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The tRNA^{Met} 4435A > G mutation in the mitochondrial haplogroup G2a1 is responsible for maternally inherited hypertension in a Chinese pedigree

Zhongqiu Lu^{1,2,6}, Hong Chen^{2,6}, Yanzi Meng^{2,6}, Yan Wang^{2,6}, Ling Xue^{2,6}, Shaoce Zhi¹, Qiaomeng Qiu¹, Li Yang³, Jun Qin Mo⁴ and Min-Xin Guan^{*,2,3,5}

Mutations in mitochondrial DNA (mtDNA) have been associated with hypertension in several pedigrees with maternal inheritance. However, the pathophysiology of maternally inherited hypertension remains poorly understood. We reported here clinical, genetic evaluations and molecular analysis of mtDNA in a three-generation Han Chinese family with essential hypertension. Eight of 17 matrilineal relatives exhibited a wide range of severity in essential hypertension, whereas none of the offsprings of the affected father had hypertension. The age-at-onset of hypertension in the maternal kindred varied from 31 to 65 years, with an average of 52 years. Sequence analysis of mtDNA in this pedigree identified the known homoplasmic 4435A > G mutation, which is located at immediately 3' end to the anticodon, corresponding to the conventional position 37 of tRNA^{Met}, and 41 variants belonging to the Asian haplogroup G2a1. In contrast, the 4435A > G mutation occurred among mtDNA haplogroups B5a, D, M7a2 and J. The adenine (A37) at this position of tRNA^{Met} is extraordinarily conserved from bacteria to human mitochondria. This modified A37 was shown to contribute to the high fidelity of codon recognition, structural formation and stabilization of functional tRNAs. However, 41 other mtDNA variants in this pedigree were the known polymorphisms. The occurrence of the 4435A > G mutation in two genetically unrelated families affected by hypertension indicates that this mutation is involved in hypertension. Our present investigations further supported our previous findings that the 4435A > G mutation acted as an inherited risk factor for the development of hypertension. Our findings will be helpful for counseling families of maternally inherited hypertension.

European Journal of Human Genetics (2011) 19, 1181–1186; doi:10.1038/ejhg.2011.111; published online 22 June 2011

Keywords: hypertension; mitochondria; tRNA metabolism; maternal inheritance; risk factor

INTRODUCTION

Hypertension is a major public health problem, affecting approximately 1 billion worldwide.¹ The etiology of hypertension is not well understood because of multi-factorial causes. Hypertension can be caused by single-gene or multi-factorial 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} Maternal transmission of hypertension has been implicated in some pedigrees, suggesting that the mutation(s) in mitochondrial DNA (mtDNA) is one of the molecular bases for this disorder.^{10–16} In particular, the 4295A > G and 4263A > G mutations

in the tRNA^{Ile} gene, the 4401A > G mutation in the junction between tRNA^{Gln} and tRNA^{Met} genes, as well as the 4435A > G mutation in the tRNA^{Met} gene were associated with essential hypertension.^{14–17}

With the aim of investigating a role of the mitochondrial genome in the pathogenesis of hypertension in the Chinese population, a systematic and extended mutational screening of mtDNA has been initiated in several cohorts of essential hypertension Chinese subjects.^{14–17} In the present study, we performed the clinical, genetic and molecular characterization of another Han Chinese family with suggestive maternally transmitted hypertension. Eight of 17 matrilineal relatives in this family exhibited variable severity and age-at-onset in essential hypertension, while none of the offspring of affected fathers had hypertension. Mutational analysis of the mitochondrial genome in this Chinese family identified the known tRNA^{Met} 4435A > G mutation, which is localized at the 3' end adjacent to the anticodon (position 37) of tRNA^{Met}.^{17–19} The adenine at this position of tRNA^{Met} is extraordinarily conserved from bacteria to human mitochondria. The mitochondrial genome in this Chinese family belonged to the Eastern-Asian haplogroup G2a1,²⁰ while the

¹Emergency Medical Department, The First Affiliated Hospital of Wenzhou Medical College, Zhejiang, China; ²Attardi Institute of Mitochondrial Biomedicine and Zhejiang Provincial Key Laboratory of Medical Genetics, Wenzhou Medical College, Wenzhou, Zhejiang, China; ³Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; ⁴Division of Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; ⁵Department of Genetics, College of Life Sciences, Zhejiang University, Zhejiang, China

*Correspondence: Dr M-X Guan, Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA. Tel: +1 513 636 3337; Fax: +1 513 636 3486; E-mail: min-xin.guan@cchmc.org or gminxin88@zju.edu.cn

⁶These authors contributed equally to this work.

Received 2 November 2010; revised 6 April 2011; accepted 26 April 2011; published online 22 June 2011

4435A>G mutation also occurs in the other mtDNA haplogroups: B5a of a Chinese family with hypertension¹⁶ and D5 of a Chinese family with LHON¹⁸ and a Japanese subject with diabetes.¹⁹ The occurrence of the 4435A>G mutation in these genetically unrelated subjects affected by the hypertension suggests that this mutation is involved in the pathogenesis of hypertension.

SUBJECTS AND METHODS

Subjects

As a part of a genetic screening program for hypertension, a Han Chinese family (Figure 1) was ascertained at the Hypertension Clinic of Wenzhou Medical College. Informed consent, blood samples and clinical evaluations were obtained from all participating family members, under the protocols approved by the ethics committee of Wenzhou Medical College and the Cincinnati Children's Hospital Medical Center Institute Review Board. Members of this family were interviewed and evaluated to identify both personal or medical histories of hypertension and other clinical abnormalities.

Clinical evaluation

Members of this Chinese family underwent a physical examination and laboratory assessment of cardiovascular disease risk factors. A physician measured the systolic and diastolic blood pressures of subjects using a mercury column sphygmomanometer and a standard protocol.^{1,21} The first and the fifth Korotkoff sounds were taken as indicative of systolic and diastolic blood pressure, respectively. The average of three such systolic and diastolic blood pressure readings was 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)¹ and the World Health Organization-International Society of Hypertension²¹ as a systolic blood pressure of ≥ 140 mm Hg and/or a diastolic blood pressure of ≥ 90 mm Hg.

These subjects then underwent a heart function evaluation by electrocardiography (ECG). Signals from the first 10 s of the conventional ECG recording were analyzed automatically in software to quantify all major intervals, axes and voltages, as well as ST segment levels. Initial candidate criteria used for defining these strictly conventional 12-lead ECGs were as follows: left ventricular hypertrophy (LVH) according to traditional Sokolow-Lyon voltage criteria²² (SV1+RV5 or RV6 ≥ 3.5 mV) or gender-specific Cornell voltage (RaVL+SV3 ≥ 2.8 mV in men or ≥ 2.0 mV in women) criteria.²³

Two-dimensional guided M-mode recording was performed from the parasternal window according to the guidelines of the American Society of Echocardiography. The following parameters on the M-mode echocardiogram were evaluated: left ventricular end diastolic diameter (LVDd, cm), interventricular septal diastolic thickness (IVSTD, cm), and left ventricular posterior wall diastolic thickness (LVPWTd, cm). Left ventricular mass (LVM) was calculated according to Devereux's adjusted formula: $LVM = 0.8 \times 1.04 \times [(LVDd + LVPWTd + IVSTD)^3 - LVDd^3] + 0.6$ g.²⁴ Left ventricular mass index (LVMI) was defined as LVM divided by body surface area (BSA) (LVM/BSA , g/m²). BSA was calculated according to the formula $BSA = 0.6 \times \text{height (m)} + 0.0128 \times \text{weight (kg)} - 0.1529$. LVMI > 125 g/m² in males or > 115 g/m² in females was diagnosed as LVH.

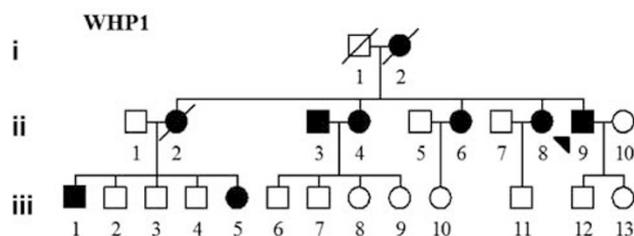


Figure 1 The Chinese pedigree with hypertension. Affected individuals are indicated by filled symbols. Arrowhead denotes the proband.

Mutational analysis of mitochondrial genome

Genomic DNA was isolated from whole blood cells of participants using Puregene DNA Isolation Kits (Gentra Systems, Minneapolis, MN, USA). The entire mitochondrial genome of the proband II-9 was PCR amplified in 24 overlapping fragments by using sets of the light-strand and heavy-strand oligonucleotide primers, as described elsewhere.²⁵ Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA, USA) using the Big Dye Terminator Cycle sequencing reaction kit. The resultant sequence data were compared with the revised consensus Cambridge sequence (GenBank accession number: NC_012920).²⁶

For the quantification of the 4435A>G mutation, the PCR segments (700 bp) were amplified using genomic DNA as template and oligodeoxynucleotides corresponding to mtDNA at positions 3861–4560, and subsequently digested with a restriction enzyme *Nla*III. In fact, the 4435A>G mutation creates a novel site for this enzyme.¹⁸ Equal amounts of various digested samples were then analyzed by electrophoresis through 7% polyacrylamide gel. The proportions of digested and undigested PCR products were determined by the Image-Quant program after ethidium bromide staining to observe whether the 4435A>G mutation is in homoplasmism in these subjects.

Phylogenetic analysis

A total of 17 vertebrate mtDNA sequences were used in the interspecific analysis. These include *Bos Taurus*, *Cebus albifrons*, *Gorilla gorilla*, *Homo sapiens*, *Hylobates lar*, *Lemur catta*, *Macaca mulatta*, *Macaca sylvanus*, *Mus musculus*, *Nycticebus coucang*, *Pan paniscus*, *Pan troglodytes*, *Pongo pygmaeus*, *Pongo abelii*, *Papio hamadryas*, *Tarsius bancanus*, and *Xenopus laevis* (Genbank). The conservation index (CI) was calculated by comparing the human nucleotide variants with those of other 16 vertebrates. The CI was then defined as the percentage of species from the list of 16 different vertebrates that have the wild-type nucleotide at that position.

Haplogroup analyses

The entire mtDNA sequence of the Chinese proband carrying the 4435A>G mutation was assigned to an Asian mitochondrial haplogroup by using the nomenclature of mitochondrial haplogroups.²⁰

RESULTS

Clinical presentation

The proband (II-9) began suffering from hypertension at the age of 54 years. As shown in Table 1, his blood pressure was 190/110 mm Hg by then. He came to the Hypertension Clinic of Wenzhou Medical College for further clinical evaluations at the age of 60 years. After the administration of ACE inhibitor, calcium channel blocker (CCB) and diuretic, his blood pressure was 132/80 mm Hg. As shown in Table 2, laboratory assessment of cardiovascular disease risk factors showed that he had a normal range of the index of liver metabolic function, the blood routine and 24-h urinary sodium. The echocardiogram (ECG) showed that his interventricular septal and posterior ventricular wall thickness (13 mm) increased with normal atrial and ventricular dimension. Physical examination showed that he did not have other clinical abnormalities, including diabetes, visual and hearing impairments, renal and neurological disorders. Therefore, he exhibited a typical essential hypertension. The family is originated from Zhejiang Province in Eastern 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 Table 1, 8 of 17 matrilineal relatives had a wide range of severity in hypertension (with blood pressure $> 140/90$ mm Hg even with treatment for hypertension), whereas only 1 of 8 nonmaternal relatives suffered from hypertension. None of the offspring of affected fathers exhibited hypertension. As shown in Table 1, the age at onset of hypertension in eight affected matrilineal relatives of the maternal kindred varied from 31 to 65 years, with an average of

Table 1 Summary of clinical data for some members in a Chinese pedigree

Subjects	Gender	Age of test (years)	Age of onset (years)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	IVST (mm) (6–12 mm)	LVMl (g/m ²)	ECG	CR (μmol/l)	UR (μmol/l)
I-2	F	65	65	165	110	—	—	—	—	—
II-2 ^a	F	63	60	165	110	—	—	—	—	—
II-3 ^a	M	70	65	170	95	9	123.77	SB	84	5.1
II-4 ^a	F	64	31	200	100	10	84.58	MI	64	6.3
II-6 ^a	F	56	46	150	96	9	77.45	N	58	5.5
II-8 ^a	F	53	53	150	100	8	85.71	MI	55	3.9
II-9 ^a	M	60	54	190	110	12	72.59	LVH	70	5.5
III-1 ^a	M	57	54	140	90	10	97.99	N	75	7.1
III-3	M	48	—	120	80	8	59.74	N	80	7.2
III-4	M	46	—	130	80	10	132.66	MI	66	5.8
III-5	F	51	51	140	95	9	89.69	MI	48	4.6
III-6	M	38	—	125	85	—	—	—	—	—
III-7	M	38	—	120	75	—	—	—	—	—
III-8	F	41	—	130	80	8	97.15	ST-E	63	5.9
III-9	F	30	—	115	85	—	—	—	—	—
III-10	F	33	—	125	80	—	—	—	—	—
III-11	M	26	—	135	75	—	—	—	—	—

Abbreviations: F, female, M, male; IVST, interventricular septal thickness; LVMl, left ventricular mass index; N, electrocardiography (ECG) was normal; LVH, ECG showed left ventricular hypertrophy; MI, myocardial ischemia; SB, sinus bradycardia; ST-E, ST segment elevation; CR, creatinine; UR, urea nitrogen.

^aThese patients had antihypertension treatment. This table shows pre-treatment blood pressures.

Table 2 Summary of laboratory examinations for some members of a Chinese family

Subjects	II-4	II-6	II-8	II-9	Chinese reference
Therapy	Yes	Yes	Yes	Yes	—
Alcohol	No	No	No	Yes	—
Tobacco	No	No	No	Yes	—
FPG, mmol/l	5.6	4.4	4.6	4.8	3.90–6.10
TC, mmol/l	5.22	4.3	5.68	3.8	2.44–5.17
TG, mmol/l	1.92	1.76	1.13	1.1	0.40–1.70
HDL, mmol/l	1.34	1.91	1.74	1.13	1.16–1.42
LDL, mmol/l	3.04	1.45	2.97	2.2	2.10–3.10
UA, μmol/l	415	268	226	358	214–488 (137–363) ^a

Abbreviations: FPG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; UA, uric acid.

^aReference value especially for females.

52 years. However, other nine unaffected matrilineal relatives, who were below 52 years, had a tendency to develop the hypertension. 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 nine matrilineal relatives and married-in subject II-3 of this family. As shown in Table 1, four (II-4, II-8, III-4 and III-5) matrilineal relatives exhibited myocardial ischemia on the ECG recorded, while subject III-8 suffered from segment elevation. In addition, none of eight matrilineal relatives, except the proband, exhibited an increased interventricular septal thickness. Furthermore, the clearance of endogenous creatinine and level of urea nitrogen were assessed in 9 matrilineal relatives and 10 control subjects. As shown in Table 1, the rates of endogenous creatinine clearance in nine subjects (II-3, II-4, II-6, II-8, II-9, III-1, III-4, III-5 and III-8) were below the standard levels, whereas the level of urea nitrogen among seven of nine matrilineal relatives were above the standard levels. These data implicated the presence of renal dysfunction in these patients. However, none of the other clinical abnormalities were observed in the maternal kindred.

mtDNA analysis

The suggestively maternal transmission of hypertension in this family implied the 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 II-6 were PCR amplified, and each fragment was purified and subsequently analyzed by direct sequence. As shown in Table 3, comparison of the resultant sequences with the revised Cambridge consensus sequence²⁶ identified the known hypertension-associated 4435A>G mutation in the tRNA^{Met} gene¹⁷ and other 41 known nucleoside changes, belonging to the Eastern-Asian haplogroup G2a1.²⁰ Of other mtDNA variants of the proband II-9, there were 12 polymorphisms in the D-loop region, 2 variants in the 12S rRNA gene, 1 variant in the 16S rRNA gene, the 5601C>T mutation in the tRNA^{Ala} gene, the 12280A>G mutation in the tRNA^{Leu(CUN)} gene, the 17 silent mutations and 6 missense mutations in protein encoding genes (<http://www.mitomap.org> or <http://www.genpat.uu.se/mtDB>).^{13,27} These missense mutations were the 4833A>G (122T>A) in the ND2 gene, the 8701A>G (59T>A) and 8860A>G (112T>A) in A6 gene, the 10398A>G (114T>A) in the ND3 gene, and the 14766C>T (7T>I) and 15326A>G (194T>A) in the Cytb gene. These variants in tRNAs, rRNAs and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from 16 vertebrates including mouse,²⁸ bovine,²⁹ and *Xenopus laevis*.³⁰ The conservation index (CI) was calculated by comparing the human nucleotide variants with 16 other vertebrates. CIs of these variants including the tRNA^{Ala} 5601C>T and tRNA^{Leu(CUN)} 12280A>G were <70% CI, which was below the threshold level to be functionally significant in terms of mitochondrial physiology, as proposed by Wallace.³¹ This suggests that these variants may not be functionally significant. Based on the nomenclature of mitochondrial haplogroups,²⁰ we used the mtDNA sequence variations of the Chinese proband to establish the haplogroup affiliation of his mtDNA. Here, mtDNA of this pedigree belonged to the Eastern-Asian haplogroup G2a1.

The known 4435A>G mutation in the tRNA^{Met} gene, as shown in Figure 2, is located at immediately 3' end to the anticodon,

Table 3 mtDNA variants in one Han Chinese subject (II-6) with hypertension

Gene	Position	Replacement	Conservation H/M/B/X ^a	Previously reported ^b	
D-Loop	73	A to G		Yes	
	152	T to C		Yes	
	263	A to G		Yes	
	310	T to CTC		Yes	
	489	T to C		Yes	
	16 223	C to T		Yes	
	16 227	A to G		Yes	
	16 272	A to G		Yes	
	16 278	C to T		Yes	
	16 319	G to A		Yes	
	16 362	T to C		Yes	
	16 519	T to C		Yes	
	12S rRNA	709	G to A	G/A/A/A	Yes
		750	A to G	A/G/A/A	Yes
1438		A to G	A/A/A/G	Yes	
16S rRNA	2706	A to G	A/A/G/A	Yes	
	4435	A to G	A/A/A/A	Yes	
tRNA ^{Met}	4769	A to G		Yes	
	4833	A to G (Thr to Ala)	T/I/I/L	Yes	
	5108	T to C		Yes	
tRNA ^{Ala}	5601	C to T	C/C/C/G	Yes	
CO1	7028	C to T		Yes	
CO2	7600	G to A		Yes	
ATP6	8701	A to G (Thr to Ala)	T/S/L/Q	Yes	
	8860	A to G (Thr to Ala)	T/A/A/T	Yes	
CO3	9377	A to G		Yes	
	9540	T to C		Yes	
	9575	G to A		Yes	
ND3	10 398	A to G (Thr to Ala)	T/T/T/A	Yes	
	10 400	C to T		Yes	
ND4	10 873	T to C		Yes	
	11 719	G to A		Yes	
tRNA ^{Leu(CUN)}	12 280	A to G	A/G/A/A	Yes	
ND5	12 705	C to T		Yes	
	13 563	A to G		Yes	
	14 034	T to C		Yes	
ND6	14 569	G to A		Yes	
CYB	14 766	C to T (Thr to Ile)	T/S/T/S	Yes	
	14 783	T to C		Yes	
	15 043	G to A		Yes	
	15 301	G to A		Yes	
	15 326	A to G (Thr to Ala)	T/M/I/I	Yes	

^aConservation of amino acid for polypeptides or nucleotide for rRNAs, in human (H), mouse (M), bovine (B), and *Xenopus laevis* (X).

^bSee <http://www.mitomap.org> and <http://www.genpat.uu.se/mtDB/>.

corresponding to the conventional position 37 of tRNA^{Met}.³² An adenine at this position is an extraordinarily conserved base in every sequenced methionine tRNA from bacteria to human mitochondria.^{32,33} The nucleotide at the position 37 is more prone to modification than those at other places of tRNA.³⁴ The nucleotide modification at this position has been shown to have a pivotal role in the stabilization of tertiary structure and the biochemical function of tRNA.³⁴ To determine if the 4435A>G mutation is present in homoplasmy, the fragments spanning the tRNA^{Met} gene were PCR-amplified and subsequently digested with *Nla*III. There was no detectable wild-type DNA in all available matrilineal relatives (data

not shown), indicating that the 4435A>G mutation was present in homoplasmy in these matrilineal relatives.

DISCUSSION

In the present study, we have performed the clinical, genetic and molecular characterization of a Han Chinese family with essential hypertension. The hypertension as a sole clinical phenotype was only present in all matrilineal relatives of this three-generation pedigree. Clinical and genetic evaluation revealed the variable severity and age at onset in hypertension. In particular, the age at onset of hypertension in the affected matrilineal relatives in this family varied from 31 to 65 years, with an average of 52 years. This result was comparable to those of other Chinese families with maternally transmitted hypertension.^{14,15,16,17} Mutational analysis of mitochondrial genome in this family identified the tRNA^{Met} 4435A>G mutation and other 35 variants belonging to the Eastern-Asian haplogroup G2a1.²⁰ On the other hand, the 4435A>G mutation also occurred in the other mtDNA haplogroups B5a, D, M7a2 and J.^{17-19,35} This suggested that the 4435A>G mutation occurred sporadically and multiplied through evolution of the mtDNA. The 4435A>G mutation was associated with essential hypertension in a Chinese family,¹⁷ and other clinical abnormalities including Leber's hereditary optic neuropathy¹⁸ and type 2 diabetes.¹⁹ The occurrence of the 4435A>G mutation in these genetically unrelated subjects affected by the hypertension suggests that this mutation is involved in the pathogenesis of hypertension.

It was anticipated that the 4435A>G mutation resulted in a deficient nucleotide modification at position 37 of tRNA^{Met}, thereby altering the structure and function of tRNA^{Met}. Functional significance of the 4435A>G mutation was supported by the fact that ~40–50% reduction in the levels of tRNA^{Met} was observed in cells carrying the 4435A>G mutation.^{17,18} As a result, a failure in the tRNA^{Met} metabolism is responsible for the reduced rate of mitochondrial protein synthesis. Subsequently, these defects led to an impairment of the mitochondrial respiration chain function, reduction of ATP production and increase of reactive oxygen species production. These mitochondrial dysfunctions may contribute to the development of hypertension.^{7,10,15,36-38} In particular, the impaired mitochondrial function could contribute to the characteristic age-related increase in blood pressure.³⁹ The homoplasmic form, mild mitochondrial dysfunction, late onset and incomplete penetrance of hypertension observed in this Chinese family carrying the 4435A>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 4401A>G mutation,¹⁵ deafness-associated 12S rRNA 1555A>G mutation⁴⁰ and Leber's hereditary optic neuropathy-associated ND4 11778G>A mutation.⁴¹ These homoplasmic mtDNA mutations only exhibited mild mitochondrial dysfunction.^{15,16,40,42} The other modifier factors such as nuclear modifier genes, environmental and epigenetic factors, and personal lifestyles^{39,43} may contribute to the development of hypertension in these subjects carrying the 4435A>G mutation. In particular, the tissue specificity of this mutation is likely attributed to tissue-specific RNA modification or the involvement of nuclear modifier genes. The 4435A>G mutation should be added to the list of inherited risk factors for future molecular diagnosis. Those who are positive for the 4435A>G mutation should be warned that they are at risk for the development of hypertension and therefore pay attention to their personal lifestyles. In conclusion, our data support the

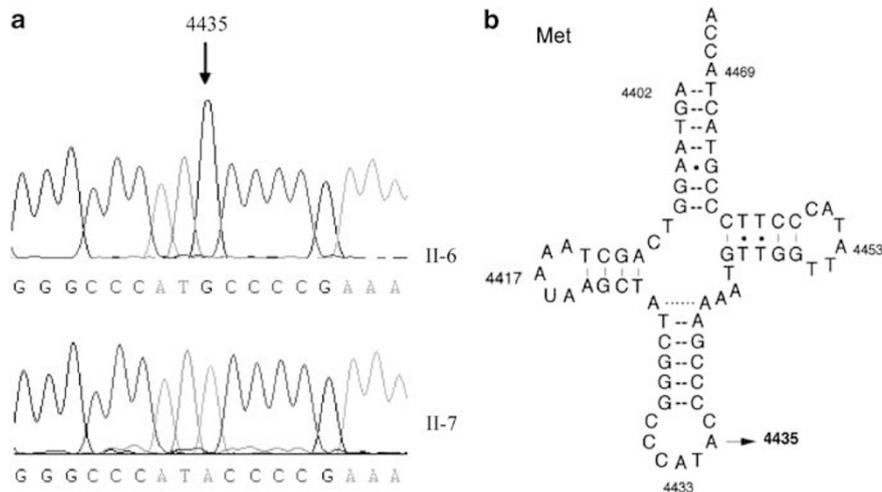


Figure 2 Identification of the 4435A>G mutation in the mitochondrial tRNA^{Met} gene. (a) Partial sequence chromatograms of the tRNA^{Met} gene from affected individual II-6 and a married-in control II-7. An arrow indicates the location of the base changes at position 4435. (b) The location of the 4435A>G mutation in the mitochondrial tRNA^{Met}. The cloverleaf structure of human mitochondrial tRNA^{Met} is derived from Florentz *et al*³². Arrowhead indicates the position of the 4435A>G mutation.

previous observation that impaired mitochondrial function caused by the 4435A>G tRNA^{Met} mutation was associated with essential hypertension. Therefore, our findings will be helpful for counseling families of maternally inherited hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by National Institutes of Health (NIH) grants RO1DC05230 and RO1DC07696 from the National Institute on Deafness and Other Communication Disorders, a start-up fund from Zhejiang University to M-XG.

- Guidelines Subcommittee: World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. *J Hypertens* 1999; **17**: 151–183.
- Lifton RP, Gharavi AG, Geller DS: Molecular mechanisms of human hypertension. *Cell* 2001; **104**: 545–556.
- Romero JC, Reckelhoff JF: State-of-the-Art lecture. Role of angiotensin and oxidative stress in essential hypertension. *Hypertension* 1999; **34**: 943–949.
- Redon J, Oliva MR, Tormos C *et al*: Antioxidant activities and oxidative stress byproducts in human hypertension. *Hypertension* 2003; **41**: 1096–1101.
- Chan SH, Wu KL, Chang AY, Tai MH, Chan JY: Oxidative impairment of mitochondrial electron transport chain complexes in rostral ventrolateral medulla contributes to neurogenic hypertension. *Hypertension* 2009; **53**: 217–227.
- Arrell DK, Elliott ST, Kane LA *et al*: Proteomic analysis of pharmacological preconditioning: novel protein targets converge to mitochondrial metabolism pathways. *Circ Res* 2006; **99**: 706–714.
- Bernal-Mizrachi C, Gates AC, Weng S *et al*: Vascular respiratory uncoupling increases blood pressure and atherosclerosis. *Nature* 2005; **435**: 502–506.
- Wallace DC: A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 2005; **39**: 359–407.
- Wisloff U, Najjar SM, Ellingsen O *et al*: Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* 2005; **307**: 418–420.
- Wilson FH, Hariri A, Farhi A *et al*: A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. *Science* 2004; **306**: 1190–1194.
- Watson Jr B, Khan MA, Desmond RA, Bergman S: Mitochondrial DNA mutations in black Americans with hypertension-associated end-stage renal disease. *Am J Kidney Dis* 2001; **38**: 529–536.
- Schwartz F, Duka A, Sun F, Cui J, Manolis A, Gavras H: Mitochondrial genome mutations in hypertensive individuals. *Am J Hypertens* 2004; **17**: 629–635.
- MITOMAP: A Human Mitochondrial Genome Database. Available at <http://www.mitomap.org>.

- Li Z, Liu Y, Yang L, Wang S, Guan MX: Maternally inherited hypertension is associated with the mitochondrial tRNA^{Ala} A4295G mutation in a Chinese family. *Biochem Biophys Res Commun* 2008; **367**: 906–911.
- Wang S, Li R, Fettermann A *et al*: Maternally inherited essential hypertension is associated with the novel 4263A>G mutation in the mitochondrial tRNA^{Leu} gene in a large Han Chinese family. *Circ Res* 2011; **108**: 862–870.
- Li R, Liu Y, Li Z, Yang L, Wang S, Guan MX: Failures in mitochondrial tRNA^{Met} and tRNA^{Gln} metabolism caused by the novel 4401A>G mutation are involved in essential hypertension in a Han Chinese Family. *Hypertension* 2009; **54**: 329–337.
- Liu Y, Li R, Li Z *et al*: The mitochondrial transfer RNA^{Met} 4435A>G mutation is associated with maternally hypertension in a Chinese pedigree. *Hypertension* 2009; **53**: 1083–1090.
- Qu J, Li R, Zhou X *et al*: The novel 4435A>G mutation in the mitochondrial tRNA^{Met} may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation. *Invest Ophthalmol Vis Sci* 2006; **47**: 475–483.
- Guo LJ, Oshida Y, Fuku N *et al*: Mitochondrial genome polymorphisms associated with type-2 diabetes or obesity. *Mitochondrion* 2005; **5**: 15–33.
- Tanaka M, Cabrera VM, Gonzalez AM *et al*: Mitochondrial genome variation in eastern Asia and the peopling of Japan. *Genome Res* 2004; **14**: 1832–1850.
- Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure: The sixth report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure. *Arch Intern Med* 1997; **157**: 2413–2446.
- Sokolow M, Lyon TP: The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. *Am Heart J* 1946; **37**: 161–186.
- Okin PM, Roman MJ, Devereux RB, Kligfield PJ: Electrocardiographic identification of increased left ventricular mass by simple voltage-duration products. *Am Coll Cardiol* 1995; **25**: 417–423.
- Devereux RB, Casale PN, Kligfield P *et al*: Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986; **57**: 450–458.
- Rieder MJ, Taylor SL, Tobe VO, Nickerson DA: Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. *Nucleic Acids Res* 1998; **26**: 967–973.
- Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N: Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* 1999; **23**: 147.
- A Human Mitochondrial Genome Database. Available at <http://www.genpat.uu.se/mtDB>.
- Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA: Sequence and gene organization of mouse mitochondrial DNA. *Cell* 1981; **26**: 167–180.
- Gadaleta G, Pepe G, De Candia G, Quagliariello C, Sbisà E, Saccone C: The complete nucleotide sequence of the *Rattus norvegicus* mitochondrial genome: cryptic signals revealed by comparative analysis between vertebrates. *J Mol Evol* 1989; **28**: 497–516.
- Roe A, Ma DP, Wilson RK, Wong JF: The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *J Biol Chem* 1985; **260**: 9759–9774.
- Wallace DC: A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 2005; **39**: 359–407.
- Florentz C, Sohm B, Tryoen-Toth P, Putz J, Sissler M: Human mitochondrial tRNAs in health and disease. *Cell Mol Life Sci* 2003; **60**: 1356–1375.

- 33 Sprinzl M, Horn C, Brown M, Ioudovitch A, Steinberg S: Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res* 1998; **26**: 148–153.
- 34 Björk GR: Biosynthesis and function of modified nucleotides; in D. Söll UL, RajBhandary (eds): *tRNA: Structure, Biosynthesis and Function*. Washington, DC: ASM Press, 1995, pp 165–206.
- 35 Herrstadt C, Elson JL, Fahy E *et al*: Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the Major African, Asian, and European haplogroups. *Am J Hum Genet* 2002; **70**: 1152–1171.
- 36 Postnov YV, Orlov SN, Budnikov YY, Doroschuk AD, Postnov AY: Mitochondrial energy conversion disturbance with decrease in ATP production as a source of systemic arterial hypertension. *Pathophysiology* 2007; **14**: 195–204.
- 37 Lopez-Campistrous A, Hao L, Xiang W *et al*: Mitochondrial dysfunction in the hypertensive rat brain: respiratory complexes exhibit assembly defects in hypertension. *Hypertension* 2008; **51**: 412–4129.
- 38 Addabbo F, Montagnani M, Goligorsky MS: Mitochondria and reactive oxygen species. *Hypertension* 2009; **53**: 885–892.
- 39 Vasan RS, Beiser A, Seshadri S *et al*: Residual lifetime risk for developing hypertension in middle-aged women and men: The Framingham Heart Study. *JAMA* 2002; **287**: 1003–1010.
- 40 Guan MX: Mitochondrial 12S rRNA mutations associated with aminoglycoside ototoxicity. *Mitochondrion* 2011; **11**: 237–245.
- 41 Qu J, Zhou X, Zhang J *et al*: Extremely low penetrance of Leber's hereditary optic neuropathy (LHON) in eight Han Chinese families carrying the ND4 G11778A mutation. *Ophthalmology* 2009; **116**: 558–564.
- 42 Hoffhaus G, Johns DR, Hurkoi O, Attardi G, Chomyn A: Respiration and growth defects in transmitochondrial cell lines carrying the 11778 mutation associated with Leber's hereditary optic neuropathy. *J Biol Chem* 1996; **22**: 13155–13161.
- 43 Djousse L, Driver JA, Gaziano JM: Relation between modifiable lifestyle factors and lifetime risk of heart failure. *JAMA* 2009; **302**: 394–400.



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