Mitochondrial *tRNA^{Met}* mutation is associated with clinical and biochemical characteristics in primary hypertension

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Abstract. Mitochondrial DNA mutations have been increasingly associated with various diseases. An association between the mitochondrial tRNA gene mutation, *tRNA^{Met}*, and primary hypertension has been suggested. In the present study, the association between the *tRNA^{Met}* mutation and the development of primary hypertension was investigated by assessing clinical and biological indicators in 800 patients with primary hypertension. General [gender, age, age of onset, body mass index (BMI) and family history] and clinical data (routine blood counts, blood biochemistry profiles and color Doppler echocardiography) were obtained. Venous blood samples were drawn from all the subjects for the separation of white blood cells (WBCs) and DNA extraction. Mitochondrial *tRNA^{Met}* was amplified using PCR, purified and sequenced; samples identified to have a mutation were sequenced in triplicate for validation. Comparisons were made between 7 hypertensive patients with mutations (0.875%) and 10 age-, gender- and medication-matched hypertensive patients without mutations (controls). A maternal history of hypertension was present in 57.1% of patients with *tRNA^{Met}* mutations and only 20.0% of patients without mutations. Notably, *tRNA^{Met}* mutations were associated with a significantly earlier age of hypertension onset, decreased red blood cell (RBC) counts and hemoglobin (Hb) levels and increased total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C) and glucose levels (all $P<0.05$). Heart structure and function differences were also assessed between the two groups. In conclusion, mitochondrial *tRNA^{Met}* mutations may induce changes in tRNA structure and function, which contributes to the pathogenesis of primary hypertension by disturbing blood lipid metabolism, the steady state of blood cells and cardiac structure and function.

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Introduction

Primary hypertension, the most common cardiovascular disease, is the leading cause of death due to cardiovascular diseases. Hypertension is associated with coronary artery disease, stroke and heart and renal failure, and exhibits a gradually increasing morbidity (1). Primary hypertension is caused by complex interactions among genetic, environmental and lifestyle factors; for example, blood pressure increases with aging, stress and high salt intake. Several genetic variants have been associated with the disease; however, the contribution of genetic factors to the development of hypertension has not yet been fully elucidated (2).

Genetic variation in the mitochondrial genome has previously been associated with several diseases, including mitochondrial myopathies, stroke and Parkinson's disease, normal human adaptations, such as superathletes, and even response to cancer treatments (3-5). Mitochondria, the organelles responsible for cellular energy, contain a genome encoding ~1,000 genes. This genome mutates more rapidly compared with the nuclear genome. Since the first disease-related mitochondrial gene mutation was reported in 1998, >200 mitochondrial variants have been associated with disease; the vast majority are located in tRNA genes (4). Mitochondrial tRNA mutations cause changes in tRNA aminoacylation characteristics, altering tRNA synthesis, structure, stability and interactions with components of the translation machinery. These changes may affect the translation of other RNAs into their corresponding protein products, thereby producing aberrant or non-functional proteins that cause disease states (5).

Such changes have been reported to occur in various forms of heart disease, including primary hypertension (6-8). A study on 20 patients with primary hypertension identified 297 mitochondrial variations, including 15 mutations in tRNA genes, particularly *tRNA^{Met}* (9). Furthermore, Fuentes *et al* (10) reported a significant correlation between hypertension in parents and their offspring, and suggested that mitochondrial

tDNA mutations may be associated with increased blood pressure. According to the Framingham Heart Study (11), 35.2% of families with hypertension had an inherited mitochondrial variant and the effects of these variants were similar for systolic and diastolic blood pressure. Although these studies implicate mitochondrial tDNA mutations in the pathogenesis of primary hypertension, the specific effects of mitochondrial tDNA mutations on the clinical characteristics of patients with primary hypertension remain unknown.

In the present study, clinical data from patients with primary hypertension were obtained and sequencing was used to screen for mitochondrial *tRNA*^{Met} mutations. The clinical features of patients with mutations were analyzed to elucidate the clinical association between mitochondrial *tRNA*^{Met} changes and primary hypertension.

Materials and methods

Study subjects. Patients with primary hypertension (n=800) diagnosed as outpatients or admitted to our hospital (The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China) between January 2010 and December 2011 were enrolled in this study. The study population consisted of 586 males and 214 females aged 20-82 years (mean age, 60.5±12.1 years). Hypertension diagnosis was based on the 1999 WHO/ISH guidelines (12) as follows: i) Patients with a clear primary hypertension history and anti-hypertensive drug administration; and ii) patients with no anti-hypertensive drug administration and systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. Individuals with secondary hypertension, diabetes, hematopoietic disease, myocardial infarction, heart failure, hypertrophic cardiomyopathy, expanding cardiomyopathy, valvular and congenital heart disease, persistent atrial fibrillation, complete atrioventricular block and arrhythmia, nervous system anomaly, chronic obstructive pulmonary disease, renal and hepatic insufficiency, hyperthyroidism or hypothyroidism, malignant tumors or autoimmune diseases were excluded.

Primary hypertensive patients with *tRNA*^{Met} mutations were selected as test cases (test group) and matched patients without *tRNA*^{Met} mutations were used as control cases (control group). Controls were matched with the test cases as follows: i) same gender and an age difference of <5 years; ii) same location, but no genetic relationship; iii) administration of the same type of antihypertensive medication, with a treatment duration difference of ≤1 year and a blood pressure control level difference of ≤10/5 mmHg. This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, and written informed consent was obtained from the patient/the patient's family.

Determination of physiological indices. Questionnaires were completed by the study subjects to provide detailed information on the following: i) general information encompassing name, gender, date of birth, heart rate, height, weight and body mass index (BMI = weight/height^2, kg/m^2); ii) previous disease history and family history of primary hypertension; and iii) auxiliary examinations including fasting blood glucose, blood lipids, total cholesterol (TC), triacylglycerol (TG), high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively), renal function (blood urea nitrogen, BUN; and creatinine, Cr), blood electrolytes (K^+, Na^+ and Mg^{2+}), routine blood (red/white blood cells, RBCs/WBCs; hemoglobin, Hb; and platelets, PLTs) and heart ultrasound (interventricular septal end diastolic thickness, IVSD; left ventricular posterior wall depth, LPWD; left ventricular end diastolic dimension, LVIDD; left ventricular end systolic dimension, LVIDS; left atrial end systolic dimension). Left ventricular mass index (LVMI) was calculated as LVMI = left ventricular weight (LVM)/body surface area (BSA). LVM was calculated as: LVM = 0.8 x [1.04 x (IVSD+LVIDD+LPWD)^3 - LVIDD^3] + 0.6 (13. BSA was calculated as BSA = 0.006 x height (cm) + 0.013 x weight (kg) - 0.15 (14). Finally, cardiac output (CO) and cardiac index (CI) were calculated as CO = stroke volume (SV) x heart rate and CI = CO/BSA, respectively.

Detection of mitochondrial tRNA^{Met} *mutations*. Venous blood samples were drawn from all the subjects for the separation of WBCs and DNA extraction. Mitochondrial DNA was extracted according to the manufacturer's instructions. Mitochondrial DNA-specific primers were designed and synthesized using the following sequences: upstream, 5'-TGGCTCCTTTAACCTCTCCA-3' and downstream, 5'-AAGGATTATGGATGCGGTTG-3'. The extracted DNA samples were amplified using PCR in a 25-μl reaction volume containing 10 μl 2X PCR Master mix and 0.3 μl of each of the 20 μM upstream and downstream primers, which was brought to the required volume with DNA and water. Thermal cycling conditions were as follows: 35 cycles of 94˚C for 30 sec, 60˚C for 30 sec, 72˚C for 45 sec and 72˚C for 10 min. PCR products were separated by 2% agarose gel electrophoresis, followed by purification and sequencing using an ABI PRISM™ 377XL DNA Sequencer (Takara Biotechnology, Dalian, China). DNA sequences were compared by BLAST (The National Center for Biotechnology Information, NCBI; http://blast.ncbi.nlm.nih.gov/Blast.cgi). PCR products identified as carrying mutations were sequenced in triplicate to exclude experimental errors.

Statistical analysis. SPSS17.0 was used for statistical analysis. Measurement data are expressed as the mean ± standard deviation (SD). A two-sample t-test was used to compare differences between measurement data and the χ^2 test was used to compare the family genetic history of the two groups of subjects. Tests were two-sided, with an α level of 0.05 and $P<0.05$ was considered to indicate a statistically significant difference.

Results

tRNA^{Met} *mutation and study population characteristics*. Following mitochondrial DNA sequencing in 800 patients with primary hypertension, 7 patients with *tRNA*^{Met} mutations were identified; 4 males and 3 females aged 45-62 years (mean age, 52.9±5.9 years). Of the 793 patients without *tRNA*^{Met} mutations, 10 age-, gender- and medication-matched individuals were selected as controls. This group comprised 6 males and 4 females aged 44-60 years (mean age, 52.5±5.5 years). Following comparisons of the general information between these two groups, a statistically significant difference in the age of hypertension onset was identified; patients with

Table I. Comparison of general characteristics between hypertensive patients with and without *tRNA^{Met}* mutations.

Variable	n	Onset age (years)	BMI (kg/m^2)	Family history of hypertension, n (%)		
				Maternal	Paternal	Denied any family history
tRNA^{Met} mutation status						
Mutation	7	40.3±2.5	26.6±0.92	4 (57.1)	0	3 (42.9)
No mutation	10	46.3±3.5	26.3±1.15	2 (20.0)	1 (10.0)	7 (70.0)
t-test/χ^2	3.891	0.622			2.825	
P-value	0.001	0.543			0.244	

Measurement data are expressed as the mean ± standard deviation. BMI, body mass index.

Table II. Blood chemistry profiles in hypertensive patients with and without *tRNA^{Met}* mutations.

Variable	n	TC (mM)	TG (mM)	HDL-C (mM)	LDL-C (mM)	Glucose (mM)	BUN (mM)	Cr (mM)	K^+ (mM)	Na^+ (mM)	Mg^{2+} (mM)
tRNA^{Met} mutation status											
Mutation	7	2.96±0.29	5.38±0.29	1.22±0.12	2.86±0.26	4.88±0.42	5.21±0.59	77.5±15.7	4.47±0.43	138.5±14.1	0.87±0.06
No mutation	10	1.28±0.17	4.76±0.21	1.02±0.11	3.08±0.30	5.41±0.29	5.10±0.36	75.5±10.8	4.53±0.36	140.8±7.5	0.93±0.10
t-test		15.050	5.193	3.608	1.572	3.117	0.497	0.314	0.295	0.423	1.472
P-value		0.001	0.001	0.003	0.137	0.007	0.627	0.758	0.772	0.678	0.162

Measurement data are expressed as the mean ± standard deviation. TC, total cholesterol; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; Cr, creatinine.

Table III. Routine blood counts in hypertensive patients with and without *tRNA^{Met}* mutations.

Variable	n	WBC count (x10^9/l)	RBC count (x10^{12}/l)	Hb level (g/l)	PLT count (x10^9/l)
tRNA^{Met} mutation status					
Mutation	7	5.39±0.16	5.04±0.12	133.0±5.0	227.9±17.6
No mutation	10	5.32±0.57	4.83±0.16	145.1±8.3	217.8±13.3
t-test		0.340	2.853	3.444	1.347
P-value		0.738	0.012	0.004	0.198

Measurement data are expressed as the mean ± standard deviation. WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelet.

Table IV. Cardiac ultrasonography measurements in hypertensive patients with and without *tRNA^{Met}* mutations.

Variable	n	LVMI (g/m^2)	IVSD (mm)	LPWD (mm)	LVIDD (mm)	LVIDS (mm)	LA (mm)	SV (ml)	EF (%)	CO (l/min)	CI (l/min/m^2)
tRNA^{Met} mutation status											
Mutation	7	106.5±9.3	11.2±0.4	10.1±0.3	48.3±0.9	35.8±1.4	33.2±2.3	66.5±2.0	59.1±2.6	5.19±0.51	2.92±0.40
No mutation	10	94.0±7.9	11.4±0.4	10.2±0.4	43.8±1.2	28.8±1.5	35.4±2.2	59.9±3.8	71.4±2.5	4.62±0.37	2.63±0.25
t-test		2.993	1.308	0.529	8.280	9.773	1.973	4.142	9.744	2.722	1.876
P-value		0.009	0.210	0.604	0.001	0.001	0.067	0.001	0.001	0.016	0.080

Measurement data are expressed as the mean ± standard deviation. LVMI, left ventricular mass index; IVSD, interventricular septal end diastolic thickness; LPWD, left ventricular posterior wall depth; LVIDD, left ventricular end diastolic dimension; LVIDS, left ventricular end systolic dimension; LA, left atrium; SV, stroke volume; EF, ejection fraction; CO, cardiac output; CI, cardiac index.

tRNA^{Met} mutations had a younger mean age of onset (P<0.05). Additionally, significant differences were observed in BMI and family hypertension history (P<0.05; Table I).

Blood chemistry differs with tRNA^{Met} mutations. To determine whether *tRNA^{Met}* mutations contribute to alterations in various biochemical indicators that have been associated with hyper-

tension, we measured and compared numerous indices between hypertensive patients with and without mutations. Notably, TC, TG, HDL-C and blood glucose levels were significantly higher in patients with $tRNA^{Met}$ mutations compared with the control patients ($P<0.05$; Table II). However, no significant differences were observed in LDL-C, BUN, Cr, K^+, Na^+ and Mg^{2+} levels between the two groups.

Additionally, comparison of routine blood counts indicated significantly lower RBC counts and Hb levels in patients with mutations in $tRNA^{Met}$ compared with the control patients ($P<0.05$; Table III). No statistical differences were observed in WBC or PLT counts between the two groups.

Heart structure and function differ in hypertensive patients according to mutational status. Ultrasonography of the hearts of hypertensive patients with and without $tRNA^{Met}$ mutations was performed to identify potential differences in heart muscle structure or function. Patients with mutations had significantly higher measures for a number of heart features, particularly LVMI, LVIDD, LVIDS, SV and CO. However, the left ventricular ejection fraction (EF) was significantly lower in patients with mutations compared with the control patients ($P<0.05$; Table IV). No statistically significant differences were observed in IVSD, LPWD, left atrium (LA) or CI between the two groups of patients.

Discussion

Mitochondrial DNA mutations have been associated with a variety of cardiovascular diseases, including hypertension (15). However, to the best of our knowledge, the present study is the first to link mitochondrial mutations, specifically in $tRNA^{Met}$, with particular features of the disease, including age of onset and biochemical and heart characteristics. Mutations in $tRNA^{Met}$ were identified to be associated with an earlier age of hypertension onset. Notably, no significant change was observed in the BMI between hypertensive patients with and without mutations, indicating that the early-onset phenomenon was not associated with BMI.

Mitochondria are maternally inherited; therefore, potential mutations in the mitochondrial DNA are inherited from the mother. A previous study identified a $tRNA^{Met}$ mutation that is associated with maternally inherited hypertension (16). Consistent with this finding, we found that a maternal history of hypertension was more common in patients with $tRNA^{Met}$ mutations compared with the control patients; a paternal history of hypertension was absent in patients without $tRNA^{Met}$ mutations.

Alterations in a number of biochemical indicators, including blood glucose, lipids, Cr and BUN, have been associated with or may be risk factors for primary hypertension (17-19). We compared measurements of numerous blood chemistry factors between hypertensive patients with and without $tRNA^{Met}$ mutations. Increases were observed in TC, TG, HDL-C and blood glucose in patients with mutations, indicating that the $tRNA^{Met}$ mutation may be associated with these changes. Previous studies have suggested that $tRNA^{Met}$ mutations affect the overall rate of cellular respiration (20) and changes in cellular respiration may affect the metabolism of cholesterol and other molecules in the blood (21).

Hypertension has also been associated with changes in RBCs (22). RBCs carry oxygen via Hb, aid the elimination of free radicals and maintain electrolyte balance. Lower RBC counts and Hb levels were found in hypertensive patients with $tRNA^{Met}$ mutations compared with the control patients without mutations. Fewer RBCs and a lower Hb concentration lead to reduced blood oxygen content, which may promote the pathogenesis of primary hypertension. Changes in heart structure and function were also identified in hypertensive patients with the $tRNA^{Met}$ mutation. LVMI, LVIDD, LVIDS, SV and CO were all higher in patients with mutations compared with the control patients, while left ventricular EF was lower. These results suggest that changes in mitochondrial $tRNA^{Met}$ structure may cause functional abnormalities that affect the structure and function of the heart.

In conclusion, the mitochondrial $tRNA^{Met}$ mutation was identified to be associated with primary hypertension; structural/functional alterations in tRNA may alter blood lipid metabolism, the steady state of blood cells and the structure and function of the heart.

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