

# tURn the Lights on: Mitochondrial Transport-RNAs and Cardiovascular Disease

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## Introduction to Mitochondrial Genome

**M**itochondria are the organelles universally known as the cellular power plants because they generate most of the cellular energy by oxidative phosphorylation. Moreover, mitochondria are involved in many other cellular activities, such as hormones and heme synthesis, calcium ions storage, and regulation of cellular proliferation and/or differentiation.<sup>1</sup> The role of mitochondrial dysfunction in cardiovascular pathophysiology, and in particular in myocardial infarction, cardiac hypertrophy, and heart failure has been extensively evaluated.<sup>2,3</sup>

Mitochondria are also the only extranuclear compartment that contains genetic material in form of deoxyribonucleic acid (DNA), probably as the result of an endosymbiotic event between ancestral eukaryotic cells and aerobic bacteria in the early evolutionary stage of eukaryotes.<sup>1</sup> Each cell contains hundreds to thousands of copies of the mitochondrial DNA (mtDNA) genome, which has a maternal inheritance. Human mtDNA is a closed circular double-stranded DNA with 16 569 base pairs (bp) encoding 37 genes: 13 for the essential subunits of respiratory complexes I, III, IV, and V; 22 for mitochondrial transport-RNAs (mt-tRNAs); and 2 for ribosomal-RNAs (mt-rRNAs).<sup>1</sup> All the RNA components necessary for mitochondrial translation are supplied in mitochondria, whereas all protein components are encoded by nuclear DNA and transported into the mitochondria after their cytoplasmic synthesis.<sup>1</sup>

The biochemical processes in mitochondria occur at much faster rates than those in the host cell. The rapidity of mtDNA replication machinery, however, is not without consequences, particularly due to the presence of reactive oxygen species (ROS) in mitochondria environment. Of consequence, mtDNA is more prone to accumulate mutations with a  $\approx 16$  times higher mutation accumulation rate than the nuclear DNA.<sup>4</sup> Mutations could initiate a cyclic process in which impaired mitochondrial functions and increased ROS cause a higher error rates of DNA polymerases with further accumulation of mutated mtDNA.<sup>4</sup> Mutations in mtDNA have been linked to various maternal inherited human diseases.<sup>1,4</sup>

A large number of mutations in mt-tRNA genes have been mapped to mt-tRNAs locus, and approximately half of mitochondrial disease is caused by mutations involving these genes (Table).<sup>1</sup> Mutations in an mt-tRNA gene may negatively influence biogenesis and functioning of tRNAs after their transcription, including processing, post-transcriptional modification, aminoacylation, association with mitochondrial elongation factors, and/or interactions with the mitoribosome during translation.<sup>1</sup> Mutations rarely provoke critical structural and/or functional alterations of tRNAs, because they generally result in a severe phenotype that is likely to be incompatible with embryogenesis and development.<sup>1</sup> The coexistence of a mixture of molecules of mutated and wild type mtDNA within mitochondria is called heteroplasmy and the percentage of mutated, pathologic DNA is a key factor for the severity of disease; on the contrary the presence of all identical copies of mtDNA, wild type or pathologic, is known as homoplasmy.

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## mt-tRNAs and Coronary Artery Disease

Only a few studies have evaluated the potential role of mt-tRNAs mutations and coronary artery disease (CAD). Jia et al were the first to assess a correlation between specific point mutations of mt-tRNA genes and CAD<sup>5</sup> by studying a 4-generation Chinese Han family with 13 of 32 members affected by maternally inherited CAD. They identified the G>A mutation at site 15927 of tRNA-Thr gene, which resulted in decreased aminoacylated efficiency of tRNA-Thr, with a 53% reduction rate of mitochondrial translation in mutant cells.<sup>5</sup> The impaired

**Table.** mt-tRNAs Most Frequent Mutations Involved in Cardiovascular Disease

Site	Sostitution	tRNA	Phenotype
586	G>A	Phe	CKD
593	C>T	Phe	AH
3243	A>G	Leu	MELAS, diabetes, AH, preeclampsia, HCM, DCM
3254	C>T	Leu	AH
3256	C>T	Leu	Atherosclerosis
3260	A>G	Leu	Heart failure, HCM
3264	T>C	Leu	Diabetes
3302	A>G	Leu	Rhythm disorders, cardiorespiratory failure
3303	C>T	Leu	HCM
4263	A>G	Lys	AH
4269	A>G	Ile	HCM, DCM
4277	T>C	Ile	HCM
4291	T>C	Ile	AH, dyslipemia, hypomagnesemia
4295	A>G	Ile	HCM, AH
4295	A>G	Ile	AH
4300	A>G	Ile	HCM
4317	A>G	Ile	HCM, DCM
4353	T>C	Met	AH
4401	A>G	Met, Gln (5')	AH
4435	A>G	Met	AH
4454	T>C	Met	AH
4454	T>C	Met	AH
5553	C>T	Trp	AH
5592	T>C	Gln	CAD
5814	T>C	Cys	AH
8344	A>G	Lys	MERRF, HCM
8348	A>G	Lys	AH
8363	G>A	Lys	HCM
9997	T>C	Gly	DCM,
12315	G>A	Leu	Atherosclerosis
15927	G>A	Thr	CAD
15928	G>A	Thr	AH

AH indicates arterial hypertension; CAD, coronary artery disease; CKD, chronic kidney disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers.

mitochondrial protein synthesis leads to defects in overall respiratory capacity, malate/glutamate-promoted respiration, succinate/glycerol-3-phosphate promoted respiration, and N, N', N'-tetramethyl-pphenylenediamine/ascorbate-promoted

respiration in immortalized lymphoblasts from subjects carrying the mutation with a concomitant increase in ROS production.<sup>5</sup>

In the present issue of *JAHA*, Qin et al investigated a total of 80 genetically unrelated Chinese subjects with coronary heart disease, whose ages were from 33 to 79 years old, and identified tRNA-Gln 5592 T>C and the previously cited tRNA-Thr 15927 G>A to be associated with a maternally inherited form of CAD.<sup>6</sup> tRNA-Gln 5592 A>G mutation is expected to lead to decreased stability of tRNA and, as a consequence, to a failure in tRNA metabolism, while tRNA-Thr 15927 G>A is suggested to reduce steady-state level of tRNA.<sup>6</sup> Such alterations, again, are supposed to impair mitochondrial translation, respiration and, in tandem, increase ROS generation.<sup>6</sup> Data from a mutation analysis performed to assess contribution of mtDNA variants or haplogroups toward the phenotypic expression of these mtDNA mutations in these Chinese pedigrees suggest that the cytochrome C oxidase subunit 1 (CO1) G6969A (I356V) variant may have a role in the phenotypic manifestation of tRNA-Thr 15927 G>A mutation.<sup>6</sup> The genetic evidence presented by Qin et al clearly demonstrated that the identified mt-tRNA mutations, even if not present in homoplasmic state, represent an inherited risk factor for CAD. Therefore, these findings might significantly impact the understanding of the different roles of mt-tRNAs in cardiovascular pathophysiology.

## mt-tRNAs and Cardiomyopathies

The 2 most significant examples of mt-tRNA-related cardiomyopathies are represented by MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), a major and well characterized mitochondrial disease caused by point mutations in mt-tRNA-Leu (UUR) genes, and MERRF (myoclonic epilepsy with ragged red fibers), a major subgroup of the mitochondrial encephalomyopathies caused by a point mutation at position 8344 in the mt-tRNA<sup>Lys</sup> gene.<sup>1</sup> These diseases are also characterized by variable cardiac involvement including hypertrophic cardiomyopathy, ventricular systolic dysfunction, and Wolff-Parkinson-White syndrome.<sup>7</sup> On the other hand, various types of isolated hypertrophic cardiomyopathy (HCM) have also been described.<sup>8,9</sup> Mutations in mt-tRNA genes have been associated to HCM in about the 10% of cases.<sup>8</sup> Dilated cardiomyopathy associated with mt-tRNA point mutations is less common than the hypertrophic phenotype.<sup>8</sup> Pathophysiology of myocardial involvement in mt-tRNA diseases is complex and probably related to multiple factors, such as a compensatory mechanism(s) to a deficient oxidative phosphorylation.<sup>8</sup> Even if such diseases generally comply with the “classic” scheme of monogenic inheritance, their penetrance may be modified by other factors such as the activity of specific tRNA synthetases.<sup>8</sup>

## mt-tRNAs and Arterial Hypertension

Mitochondrial genomic alterations have also been linked to multifactorial cardiovascular disease, such as arterial hypertension. Zhang et al have previously shown that a nucleotide mutation in Leu mt-tRNA gene decreased cytochrome C oxidase activity and led to mitochondrial and placental dysfunction in preeclampsia.<sup>10</sup> Schwartz et al identified mutations in mt-tRNA genes in 14 of 20 hypertensive subjects screened for mutations in mitochondrial genome, some of which have previously been associated with HCM.<sup>11</sup> Interestingly, the association between mt-tRNA genetic pedigree and hypertensive phenotype has been extensively investigated only in Chinese populations.<sup>12–14</sup> Pathogenic mechanisms of mt-tRNA mediated form of essential hypertension probably involve ROS production, impaired ATP production, modification in cytosolic calcium handling and, possibly, apoptotic cell death. Other mutations at the same promoter of tRNA<sup>Leu</sup> gene have also been linked to a maternally inherited form of hypertension, dyslipidaemia, and hypomagnesaemia<sup>15</sup> and to diabetes mellitus.<sup>16</sup>

## Conclusive Remarks

Studies on mitochondrial genomic mutations and cardiovascular diseases are a recent, fascinating field of research. While mitochondriopathies are usually considered to cause severe multi-organ involvement and rare diseases, it is crucial to note that also for common diseases such as hypertension, metabolic syndrome and CAD, several mutations may not have a disease-causative role, but may predispose and determine the genetic susceptibility to these diseases. mt-tRNAs abnormalities may lead to various degree of mitochondrial dysfunction that could be reflected in different phenotypic presentations of disease. One of the major limits of all these studies regards the paucity of data on the pathogenic mechanisms activated by mt-tRNAs mutations. Moreover, the complexity of mt-tRNAs genetic analysis, and the difficulties of experimental manipulation of mtDNA sequences represent additional technical issues in these studies. However, the evidence that mt-tRNA mutations impair the efficiency of mitochondrial translation machinery, worsen oxidative phosphorylation and fatty acid metabolism and increase ROS production, further highlight the well-known role of mitochondrial dysfunction in cardiovascular pathophysiology.<sup>2,3</sup>

The study from Qin and coworkers, published on this issue of *JAHA*, describes a correlation between mt-tRNA mutations and maternal inheritance of CAD.<sup>6</sup> Therefore, results of this study may lay the ground for further studies evaluating the role of mt-tRNA genetic instability on larger populations, preferably of different ethnicity. The goal of future studies will

be to assess the role of mt-tRNAs mutations in the complex, multifactorial determinism of coronary heart disease.

## Disclosures

None.

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