Mitochondrial tRNA mutations in Chinese Hypertensive

Individuals

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ABSTRACT

Purpose: Hypertension is a very important risk factor for cardiac vascular disease. The previous studies showed that mitochondrial DNA mutations are associated with cardiovascular disease, including hypertension.

Methods: In this study we did systematical analysis on the total 22 mitochondrial tRNAs and the clinical, genetic and molecular changes of 140 Chinese hypertension and 124 controls.

Results: This analysis identified 22 nucleotide changes among 15 different tRNA genes. There are 15 mutations with CI (Conservation index) larger than 75%. Of these, there are 26 patients with CI larger than 75% in the HTN group, higher than the 6 subjects in the control group ($P=0.00$). The tRNA^{Phe} G586A, tRNA^{Lys} G8313A and tRNA^{His} G12147A mutations create highly conservative base-pairings on the D-stem, tRNA^{Lys} G8342A on the T-stem, tRNA^{Phe} T616C, tRNA^{Ala} T5628C, tRNA^{Tyr} G5856A and tRNA^{Thr} A15924G on the AC stem, tRNA^{Leu(CUN)} G12300A on the AC loop, tRNA^{Met} C4467T, tRNA^{Trp} T5578C, tRNA^{Lys} A8296G, tRNA^{Arg} T10463C and tRNA^{Thr} C15891T on ACC stem, and tRNA^{Ser(UCN)} C7492T on D-A junction, while the other tRNA variants were polymorphisms. The pedigrees of PLAH₇₈ carrying the T5578C, PLAH₈₄ carrying the C4467T, PLAH₆₀ carrying the T5628C and PLAH₁₁₈ carrying the C7492T mutation exhibited maternal transmission of essential hypertension. Sequence analysis of their mitochondrial genomes revealed the presence of T5578C, C4467T, T5628C or C7492T mutations but the absence of other functionally significant mutations in all matrilineal relatives of these families.

Conclusions: These tRNAs mutations, associated with altered structures of tRNAs

and mitochondrial dysfunction, may contribute to the hypertension in Chinese population. A lot of work still should be done for the mechanism and functional effect of the mtDNA mutation on hypertension.

Key words: Hypertension; maternal transmission; mitochondrial tRNAs; mutation

1.Introduction

Hypertension (HTN) is a major public health problem, affecting approximately 1 billion worldwide. [Guidelines Subcommittee, 1999] Hypertension is also a major risk factor for coronary heart disease, stroke, congestive heart failure and renal disease. [Chobanian, et al., 2003] Currently essential hypertension (EH) are regarded as a multifactorial disease, and the onset and severity of which are influenced by both genetic and environmental factors. The role of genetic factors in the etiology of hypertension is supported by cross-sectional studies that document familial aggregation of the disorder despite different environmental factors. [Zinner, et al., 1971; Havlik, et al., 1982] Estimates of genetic variance range from 20% to 50%. [Rice, et al., 1989; Longini, et al., 1984; Hunt, et al., 1989] Significant blood pressure (BP) correlations between mothers and offspring or excess maternal transmission of hypertension have been noted in several early [Bengtsson, et al., 1979] and more recent [DeStefano, et al., 2001] investigations, whereas such correlations between fathers and offspring were either marginal or absent in these studies. Our previous studies reported a finding of excess maternal transmission of HTN in hypertensive families. [Liu, et al., 2008; Li, et al., 2007; Liu, et al., 2009; Li, et al., 2009; Shiwen, et al., 2011] By far the most widely studied class of mtDNA point mutations are those affecting single tRNA genes. At the present time, mutations in 19 of the 22 human mitochondrial tRNA gene have been reported in association with disease, although not every one has been validated by mechanistic studies. To investigate the role of mitochondrial genetic defects in the development of hypertension, we carried out a systematic and extended mutational screening of 22 tRNA genes in a cohort of 140

Han Chinese subjects with HTN. Mutational analysis of these tRNA genes in these subjects identified 22 nucleotide changes in 15 different tRNA genes, especially including 3 novel and highly conservative mutations.

2. Materials and methods

2.1. Subjects

There're 264 subjects, including 140 hypertension patients and 124 controls, ascertained at the Institute of Geriatric Cardiology of Chinese PLA General Hospital, as a part of genetic screening program for hypertension. Hypertension was defined according to the recommendation of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC VI) [The sixth report of JNC, 1997] as a systolic blood pressure of 140 mmHg or higher and /or a diastolic blood pressure of 90 mmHg or greater., including patients with history of HTN took antihypertension drugs.

All the subjects were interviewed and evaluated to identify both personal or medical histories of hypertension and other clinical abnormalities. The past history, including coronary heart disease (CHD), cerebrovascular disease, diabetes, hyperlipidemia, renal disease, was also evaluated. Coronary heart disease (CHD) is diagnosed on the medical and family histories, the risk factors for CHD, a physical exam, and the results from tests and procedures by the cardiac physicians. Cerebrovascular disease is defined as a neurologic symptom or symptom complex caused by cerebral ischemia or hemorrhage proved by further examination. Diabetes mellitus was defined as hyperglycemia, requiring anti-diabetic drugs or fasting blood sugar over 126 g/dL.

Hyperlipidemia was defined as plasma low-density lipoprotein cholesterol (LDL-C) >130 mg/dL or total cholesterol (TC) >200 mg/dL or using lipid-lowering drugs at the time of investigation. Renal disease usually is made by blood tests measuring BUN, creatinine, and glomerular filtration rate (GFR) less than 90ml/min. Patients reporting cigarette use within one year prior to examination were considered as smokers. BMI defined as the individual's body mass divided by the square of their height. BMI of 18.5 to 25 kg/m² indicates optimal weight, a BMI lower than 18.5 kg/m² suggests the person is underweight, a number above 25 kg/m² is overweight. DNA samples of the family and 124 healthy controls were acquired for sequence analysis. Informed consents were obtained from all participating members, under protocols approved by the ethics committee of the Chinese PLA General Hospital.

2.2 mtDNA Sequencing and Sequence Analysis

Genomic DNA was isolated from whole blood cells of participants using Puregene DNA Isolation Kits (Gentra Systems, Minneapolis, MN, USA). The entire mitochondrial tRNAs of the HTN and controls were PCR amplified in 24 overlapping fragments by use of sets of the light-strand and the heavy strand oligonucleotide primers, as described elsewhere. [M.J., et al., 1998]. 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 entire mitochondrial genomes of four probands (PLAH_{78-III-2}, PLAH_{84-III-10} and PLAH_{60-II-2}, PLAH_{118-III-8}) were also PCR amplified in 24 overlapping fragments as previous described. The resultant sequence

data were compared with the revised consensus Cambridge sequence (GenBank accession No.NC-012920). [S. Anderson, et al., 1981]

All 22 tRNA mutations were individually analyzed using the published secondary structures for the tRNAs with the stem and loop structure. [Suzuki, et al., 2011; Ruiz-Pesini, et al., 2006]

2.3. Phylogenetic analysis

A total of 17 vertebrates mitochondrial DNA 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*, *Papio hamadryas*, *Pongo abelii*, *Pongo pygmaeus*, *Tarsius bancanus*, and *Xenopus laevis* (Genbank) (Table 2). The conservation index (CI) was calculated by comparing the human nucleotide variants with other 16 vertebrates. The CI was then defined as the percentage of species from the list of 17 different vertebrates that have the wild-type nucleotide at that position.

2.4. Statistical analysis

Statistical analyses were performed with the Statistical Package for Social Sciences software (SPSS version 16.0). Continuous variables with normal distributions were expressed as means $\pm$ SD and compared using a *t* test. Categorical variables were compared using the chi-square test where appropriate.

3. Results

3.1. Clinical evaluation

The clinical data showed in table 1. The average age was 49.2 ± 15.2 years in the HTN group (range from 19 to 88 years) versus 56.5 ± 11.8 years in the control group (range from 28 to 82 years) with significant difference ($P=0.00$). The onset age of hypertension was 45.2 ± 11.9 years, range from 19 to 76 years old. The BMI of the HTN group was higher than the control group (26.5 ± 4.3 versus 23.7 ± 3.2 , $P=0.00$). The SBP and DBP of the HTN were higher than that of the control, 142.6 ± 17.3 versus 118.9 ± 10.9 and 92.1 ± 11.2 versus 76.8 ± 7.1 , respectively, both $P<0.01$. The data also showed that there are 39 hypertension patients complicated with CHD, 41 with cerebrovascular disease, 16 with diabetes, 33 with hyperlipidemia and 5 with renal disease. The laboratory test showed no difference of FBS, sodium, potassium, triglyceride, HDL-c, while TC, LDL-cholesterol and creatinine were higher than that of the control group ($P>0.05$). There're 24 smokers in the hypertension group with no significant difference compared with the control group.

Table 1 clinical data for the hypertensions and the controls

	Hypertensions (n=140)		Controls (n=124)		P value
	Mean $\pm$ SD	(Range)	Mean $\pm$ SD	(Range)	
Men, n (%)	151		30		0.00
Age (years)	49.2 ± 15.2	(19~88)	56.5 ± 11.8	(28~82)	0.00
Onset age(years)	45.2 ± 11.9	(19~76)			
BMI (kg/m ²)	26.5 ± 4.3	(16.02~38.29)	23.7 ± 3.2	(18.07~30.48)	0.00
Systolic BP (mmHg)	142.6 ± 17.3	(100~220)	118.9 ± 10.9	(105~138)	0.00
Diastolic BP (mmHg)	92.1 ± 11.2	(60~128)	76.8 ± 7.1	(54~78)	0.00
<i>Past disease</i>					
CHD	39				
Cerebrovacular disease	41				
Diabetes	16				
Hyerlipidemia	33				
Renal disease	5				
Alcohol	10		9		0.84
Smoking	24		17		0.54

<i>Laboratory test</i>					
FSB (mmol/L)	5.74±1.47	(3.62~19.08)	5.5±1.8		0.16
			(3.94~18.95)		
TC (mmol/L)	4.78±0.97	(2.29~7.55)	4.16±1.0	(2.36~6.84)	0.00
Sodium (mmol/L)	140.57±3.98	(127.0~156.7)	140.4±3.5		0.68
			(130.3~148.4)		
Potassium (mmol/L)	4.13±0.39	(3.18~5.78)	4.37±0.52	(3.46~5.72)	0.00
Triglyceride (mmol/L)	1.56±0.70	(0.59~5.87)	1.56±0.89	(0.74~4.98)	0.99
HDL (mmol/L)	1.20±0.31	(0.49~2.22)	1.21±0.30	(0.70~2.20)	0.76
LDL (mmol/L)	2.79±0.88	(0.74~5.50)	2.11±0.71	(1.08~4.21)	0.00
Creatinine (mol/L)	69.35±15.59	(38.0~133.8)	59.06±11.76		0.00
			(32.3~122.3)		

CHD, coronary heart disease; FSB, fasting blood glucose; TC, total cholesterol; HDL, High-density lipoprotein; LDL, low-density lipoprotein; UN, Urea nitrogen.

3.2. Mutational analysis of mitochondrial 22 tRNA genes

We sequenced the 22 tRNA genes in probands from 140 hypertension patients and 124 healthy controls. All the variants were compared with the data of mitomap (<http://www.mitomap.org>) and mtDB (<http://www.genpat.uu.se/mtDB>). [Wallace, et al., 2003] Comparison with the “Cambridge” reference sequence (CRS) [Anderson, et al., 1981; Andrews, et al., 1999] revealed a total of 22 (9 novel and 13 reported) nucleotide changes in the 15 different tRNA genes (table 2). The novel variants included G586A and T616C in the tRNA^{Phe}, A5823G tRNA^{Cys}, G5856A tRNA^{Tyr}, C7492T tRNA^{Ser(UCN)}, T10031C in the tRNA^{Gly}, C15891T and G15930A in the tRNA^{Thr}, and T16017C in the tRNA^{Pro}, while the known variants were C4467T in the tRNA^{Met}, T5578C in the tRNA^{Trp}, T5628C in the tRNA^{Ala}, A8296G, G8313A, G8342A in the tRNA^{Lys}, T10463C in the tRNA^{Arg}, G12147A in the tRNA^{His}, C12246A in the tRNA^{Ser(AGY)}, G12300A in the tRNA^{Leu(CUN)}, A15924G, G15927A and A15951G in the tRNA^{Thr}. All the nucleotide changes were verified by sequence analysis to be homoplasmic. Among 140 subjects with HTN, 26 probands carried at

least one tRNA variant.

Table 2. Variants in the mitochondrial tRNA genes in 140 Chinese subjects with EHT and 126 controls.

tRNA	Position	Conservation index(%) ^a	Replacement	Watson-Crick basepairs	Numbering of nucleotides in tRNAs ^b		No. of 140 patients(%)	No. of 124 controls(%)	χ^2 p value	Previously reported ^d
tRNA ^{Phe}	586	100	G to A	G-C↓	10	D-stem	1(0.71)	1 (0.79)	1	Yes
	616	100	T to C	A-U↓	39	AC stem	2(1.43)	1 (0.79)	0.690	Yes
tRNA ^{Met}	4467	100	C to T	G-C↓	71	ACC stem	2(1.43)	0(0.00)	0.499	No
tRNA ^{Trp}	5578	100	T to C	A-U↓	72	ACC stem	2(1.43)	0(0.00)	0.499	No
tRNA ^{Ala}	5628	93.8	T to C	A-U↓	31	AC stem	2(1.43)	1(0.79)	0.927	Yes
tRNA ^{Cys}	5823	50	A to G	C-G↑	4	ACC stem	2(1.43)	0(0.00)	0.499	No
tRNA ^{Tyr}	5856	100	G to A	G-C↓	40	AC stem	1(0.71)	0(0.00)	1	No
tRNA ^{Ser(UCN)}	7492	81.2	C to T	A-U↑	27a	D-A junction	2(1.43)	0(0.00)	0.499	Yes
tRNA ^{Lys}	8296	100	A to G	A-U↓	2	ACC stem	1(0.71)	1 (0.81)	1	Yes
	8313	100	G to A	C-G↓	24	D-stem	2(1.43)	1 (0.81)	0.927	Yes
	8342	93.8	G to A	C-G↓	53	T-stem	3(2.14)	1 (0.81)	0.690	Yes
tRNA ^{Gly}	10031	43.8	T to C		44	Variable loop	2(1.43)	0(0.00)	0.499	No
tRNA ^{Arg}	10463	93.8	T to C	U-A↑	67	ACC stem	1(0.71)	0(0.00)	1	Yes
tRNA ^{His}	12147	100	G to A	G-C↓	10	D-stem	2(1.43)	0(0.00)	0.499	Yes
tRNA ^{Ser(AGY)}	12246	50	C to A		54	T-loop	1(0.71)	0(0.00)	1	Yes
tRNA ^{Leu(CUN)}	12300	100	G to A		36	AC loop	1(0.71)	0(0.00)	1	Yes
tRNA ^{Thr}	15891	87.5	C to T	C-G↓	4	ACC stem	1(0.71)	0(0.00)	1	No
	15924	81.2	A to G	U-A↓	39	AC stem	3(2.14)	0(0.00)	0.284	Yes
	15927	56.3	G to A	C-G↓	42	AC stem	1(0.71)	5(4.03)	0.170	Yes
	15930	12.5	G to A		45	Variable loop	2(1.43)	2(1.61)	0.690	No
	15951	62.5	A to G	U-A↓	71	ACC stem	1(0.71)	2(1.61)	0.927	Yes
tRNA ^{Pro}	16017	56.3	T to C	A-U↓	7	ACC stem	1(0.71)	0(0.00)	1	No
CI>75%							26(18.6)	6(4.8)	0.00	

^a The conservation index (CI) was then defined as the percentage of the human nucleotide variants with other 16 vertebrates that have the wild-type nucleotide at that position.

^b Numbers represent the nucleotide positions according to the tRNAdb numbering system (Sprinzl et al., Nucleic Acids Res., 1998, 26(1):148-53) and mitotRNAdb <http://mttrna.bioinf.uni-leipzig.de/mtDataOutput>. Symbol “-” represents nucleotides that are not conserved in the numbering system for canonical tRNAs. ACC, Acceptor; AC, Anticodon; A-D Junction, nucleotides between acceptor stem and D-stem; D-A Junction, nucleotides between D-stem and anticodon stem.

^c See <http://www.genpat.uu.se/mtDB>

^d According to MITOMAP (<http://www.mitomap.org/MITOMAP>). Database of mtDNA Coding Region & RNA Sequence Variants was last edited on Dec 11, 2013, while that of

Reported Mitochondrial DNA Base Substitution Diseases: rRNA/tRNA mutations was last edited on Dec 18, 2013.

3.3.Evaluation of mitochondrial tRNA variants

To identify putative deleterious mutation, these variants were further evaluated using following three criteria: 1). Present <1% in the controls; 2). CI is >75%, proposed by Ruiz-Pesini and Wallace [Ruiz-Pesini, et al., 2006]; 3). Potential structural and functional alterations. First, we used the secondary structure of tRNAs to localize each variant with either a stem or a loop and to analyze if the base changes within stems alter classic Watson-Crick (WC) base pair. There are 17 variants located at the stems of tRNAs, while 5 variants occurred in the loops of tRNAs (figure 1). Here, we identified the A5823G variant in the tRNA^{Cys} contributed to a putative C-G base-pairing and T10463C in the tRNA^{Arg} created to a putative U-A base-pairing, while the other variants in the stem of tRNAs abolished a putative base pairing(s) (Table 2 and Figure 1). In addition, we also performed a phylogenetic analysis by comparing the human tRNA nucleotide variants with those in other 16 vertebrates. As shown in Table 2, CI among the variants ranged from 12.5% (tRNA^{Thr} G15930A variant) to 100% (G586A and T616C in the tRNA^{Phe}, tRNA^{Met} C4467T, tRNA^{Trp} T5578C, tRNA^{Tyr} G5856A, A8296G and G8313A in the tRNA^{Lys}, tRNA^{His} G12147A and tRNA^{Leu(CUN)} G12300A variants). There are 15 variants with CI >75%, 5 variants with CI between 75% and 50% and 2 variants with CI <50%. These variants were then evaluated by examining the allelic frequency in 124 Han Chinese control population. In the 15 variants with CI >75%, there were 9 variants absent in these Chinese control population, including tRNA^{Met} C4467T, tRNA^{Trp} T5578C,

tRNA^{Tyr} G5856A, tRNA^{Ser(UCN)} C7492T, tRNA^{Arg} T10463C, tRNA^{His} G12147A, tRNA^{Leu(CUN)} G12300A, C15891T and A15924G.. Based on these criteria, these 15 variants may have functional significance, including the G586A and T616C in tRNA^{Phe}, tRNA^{Met} C4467T, tRNA^{Trp} T5578C, tRNA^{Ala} T5628C, tRNA^{Tyr} G5856A, tRNA^{Ser(UCN)} C7492T, A8296G, G8313A and G8342A in the tRNA^{Lys}, tRNA^{Arg} T10463C, tRNA^{His} G12147A, tRNA^{Leu(CUN)} G12300A, C15891T and A15924G in the tRNA^{Thr}. The higher *p*-value showed no difference between the hypertension and the control group, due to small size of subjects. But there are 26 patients with CI larger than 75% in the HTN group, higher than the 6 subjects in the control group (*P*=0.00).

Figure 1. Mitochondrial tRNA variants in Chinese subjects with hypertension. Cloverleaf structures of canonical tRNA and nine mitochondrial tRNAs are shown. Circled numbers represent the nucleotide positions according to the conventional tRNA numbering system. Tertiary interactions between nucleotides are indicated by dotted lines. Arrows indicate the position of the tRNA mutations.

3.4. Clinical and genetic evaluation of 4 Chinese Han families presented with maternally inherited hypertension

According to the results of the structure and function analysis of tRNAs, we identified 13 variants might be associated with hypertension, involved 19 families. In these families, there are 4 families presented with maternally inherited hypertension with other families no family history of hypertension or inconsistent with maternal pattern, which was substantiated with the method by Sun et al [Sun, et al., 2003]. The four probands included PLA_{H84} with tRNA^{Met} C4467T, PLA_{H78} with tRNA^{Trp} T5578C, PLA_{H60} with tRNA^{Ala} T5628C, PLA_{H118} with tRNA^{Ser(UCN)} C7492T. The four probands and their family members underwent further physical examination, and laboratory tests. The proband PLA_{H78} was female and her mother (II-2), her sister (III-6) and the son of her sister (IV-6) also presented with hypertension. None of her brother (III-3) or his children (IV-3 and IV-5) affected. The proband PLA_{H84} was male and none of his children (IV-7 and IV-8) presented with hypertension. His mother (II-2), all of his sisters (III-2, III-4, III-6, III-8 and III-9) and one daughter of his sister (IV-3) presented with hypertension. None of his children (IV-7 and IV-8) or his uncle's children (III-12 and III-14) presented with hypertension. The PLA_{H60} was a female and her mother (I-2) and one daughter (III-1) presented with hypertension. Although one of her brothers (II-3) presented with hypertension, none of his children (III-3 and III-4) affected. The proband PLA_{H118} was a female and her mother (II-2), two brother (III-1 and III-2), and three out of her sisters (III-6, III-10 and III-12) presented with hypertension. Her daughter (IV-12) and one son of her sisters (IV-13) also presented with hypertension. None of her brothers' children or her uncle's children affected. All of these four families presented with maternally inherited

pattern.

Figure 2. Four Han Chinese pedigrees with hypertension. Affected individuals are indicated by filled symbols. An arrow denotes probands.

3.5. Mutational analysis of mitochondrial genomes

To evaluate the contribution of mtDNA variants or haplogroups to the phenotypic expression of these putative mtDNA mutations in these Chinese pedigrees, a PCR-amplification of fragments spanning entire mtDNA and subsequent DNA sequence analysis was performed in 4 probands, including PLAH₈₄ with tRNA^{Met} C4467T, PLAH₇₈ with tRNA^{Trp} T5578C, PLAH₆₀ with tRNA^{Ala} T5628C, PLAH₁₁₈ with tRNA^{Ser(UCN)} C7492T. The sequence results from these Chinese subjects were aligned with the updated consensus Cambridge sequence (see table 3). The probands from the four families exhibited distinct sets of mtDNA polymorphisms. These included 23 variants in the D-loop region, 4 known variants in the 12S rRNA gene, 5 known variants in the 16S rRNA gene, the known tRNA^{Ala} T5628C and tRNA^{Ser(UCN)} C7492T, the unknown tRNA^{Met} C4467T and tRNA^{Trp} T5578C, and other 29 known mutations in the polypeptide encoding genes. [Ruiz-Pesini, et al., 2006] All the variants in the RNAs were further evaluated by phylogenetic analysis of these variants and sequences from other 16 organisms including mouse, [Bibb, 1981] bovine, [Gadaleta, 1989] and *Xenopus laevis*. [Roe, et al., 1985] Only the known 12S rRNA T1005C variant of subject PLAH118 had been reported associated with hearing loss with high conservation. These data suggest that the 12S rRNA T1005C variant may have a interaction with the tRNA^{Ser(UCN)} C7492T mutation in the phenotypic manifestation of hypertension. By contrast, none of other variants showed both evolutionary conservation and <1% frequency of 2704 mtDNAs (See <http://www.genpat.uu.se/mtDB>)

Table 3. mtDNA variants in three Han Chinese probands with coronary heart disease

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS	PLAH-78	PLAH-84	PLAH-60	PLAH-118	Previous reported
D-loop	64	C to T		C	T		T		Yes
	73	A to G		A		G	G	G	Yes
	146	T to C		T			C		Yes
	152	T to C		T		C			Yes
	186	C to G		C	G		G		Yes
	204	T to G		T	G				Yes
	235	A to G		A				G	Yes
	248	A to Del		A	Del		Del	Del	Yes
	263	A to G		A	G	G	G	G	Yes
	310	T to C		T		C	C	C	Yes
	489	T to C		T		C			Yes
	494	C to Del		C	Del				Yes
	515	A to Del		A	Del				Yes
	522	C to Del		C	Del				Yes
12SrRNA	750	A to G	A/A/G/-	A	G			G	Yes
	1005	T to C	T/T/T/T	T				C	Yes
	1382	A to C	A/A/A/G	A		C			Yes
16SrRNA	1438	A to G	A/A/A/G	A	G	G	G	G	Yes
	1734	C to T	C/A/T/A	C			T		Yes
	2706	A to G	A/G/A/A	A	G	G	G	G	Yes
	2755	A to G		A			G		Yes
	3010	G to A	G/G/A/A	G		A			Yes
	3107	C to Del	C/T/T/T	C	Del	Del	Del	Del	Yes
ND1	3970	C to T		C	T	T		T	Yes
	4086	C to T		C	T				Yes
	4260	C to A		C		A			Yes
tRNA ^{Met}	4467	C to T				T			No
ND2	4769	A to G		A				G	Yes
tRNA ^{Trp}	5578	T to C		T	C				No

tRNA ^{Ala}	5628	T to C	T//T/T/T	T			C		Yes
COI	6962	G to A		G	A		A		Yes
	7028	C to T		C	T		T	T	Yes
tRNA ^{Ser}	7492	C to T		C				T	Yes
ATPase 8	8414	C to T	L/F/M/L	C		T			Yes
ATPase 6	8701	A to G	T/S/L/Q	A		G			Yes
	8860	A to G	T/A/A/T	A			G	G	Yes
Cyt C III	9824	T to A		T		A			Yes
NADHase 3	10310	G to A		G	A		A	A	Yes
	10398	A to G	T/T/T/A	A		G			Yes
	10400	C to T		C		T			Yes
ND4L	10586	G to A		G				A	Yes
	10609	T to C	M/T/T/T	T	C		C		Yes
NADH4	10873	T to C		T		C			Yes
	11719	G to A		G	A	A	A	A	Yes
ND5	12338	T to C		T				C	Yes
	12406	G to A	V/F/S/F	G	A		A		Yes
	13708	G to A		G				A	Yes
	13928	G to C	S/T/S/T	G	C		C	C	Yes
ND6	14587	A to G		A		G			Yes
	14668	C to T		C		T			Yes
Cyt b	14783	T to C		T		C			Yes
	15043	G to A		G		A			Yes
	15301	G to A		G		A			Yes
	15326	A to G	T/M/I/I	A	G	G		G	Yes
	15823	C to T		C	T				Yes
	15824	A to G		A	G				Yes
D-loop	16129	G to A		G	A				Yes
	16172	T to C		T	C				Yes
	16189	T to C		T			C		Yes
	16223	C to T		C		T			Yes
	16258	A to C		A			C		Yes

16293	A to C	A		C	Yes
16311	T to C	T	C	C	Yes
16362	T to C	T	C		Yes
16519	T to C	T	C	C	Yes

*Conservation of amino acid for polypeptides or nucleotide for RNAs in human (H), bovine (B), mouse (M), and *Xenopus laevis* (X);

†CRS: Cambridge reference sequence;⁵

‡See the online mitochondrial genome database <http://www.mitomap.org> and <http://www.genpat.uu.se/mtDB/>

4. Discussion

In this study, we did complete sequencing analysis of the whole 22 mitochondrial tRNAs and the clinical, genetic and molecular characterization of 140 hypertension and 124 healthy controls. The clinical data showed that the HDL-cholesterol of the hypertension subjects were lower than that of control. Similarly, Wilson et al reported in a large kindred with a metabolic disorder including hypertension, hypercholesterolemia, and hypomagnesemia [Frederick, et al., 2004]. Mutational analysis of mitochondrial tRNA genes identified 22 variants. These variants were further evaluated for the evolutionary conservation, lower frequency in the controls and 2704 mtDNAs, the potential structural and functional alterations and maternal transmission of hypertension in matrilineal relatives carrying one of tRNA mutations. Of these variants, tRNA^{Met} C4467T, tRNA^{Trp} T5578C, tRNA^{Ala} T5628C and tRNA^{Ser(UCN)} C7492T were highly conserved and with potential structural and functional alterations, suggesting that these mutations may be associated with hypertension.

All the probands' families carried with 22 tRNAs were evaluated. Four of them were consistent with maternal pattern. Maternal influences on BP can be explained by X-chromosomal inheritance, chromosomal imprinting [Reik, et al., 2001], gestational [Miller, et al., 1987; Alexander, et al., 2006] mechanisms and mitochondrial disorders [Giles, et al., 1980]. For autosomal inheritance, the morbidity of offspring of affected mothers should be equal to that of the affected fathers. However, none of the offsprings of affected fathers from these families had hypertension, so the autosomal inheritance could be rejected. For X-linked inheritance, only the female could be affected, but all these families had both male and female patients, so X-linked

inheritance could be rejected. As for gestational mechanisms, could be excluded since the ratio of affected offspring of hypertension mother should be less than 50 percent, while those of these families were larger or equal to 50 percent. By far the mitochondrial DNA abnormal is the most likely explanation of etiology of hypertension for these pedigrees.

Human (mammalian) mtDNA encodes 13 essential subunits of the inner membrane complex responsible for OXPHOS. These mRNAs are translated by the mitochondrial protein synthesis machinery, which uses the 22 species of mitochondrial tRNAs (mt tRNAs) encoded by mtDNA. The unique structural features of mt tRNAs distinguish them from cytoplasmic tRNAs bearing the canonical cloverleaf structure. The genes encoding mt tRNAs are highly susceptible to point mutations, which are a primary cause of mitochondrial dysfunction and are associated with a wide range of pathologies. Approximately 200 pathogenic mutations have been mapped to mt tRNA genes (<http://www.mitomap.org/MITOMAP>), emphasizing the importance of mt tRNAs for mitochondrial function. The structure of tRNA can be decomposed into its primary structure, usually visualized as the cloverleaf structure. The acceptor stem with 7-base pair (bp) stem is made by the base pairing of the 5'-terminal nucleotide with the 3'-terminal nucleotide (which contains the CCA 3'-terminal group used to attach the amino acid). The acceptor stem may contain non-Watson-Crick base pairs. The anticodon arm is a 5 to 6 bp stem whose loop contains the anticodon. The D arm is a 4 bp stem ending in a loop that often contains dihydrouridine. The T arm is a 3-5 bp stem containing the sequence TΨC where Ψ is a pseudouridine.

In this study, the C4467T mutation is first reported, which is localized at a highly conserved cytosine (71C) on the acceptor stem of the tRNA^{Ala}. The position is important for the stability and identity of tRNA. The C-to-T transition at this position

by the C4467T mutation is expected to alter the secondary structure and stability of tRNA^{Ala}. Similarly, previous study reported that A3302G at the highly conserved (2U-71A) aminoacyl stem of tRNA^{Leu(UUR)} was associated with abnormal mitochondrial RNA processing and contributed to mitochondrial myopathy. [Maniura-Weber, et al., 2006] We also identified the novel T5578C mutation was localized at a highly conserved thymine (72T) on the acceptor stem of the tRNA^{Trp}, which is important to the secondary structure and stability of tRNA^{Trp}. Silvestri et al [Silvestri, et al., 1994] reported an infant with a maternally inherited cardiomyopathy was associated with tRNA^{Leu(UUR)} C3303T (destablized a highly conservative base-pairing (1G-72C) on the acceptor stem of this tRNA). The other novel reported mutation, tRNA^{Ser(UCN)} C7492T, was localized at a highly conserved 27a D-A junction, where the position is expected to create a highly conservative base-pairing (27U-43A) on the acceptor stem of this tRNA, alter the secondary structure of this tRNA. Finally, tRNA^{Ala} T5628C had been reported before that was associated with chronic progressive external ophthalmoplegia, dysphagia and mild proximal myopathy, which was located on the highly conserved A-U base pair within the anticodon stem of the tRNA^{Ala}. [Spagnolo, et al., 2001] These three novel muatations, as tRNA^{Met} C4467T, tRNA^{Trp} T5578C and tRNA^{Ser(UCN)} C7492T fulfills the suggested criteria for the definition of disease-related nucleotide base change: i) disrupt an evolutionary highly conserved base pair located in the anticodon stem ii) demonstrate a situation of heteroplasmy in patient's blood iii) the mutation was absent in controls and in patients with mitochondrial disorders associated with known mtDNA mutations.

5.Conclusion

The major findings of this study are that tRNAs mutations, associated with altered

structures of tRNAs and mitochondrial dysfunction, may contribute to the hypertension in Chinese population. These variants, tRNA^{Met} C4467T, tRNA^{Trp} T5578C, tRNA^{Ala} T5628C and tRNA^{Ser(UCN)} C7492T were highly conserved and altered structure and function of their tRNAs, thereby causing mitochondrial dysfunctions. These four mutations may be the inherited risk factors for hypertension. Thus, our findings may provide new insights into the understanding of pathophysiology and valuable information for management and treatment of hypertension.

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