

Molecular characterization of mitochondrial transferRNA^{Gln} and transferRNA^{Met} A4401G mutations in a Chinese family with hypertension

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Abstract. Mutations in mitochondrial (mt)transfer (t)RNA (mt-tRNA) have been reported to serve important roles in hypertension. To determine the underlying molecular mechanisms of mt-tRNA mutations in hypertension, the present study screened for mt-tRNA mutations in a Chinese family with a high incidence of essential hypertension. Sequence analysis of the mt-tRNA genes in this family revealed the presence of an A4401G mutation in the glycine-and methionine-tRNA genes, and a G5821A mutation in the cysteine-tRNA (tRNA^{Cys}) gene. The G5821A mutation was located at a position conserved in various species, and disrupted G6-C67 base-pairing. It was hypothesized that the G5821A mutation may decrease the baseline expression levels of tRNA^{Cys}, and consequently result in failure of tRNA metabolism. The A4401G mutation was reported to cause the mitochondrial dysfunction responsible for hypertension. Thus, the combination of G5821A and A4401G mutations may contribute to the high incidence of hypertension in this family. Mt-tRNA mutations may serve as potential biomarkers for hypertension.

Introduction

Hypertension is a primary public health problem, affecting ~1 billion individuals worldwide (1). To date, the etiology of hypertension remains to be fully elucidated due to its multi-factorial nature. Hypertension may be due to one or numerous hereditary, environmental and individual factors. A maternal inheritance pattern of hypertension has previously been identified in certain families, suggesting that a mutation

in mitochondrial (mt) DNA is a cause of this disease (2-4). Previously, various mtDNA point mutations have been associated with hypertension, including the A4435G mutation in the methionine-transfer RNA (tRNA^{Met}) gene (5), A4295G and A4263G mutations in the isoleucine-tRNA (tRNA^{Ile}) gene (6,7), anA1555G mutation in the 12S ribosomal RNA gene (8), a T3308C mutation in the nicotinamide adenine dinucleotide dehydrogenase (ND), subunit 1 gene and a C3303T mutation in the leucine-tRNA, codons UUA/G gene (9,10). Leucine-tRNA, codons CUN A12330G combined with ND, subunit 5 T12338C mutations have previously been reported to associate with hypertension in a Han Chinese family (11).

The present study investigated the involvement of the mitochondrial genome in the pathogenesis of hypertension in the Chinese population. During a systematic and extended mutational screening of mtDNA in a large cohort of subjects admitted to The First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) with hypertension, a single Chinese family with maternally inherited hypertension was identified. The mutational screening of the mt-tRNA gene led to the identification of a homoplasmic A4401G mutation in the conjunction of glycine-tRNA(tRNA^{Gln}) and tRNA^{Met}, and a G5821A mutation in cysteine-tRNA (tRNA^{Cys}) was additionally identified.

Materials and methods

Subjects. As part of genetic screening program for hypertension, a Han Chinese family (Fig. 1) was recruited from The First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China). Informed consent, blood samples and clinical evaluations were obtained from all participating family members, under protocols approved by the ethics committee of Wenzhou Medical University. A total of 300 control DNA samples were obtained from a panel of unaffected Han Chinese individuals from the same area. Members of this family were interviewed and evaluated to determine personal and medical histories, including hypertension and other clinical abnormalities. In addition, a physical examination, laboratory assessment of cardiovascular disease risk factors and routine electrocardiography were performed. A physician

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Table I. Primers for polymerase chain reaction amplification of the mt-tRNA genes.

Target gene	Primer name	Primer sequence (5'-3')	Product size (bp)
tRNA ^{Phe}	MT-1F	CTCCTCAAAGCAATACACTG	802
	MT-1R	TGCTAAATCCACCTTCGACC	
tRNA ^{Val}	MT-2F	CGATCAACCTCACCACCTCT	802
	MT-2R	TGGACAACCAGCTATCACCA	
tRNA ^{Leu(UUR)}	MT-4F	AAATCTTACCCCGCTGTTT	887
	MT-4R	AGGAATGCCATTGCGATTAG	
tRNA ^{Ile}	MT-6F	TGGCTCCTTTAACCTCTCCA	898
tRNA ^{Gln}			
tRNA ^{Met}	MT-6R	AAGGATTATGGATGCGGTTG	814
tRNA ^{Ala}	MT-8F	CTAACCGGCTTTTTTGCCC	
tRNA ^{Asn}			987
tRNA ^{Cys}	MT-8R	ACCTAGAAGGTTGCCTGGCT	
tRNA ^{Ser(UCN)}	MT-11F	ACGCCAAAATCCATTTCACCT	900
tRNA ^{Asp}	MT-11R	CGGGAATTGCATCTGTTTTT	
tRNA ^{Lys}	MT-12F	ACGAGTACACCGACTACGGC	891
	MT-12R	TGGGTGGTTGGTGTAATGA	
tRNA ^{Gly}	MT-15F	TCTCCATCTATTGATGAGGGTCT	866
tRNA ^{Arg}	MT-15R	AATTAGGCTGTGGGTGGTTG	
tRNA ^{His}	MT-18F	TATCACTCTCCTACTTACAG	938
tRNA ^{Ser(AGY)}			
tRNA ^{Leu(CUN)}	MT-18R	AGAAGGTTATAATTCCTACG	1,162
tRNA ^{Glu}	MT-21F	GCATAATTAACTTTACTTC	
	MT-21R	AGAATATTGAGGCGCCATTG	
tRNA ^{Thr}	MT-22F	TGAAACTTCGGCTCACTCCT	
tRNA ^{Pro}	MT-22R	GAGTGGTTAATAGGGTGATAG	

F, forward; R, reverse; mt, mitochondrial; t, transfer; Leu, leucine; UUR, codons UUR; Phe, phenylalanine; Val, valine; Ile, isoleucine; Gln, glutamine; Met, methionine; Ala, alanine; Asn, asparagine; Cys, cysteine; Ser, serine; UCN, codons UCN; AGY, codons AGY; Asp, aspartic acid; Lys, lysine; His, histidine; Thr, threonine; Pro, proline; CUN, codons CUN.

measured the systolic and diastolic blood pressures of subjects using a mercury column sphygmomanometer, following a standard protocol. The first and the fifth Korotkoff sounds were measured as indicators of systolic and diastolic blood pressure, respectively. Hypertension was defined according to the recommendation of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure 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 (12,13).

Genotype analysis of mt-tRNA mutations. Genomic DNA was isolated from the whole blood of subjects using a Gentra Puregene Blood kit (Qiagen, Inc., Valencia, CA, USA). DNA fragments spanning the mt-tRNA genes were amplified by polymerase chain reaction (PCR) using oligodeoxynucleotides, as previously described (14) (Table I). Each fragment was purified and subsequently analyzed by direct sequencing using the ABI PRISM[®]3700 Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and BigDye Terminator version 3.1 Cycle Sequencing kit (Thermo Fisher Scientific, Inc.). The resultant sequence

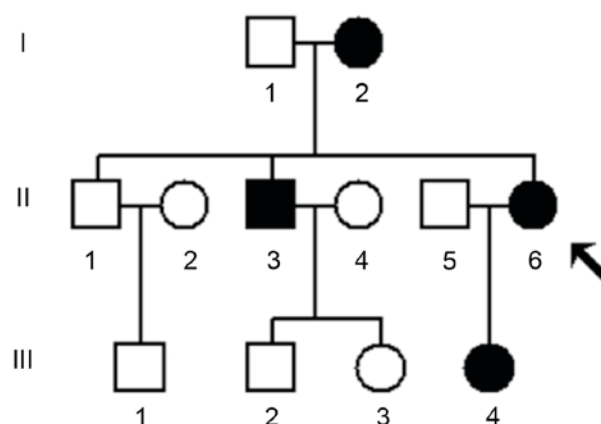


Figure 1. Pedigree of hypertension inheritance patterns in a three-generation Han Chinese family. Individuals with hypertension are represented by filled symbols. The proband is indicated by the arrow. Squares, males; circles, females.

data were compared with the updated Cambridge Reference Sequence (GenBank accession no. NC_012920).

Table II. Clinical features of the Han Chinese family with essential hypertension.

Subject	Gender	Age at onset	Age when assessed	Systolic pressure (mm/Hg)	Diastolic pressure (mm/Hg)
I-2	Female	70	83	145	95
II-6	Female	50	59	140	85
II-3	Male	51	57	150	95
III-4	Female	33	35	140	90

Assigning pathogenicity to the mt-tRNA mutation. The pathogenicity classifications of the A4401G and G5821A mutations were assigned using an updated version of a previously validated scoring system (15). This pathogenicity scoring system uses weighted criteria covering a variety of molecular and genetic data, from which an overall pathogenicity score was obtained. The scoring system for variants was as follows: ≥ 11 points, 'definitely pathogenic'; 7-10, 'possibly pathogenic' and < 6 , 'neutral polymorphism'.

Results

Clinical features. The proband (II-6) was a 59-year-old woman from Wenzhou, Zhejiang. She began to suffer from hypertension at age 50. Her blood pressure was 145/95 mmHg and she attended The First Affiliated Hospital of Wenzhou Medical University for treatment. A physical examination revealed the absence of other clinical abnormalities, including diabetes mellitus, loss of vision or deafness. As presented in Fig. 1, the family of the proband exhibited a typical pattern of maternally-inherited hypertension, with a penetrance of 80%. The clinical features of this Han Chinese family are listed in Table II.

mt-tRNA analysis. The maternal inheritance pattern of hypertension suggested the involvement of mitochondria; therefore, the mitochondrial genome of matrilineal relatives was investigated. As mt-tRNA genes have previously been demonstrated to be hotspots for pathogenic mutations associated with hypertension (16), the present study focused on mt-tRNA variants. As presented in Table I, 11 primers that spanned the entire mt-tRNA genome were designed. Following this, putative mt-tRNA mutations were screened; the comparison of the resultant sequences with the reversed Cambridge consensus sequences identified two candidate tRNA mutations: The A4401G mutation in the conjunction of tRNA^{Gln} and tRNA^{Met} and a mutation G5821A in the tRNA^{Cys} gene (Figs. 2 and 3). These tRNA mutations were further assessed by phylogenetic analysis of sequences from other organisms, including mouse (17), bovine (18) and *Xenopus Laevis* (19). The present study identified that these mutations are highly evolutionarily conserved and may therefore possess significant functions.

Pathogenicity scoring of the A4401G and G5821A mutations. According to the pathogenicity scoring system (15), the present study classified the A4401G mutation as 'definitely pathogenic' with a total score of 15 points, whereas the G5821A mutation was identified to be 'possibly pathogenic' with a total score of 8 points (Table III).

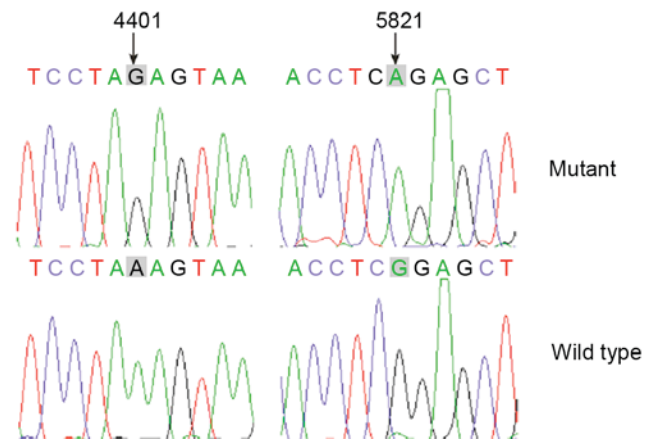


Figure 2. Identification of A4401G and G5821A mutations in the mitochondrial genome. Partial sequence chromatograms of mitochondrial genes from the affected proband (II-6) and a control (II-5). Arrows indicate the location of base changes at positions 4401 and 5821.

Discussion

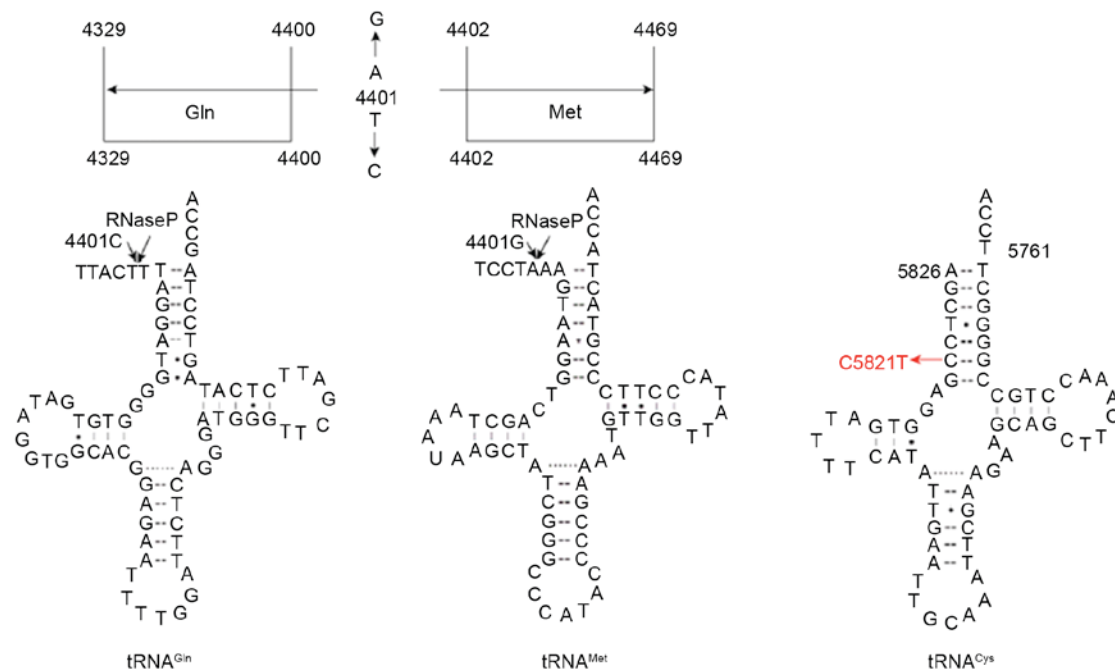
The present study conducted clinical, genetic and molecular analysis of a three-generation Han Chinese family with a high incidence of essential hypertension. Hypertension, with no accompanying conditions, was identified in matrilineal relatives of this family, suggesting that mtDNA mutations were the underlying molecular basis. The age-of-onset of hypertension varied from 33 to 70 years, with an average of 51 years, whereas matrilineal relatives exhibited early-onset hypertension, suggesting that mitochondrial sequence variants may be the risk factor for this disease.

Screening for mt-tRNA mutations identified homoplasmic mutations in the A4401G and G5821A genes. The absence of A4401G and G5821A mutations in the 300 healthy controls indicated that these mutations may be involved in the pathogenesis of hypertension. Notably, the A to G transition at position 4401 was located at the 5' end of the tRNA^{Met} and tRNA^{Gln} genes (20,21). A at the 4401 position is highly conserved among various primates. In addition, this mutation was present only in matrilineal relatives of this family but not in healthy controls, indicating that it may contribute to the pathogenesis of essential hypertension. The A4401G mutation was first described in a patient with left ventricular hypertrophy (22). Functional analysis demonstrated that cytoplasmic hybrid cells containing the A4401G mutation have a 30% reduction in tRNA^{Met} and tRNA^{Gln} expression levels. The reduced levels of tRNA^{Met} and tRNA^{Gln} in cells with the A4401G mutation potentially results from a defect in the

Table III. Pathogenicity scoring system for the A4401 and G5821A mutations.

Scoring criteria	A4401G mutation	Score/20	G5821A mutation	Score/20
More than one independent report	Yes	2	Yes	2
Evolutionary conservation of the base pair	No changes	2	No changes	2
Variant heteroplasmy	No	0	No	0
Segregation of the mutation with disease	Yes	2	Yes	2
Histochemical evidence of mitochondrial disease	Strong evidence	2	No evidence	0
Biochemical defect in complex I, III or IV	Yes	2	No	0
Evidence of mutation segregation with biochemical defect from single-fiber studies	No	0	No	0
Mutant mt-tRNA steady-state level or evidence of pathogenicity in trans-mitochondrial cybrid studies	Strong evidence	5	Weak evidence	2
Maximum score	Definitely pathogenic	15	Possibly pathogenic	8

Scoring system classification: ≥11 points, definitely pathogenic; 7-10 points, possibly pathogenic; ≤6 points, neutral polymorphisms.

Figure 3. Cloverleaf structures of tRNA^{Met}, tRNA^{Gln} and tRNA^{Cys}. Arrows indicate the positions of the 4401C and 4401G mutations. tRNA^{Cys} is localized in the light chain of the mitochondrial genome, thus the G5821A mutation is also known as the C5821T mutation, as indicated in red. tRNA, transfer RNA.

processing of tRNA^{Met} and tRNA^{Gln} precursors at the 5' end. Therefore, A4401G may be regarded as a pathogenic mutation associated with cardiovascular diseases.

Sequence analysis of the mt-tRNA genes identified the presence of a homoplasmic G5821A mutation in the tRNA^{Cys} gene. The G5821A mutation was located in extremely conserved nucleotides in the acceptor arm of tRNA^{Cys}, and disrupted the highly conserved base-pairing (G6-C67). A failure in tRNA^{Cys} function may aggravate the mitochondrial dysfunction caused by the A4401G mutation. A decrease in tRNA^{Met}, tRNA^{Gln} and tRNA^{Cys} may result in reduced mitochondrial protein synthesis, decreased activities of the mitochondrial respiration chain, and consequently a reduction of adenosine triphosphate production and an increase of reactive oxygen species generation. In

conclusion, the results of the present study suggested that the tRNA^{Cys} G5821A gene mutation may regulate the phenotypic expression of hypertension-associated A4401G mutations in tRNA^{Met} and tRNA^{Gln}. mt-tRNA gene mutations may therefore be useful biomarkers for the molecular detection or prevention of maternally inherited hypertension. Further studies involving the use of additional samples are required to verify this conclusion.

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