Mitochondrial DNA mutations and essential hypertension (Review)

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Received May 1, 2013; Accepted July 22, 2013

DOI: 10.3892/ijmm.2013.1459

Abstract. Essential hypertension (EH) is a frequent, chronic, age-related disorder, which remains a major modifiable risk factor for cardiovascular disease despite important advances in our understanding of its pathophysiology. Previous studies have noted a consistent maternal effect on blood pressure (BP). Consequently, mutations in mitochondrial DNA (mtDNA) have become an additional target of investigations on the missing BP heritability. Among these mutations, mt-transfer RNA (tRNA) is a hot mutational spot for pathogenic mutations associated with EH. Mutant mtDNA aggravates mitochondrial dysfunction, pivotally contributing to the clinical phenotype. Moreover, the damaged mitochondria, due to their inability to provide the high-energy requirements for cells, generate reactive oxygen species (ROS) and induce mitochondrial-mediated cell death pathways. Therefore, mitochondrial dysfunction plays a critical role in the pathogenesis of EH. This review summarizes the basic knowledge of mitochondrial genetics and EH-associated mtDNA mutations and further discusses the molecular mechanisms behind these mtDNA mutations in clinical manifestations of EH.

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1. Introduction

Hypertension is an established risk factor for coronary heart disease, stroke, congestive heart failure and renal dysfunction. Despite significant advances in our understanding of the pathophysiology of hypertension, it remains to be one of the world’s greatest public health issues (1). It is estimated that one third of the world’s adult population will be hypertensive by 2025 (2). In particular, in industrialized countries, the risk of becoming hypertensive [blood pressure (BP) >140/90 mmHg] during a lifetime exceeds 90% (3). Essential hypertension (EH), or hypertension due to undetermined causes, accounts for >90% of cases of hypertension. It is a heterogeneous disorder, with different patients having different causal factors that lead to high BP.

To date, the etiology of EH is not well understood due to multifactorial causes. It is generally believed that EH is a multifactorial trait involving interactions among genetic, environmental and demographic factors (4). Of these, hereditary factors account for 30 to 50% of BP variability (5). Variations in a variety of genes have shown an association with hypertension in certain studies; however, these associations are often not reproducible in studies on other populations. Improved techniques of genetic analysis, particularly genome-wide linkage analysis, have enabled the search for genes that contribute to the development of primary hypertension in the population (6,7). However, the majority of the reported genetic variants were identified in studies of the nuclear genome (8-10); only limited insights have been gained from the investigation of the mitochondrial genome.

This review provides a detailed introduction of the mitochondrial genome, summarizes the results of studies on the role of mitochondrial DNA (mtDNA) mutations associated with EH published thus far, and highlights some of the general conclusions that have become apparent.

2. Mitochondrial genome and mitochondrial genetics

Mitochondria are bacterium-sized organelles found in all nucleated cells (11). Uniquely, they contain their own genome (mtDNA) and it is widely accepted that mitochondria originate from aerobic bacteria engulfed by an anaerobic eukaryotic cell.
Mammalian mtDNA encodes 13 proteins that are subunits of the oxidative phosphorylation (OXPHOS) system and 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs) (Fig. 1). The mitochondrial OXPHOS complexes are assembled from genes distributed between mtDNA and nuclear DNA (nDNA) (12). Unlike nDNA, mtDNAs are maternally inherited and are present in multiple copies/cell. The numbers vary according to the bioenergetic needs of each unique tissue and can range over three orders of magnitude, depending on the cell type (13).

Each mammalian cell contains hundreds of mitochondria and thousands of mtDNAs. Since mtDNA is in the proximity of reactive oxygen species (ROS) generation sites and mitochondria have less sophisticated DNA protection and repair systems, mtDNA is hence vulnerable to a high mutation rate (14). The most prominent is polyploidy. Polyploidy is the basis of heteroplasmy (alternations of mtDNA may be present in some of the mtDNA molecules) and homoplasmy (mutations in all of the molecules). Neutral polymorphisms are usually homoplasmic, whereas pathogenic mutations are usually heteroplasmic in nature, and EH-associated mtDNA mutations are commonly homoplasmic or almost homoplasmic.

3. Mitochondrial function and dysfunction

Mitochondria exert both vital and lethal functions in physiological and pathological conditions. On the one hand, they provide the majority of cellular energy in the form of adenosine-5'-triphosphate (ATP) through OXPHOS (15). Additionally, they are involved in a variety of processes, including regulation of the cell cycle, cell signaling, apoptosis and calcium (Ca²⁺) buffering (12,16,17).

Oxygen radicals, such as ROS, are also generated during OXPHOS as a toxic byproduct in the mitochondria, which may damage the mitochondrial and cellular DNA, protein, lipids and other molecules, leading to oxidative stress and mitochondrial dysfunction (18,19). An impairment of normal mitochondrial function leads to an excessive production of ROS and a general decrease in ATP levels. Moreover, there is a concomitant loss of mitochondrial transmembrane potential (20). Excessive ROS and Ca²⁺ production lead to mitochondrial outer membrane permeabilization and to the release into the cytosol of cytotoxic proteins normally confined within the mitochondrial intermembrane space. As a result, this process induces apoptosis or necrosis (21).

4. Oxidative stress and hypertension

In animal models, oxidative stress has been observed in spontaneously hypertensive rats (22), renovascular hypertension (23) and salt-sensitive hypertension (24). Although its pathogenesis is complex and multifactorial, human hypertension is considered as a state of increased oxidative stress (25). An excessive endothelial production of ROS and nitric oxide synthase (NOS) may lead to impaired endothelium-dependent vasorelaxation in human internal mammary arteries and the saphenous vein (26), contributing to vascular pathophysiology by promoting increased vascular tone, cell growth, as well as the activation of matrix metalloproteinases and the deposition of extracellular matrix proteins, which are processes associated with the vascular phenotype of hypertension (27).

5. Role of mtDNA mutations in EH

12S rRNA A1555G mutation. The A1555G mutation in the 12S rRNA gene has been associated with aminoglycoside-induced and non-syndromic hearing loss in various ethnic populations (28-31). Chen et al (32) described two Han Chinese families with hearing loss and hypertension carrying the homoplasmic A1555G mutation. An A to G transition at this position in the 12S rRNA gene has been predicted to encode an aminoglycoside binding based on sequence similarity to Escherichia coli (33) and alters mitochondrial ribosomal function and translation (34-36), which in turn causes OXPHOS defects that are thought to contribute to the clinical pathology of hypertension. Insufficient metabolism caused by mitochondrial dysfunction may lead to the elevation of systolic BP and may be involved in the development of hypertension (37).

ND1 T3308C mutation. The homoplasmic ND1 T3308C mutation is a known disease-associated mutation. This mutation has been suggested to contribute to the higher penetrance of hearing loss in a large African family than Japanese and French pedigrees carrying the tRNA_Ser(UCA) T7511C mutation (38,39). Liu et al (40) reported a Han Chinese family carrying the ND1 T3308C mutation with EH. The T to C transition at position 3308 causes translation-initiating methionine with a threonine in ND1. Thus, the ND1 mRNA is shortened by two amino acids (41). Moreover, the T3308C mutation is also located in two nucleotides adjacent to the 3' end of the tRNA_Leu(UUR) gene; the T3308C mutation also affects the processing of the H-strand polycistronic RNA precursors (42).

ND5 T12338C mutation. The well-known T12338C mutation in the ND5 gene, combined with tRNA_Ser(UCA) A12330G mutation, has been found in a three generation Han Chinese
family with high penetrance of EH (43). Moreover, this mutation was also shown to be present in a Chinese family with hypertrophic cardiomyopathy (44). In fact, the ND5 T12338C mutation, which is similar to the NDI T3308C mutation, causes a replacement of the first amino acid, translation-initiating methionine with a threonine in the ND5 polypeptide, and decreases ND5 mRNA levels and alters the processing of RNA precursors. In addition, this mutation is located in two nucleotides adjacent to the 3' end of the tRNA<sup>Leu</sup>(CUN), and it is anticipated that the T12338C mutation will lead to a reduction in tRNA<sup>Leu</sup>(CUN) levels, whereas the A12330G mutation disrupts the highly conserved base pairing (6T-67A) in the acceptor arm of tRNA<sup>Leu</sup>(CUN) (45). Therefore, the combination of T12338C and A12330G mutations may have contributed to the high penetrance of EH in this Chinese family (43).

**ND6 T14484C mutation.** The T14484C mutation in the ND6 gene is one of the primary mutations associated with Leber's hereditary optic neuropathy (LHON) (46). In a recent study, a large Han Chinese family with maternally transmitted EH but not presenting any LHON phenotype, was found to be associated with the T14484C mutation (47). Analysis of the complete mtDNA sequence of the proband showed the absence of additional pathogenic mutations, apart from the T14484C mutation. Moreover, analysis of the mitochondrial function of lymphoblastoid cell lines established from the family members showed that mitochondrial respiration rate and membrane potential were significantly reduced when compared with the control cell lines. In addition, there was an increase in the levels of ROS and mitochondrial mass in the mutant cell lines. These data suggest that ND6 T14484C also plays an important role in the pathogenesis of EH and is also a pathogenic mutation associated with EH (47).

**CyB G15059A mutation.** The heteroplasmic G15059A mutation in CyB gene was first described in a patient with mitochondrial myopathy (48). This substitution results in the replacement of a glycine at amino acid position 190 of CyB with a stop codon leading to a truncated protein that misses 244 amino acids at the C-terminus of CyB (49). Nikitin et al (50) examined the role of the CyB G15059A mutation in patients with type 2 diabetes (T2D) with EH. In addition, the G15059A heteroplasmic level exceeding 39% was associated with an increased risk of EH, indicating a direct pathogenic role for this mutation in EH (50).

**50-bp deletion.** The 50-bp deletion (m.298_347del50) in the mtDNA control region removes the conserved sequence block II (CSBII) and the replication primer location (51-53). This deletion, co-occurring with two novel mutations (ND1 C3519T and ND5 G13204A), accounted for the complex clinical traits, including hypertension, T2D and coronary artery disease (CAD) in an Indian family (54). Of note, two short homologous direct repeats of CCAAACCCC flanked the 50-bp deletion. The CSBII directs transcription termination and primer formation in mtDNA replication (55). Thus, it can be predicted that the 50-bp deletion may reduce the mtDNA copy number and decrease the levels of cellular energy. However, conflicting reports have shown that this deletion does not affect the mtDNA copy number (53); its functional role requires further elucidation in future studies.

tRNA<sup>Leu</sup> A4295G mutation. The homoplasmic A4295G mutation in the tRNA<sup>Leu</sup> gene was identified in a three generation Han Chinese family with maternally inherited EH (56). An A to G transition at nucleotide position 4295 was shown to be highly evolutionarily conserved, and was not present in the healthy controls (57). The A4295G mutation is localized at the 3' end adjacent to the anticodon (position 37) of tRNA<sup>Leu</sup> (45); nucleotide at position 37 is responsible for the stabilization of functional tRNA (58). Thus, it has been suggested that this mutation reduces the efficiency with which tRNA<sup>Leu</sup> can be processed by 3'-tRNase, reducing the level of functional tRNA<sup>Leu</sup> (59). The functional characterization of the A4295G mutation shows that this mutation induces a significant decrease in complex III protein levels, leading to a decrease in the activity of this complex, which is reflected by the decline in mitochondrial respiration (60).

tRNA<sup>Leu</sup> A4263G mutation. The A4263G mutation changes the stop codon TAA of the NDI mRNA to an equivalent TAG stop codon. This mutation causes an A to G transition at the 5' end of the tRNA<sup>Leu</sup> gene (61,62). Cybrid cells derived from the proband carrying A4263G mutation show a reduction in tRNA<sup>Leu</sup> steady-state levels, as well as in the rate of mitochondrial protein translation. Increased ROS and the decreased efficiency of 5' end processing of tRNA<sup>Leu</sup> precursor indicated that this mutation caused mitochondrial dysfunction that was responsible for EH in this Han Chinese family (61).

tRNA<sup>Leu</sup> T4291C mutation. The homoplasmic T4291C mutation in the tRNA<sup>Leu</sup> gene has been associated with a cluster of metabolic defects, including hypertension, hypercholesterolemia and hypomagnesaemia in a large family (63). The T4291C mutation occurs immediately 5' to the tRNA<sup>Leu</sup> anticodon (position 33), and is conserved in every sequenced tRNA<sup>Leu</sup> from bacteria to human mitochondria (64). Biochemical studies with anticodon stem-loop analogs of tRNAs have been performed and have indicated that the substitution of cytidine for uridine at this position markedly impairs ribosomal binding (65), providing evidence of the functional importance of this mutation.

tRNA<sup>Met</sup> A4435G mutation. The presence of the A4435G mutation with chronic progressive external ophthalmoplegia (CPEO) was initially reported in the study by Jakusch et al (66). Later on, this mutation was found in patients with LHON (67), as well as in a Japanese subject with diabetes (68). In addition, the East Asian haplogroup G2a1-specific A4435G mutation (69) has also been associated with EH in two Chinese families (70,71). The homoplasmic A4435G mutation, which is located at immediately 3' end to the anticodon of tRNA<sup>Met</sup> (nucleotide position 37), is extremely conserved from bacteria to human mitochondria (45). In fact, as shown in previous studies, the A4435G mutation causes ~40-50% reduction in the steady-state level of tRNA<sup>Met</sup> and consequently results in the failure of mt-tRNA metabolism (67,70). Impaired mt-tRNA metabolism subsequently worsens the mitochondrial protein synthesis, decreases ATP production and increases ROS levels. Thus, mitochondrial dysfunction may contribute to the development of EH in these families carrying the A4435G point mutation (37,63,72).
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A4401G mutation in tRNA^Met and tRNA^Gln. The homoplasmic A4401G mutation was originally described in a three generation Chinese family with left ventricular hypertrophy (LVH) (73). This mutation was also present in a five generation Chinese pedigree with EH (74). The A4401G mutation is localized at the junction of tRNA^Met at the H-strand and tRNA^Gln at the L-strand (75). Thus, it is anticipated that this mutation may lead to defective tRNA^Met 5' end processing in the H-strand transcripts and reduce the efficiency of tRNA^Gln precursor 5' end cleavage in the L-strand transcripts. Functional characterization of cell lines derived from the proband carrying the A4401G mutation shows virtually ~30% reduction in the levels of tRNA^Ile and tRNA^Gln (74). In addition, the mutant cell lines present a significant decrease in the oxygen consumption rate (73). These findings indicate that the A4401G mutation is involved in the pathogenesis of EH in Han Chinese families.

T4353C mutation in tRNA^Gln. The T4353C mutation, in conjunction with the tRNA^Trp C593T mutation, has been shown to account for the high penetrance of EH in a Han Chinese family (76). Clinical evaluation of this family showed a typical

Table I. Summary of cardiovascular diseases associated mt-tRNA mutations.

<table>
<thead>
<tr>
<th>Position</th>
<th>tRNA species</th>
<th>Allele</th>
<th>Homoplasmy/heteroplasmy</th>
<th>Clinical features</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>tRNA^{Val}</td>
<td>C1628T</td>
<td>Homoplasmy</td>
<td>Cardiomyopathy</td>
<td>(80)</td>
</tr>
<tr>
<td>14</td>
<td>tRNA^{Leu(UUR)}</td>
<td>A3243G</td>
<td>Heteroplasmy</td>
<td>Cardiomyopathy</td>
<td>(81)</td>
</tr>
<tr>
<td>29</td>
<td>tRNA^{Leu(UUR)}</td>
<td>A3260G</td>
<td>Heteroplasmy</td>
<td>Myopathy, cardiomyopathy, MELAS</td>
<td>(82-84)</td>
</tr>
<tr>
<td>72</td>
<td>tRNA^{Leu(UUR)}</td>
<td>C3303T</td>
<td>Homoplasmy</td>
<td>Cardiomyopathy, myopathy, HCM</td>
<td>(85-87)</td>
</tr>
<tr>
<td>1</td>
<td>tRNA^{ile}</td>
<td>A4263G</td>
<td>Homoplasmy</td>
<td>Hypertension</td>
<td>(61)</td>
</tr>
<tr>
<td>7</td>
<td>tRNA^{ile}</td>
<td>A4269G</td>
<td>Homoplasmy</td>
<td>Cardiomyopathy</td>
<td>(88)</td>
</tr>
<tr>
<td>15</td>
<td>tRNA^{ile}</td>
<td>T4277C</td>
<td>Homoplasmy</td>
<td>Cardiomyopathy</td>
<td>(89)</td>
</tr>
<tr>
<td>33</td>
<td>tRNA^{ile}</td>
<td>T4291C</td>
<td>Homoplasmy</td>
<td>Hypertension, hypercholesterolemia, hypomagnesaemia</td>
<td>(63)</td>
</tr>
<tr>
<td>37</td>
<td>tRNA^{ile}</td>
<td>A4295G</td>
<td>Homoplasmy</td>
<td>HCM, hypertension</td>
<td>(56,57)</td>
</tr>
<tr>
<td>42</td>
<td>tRNA^{ile}</td>
<td>A4300G</td>
<td>Homoplasmy</td>
<td>HCM</td>
<td>(90)</td>
</tr>
<tr>
<td>37</td>
<td>tRNA^{Met}</td>
<td>A4435G</td>
<td>Homoplasmy</td>
<td>LHON, CPEO, hypertension</td>
<td>(66-68,70,71)</td>
</tr>
<tr>
<td>1</td>
<td>tRNA^{Glu} and tRNA^{Met}</td>
<td>A4401G</td>
<td>Homoplasmy</td>
<td>LVH, hypertension</td>
<td>(73,74)</td>
</tr>
<tr>
<td>49</td>
<td>tRNA^{Gln}</td>
<td>T4353C</td>
<td>Homoplasmy</td>
<td>Hypertension</td>
<td>(76)</td>
</tr>
<tr>
<td>58</td>
<td>tRNA^{Thr}</td>
<td>A5600T</td>
<td>Heteroplasmy</td>
<td>DCM</td>
<td>(91)</td>
</tr>
<tr>
<td>54</td>
<td>tRNA^{Thr}</td>
<td>A8348G</td>
<td>Heteroplasmy</td>
<td>Cardiomyopathy</td>
<td>(92)</td>
</tr>
<tr>
<td>7</td>
<td>tRNA^{Gly}</td>
<td>T9997C</td>
<td>Heteroplasmy</td>
<td>HCM</td>
<td>(93)</td>
</tr>
<tr>
<td>59</td>
<td>tRNA^{His}</td>
<td>G12192A</td>
<td>Homoplasmy</td>
<td>Cardiomyopathy</td>
<td>(94)</td>
</tr>
<tr>
<td>67</td>
<td>tRNA^{Leu(UUR)}</td>
<td>A12330G</td>
<td>Homoplasmy</td>
<td>Hypertension</td>
<td>(43)</td>
</tr>
<tr>
<td>37</td>
<td>tRNA^{Glu}</td>
<td>T14709C</td>
<td>Homoplasmy</td>
<td>Cardiomyopathy</td>
<td>(95)</td>
</tr>
<tr>
<td>2</td>
<td>tRNA^{Thr}</td>
<td>T15889C</td>
<td>Heteroplasmy</td>
<td>DCM</td>
<td>(91)</td>
</tr>
<tr>
<td>16</td>
<td>tRNA^{Thr}</td>
<td>A15902G</td>
<td>Heteroplasmy</td>
<td>DCM</td>
<td>(91)</td>
</tr>
<tr>
<td>38</td>
<td>tRNA^{Thr}</td>
<td>T15924C</td>
<td>Homoplasmy</td>
<td>DCM</td>
<td>(96)</td>
</tr>
<tr>
<td>51</td>
<td>tRNA^{Thr}</td>
<td>A15935G</td>
<td>Heteroplasmy</td>
<td>DCM</td>
<td>(91)</td>
</tr>
</tbody>
</table>

MELAS, mitochondrial encephalomyopathy; lactic acidosis and stroke-like episodes; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy; LHON, Leber’s hereditary optic neuropathy; CPEO, chronic progressive external ophthalmoplegia; DCM, dilated cardiomyopathy.

Figure 2. Cloverleaf structure of mt-tRNA with standard nucleotide numbering. The nucleotides in the variable stem have the prefix 'e' and are located between positions 45 and 46, obeying the base-pairing rules. The nucleotides in the 54-strand and 34-strand are numbered by e11, e12, e13; the second digit identifies the base-pair.
maternally transmitted pattern. Analysis of the complete mtDNA sequence identified the homoplasmic T4353C mutation. This mutation alters a conservative base-pairing (49A-65U) on the T arm of tRNA\textsuperscript{Gin}, thereby affecting tRNA metabolism (45). Moreover, the T4353C mutation reduces the steady-state level of tRNA\textsuperscript{Gin}, mutant tRNA\textsuperscript{Gin} and tRNA\textsuperscript{Gin} may be metabolically less stable and more subject to degradation. The cybrid cell lines carrying the T4353C mutation present mitochondrial protein synthesis defects. As a result, this mutation causes the mitochondrial dysfunction responsible for EH.

6. Molecular mechanisms behind mtDNA mutations associated with EH

In previous studies, we noticed that several hypertension-associated mitochondrial pathogenic mutations were located in tRNA genes. Mt-tRNA mutations have structural and functional effects, including destabilization of the tRNA tertiary structure, altered processing of RNA precursors, loss of nucleotide modification and deficient aminoacylation (Fig. 2 and Table I). In particular, these pathogenic mutations may lead to deficiencies in tRNA\textsuperscript{3' end} metabolism including 3' end cleavage, CCA addition, aminoacylation or impairment of critical subunits of respiratory chain functions. Failures in mt-tRNA metabolism subsequently lead to the impairment of mitochondrial protein (77-79). However, mutations in protein coding genes may have the potential to affect OXPHOS and in turn cellular death, resulting in a failure in mitochondrial protein synthesis. The increased bioavailability of ROS results in oxidative stress, which leads to cardiovascular and renal damage, thus, contributing to EH.

7. Conclusion

EH is a multifactorial syndrome that is characterized by abnormal energy metabolism and high BP. Recent studies have identified the mitochondria as the target and origin of major pathogenic pathways which lead to the progression of hypertension (97-99). Although existing therapies have been beneficial, there is a clear need for new approaches to treatment. Pharmacological targeting of the cellular stresses underlying mitochondrial dysfunction is showing promise. In addition, screening for mtDNA mutations for the clinical expression of hypertension may provide further insight into the understanding of the pathophysiology for maternally inherited hypertension.

Acknowledgements

This study was supported by grants from the Ministry of Public Health of Zhejiang Province (2013KYA158), Nanjing Medical University (no. 2010NJMU011), Hangzhou Bureau of Science and Technology (no. 20120533Q03) and the Specialized Research Fund for the Doctoral Program of Higher Education (no. 20124323120004).

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