16	French-Canadian LS	AR	[41]
Complex V				
<i>ATPAF2</i>	17p11.2	Early-onset encephalopathy, lactic acidosis	AR	[7]

AR, autosomal-recessive; AD, autosomal-dominant.

identified a homozygous C182T mutation of *B17.2L*, resulting in a premature stop codon, in a patient with progressive encephalopathy. *CIA30* (*NDUFAF1*) encodes complex I assembly factor 1. Low levels of *CIA30* in patient mitochondria correlated with decreased levels of assembled complex I and also decreased enzymatic activity. A *CIA30* mutation was identified in a patient with cardioencephalomyopathy [29]. A missense mutation in a conserved residue of *C6orf66* (chromosome 6 open reading frame 66) was identified from a consanguineous family with infantile encephalomyopathy, which resulted in a decrease of the protein in muscle and severely reduced levels of complex I activity [30]. A homozygous missense mutation in *C20orf7* (chromosome 20 open reading frame 7) was identified from an Egyptian family with lethal neonatal mitochondrial disease. *C20orf7* patient fibroblasts showed an almost complete absence of complex I holoenzyme and were defective at an early stage of complex I assembly, but in a manner distinct from the assembly defects caused by mutations in the assembly factor *NDUFAF1* [31].

BCSIL encodes a *bcs1*-like protein as an assembly factor of complex III. de Lonlay *et al.* [32] reported that mutations in *BCSIL* are associated with tubulopathy,

encephalopathy, and liver failure. Pathogenic expression of the *BCSIL* mutation also displays another different phenotype, GRACILE (growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death) syndrome, which is a recessively inherited lethal disease characterized by fetal growth retardation, lactic acidosis, aminoaciduria, cholestasis, and abnormalities in iron metabolism [33].

Nuclear-gene defects of complex IV are caused by mutations in assembly factors of the enzyme, including *SURF1* [34,35], *SCO1* [36], *SCO2* [37], *COX10* [38], *COX15* [39,40], and *LRPPRC* [41]. Patients with COX deficiencies can present with a number of different clinical phenotypes, including LS, French Canadian LS, cardiomyopathy, neonatal hepatic failure, tubulopathy and encephalopathy, and early onset hypertrophic, encephalopathy, lactic acidosis.

No mutation in any of the nuclear-encoded subunits of complex V has been described. Only a homozygous mutation of *ATP12*, encoding the ATPase assembly factor, was reported by De Meirleir *et al.* [7] in 2004. Probably, the *Atp12p* activity is severely compromised when a neutral polar amino acid (tryptophan) is changed into a basic one (arginine) and is no longer able to mediate proper F_1 assembly.

Pathogenic Mutations of Nuclear Genes Encoding Mitochondrial Translational Machinery

Mitochondria contain a separate translational machinery to produce the mtDNA-encoded polypeptides using mtDNA-encoded tRNA. rRNA encoded by the mtDNA is combined with nuclear-coded proteins to generate mitochondrial ribosomes (mitoribosomes), which is composed of two subunits: the small subunit (SSU) consists of the 12S rRNA and 29 proteins and the large subunit consists of the 16S rRNA and 48 proteins. Synthesis of mitochondrial proteins requires a number of initiation, elongation, and termination (or release) factors and enzymes for mitochondrial rRNA and tRNA maturation (RNA processing and base-modification), all of which are encoded by nuclear genes [42].

Initial factor IF2 promotes the binding of formyl methionyl-transfer RNA (fMet-tRNA) to the small ribosomal subunit in the presence of guanosine triphosphate (GTP) and a template, whereas IF3 promotes the dissociation of the two ribosomal subunits, which produces free SSUs for the initiation of translation. The mammalian elongation factor Tu participates in the formation of the ternary complex that includes EFTu, GTP, and aminoacyl-tRNA, which delivers the aminoacyl-tRNA to the acceptor site of the ribosome. The energy required for this process is supplied by the hydrolysis of GTP, which is followed by the release of EFTu from the ribosome as an EFTu-guanosine diphosphate (GDP) complex. The exchange of GDP for GTP, which regenerates

EFTu-GTP, is accomplished by EFTs. EFG catalyzes the translocation of peptidyl-tRNA from the ribosomal-acceptor site to the peptidyl site after peptide-bond formation. Concomitant movement of mRNA exposes the next codon in the acceptor site. Release factor RF1 recognizes stop codons and promotes the releases of the completed protein chain [43].

Several mutations in nuclear genes influencing mitochondrial translational machinery have been identified, as summarized in **Table 3**.

MRPS16 encodes a protein of the mitoribosomal SSU. The MRPS16 protein is located in a narrow crevice on the SSU and has many contacts with the rRNA, being surrounded by about five rRNA double helices. The binding of the *Thermus thermophilus* ribosomal protein S16 is an important step in the assembly of the SSU of this organism. A homozygous mutation C331T of *MRPS16*, predicting a premature stop codon Arg111Ter, was identified in one infant with severe lactic acidosis, developmental defects in the brain, and facial dysmorphism [44]. *MRPS22* encodes a mitochondrial ribosomal protein S22. A mutation in the *MRPS22* gene was identified, leading to a reduction of 12S rRNA in fibroblasts, from patients with fatal neonatal hypertrophic cardiomyopathy and kidney tubulopathy [45].

PUS1 (pseudouridine synthase 1) converts uridine into pseudouridine in several positions of tRNAs synthesized in both nuclear and mitochondrial compartments. A homozygous missense mutation of *PUS1* was identified in Persian Jewish families affected by myopathy, lactic acidosis, and sideroblastic anemia. The amino

Table 3 Pathogenic mutations of nuclear genes influencing mitochondrial translational machinery

Gene	Chromosome	Clinical phenotype	Inheritance	Reference
Mitoribosomal protein				
<i>MRPS16</i>	10q22.1	Agenesis of corpus callosum, dysmorphism, and fatal neonatal lactic acidosis	AR	[44]
<i>MRPS22</i>	3q23	Antenatal skin edema, hypotonia, cardiomyopathy, and tubulopathy	AR	[45]
Mt tRNA processing				
<i>PUS1</i>	12q24.33	Myopathy, lactic acidosis, and sideroblastic anemia (MLASA)	AR	[46,47]
Elongation factors				
<i>EFTu</i>	16p11.2	Infantile macrocystic leukodystrophy with micropolygyria	AR	[48]
<i>EFTs</i>	12q13–q14	Encephalomyopathy, hypertrophic cardiomyopathy	AR	[49]
<i>EFG1</i>	3q25.1–q26.2	Severe hepatoencephalopathy and lactic acidosis	AR	[43,50]

AR, autosomal-recessive.

acid change (Arg656Try) appears to be in the catalytic center of the protein PUS1p [46,47].

EFTu (elongation factor Tu) brings aminoacylated transfer RNAs to the ribosomal A site as a ternary complex with guanosine triphosphate. EFTs (elongation factor Ts) functions as a guanine nucleotide exchange factor for EFTu. The first mutation of *EFTu* was identified in a patient with a severe infantile macrocystic leukodystrophy with micropolygyria [48]. A homozygous mutation (C997T) of EFTs was found in patients with hypertrophic cardiomyopathy [49]. Antonicka *et al.* investigated the tissue specificity in patients with fatal hepatopathy due to *EFG1* mutations. Liver was the most severely affected tissue, with <10% residual assembly of complexes I and IV and a 50% decrease in complex V. Skeletal muscle showed a 50% reduction in complex I, and complexes IV and V were 20% of the control. In fibroblasts, complexes I and IV were 20% of the control, and there was a 40–60% reduction in complexes III and V. In contrast, except for a 50% decrease in complex IV, all other complexes were nearly normal in the heart [50].

Nuclear Modifier Genes Modulate the Phenotypic Expression of mtDNA Mutations

mtDNA mutations are responsible for a number of maternally inherited diseases, but not sufficient to account for the variable penetrance, implying that there must be some modifiers involved. These reasonable modifiers include mtDNA haplotype background, environmental factor, and nuclear modifier gene. The nuclear modifier does not induce any pathology *per se*, but it contributes to the pathogenic effect of the mitochondrial mutation. The nuclear modifier could be a common functional polymorphism in a tissue-specific protein, possibly with mitochondrial location [51].

In 1993, Prezant *et al.* [52] found that a homoplasmic mtDNA A1555G mutation for 12S rRNA was in a large Arab-Israeli pedigree with maternally inherited non-syndromic sensorineural deafness. Matrilineal inter-family and intra-family relatives carrying the A1555G mutation exhibited variable penetrance and expression, including severity and age-of-onset in hearing impairment, ranging from profound congenital deafness to severe and moderate progressive hearing loss of late onset or to completely normal hearing. Functional characterization demonstrated more-severe biochemical defects in the mutant lymphoblastoid cell lines derived

from symptomatic individuals carrying the A1555G or C1494T mutation than from those of cell lines derived from asymptomatic individuals in the same family [53,54]. However, under a constant nuclear background, a nearly identical degree of mitochondrial dysfunction was observed in cybrid cell lines derived from symptomatic and asymptomatic individuals from this family [54,55]. These findings strongly indicated that the A1555G or C1494T mutation is a primary factor underlying the development of deafness, but it is insufficient to produce a clinical phenotype. Extensive genome-wide linkage studies of Arab-Israeli and European (Italian and Spanish) families revealed that the phenotypic expression of the A1555G mutation is, in fact, influenced by the complex inheritance of multiple nuclear-encoded modifier genes. Despite statistical support for the linkages of several putative modifier loci, including one locus localized to chromosome 8p23.1, no mutations in these modifier genes have been identified.

An interesting model for nuclear–mtDNA interaction for the phenotypic expression of the A1555G or C1494T mutation has been proposed. In the yeast *Saccharomyces cerevisiae*, the mutant alleles of *MTO1*, *MSS1*, or *MTO2* that encodes mitochondrial proteins manifest a respiratory-deficient phenotype only when coupled with the paromomycin-resistance mitochondrial 15S rRNA C1409G mutation (P_{454}^R or P^R), corresponding to human deafness-associated 12S rRNA A1555G and C1494T mutations [56–58]. These observations strongly indicated that Mss1p, Mto1p, or Mtop2 protein products affect the phenotypic expression of the C1409G mutation by functionally interacting with the region of the C1409G mutation in mitochondrial 15S rRNA. 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, aminoacylation, and codon recognition at the decoding site of small ribosomal RNA.

Recently, we identified the nuclear modifier gene *TRMU*, which encodes a highly conserved 5-methylaminomethyl-2-thiouridylate-methyltransferase responsible for the biosynthesis of 5-taurinomethyl-2-thiouridine (π m5s2U) of mt tRNA^{Lys}, tRNA^{Glu}, and tRNA^{Gln} in the wobble position. The π m5s2U is further modified to mnm5s2U34 in the same position of those tRNAs in *Escherichia coli* and human mitochondria. This modified nucleotide contributes to the high fidelity of codon recognition and the structural formation and stabilization of functional tRNAs [59]. We demonstrated that isolated human *TRMU* cDNA partially

restored the respiratory-deficient phenotype of yeast *Mto2* cells carrying the C1409G mutation, and there was highly suggestive linkage and linkage disequilibrium between microsatellite markers adjacent to *TRMU* and the presence of deafness [45]. Genotyping analysis of *TRMU* in 613 subjects from one Arab-Israeli kindred, European (Italian and Spanish pedigrees) families, and 31 Chinese pedigrees 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 the TRMU protein. Interestingly, all 18 Arab-Israeli/Italian-Spanish matrilineal relatives carrying both the TRMU A10S and 12S rRNA A1555G mutations exhibited prelingual profound deafness. Functional analysis showed that this mutation did not affect importation 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 tRNA. 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, modulates the phenotypic manifestation of the deafness-associated 12S rRNA mutations [60].

Indeed, the A1555G or C1494T mutation produces about 30 or 40% decrease in the rate of mitochondrial translation, respectively. The exposure to aminoglycosides yielded an additional 30% decrease in the rate of mitochondrial-protein synthesis in cells carrying the A1555G mutation. In fact, aminoglycosides are concentrated in the perilymph and endolymph of the inner ear, but are rapidly cleared in other tissues or organs [53,55]. Therefore, a 50–60% decrease in the rate of mitochondrial translation, caused by the combination of the A1555G mutation with aminoglycosides, leads to cell dysfunction or death of the auditory system, thereby inducing or worsening hearing loss. Here, a failure in tRNA metabolism, caused by the homozygous TRMU A10S mutation, accounted for a >20% decrease in the rate of mitochondrial-protein synthesis [60]. Indeed, about 50% decrease in the rate of mitochondrial translation observed in cells derived from symptomatic individuals V-1 and V-3 of the Arab-Israeli family was the consequence of a faulty interaction between unmodified tRNAs caused by the TRMU A 10S mutation and mitochondrial ribosomes carrying the 12S rRNA A1555G mutation [60]. Defects in the mitochondrial translation consequently led to a respiratory phenotype and a

decline in ATP production below the threshold level required for normal cell function in the auditory organs, including cochlea, thus producing the deafness phenotype.

Concluding Remarks

In conclusion, mitochondrial functions are under two separate genomes: the mitochondrial and the nuclear genomes. Mitochondrial disorders are complex and often poorly understood. In contrast to hundreds of mtDNA mutations, nuclear mutations have been found only in 19 genes encoding OXPHOS complex structural subunits. Identification of pathogenic mutations in complex assembly factors and translational machinery components will open a new field for mitochondrial disorders. Nuclear modifier genes play a role in the clinic expression of mtDNA mutations. Based on the correlation between genotype and phenotype, more attention should be paid to clarify the molecular pathogenic mechanisms of mitochondrial disorders in the future.

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