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REVIEW

Potential Roles of mtDNA Mutations in PCOS-IR: A Review

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Abstract: Polycystic ovary syndrome (PCOS) is the most common heterogeneous endocrine disease that affecting females in reproductive age. Insulin resistance (IR), an important molecular basis for PCOS, accounts for at least 75% of women carrying this syndrome. Although there have been many studies on PCOS-IR, the detailed mechanisms are not fully understood. As essential hub for energy generation, mitochondria are critical to insulin secretion and normal function, whereas mutations in mitochondrial DNA (mtDNA) result in mitochondrial dysfunctions contributing to the pathophysiology of PCOS-IR via the regulation of balance of oxidative stress (OS), energy deficiency, or hormone metabolism. In the current review, we summarize the clinical and molecular features of PCOS-IR and discuss molecular mechanisms related to mtDNA mutations.

Keywords: PCOS-IR, mitochondrial dysfunction, mtDNA mutations

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disease occurring during reproductive years. It was a kind of endocrine and metabolic disorders that results in obesity, irregularity of menstruation, OS, hyperinsulinemia, hyperandrogenism, infertility, and sterility.[1,2] First identified in 1935, PCOS was also recognized as Stein–Leventhal syndrome.[3] Diagnosis of PCOS in adults can be made when at least two of three criteria are met: impairment of ovarian function, clinical and/or biochemical hyperandrogenism, and polycystic ovaries.[4,5] Despite significant progress in diagnostic criteria for PCOS, the syndrome is still underdiagnosed or misunderstood by many practitioners.[6]

In the early stage, PCOS is often complicated with infertility and adverse pregnancy outcomes, while in the long term, the incidence of endometrial cancer, type 2 diabetes mellitus (T2DM), and cardiovascular diseases gradually increase, seriously harming women's physical and mental health. Based on the National Institutes of Health's diagnostic criteria, the geographical prevalence of this disease is 8.7%, 17.8%, and 12% based on the definition proposed by the Androgen Excess and PCOS Society.[7] In a 2019 study, the prevalence of PCOS in Chinese women of reproductive age was 5.6%, which was consistent with other studies,[8] while its incidence in Indian women was 9.13% according to a recent study.[9] Interestingly, PCOS seems to be more frequent in black women (8.0%) than white women (4.8%), with an incidence of 6.6%.[10] The prevalence of oligoanovulation and hyperandrogenemia is 56.6% and 60% among women with PCOS, respectively.[11]

Multiple morbidities are linked to PCOS, such as infertility, impairment of glucose tolerance, T2DM, coronary heart disease, depression, gynecological oncology, and nonalcoholic fatty-liver disease.[12] Despite these well-characterized phenotypes, the pathogenesis of PCOS remains unclear. Increasing evidence suggests that genetic, epigenetic, and environmental factors contribute to PCOS progression.[13] However, it was generally accepted that IR and hyperandrogenism play key roles in its etiology.[14]

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The Role of IR in PCOS

Insulin is the master regulator of glucose metabolism. This hormone works under the condition of glucose uptake by insulin-sensitive tissue (muscle, liver, and adipose).[15,16] IR is caused by defects in insulin signaling, reducing the ability of insulin to stimulate glucose utilization, and can thus lead to high insulin levels (hyperinsulinemia). It has been suggested that >75% of patients have associated IR.[17]

At the molecular level, IR and hyperinsulinemia may stimulate P450c17α and influence the activity of 17-hydroxylase and 17,20-lyase.[18] Subsequently, these biochemical processes promote the secretion of androgen, increase free-androgen levels, and inhibit insulin signal transduction and translocation of glucose transporter 4, which affects glucose and lipid metabolism.[19] Androgens can produce IR by directly affecting insulin action in skeletal muscle and adipose tissue, changing adipokine secretion and increasing visceral adiposity. Moreover, insulin and IGF1[20] synergize with luteinizing hormone (LH).[21] Hyperinsulinemia enhances LH binding and androgen-producing response to LH.[22] Hyperinsulinemia also reduces hepatic sex hormone–binding globulin,[23–25] increasing free-testosterone levels in the blood and thus contributing to PCOS phenotypes (Figures 1 and 2).

Mitochondrial Structure and Function

Mitochondria are very important organelles consisting an of outer membrane, intermembrane space, and inner membrane that surround the matrix. Structurally, the inner membrane is **tightly**folded and is the major site for electron-transport chain (ETC) (complexes I–IV), which are essential for oxygen consumption in mammalian cells.[26] Among these, complex I — nicotinamide adenine dinucleotide Q (NADH-Q) oxidoreductase, comprises enzymes consisting of iron sulfur and flavin mononucleotide.[27] Complex II, also known as succinate dehydrogenase (SDH), contains four nuclear encoded subunits: SDHA, SDHB, SDHC, and SDHD. Interestingly, complex II has a dual role, ie, in the ETC. and the tricarboxylic acid cycle, linking the two essential energy-producing processes of the cell.[28,29] Complex I and II oxidize NADH and flavin adenine dinucleotide 2, respectively, transferring the resulting electrons to ubiquinol, which carries electrons to complex III. Complex III shunts the electrons across the intermembrane space to cytochrome C, which brings electrons to complex IV.[30,31] Complex IV then uses the electrons to reduce oxygen to water. There are many enzymes located within the mitochondrial matrix that are critical for metabolic pathways, including tricarboxylic acid cycle or β-oxidation.

Mitochondria are also important for the maintenance of cellular energy homeostasis. They are often called the powerhouses of the cell because of their significant role in the supplementation of ATP via oxidative phosphorylation (OxPhos). In contrast, mitochondria also generate reactive oxygen species (ROS) through ETC complexes, and excess

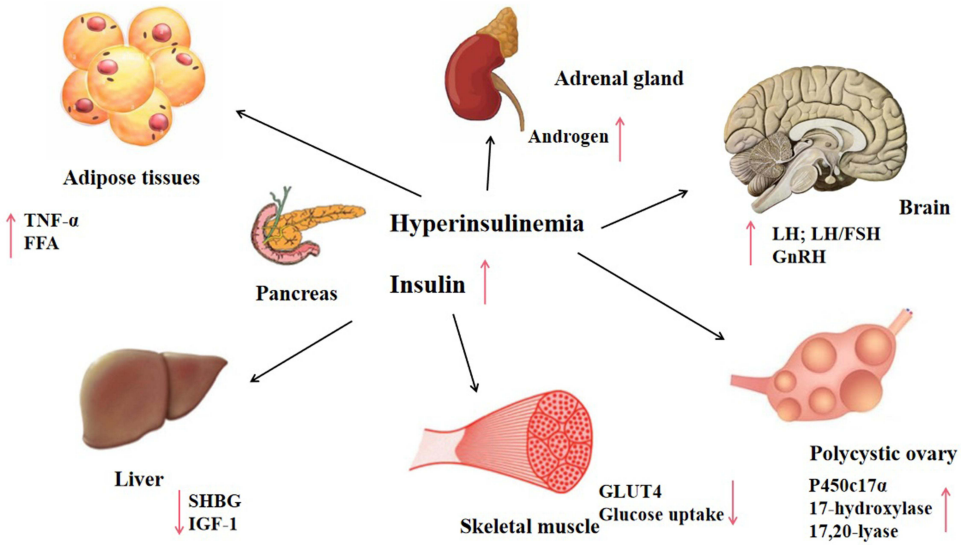

Figure 1 Influence of hyperinsulinemia on various human organs.

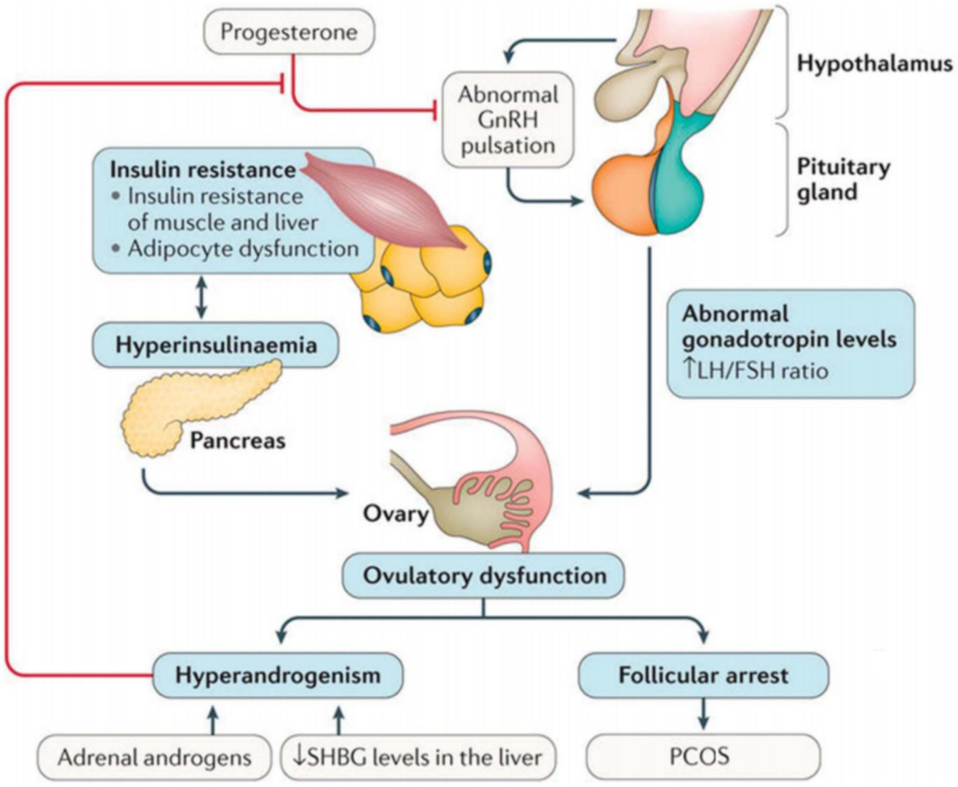

Figure 2 Summarized scheme of the pathophysiology of PCOS.
Abbreviations: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicule-stimulating hormone; SHBG, sex hormone –binding globulin.

ROS production will induce OS and cause mitochondrial dysfunctions.[32] Mitochondria contain their own genetic material, mtDNA, which encodes seven genes of the ETC complex I: one for ETC complex III, three for ETC complex IV, and two for ETC complex V.[33] The rest of the mitochondrial proteins are encoded by nuclear genes (Figure 3).[34]

Homoplasmy and Heteroplasmy

mtDNA has a very high sequence-evolution rate, in part because it is exposed to ROS. mtDNA mutations include point mutations, deletions, and insertions. mtDNA mutations can be either homoplasmic or heteroplasmic when just one or more than two variants exist, respectively.[35,36] The heteroplasmic level of a certain mtDNA mutation is critical in clinical phenotypes.[36]

Heteroplasmic mtDNA mutations are frequently associated with human pathologies because they cause more severe mitochondrial dysfunction than homoplasmic mtDNA mutations. Under normal conditions, mtDNA can "repair" mitochondrial dysfunction. When it comes to a certain heteroplasmic level, nevertheless, such compensation will be insufficient and lead to clinical expression of disease (Figure 4).[37,38]

OS and PCOS-IR

Because mitochondria are the major sites for ROS generation, overproduction of ROS will lead to OS and consequent imunbalance between the oxidant and antioxidant systems.[39] This imbalance may be caused by several metabolic activities, including obesity, hyperinsulinemia, and dyslipidemia.[40] The predominant ROS in the mitochondria are superoxide anions (O_2^-), which are produced by the leakage of electrons from the ETC, which can then react with O_2.[41]

Endometrial IR is linked to hyperandrogenemia, obesity, and inflammation and strongly associated with OS, resulting in an upregulation of OS caused by excessive ROS and pregnancy impairment.[42] OS can lead to IR through the impairment of insulin signaling and causing adipokine dysregulation.[43] OS also regulates some classical signaling pathways, such as NFκB and JNK, which in turn phosphorylate insulin-receptor substrate proteins and lead to their degradation.[44] Overproduction of ROS also suppresses GLUT4 translocation in cells via affecting insulin signaling.[45]

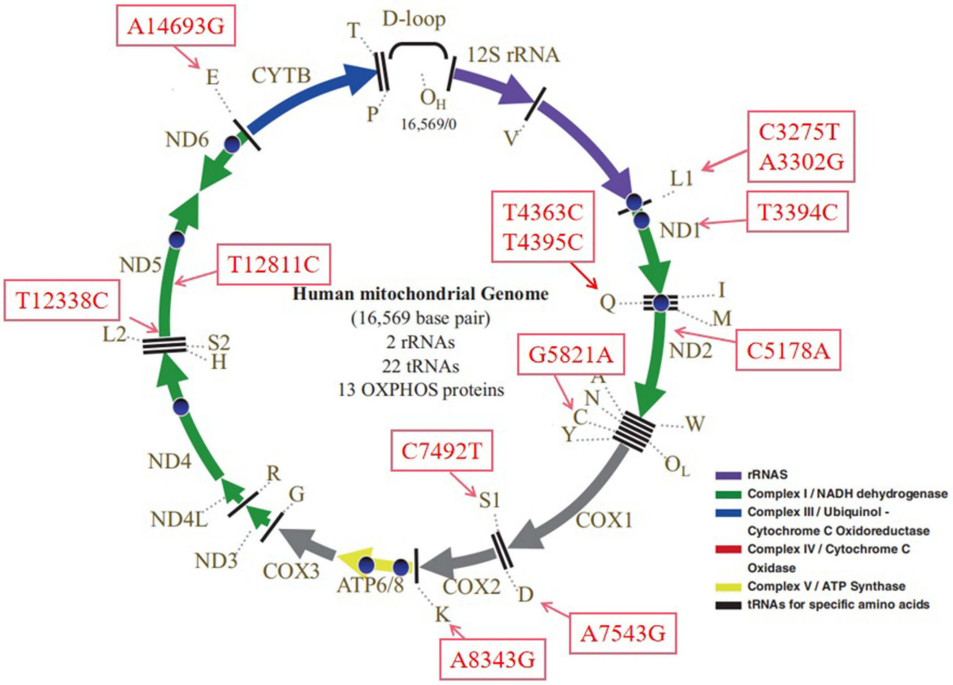

Figure 3 Genetic map of human mitochondrial genome, which has a 16,569 bp sequence. Red boxes indicated PCOS-IR–associated mtDNA mutations.

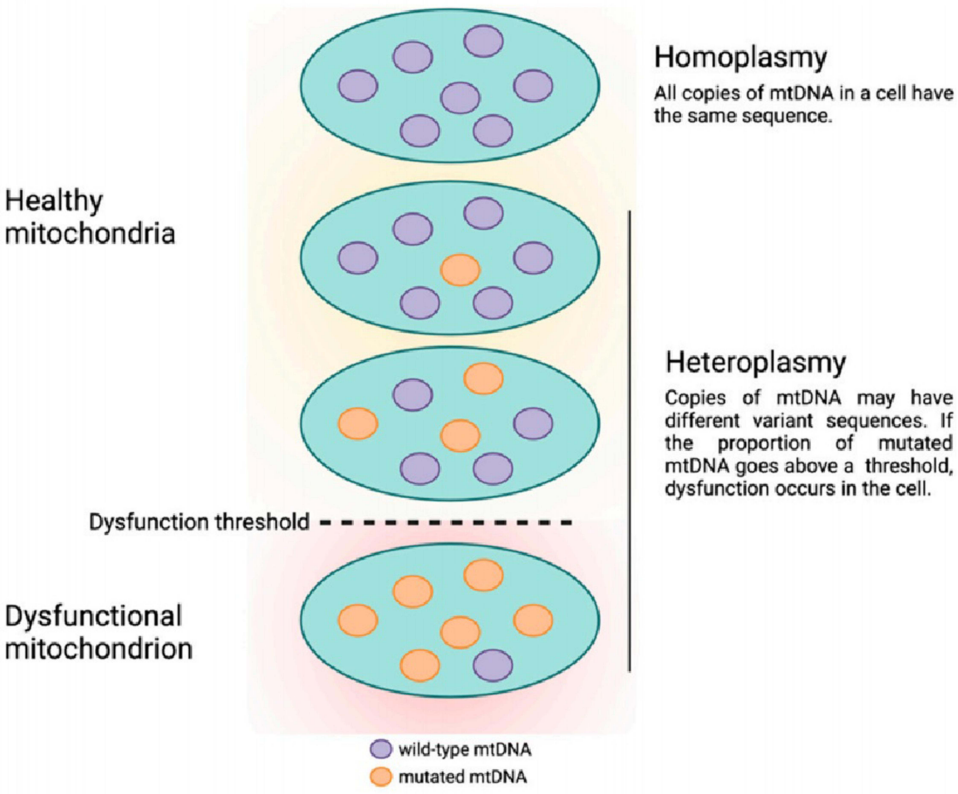

Figure 4 Heteroplasmy and the threshold effect.

PCOS-IR–Associated mtDNA Mutations

ND1 T3394C Mutation

The *ND1* T3394C (p.Y30H) mutation changes an amino acid (AA) that is extremely conserved in >90% of mammalian mtDNAs and believed to be associated with PCOS-IR.[46,47] Functional analysis has revealed that this mutation affects the stability of *ND1* mRNA, as well as complex I assembly and activity, decreases ATP levels and mitochondrial membrane potential (MMP), and enhances ROS production.[47,48]

ND2 C5178A Mutation

The m.C5178A mutation causes the alternation of leucine to methionine at position 237 of the corresponding AA, which occurrs within the *ND2* gene in complex I and is associated with PCOS-IR,[46] longevity,[49] and acute myocardial infarction.[50] Markedly decreased ATP, MMP, superoxide dismutaseand significantly increased ROS, malondialdehyde, and 8-hydroxydeoxyguanosine have been identified in polymononuclear leukocytes derived from subjects harboring this mutation, suggesting that the m.C5178A mutation may cause OS and result in mitochondrial dysfunction.[51]

ND5 T12338C and T12811C Mutations

We previously identified homoplasmic m.T12338C (p.M1T) together with $tRNA^{Ser(UCN)}$ C7492T mutation in a patient with PCOS-IR.[52] At the molecular level, m.T12338C altered well-conserved methionine with threonine; therefore, the *ND5* mRNA was expected to be shortened by two AAs.[53] Using cybrid cell models, the m.T12338C mutation decreased the stability of the *ND5* polypeptide, affecting the assembly and activity of respiratory chain complexes.[54] Therefore, m.T12338C causes a mitochondrial dysfunction that plays a key role in PCOS-IR.

The m.T12811C (p.Y159H) mutation occurs at extremely conserved residues in *ND5*, which is essential for the functions of complex I.[55] The alternation of tyrosine to histidine is believed to affect the structure and function of the transmembrane region of the ND5 protein.[56] Since *ND5* plays a a putative role in maintening the functions of complex I, the m.T12811C mutation may affect the *ND5* polypeptide and influence ETC activities.[57]

D-loop Mutations

The D-loop region is where mtDNA replication and transcription occur, and is important for transcription of both heavy and light strands.[58] A recent case–control study by Deng et al suggested that variants m.G207A, m.16036GGins, and m.16049Gins may decrease the risk of PCOS in a Chinese population.[59] The m.G207A substitution was located at the heavy strand, which is critical for mtDNA replication, suggesting that m.G207A may affect the binding affinity and influence the replication of mtDNA.[60] While m.16036GGins and m.16049Gins both occurred at hypervariable region 1, notably they were found to reduce the risk of endometriosis,[61] highlighting the importance of these mutations in maintaining mitochondrial functions.

4977-bp Deletion

The 4977-bp deletion is one of the most common deletions of mtDNA, spanning approximately a third of the entire mitochondrial genome (nucleotides 8470–13,447), and is regarded as a pathogenic deletion in PCOS.[62,63] The 4977-bp deletion removes five tRNAs and seven genes encoding respiratory chain complexes that are important for normal OxPhos functions. The 4977-bp deletion results in an impairment of protein synthesis and reduces ATP and mtDNA copy number.[64]

$tRNA^{Leu(UUR)}$ Mutations

We previously described a Chinese pedigree with PCOS-IR that harbored a heteroplasmic $tRNA^{Leu(UUR)}$ A3302G mutation.[65] The proband's mother and grandmother were diagnosed with T2DM. The m.A3302G mutation occurred at two nucleotides from the 5' end of $tRNA^{Leu(UUR)}$, which is evolutionarily conserved from various species (Figure 5A). As such, it can be anticipated that m.A3302G mutation may influence 5' end processing.[66] Biochemical analysis has revealed that this mutation leads to severe complex I and IV deficiencies. A marked decreased in the stability of $tRNA^{Leu(UUR)}$ was identified in cybrids with this mutation. In addition, the m.A3302G mutation led to abnormal

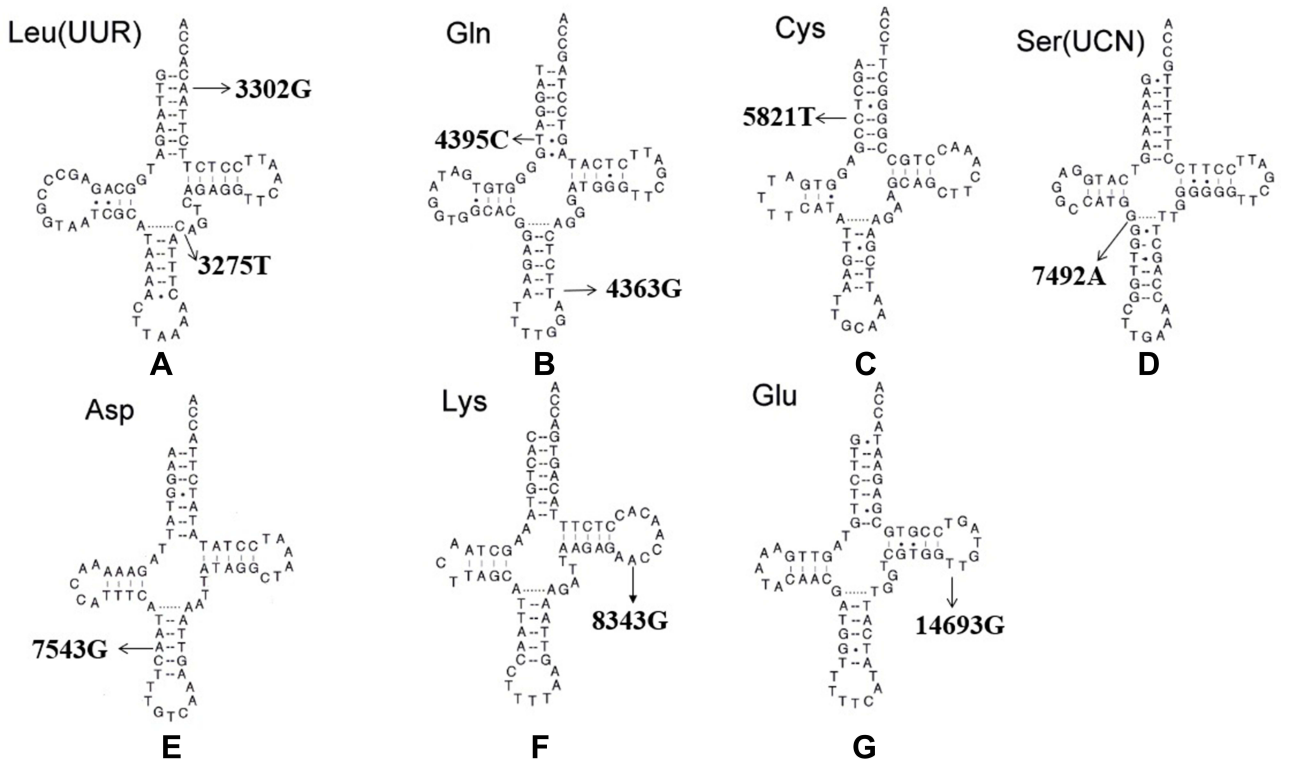

Figure 5 (**A-G**) Cloverleaf structure of mt-tRNA genes, arrows indicate the positions of PCOS-IR related mutations.

processing of RNA19, an unprocessed RNA intermediate comprising mt-16S rRNA, mt-tRNA$^{Leu(UUR)}$, and mtND1.[67,68] Therefore, the m.A3302G mutation can cause mitochondrial dysfunctions involved in PCOS-IR.

Another PCOS-IR–associated mutation is m.C3275T in tRNA$^{Leu(UUR)}$, which has been reported in another family with PCOS and metabolic syndrome.[69] The m.C3275T mutation was located in a well-conserved position in the variable region of tRNA$^{Leu(UUR)}$ (Figure 5A). A recent study revealed that the m.C3275T mutation was a risk factor of Leber's hereditary optic neuropathy.[70] Intriguingly, m.C3275T disrupted Watson–Crick base-pairing (28A–46C) and may have caused a failure in tRNA$^{Leu(UUR)}$ metabolism.

tRNA^{Gln} Mutations

By mutational screening for mt-tRNA genes in 80 patients with PCOS-IR and 50 healthy controls, we identified a set of tRNA mutations.[71] Among these, m.T4363C mutation occurred at the anticodon stem of tRNA^{Gln} (conventional position 38). A mutation at position 38 needed to be modified and played important roles in tRNA structure and function (Figure 5B).[72] Bioinformatic analysis indicated that the m.T4363C mutation caused the thermodynamic change of tRNA^{Gln}[69]. The m.T4363C mutation has been identified in a Chinese pedigree with hypertension, but detailed molecular mechanisms remain unexplored.[73] While the m.T4395C mutation occurs at the sixth base of mt-tRNA^{Gln}-accept arm, adjacent to the 5' end of tRNA^{Gln} (Figure 5B), interestingly the mutant 4395C formed a novel (6C–64G) base-pairing that was associated with essential hypertension.[74] The secondary structure altered by the m.T4395C mutation may influence tRNA function and impair mitochondrial translation.

tRNA^{Cys} Mutation

The homoplasmic m.G5821A mutation resides at the acceptor arm of tRNA^{Cys} gene (position 6). This mutation has been reported to be associated with cardiomyopathy,[75] and is also a risk factor in clinical expression of deafness-related m. A1555G mutation.[76] At the molecular level, m.G5821A abolishes conserved base-pairing (6G–67C); therefore, it may lead to failure of tRNA metabolism via the alternation of its structure (Figure 5C).[77]

$tRNA^{Ser(UCN)}$ Mutation

The m.C7492T mutation in homoplasmy is located at the anticodon stem of $tRNA^{Ser(UCN)}$ (position 26). Notably, the cytosine at that position is conserved from bacteria to human mitochondria, emphasizing the importance of m.C7492T mutation to tRNA function (Figure 5D).[78] A heteroplasmic mutation (m.T4295C) occurring at the same conventional position in $tRNA^{Ile}$ has been reported to cause chronic progressive external ophthalmoplegia.[79] Therefore, the m.C7492T mutation may have the same impact on tRNA function.

$tRNA^{Asp}$ Mutation

Adenine-to-guanine alternation at position 7543 affects a well-conserved adenosine in the anticodon stem of $tRNA^{Asp}$, which may influence the posttranscriptional modification of this tRNA (Figure 5E).[80] Yeast genes harboring C-to-T transition at position 28 are transcribed and can be further processed to form the maturation of 4S-size $tRNA^{Asp}$, whereas mutant tRNA may not be charged with radiolabeled aspartate.[81] Subjects with m.A7543G mutation have shown partial cytochrome C oxidase deficiency, suggesting the potential pathogenicity of this mutation in mitochondrial dysfunction.[82]

$tRNA^{Lys}$ Mutation

The homoplasmic A8343G mutation affects the first adenine in the TψC loop of $tRNA^{Lys}$ (position 54). The nucleotide at that position is often modified, thus playing an important role in the structure and function of tRNA.[83] This mutation may affect tRNA aminoacylation ability and binding affinity with mitochondrial elongation factor Tu, which is critical for mitochondrial protein synthesis (Figure 5F).[84,85] Therefore, the m.A8343G mutation is pathogenic in PCOS-IR.

$tRNA^{Glu}$ Mutation

The well-known m.A14693G mutation occurs at a conserved position of the TΨC loop of $tRNA^{Glu}$ (Figure 5G). The nucleotide at position 54 (m.A14693G) of $tRNA^{Glu}$ is often modified, thus having an impact on tRNA functions.[86] It has been proposed that the m.A14693G mutation can cause failure in $tRNA^{Glu}$ metabolism and impair mitochondrial protein synthesis.[87]

Mechanism of PCOS-IR–Associated mtDNA Mutations

Mutations in mtDNA have structural and functional consequences, such as affecting OxPhos complexes and influencing mitochondrial protein synthesis. Mmost of these mtDNA mutations occurred with mt-tRNA genes (Figure 5 and Table 1). mt-tRNA mutations may destabilize tRNA tertiary structure, alter RNA processing, and lead to defects in

Table 1 Summary of PCOS-IR–associated Mt-tRNA mutations

tRNA species	Allele	Nucleotide position in tRNAs	Structure location	Homoplasmy/ heteroplasmy	Aberrant tRNA Biology	References
$tRNA^{Leu(UUR)}$	C3275T	44	Variable region	Homoplasmy	Disrupts conserved base-pairing	[69]
	A3302G	71	Acceptor arm	Heteroplasmy	Affects 3'-end processing	[65]
$tRNA^{Gln}$	T4363C	38	Anticodon stem	Homoplasmy	Affects tRNA posttranscriptional modification	[69]
	T4395C	6	Acceptor arm	Homoplasmy	Creates new base-pairing	[69]
$tRNA^{Cys}$	G5821A	6	Acceptor arm	Homoplasmy	Disrupts conserved base-pairing	[75,76]
$tRNA^{Ser(UCN)}$	C7492T	26	Anticodon stem	Homoplasmy	Creates new base-pairing	[52]
$tRNA^{Asp}$	A7543G	29	Anticodon stem	Heteroplasmy	Affects tRNA aminoacylation and steady-state level	[71]
$tRNA^{Lys}$	A8343G	54	TψC loop	Homoplasmy	Affects tRNA posttranscriptional modification	[69]
$tRNA^{Glu}$	A14693G	54	TψC loop	Homoplasmy	Affects tRNA posttranscriptional modification	[46,71]

nucleotide modification. Subsequently, these mutations lead to failures in tRNA metabolism. These mitochondrial protein-synthesis defects resulte in decreased in ATP production in granulosa cells or pancreatic cells, thus contributing to PCOS clinical phenotypes.

Conclusion

Mutations in mtDNA important contributors to PCOS-IR. Genetic variants in mitochondrial genomes can perturb OxPhos and are thought to contribute to the clinical pathology of PCOS. mtDNA damage is believed to increase OS and create a proinflammatory state, which could accelerate the progression of PCOS.[88] Therefore, mtDNA may offer a viable alternative target for genetic studies tackling this complex but common disease and attempting to explain the discrepancies in clinical phenotype and progression of PCOS.

Abbreviations

PCOS, polycystic ovary syndrome; IR, insulin resistance; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; OS, oxidative stress; T2DM, type 2 diabetes mellitus; LH, luteinizing hormone; ATP, adenosine triphosphate; OxPhos, oxidative phosphorylation; nDNA, nuclear DNA; ETC, electron transport chain; NADH-Q, nicotinamide adenine dinucleotide Q; SDH, succinate dehydrogenase; AA, amino acid; MMP, mitochondrial membrane potential.

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Disclosure

The authors report no conflicts of interest in this work.

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