Maternally Inherited Essential Hypertension Is Associated With the Novel 4263A>G Mutation in the Mitochondrial tRNA^{Ile} Gene in a Large Han Chinese Family

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Rational: Despite maternal transmission of hypertension in some pedigrees, pathophysiology of maternally inherited hypertension remains poorly understood.

Objective: To establish a causative link between mitochondrial dysfunction and essential hypertension.

Method and Results: A total of 106 subjects from a large Chinese family underwent clinical, genetic, molecular, and biochemical evaluations. Fifteen of 24 adult matrilineal relatives exhibited a wide range of severity in essential hypertension, whereas none of the offspring of affected fathers had hypertension. The age at onset of hypertension in the maternal kindred varied from 20 years to 69 years, with an average of 44 years. Mutational analysis of their mitochondrial genomes identified a novel homoplasmic 4263A>G mutation located at the processing site for the tRNA^{Ile} 5'-end precursor. An in vitro processing analysis showed that the 4263A>G mutation reduced the efficiency of the tRNA^{Ile} precursor 5'-end cleavage catalyzed by RNase P. tRNA Northern analysis revealed that the 4263A>G mutation caused a 46% reduction in the steady-state level of tRNA^{Ile}. An in vivo protein-labeling analysis showed a 32% reduction in the rate of mitochondrial translation in cells carrying the 4263A>G mutation. Impaired mitochondrial translation is apparently a primary contributor to the reductions in the rate of overall respiratory capacity, malate/glutamate-promoted respiration, succinate/glycerol-3-phosphate-promoted respiration, or N,N,N',N'-tetramethyl-p-phenylenediamine/ascorbate-promoted respiration and the increasing level of reactive oxygen species in cells carrying the 4263A>G mutation.

Conclusions: These data provide direct evidence that mitochondrial dysfunction caused by mitochondrial tRNA^{Ile} 4263A>G mutation is involved in essential hypertension. Our findings may provide new insights into pathophysiology of maternally transmitted hypertension. (Circ Res. 2011;108:862-870.)

Key Words: genetics ■ hypertension ■ mitochondria ■ transcription ■ processing

Hypertension is a major public health problem, affecting approximately 1 billion worldwide.1 The etiology of hypertension is not well understood because it is often a multifactorial condition. Hypertension can be caused by single-gene or multifactorial conditions, resulting from interactions between the environment and inherited risk factors.2 In fact, human hypertension is a condition associated with endothelial dysfunction and oxidative stress.3,4 Mitochondrial dysfunction has been potentially implicated in both human and experimental hypertension.5–7 Specifically, abnormal mitochondrial respiration results in oxidative stress, uncoupling of the oxidative pathways for ATP synthesis, and subsequent failure of cellular energetic processes.8 An inefficient metabolism caused by mitochondrial dysfunctions in skeletal and vascular smooth muscles may lead to the elevation of systolic blood pressure and therefore may be involved in the development of hypertension.6,7,9,10 In particular, maternal transmission of hypertension has been implicated in some pedigrees, suggesting that the mutation(s) in mitochondrial (mt)DNA is one of the molecular bases for this disorder.10–16 However, molecular pathogenesis of maternally inherited hypertension remains poorly understood.
As the part of a genetic screening program for essential hypertension in the Chinese population, we performed clinical, genetic, molecular, and biochemical characterization of a large Han Chinese family with maternally transmitted hypertension. Fifteen of 24 adult matrilineal relatives in this 5-generation family exhibited variable severity and age at onset of hypertension. Mutational analysis of the mitochondrial genome has identified the novel 4263A>G mutation in this Chinese family. This 4263A>G mutation is localized at the processing site for the tRNA\textsuperscript{ile} 5′-end precursor, which is catalyzed by the RNase P. Thus, it is anticipated that the 4263A>G mutation affects the 5′-end processing of precursor in the mitochondrial tRNA\textsuperscript{ile}, thereby causing the mitochondrial dysfunction. Functional significance of the 4263A>G mutation was evaluated by examining processing efficiency of tRNA\textsuperscript{ile} precursor, steady-state levels of mitochondrial tRNAs, including tRNA\textsuperscript{ile} by using lymphoblastoid cell lines derived from 3 affected matrilineal relatives carrying the 4263A>G mutation and from 3 control individuals lacking the mtDNA mutation. These cell lines were further assessed for the effects of the 4263A>G mutation on mitochondrial protein synthesis, endogenous respiration, and substrate-dependent respiration as well as the production of reactive oxygen species (ROS).

**Methods**

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

**Subjects**

A Han Chinese family (Figure 1) was ascertained at the Institute of Geriatric Cardiology of Chinese PLA General Hospital, Beijing. Informed consent, blood samples, and clinical evaluations were obtained from all participating family members, under protocols approved by the ethics committee of the Chinese PLA General Hospital and the Cincinnati Children’s Hospital Medical Center Institute Review Board. Members of this family were interviewed and evaluated to identify both personal and medical histories of hypertension and other clinical abnormalities. The 342 control DNA samples were obtained from a panel of unaffected Han Chinese individuals from the same area. Members of this Chinese family underwent a physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography. A physician measured the systolic and diastolic blood pressures of subjects using a mercury column sphygmomanometer and a standard protocol. The first and the fifth Korotkoff sounds were taken as the examination blood pressure. Hypertension was defined according to the recommendation of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC VI) and the World Health Organization–International Society of Hypertension as a systolic blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of ≥90 mm Hg.

**Clinical Presentation**

The proband (III-14) developed hypertension at the age of 45 years. She presented to the Geriatric Cardiology Clinic of Chinese PLA General Hospital for further clinical evaluations at the age of 48 years. Her blood pressure was 140/90 mm Hg. Physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography showed no other clinical abnormalities, including diabetes, vision or hearing impairments, or renal or neurological disorders. Therefore, she exhibited a typical essential hypertension. The family originated from Shanxi in Northern China. All members of this family were interviewed and/or evaluated to identify both personal and medical histories of hypertension and other clinical abnormalities. As shown in Figure 1 and the Table, 15 of 27 matrilineal relatives had a wide range of severity in hypertension (with blood pressure greater than 140/90 mm Hg, even with treatment for hypertension), whereas only 7 of 81 nonmaternal relatives had hypertension. None of the offspring of affected fathers exhibited hypertension. As shown in the Table, the age at onset of hypertension in the maternal kindred varied from 20 years to 69 years, with an average of 44 years. Notably, the average age at onset of hypertension in this family has changed from 62 years (generation II) to 46 years (generation

**Results**

![Figure 1. The Chinese pedigree with hypertension. Affected individuals are indicated by filled symbols. Arrowhead denotes proband.](Image 61x70 to 541x172)
III) to 23 years (generation IV) (Table). There was no evidence that any member of this family had any other cause to account for hypertension. We further examined the end organ damage on the heart and kidney among 23 matrilineal relatives of this family. As shown in the Table, 4 (II-2, II-7, III-18, and IV-7) matrilineal relatives exhibited left ventricular hypertrophy on the ECG recorded, whereas subject III-11 had premature ventricular contraction. In addition, 7 matrilineal relatives exhibited an increased interventricular septal thickness. Furthermore, the clearance of endogenous creatinine was assessed in 20 matrilineal relatives and 10 control subjects. As shown in the Table, the rates of endogenous creatinine clearance in 3 subjects (II-2, II-5, and II-7) were below the standard levels, implicating the renal dysfunction in these patients. However, none of other clinical abnormalities was observed in the maternal kindred.

**Identification of the 4263A>G Mutation in the Mitochondrial tRNA^ile Gene**

The maternal transmission of hypertension in this family suggested mitochondrial involvement and led us to analyze the mitochondrial genome of matrilineal relatives. For this purpose, the DNA fragments spanning the entire mtDNA of the proband III-14 were PCR-amplified, and each fragment was purified and subsequently analyzed by direct sequence. As shown in Online Table I, we identified 40 variants belonging to the Eastern Asian haplogroup D4j on the maternal lineage.13 Thirty-nine variants were the known polymorphisms, whereas a novel adenine-to-guanine substitution at position 4263 (4263A>G) altered the stop codon TAA of the ND1 mRNA to an equivalent TAG stop codon,21 and, at the same time, caused an A-to-G transition at the 5' end of the tRNA^ile gene (Figure 2B).22 In fact, the 4263A>G mutation lies in the processing site for the tRNA^ile 5' end precursor, which is catalyzed by the RNase P23 and is important for tRNA identity.24 The processing of precursors in mitochondrial tRNAs requires the precise endonucleolytic cleavage at both 5' and 3' ends.17,18 Thus, the 4263A>G mutation may affect the reaction efficiency of the RNase P involved in tRNA^ile 5' end metabolism.

To determine whether the 4263A>G mutation is present in homoplasmy in all matrilineal relatives, the fragments spanning the tRNA^ile gene were PCR-amplified and subsequently digested with Styl because the 4263A>G mutation created...
the site for this restriction enzyme. As shown in Figure 2C, there was no detectable wild type DNA in all available matrilineal relatives, indicating that the 4263A→G mutation was present in homoplasmy in these matrilineal relatives. In addition, this mutation was absent in 342 Han Chinese controls and 100 affected matrilineal relatives from other 50 Chinese families with maternally transmitted essential hypertension. To examine if the 4263A→G mutation affects the tRNAile coding sequence, the 5' and 3' ends of the mitochondrial tRNAile from a Chinese control subject A32 and the proband III-14 cell lines were sequenced after cDNA synthesis, PCR amplification, and cloning as described elsewhere. Indeed, both sequences of tRNAile carried either adenine or guanine at position at 4263, indicating that the 4263A→G mutation did not affect the tRNAile coding sequence.

Alteration in the 5'-End Processing of Mitochondrial tRNAile Precursor

We used an in vitro processing system to determine whether the primary defect arising from the 4263A→G mutation is the perturbed processing of the 5' end of the tRNA by RNase P. The wild-type and mutant tRNAile precursors corresponding to mtDNA at positions 4235 to 4350 (Figure 3A) were prepared by in vitro transcription. Mitochondrial RNase P was reconstituted from purified recombinant proteins MRPP1, MRPP2, and MRPP3. The in vitro processing kinetics of the wild-type and mutant substrates were determined as detailed elsewhere. No qualitative processing alteration of the mutant tRNAile precursor was observed (data not shown). However, the processing efficiency of the mutant tRNAile transcript decreased to ~70% of that of the wild type (Figure 3B). These data indicate that the 4263A→G mutation has a quantitative effect on the 5' end processing of tRNAile precursor, but does not lead to any aberrant processing product.

Marked Decrease in the Level of Mitochondrial tRNAile

To investigate whether the impaired processing of the tRNAile precursor caused by the 4263A→G mutation affects this steady-state level of tRNA, we subjected total mitochondrial RNA from lymphoblastoid cell lines to Northern blots and hybridized them with a digoxigenin (DIG)-labeled oligodeoxynucleotide probes specific for tRNAile and other tRNAs. The other probes were specific for tRNAleu(UUR), tRNAleu(CUN), tRNALys, and tRNAMet as representatives of the whole Heavy (H)-strand transcription unit and tRNASer(UCN) and tRNAGln derived from the Light (L)-strand transcription unit. As shown in Figure 4A, the amount of tRNAile in mutant cells was markedly decreased, as compared to those in control cells. For comparison, the average level of each tRNA in control and mutant cell lines was normalized to the average levels in the same cell line for reference 5S RNA encoded by nuclear genome. As shown in Figure 4B, ~46% reduction in the steady-state level of tRNAile was observed in the mutant III-14 cell line carrying the 4263A→G mutation, as compared with that of a wild type A32 cell line belonging to the same mtDNA haplogroup. In contrast, the average steady state levels of tRNAglu, tRNALeu(UUR), tRNALeu(CUN), tRNASer(UCN), tRNAGln, tRNAile, and tRNAlys in the mutant cell line ranged from 75% to 90% of those in the wild-type cell line.

Mitochondrial Protein Synthesis Defect

To examine whether the failure in tRNA metabolism caused by the 4263A→G mutation impairs mitochondrial translation, cells from cell lines derived from the proband and 2 affected matrilineal relatives carrying the 4263A→G mutation and 3
controls were labeled for 30 minutes with [35S]methionine–[35S]cysteine in methionine-free regular DMEM medium in the presence of 100 μg/mL of emetine which was used to inhibit cytosolic protein synthesis.27 Figure 5A shows typical electrophoretic patterns of the mitochondrial translation products of the mutant and control cell lines. Patterns of the mtDNA-encoded polypeptides of the cells carrying the 4263A>G mutation were qualitatively identical to those of the control cells, in terms of electrophoretic mobility of the various polypeptides. However, cell lines carrying the 4263A>G mutation trended to a decrease in the total rate of labeling of the mitochondrial translation products relative to those of the control cell line. Figure 5B shows a quantification of the results of a large number of labeling experiments and electrophoretic runs, which were carried out using Image-Quant program analysis of appropriate exposures of the fluorograms and normalization to data obtained for the 143B.TK sample. In fact, the overall rates of labeling of the mitochondrial translation products in the cell lines derived from 3 affected individuals (III-11, III-14, III-18) carrying the 4263A>G mutation were decreased 25%, 30% and 40%, with an average of 32% (P=0.026) relative to the mean value measured in the control cell lines.

**Respiration Deficiency**

The endogenous respiration rates of cell lines derived from 3 affected individual (III-11, III-14, III-18) carrying the 4263A>G mutation and 3 controls were measured by determining the O2 consumption rate in intact cells, as described previously.28 As shown in Figure 6A, the rate of total O2 consumption in the lymphoblastoid cell lines derived from 3 affected individuals ranged between 74.9% and 80.6%, with an average reduction of 77.8% (P=0.003) relative to the mean value measured in the control cell lines. To investigate which of the enzyme complexes of the respiratory chain was affected in the mutant cell lines, O2 consumption measurements were carried out on digitonin-permeabilized cells, using different substrates and inhibitors.29 As shown in Figure 6B, in the cell lines derived from 3 affected individuals, the rate of malate/glutamate-driven respiration, which depends on the activities of NADH:ubiquinone oxidoreductase (complex I), ubiquinol–cytochrome c reductase (complex III), and cytochrome c oxidase (complex IV), but usually reflects the rate-limiting activity of complex I,29 was very significantly decreased, relative to the average rate in the control cell lines, by 77% to 80% (78% on the average; P=0.009). Similarly, the rate of succinate/glycerol-
3-phosphate (G3P)-driven respiration, which depends on the activities of complexes III and IV, but usually reflects the rate-limiting activity of complex III, was significantly affected in the mutant cell lines, relative to the average rate in the control cell lines, by 76% to 81% (≈78% on the average; \( P = 0.0063 \)). Furthermore, the rate of \( N,N,N',N''-\text{tetramethyl-p-phenylenediamine} \) (TMPD)/ascorbate-driven respiration, which reflects the activity of complex IV, exhibited a 78% to 82% reduction in complex IV activity (≈80% on the average) in the mutant cell lines relative to the average rate in the control cell lines.

**ROS Production Increases**

The levels of the ROS generation in the vital cells derived from 3 affected matrilineal relatives carrying the 4263A>G mutation and 3 control individuals lacking the mutation were measured with flow cytometry under normal and \( \mathrm{H}_2\mathrm{O}_2 \) stimulation. Geometric mean intensity was recorded to measure the rate of ROS of each sample. The ratio of geometric mean intensity between unstimulated and stimulated with \( \mathrm{H}_2\mathrm{O}_2 \) in each cell line was calculated to delineate the reaction on increasing level of ROS under oxidative stress. As shown in Figure 7, the levels of ROS generation in the lymphoblastoid cell lines derived from 3 affected individuals carrying 4263A>G mutation ranged from 113% and 121%, with an average 117% (\( P = 0.0007 \)) of the mean value measured in the control cell lines.

**Discussion**

In the present study, we performed clinical, genetic, and molecular characterizations of a five-generation large Chinese family with essential hypertension. Hypertension as the molecular characterizations of a five-generation large Chinese family with essential hypertension. **11,15,16** This suggests that a mtDNA mutation is the molecular basis for this disorder. Molecular analysis of the mitochondrial genome identified a homoplasmic 4263A>G mutation in the tRNA^{Ile} gene. The following evidence suggests that the 4263A>G mutation is a pathogenic mtDNA mutation that causes a genetic predisposition to essential hypertension. This mutation is only present in the maternal lineage of this pedigree but not in other members of this family and 342 Chinese controls. The 4263A>G mutation is localized at the processing site for the tRNA^{Ile} 5'-end precursor, perturbing the processing of the tRNA^{Ile} 5'-end precursor. Finally, lymphoblastoid cell lines derived from 3 affected matrilineal relatives of the Chinese family carrying the 4263A>G mutation, compared with 3 wild-type cell lines, exhibited marked reduction in the steady state level of affected tRNA^{Ile}, impairment of mitochondrial translation and deficient respiration.

All 22 human mitochondrial tRNAs including tRNA^{Ile} are transcribed from the precursors of the H- or L-strand polycistronic transcripts. The processing of precursors in mitochondrial tRNAs requires the precise endonucleolytic cleavage at both 5' and 3' ends. Extra nucleotides at their 5' termini are removed by RNase P, whereas the excision of tRNAs from primary polycistronic mitochondrial transcripts at their 3' end is catalyzed by the 3' endonuclease. Thus, it was anticipated that the A-to-G transition at position 4263 in the H-strand led to defective tRNA^{Ile} 5'-end processing in the H-strand transcripts. The observation that the 4263A>G mutation caused ≈30% reduction in the efficiency of the 5'-end processing of tRNA^{Ile} precursor strongly indicated that the primary defect arising from the 4263A>G mutation was the perturbed processing of the tRNA^{Ile} 5'-end precursor. There is increasing evidence showing that the 5'- and 3'-end processing defects arising from pathogenic mitochondrial tRNA mutations could contribute to clinical abnormalities. The deafness-associated 7445T>C mutation in the precursor of the tRNA^{Ser(UCC)} gene and the cardiomyopathies-associated 4269A>G and 4295A>G mutations in the tRNA^{Ile} gene altered 3'-end processing efficiency of corresponding tRNAs. Similarly, the mitochondrial encypha-
lomyopathy, lactic acidosis, stroke-like symptoms (MELAS)-associated 3243A/H11022G and 3271T/H11022C mutations and mitochondrial myopathy-associated 3302A/H11022G mutation in the tRNA Leu(UUR) led to the tRNA 5'/H11032-end processing defects.36–38

In the present investigation, 46% reduction in the steady-state level of tRNAIle was observed in the mutant cell line carrying the 4263A>G mutation, relative to that of a wild type cell line belong to the same mtDNA haplogroup. The reduced level of tRNAIle in cells carrying the 4263A>G mutation most likely resulted from a defect in 5'-end processing of tRNAIle precursor, similar to the 4401A>G mutation in the junction between tRNA Met and tRNAGln genes.15 Alternatively, the mutant tRNAIle may be metabolically less stable and more subject to degradation, thereby lowering the steady state level of tRNAIle. It is interesting to note that the steady state levels of tRNAGln, tRNALeu(UUR), tRNALeu(CUN), tRNASer(UCN), tRNAMet, and tRNALys in the mutant cell line reduced from 10% to 25%, as compared with those in the wild-type cell line. It is likely that the reduced level of tRNAIle may mediate mitochondrial tRNA metabolism, thereby lowering the levels of those mitochondrial tRNAs, as in the case of the tRNALeu(UUR) A3243G mutation.38 Furthermore, the mutation in TRMU, encoding a 5-methylaminomethyl-2-thiouridylate-methyltransferase responsible for the biosynthesis of 5-taurinomethyl-2-thiouridine (m5s2U) of mitochondrial tRNALys, tRNAGlu, and tRNAGln in the wobble position, not only lowered the steady-state levels of tRNALys, tRNAGlu, and tRNAGln but also those of other tRNAs, such as tRNALeu(UUR), tRNASer(UCN), tRNAMet, and tRNALys.39

A shortage of the tRNAIle leads to the reduced rate of mitochondrial protein synthesis. These defects appear to be responsible for the reduced activities of the mitochondrial respiration chain. Subsequently, these defects result in the reduction of ATP production and an increase of reactive oxygen species production. These mitochondrial dysfunctions may contribute to the development of hypertension.7,10,40–44

The homoplasmic form, mild mitochondrial dysfunction, late onset, and incomplete penetrance of hypertension observed in this Chinese family carrying the 4263A>G mutation suggest that the mutation is an inherited risk factor necessary for the
development of hypertension but may by itself be insufficient to produce a clinical phenotype. Indeed, the incomplete penetrance of other clinical abnormalities arises from homoplasmic mtDNA mutations such as hypertension-associated mtDNA 4435A>G and 4401A>G mutations,\textsuperscript{15,16} deafness-associated 12S rRNA 1555A>G mutation,\textsuperscript{45} and Leber’s hereditary optic neuropathy-associated ND4 11778G>A mutation.\textsuperscript{46} These homoplasmic mtDNA mutations only exhibited mild mitochondrial dysfunction.\textsuperscript{15,16,45,47} The other modifier factors such as nuclear modifier genes, environmental and epigenetic factors, and personal lifestyles\textsuperscript{43,48} may also contribute to the development of hypertension in these subjects carrying the 4263A>G mutation. In particular, the tissue specificity of this mutation is likely attributable to tissue-specific RNA processing or the involvement of nuclear modifier genes.

In summary, the present study provides the clinical, genetic, molecular, and biochemical evidence that the novel mitochondrial tRNA\textsuperscript{Ile} 4263A>G mutation is associated with essential hypertension in a Chinese family. However, the tissue specificity of this mutation is likely attributable to the tissue-specific RNA processing or the contribution of nuclear modifier genes. The 4263A>G mutation should be added to the list of inherited risk factors for future molecular diagnosis. Thus, our findings may provide the new insights into the understanding of pathophysiology and valuable information for management and treatment of maternally inherited hypertension. Future research should further explore the emerging link among hypertension, mitochondrial dysfunction, and their cause/effect relationship.

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Disclosures

None.

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**Novelty and Significance**

**What Is Known?**

- Maternal transmission of hypertension has been observed in a subset of familial systemic arterial hypertension.
- Matrilineal pattern of transmission is attributable to the inheritance of mitochondria from oocyte during fertilization.
- Mitochondrial dysfunction may be involved in the development of systemic hypertension.

**What New Information Does This Article Contribute?**

- We describe a large Chinese family with a matrilineal pattern of inheritance of hypertension and show that 65% of the adults exhibited systemic hypertension as the sole clinical phenotype.
- We identified a new tRNA<sup>Thr(UCN)</sup> 4263A→G mutation that perturbed the processing of the tRNA<sup>Thr(UCN)</sup> 5′-end precursor and was associated with a lower level of tRNA<sup>Thr(UCN)</sup>.

- Reduced tRNA<sup>Thr(UCN)</sup> level was associated with impaired protein translation in the mitochondria and reduced activity of the mitochondrial respiration chain. These defects were associated with reduced ATP production and an increase in the production of reactive oxygen species.

Although maternal transmission of systemic hypertension has been observed in some pedigrees, the pathophysiology of maternally inherited hypertension remains poorly understood. Here, we provide direct evidence that mitochondrial dysfunction caused by the mitochondrial tRNA<sup>Thr(UCN)</sup> 4263A→G mutation is responsible for systemic hypertension in a single large Chinese family. These data may provide new insights into the understanding of pathophysiology and valuable information for management and treatment of maternally inherited systemic arterial hypertension.
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