Original Article
Mitochondrial tRNA[Glu] A14683G may be a novel mutation associated with inherited hypertension

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Abstract: Mutations in mitochondrial genome have been found to play important roles in the pathogenesis of hypertension. We reported here clinical, genetic and molecular characterization of a three-generation Han Chinese family with maternally inherited hypertension. Most strikingly, this family exhibited a high penetrance and expressivity of hypertension, and seven matrilineal relatives in this pedigree manifestated variable degrees of hypertension. Sequence analysis of the complete mitochondrial genome from the affected individuals showed the presence of a novel tRNA[Glu] A14683G mutation and a set of genetic polymorphisms belonging to mitochondrial haplogroup M9a. Interestingly, the homoplasmic A14683G mutation was localized at the highly conserved TψC stem of tRNA[Glu] gene (position 64), which was highly conserved from bacteria to human mitochondria. Moreover, the A14683G mutation created a new Watson-Crick base-paring (64A-50G) and may result a failure in mitochondrial tRNA metabolism. Therefore, it can be speculated that the A14683G mutation may cause the mitochondrial dysfunction that was responsible for hypertension. Taken together, our data indicated that the mitochondrial A14683G mutation should be regarded as a risk factor for hypertension. Thus, our findings provided valuable information for the early detection, management and prevention of maternally transmitted hypertension.

Keywords: Hypertension, mitochondrial tRNA[Glu], A14683G mutation, Chinese family

Introduction

Essential hypertension (EH [MIM 145500]) is one of the most common complex disorders, and genetic factor is a well-known risk factor account for blood pressure (BP) variability [1]. To date, the etiology of EH is not fully understood because of its multifactorial causes. Familial aggregation of high BP, despite different environmental factors, suggests that genetic factors are involved in the etiology of this disorder [2, 3]. In fact, human hypertension is a condition associated with endothelial dysfunction and oxidative stress [4]. Mitochondrial dysfunction has been potentially implicated in both human and experimental hypertension [5]. In particular, abnormal mitochondrial respiration results in oxidative stress, uncoupling of the oxidative pathways for ATP synthesis and subsequent failure of cellular energetic processes [6]. An inefficient metabolism caused by mitochondrial dysfunctions in skeletal and vascular smooth muscles may lead to the elevation of systolic BP and, therefore, may be involved in the development of EH [7, 8]. Maternally transmitted EH is an inherited mode of EH occasionally observed in clinic, and mitochondrial DNA (mtDNA) mutations are suggested to be involved in the genetic risk of EH [9]. Among these mutations, mitochondrial transfer RNA (mt-tRNA) is a hotspot for pathogenic mutations [10]. These mutations included the A4263G in tRNA[Ile] gene [11], A4435G in tRNA[Met] gene [12] and the A4401G mutation in the junction between tRNA[Gln] and tRNA[Met] genes [13].

To understand the molecular basis of mt-tRNA mutations for EH, we recently initiated a systematic and extensive mutational screening for 22 mt-tRNAs in patients with EH. In this study, mutational screening for the mitochondrial genome led us to identify a novel A14683G mutation in tRNA[Glu] gene.
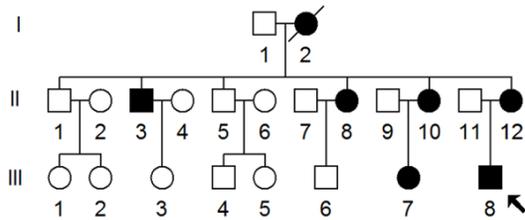
Figure 1. One Han Chinese family with maternally transmitted hypertension. Hypertension individuals are indicated by filled symbols. The arrow indicates the proband.

Materials and methods

Patients

As a part of a genetic screening program for EH, a Han Chinese family (**Figure 1**) was ascertained at the department of Cardiology, Henan Provincial People's Hospital. Informed consent, blood samples and clinical evaluations were obtained from all participating family members, under the protocols approved by the ethics committee of Henan Provincial People's Hospital. Members of this pedigree were interviewed and evaluated to identify both personal or medical histories of hypertension and other clinical abnormalities. In addition, 300 healthy subjects with age- and gender-matched were recruited from the same area. Members of this Chinese family underwent a comprehensive physical examination, laboratory assessment of cardiovascular disease risk factors and routine electrocardiography. A physician measured the systolic and diastolic BP of subjects using a mercury column sphygmomanometer and a standard protocol. The first and the fifth Korotkoff sounds were taken as indicators of systolic and diastolic BP, respectively. The average of 3 such systolic and diastolic BP readings was taken as the examination BP. Notably, hypertension was defined according to the recommendations of the Joint National Committee on Detection, Evaluation and Treatment of High BP (JNCVI) as a systolic BP of 140 mmHg or higher and/or a diastolic BP of 90 mmHg or greater [14].

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 (III-8) and the matrilineal relatives (II-3, II-8, II-10, II-12, III-7) were PCR amplified in 24-overlapping fragments by using sets of the light-strand and heavy-strand oligonucleotide primers, as described in a previous study [15]. 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_012-920) [16].

Evolutionary conservation analysis

A total of 13 vertebrate mtDNA sequences were used in the interspecific analysis. These included Cavia porcellus, Mus musculus, Myoxus glis, Gorilla gorilla, Rattus norvegicus, Homo sapiens, Pan troglodytes, Papio hamadryas, Tarsius bancanus, Lemur catta, Macaca sylvanus, Pongo pygmaeus and Vombatus ursinus. The conservation index (CI) was calculated by comparing the human nucleotide variants with the other 12 vertebrates. The CI was then defined as the percentage of species from the list of 12 vertebrates that have the wild-type nucleotide at that position. We regarded the CI ≥70% as having functional potential [17].

Determining the pathogenicity

To evaluate the potential deleterious role of the tRNA^{Glu} A14683G mutation, we used the following criteria: (1) present in <1% of the health controls; (2) CI ≥70%, proposed by Ruiz-Pesini and Wallace [17]; (3) potential structural and functional alternations for the corresponding tRNA gene.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Differences in categorical variables were assessed with Fisher's exact test. We considered $P < 0.05$ as statistically significant.

Results

Clinical features of the Chinese family with EH

The proband (III-8) was a 42-year-old man who came from Zhengzhou area of Henan Province.

Table 1. Summary of clinical data of several members in this family with hypertension

Subjects	Gender	Age at onset (Year)	Age at test (Year)	Systolic pressure (mmHg)	Diastolic pressure (mmHg)
II-3	Male	55	61	145	90
II-8	Female	57	65	155	80
II-10	Female	61	69	160	95
II-12	Female	61	71	155	85
III-7	Female	37	41	140	100
III-8	Male	31	42	150	95
III-6	Male	/	39	135	70

Table 2. MtDNA sequence variants in this family with EH

Gene	Position	Replacement	Conservation (H/B/M/X)[a]	rCRS	Previously reported[b]
D-loop	73	A to G		A	Yes
	150	C to T		C	Yes
	263	A to G		A	Yes
	16159	A to G		A	Yes
	16363	T to C		T	Yes
12S rRNA	750	A to G	A/A/A/-	A	Yes
	1438	A to G	A/A/A/G	A	Yes
16S rRNA	2706	A to G	A/G/A/A	A	Yes
ND1	3522	C to T		C	Yes
	4491	G to A (Val to Ile)		G	Yes
	4769	A to G		A	Yes
CO1	7028	C to T		C	Yes
A6	8701	A to G (Thr to Ala)		A	Yes
	8860	A to G (Thr to Ala)	T/A/A/T	A	Yes
CO3	9540	T to C		T	Yes
ND3	10398	A to G (Thr to Ala)	T/T/T/A	A	Yes
	10400	C to T		C	Yes
ND4	10873	T to C		T	Yes
	11719	G to A		G	Yes
ND5	12705	C to T		C	Yes
tRNA[Glu]	14683	A to G	A/A/A/A	A	No
CytB	14766	C to T (Thr to Ile)	T/S/T/S	C	Yes
	14783	T to C		T	Yes
	15301	G to A		G	Yes
	15326	A to G (Thr to Ala)	T/M/I/I	A	Yes

[a]Concervation of amino acid for polypeptides or nucleotide for rRNAs, in human (H), mouse (M), bovine (B), and *Xenopus laevis* (X). [b]See http//www.mitomap.org and http://www.genpat.uu.se/mtDB/.

He began to suffer EH when he was 31-year-old, and his BP was 150/95 mmHg. He came to the Department of Cardiology in Henan Provincial People's Hospital for further clinical evaluation. Physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography showed no other clinical abnormalities, including diabetes, vision and hearing impairments, renal and neurological disorders. Therefore, he exhibited a typical EH. As shown in **Figure 1** and **Table 1**, this familial history was consistent with a maternal inheritance, in addition, the age at onset of hypertension in this family varied from 31 to 61 years, with an average of 50 years.

MtDNA mutations analysis

The maternal transmission of hypertension in this family suggested the mitochondrial involvement and led us to analyze the complete mitochondrial genomes of the proband and matrilineal relatives (II-3, II-8, II-10, II-12, III-7). 24-overlapping fragments, including 37 genes, as well as 22 tRNAs were PCR-amplified, purified and subsequently analyzed by DNA sequencing. All variants were compared with the data in Mitomap (www.mitomap.org) [18] and Phylotree (http://www.phylotree.org/). As shown in **Table 2**, the comparison of the resultant sequences with the Cambridge consensus sequence identified a number of nucleoside changes, belonging to the Eastern Asian haplogroup M9a [19]. Of these nucleoside changes, there were 5 polymorphisms in the D-loop region, 2 variants in the 12S rRNA gene, 1 variant in the

Table 3. Sequence alignment of mt-tRNA^{Glu} gene from different species, arrow indicated the position 64, corresponding to the A14683G mutation

Organism	Acc-stem	D-stem	D-loop	D-stem	Ac-Stem	Anticd-loop	Ac-stem	V-region	T-stem	T-loop	T-stem	Acc-stem
Cavia porcellus	GTTTCTGTA	GTTG	AATTA	CAACA	GTGGT	TTTTCAT	ACCAC	TAGT	CATGG	TTAAACT	CCATG	TAGGAATT
Mus musculus	GTTTCTGTA	GTTG	AATTA	CAACG	ATGAT	TTTTCAT	GTCAT	TGGT	CGCAG	TTGAATG	CTGTG	TAGAAATA
Myoxus glis	GTTTTTATA	GTTG	AAATA	CAACG	ATGAT	TTTTCAT	GTCAT	TAGT	CATGG	TTTAATT	CCATG	TAAAAACT
Gorilla gorilla	GTTCTTGTA	GTTG	AAGTA	CAACG	ATGGT	TTTTCAT	ATCAT	TAGT	CGCGG	TCGTGGT	CCGTG	CGAGAATG
Rattus norvegicus	GTTTCTATA	GTTG	AATTA	CAACG	ATGAT	TTTTCAT	GTCAT	TAGT	CACAG	TTAAATG	CTGTG	TAGAAATA
Homo sapiens	GTTCTTGTA	GTTG	AAATA	CAACG	ATGGT	TTTTCAT	ATCAT	TGGT	CGTGG	TTGTAGT	CCGTG	CGAGAATA
Pan troglodytes	GTTCTTGTA	GTTG	AAATA	CAACG	ATGGT	TTTTCAT	ATCAT	TGGT	CGTGG	TTGTAGT	CCGTG	CGAGAATA
Papio hamadryas	GCTTTGTA	GTTG	AAATA	CAGCG	ATGGT	TTTTCGT	ATCAT	TGGT	TATGG	TTAGAGT	CCATA	TGAAAGCA
Tarsius bancanus	GTTCTTATA	GTTG	AATAA	CAACG	GTGAT	TTTTCAG	GTCAT	TAAT	CATGG	TTTAAGT	CCATG	TAGGAACT
Lemur catta	ATTTTTATA	GTTG	AAGTA	CAACG	ATGGT	TTTTCAT	ATCAT	TAGT	CATGG	TTAAAGT	CCATG	TAAGAATT
Macaca sylvanus	GCTTTTATA	GTTG	AAGTA	CAACG	ATGGC	TTTTCAT	ATCAT	TGGT	TATGG	TTGGAGT	CCATA	TGGAAGCA
Pongo pygmaeus	GTTCTTGTA	GTTG	AGATA	CAACG	ATGTT	TTTTCAT	ATCAT	TAGT	CACAG	TTACAGT	CTATG	CGAGAACA
Vombatus ursinus	GTTTTTGTA	GTTG	AATGA	CAACA	ATGGT	TTTTCAT	GCCAT	AGGT	TATGG	TTAGAGT	CCATG	CTAAAATA

Table 4. The tRNA^{Glu} A14683G mutation in affected individuals and controls

Gene	Position	Replacement	Number of patients (%)	Number of 300 controls (%)	*P* Value
tRNA^{Glu}	14683	A to G	6 (85.7)	3 (1)	<0.001
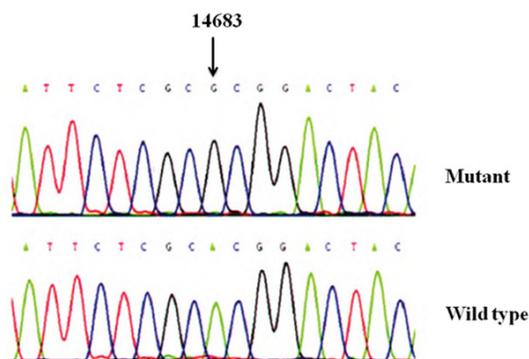
Figure 2. Identification of the A14683G mutation in the mt-tRNA^{Glu} gene. Partial sequence chromatograms of the mt-tRNA^{Glu} gene from affected individual and the healthy control. An arrow indicates the location of the base changes at position 14683.

16S rRNA gene, a novel A14683G mutation in tRNA^{Glu} gene, while other mutations were mainly located at protein coding genes. These missense mutations included the *ND1* G4491A (Val→Ile), *A6* A8701G (Thr→Ala) and A8860G (Thr→Ala), *ND3* A10398G (Thr→Ala), *CytB* C14766T (Thr→Ile) and A15326G (Thr→Ala). These variants in RNAs and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from other 17 vertebrates including Mouse [20], Bovine [21] and *Xenopus laevis* [22]. However, none of these variants showed evolutionary conservation excepted for the A14683G mutation (**Table 3**). This suggested that the A14683G mutation may have functional significance. Moreover, the Fisher's exact frequency difference test showed that the A14683G mutation was significant with the *P*<0.05 (**Table 4**).

The A14683G may be a novel mutation associated with hypertension

We proposed the A14683G as a pathogenic mutation associated with hypertension according to the following criteria: firstly, this mutation was present in the hypertensive persons and healthy controls with the allele frequency ≤1%; secondly, the A14683G mutation was extremely conserved from different species (CI =100%); thirdly, this mutation may change the secondary structure of tRNA^{Glu} and lead to failure in tRNA metabolism, and cause the mitochondrial dysfunction that was responsible for hypertension.

Discussion

In the present study, we have performed clinical, genetic and molecular characterization of a Han Chinese family with maternally transmitted EH. 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
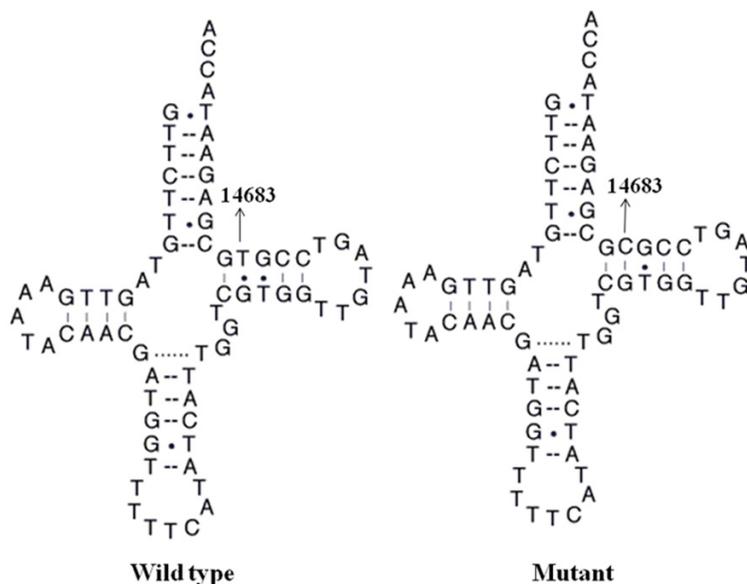
Figure 3. Location of the A14683G mutation in mt-tRNAGlu gene. The cloverleaf structure of mt-tRNAGlu gene is derived from the Mitomap database (http://www.mitomap.org/MITOMAP), arrow indicates the A14683G mutation.

affected matrilineal relatives in this family varied from 31 to 61 years, with an average of 50 years. Moreover, members in this family had earlier age at onset of EH (**Table 1**), indicating that screening for the pathogenic mtDNA mutations may be useful to early diagnosis and prevention for hypertension.

Analysis of the entire mitochondrial genome identified 25 polymorphisms belonging to human mitochondrial haplogroup M9a. Of these, the novel tRNAGlu A14683G mutation was of special interest (**Figure 2**). This mutation was only presented in matrilineal relatives with EH and showed statistically significant (**Table 4**). The A14683G mutation was localized at the TψC stem of tRNAGlu gene (position 64) (**Figure 3**), created a new base-pairing (64A-50G) and may change the secondary structure of tRNAGlu. In fact, nucleotide at this position was highly conserved between different species (**Table 3**). Notably, the T15965C point mutation in the mt-tRNAPro gene occurred at the same position of the TψC stem had been associated with Parkinson diseases [23]. The corresponding nucleotide in bacterial aminoacyl-tRNAs was reported to be involved in the interaction with elongation factor Tu (EF-Tu) [23]. Thus, it can be anticipated that the A14683G mutation, which was similar to the T15965C mutation, may cause the failure in tRNA metabolism and consequently led to mitochondrial dysfunction. Defects in mitochondrial translation would subsequently cause the respiratory chain dysfunction and a decline in ATP production below the threshold level required for endothelial cell function [24]. These mitochondrial dysfunction may contribute to the development of EH [25]. The homoplasmic form, mild mitochondrial dysfunction, late onset and incomplete penetrance of hypertension observed in this Chinese family carrying the A14683G mutation suggest that the mutation was an inherited risk factor necessary for the development of hypertension, but the mutation itself may be insufficient to produce a clinical phenotype. The other modifier factors such as nuclear modifier genes, environmental and epigenetic factors, and personal lifestyles may contribute to the development of hypertension in these subjects carrying the A14683G mutation.

In conclusion, our data indicated that the novel tRNAGlu A14683G mutation in mitochondrial genome may be a risk factor for hypertension; our findings will be helpful for counseling families of maternally inherited hypertension.

Disclosure of conflict of interest

None.

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