Original Article

Analysis of mitochondrial tRNA\textsuperscript{Thr} variants in patients with essential hypertension

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Abstract: Mutations in mitochondrial tRNA (mt-tRNA) are associated with maternally transmitted hypertension. However, the molecular mechanism underlying mt-tRNA mutations and hypertension remain largely undetermined. To see the allele frequency of hypertension-associated mt-tRNA\textsuperscript{Thr} variants, a systematic and extensive mutational analysis of mt-tRNA\textsuperscript{Thr} in 500 hypertensive individuals and 300 controls was performed. As a result, three potential pathogenic mutations were identified: G15927A, A15951G and A15924G, that occurred at highly conserved nucleotides of mt-tRNA\textsuperscript{Thr}. These may cause structural and functional alterations. Among these, only one patient carrying the G15927A mutation had an obvious family history of hypertension. Most strikingly, this family exhibited a high penetrance of hypertension. Sequence analysis of complete mitochondrial DNA (mtDNA) genes showed the presence of ND1 T3394C and G15927A mutations, as well as a set of polymorphisms belonging to human mitochondrial haplogroup B5b1. Notably, T3394C mutation, which was located at highly conserved nucleotide of ND1 gene, had been regarded as a pathogenic mutation associated with Leber’s hereditary optic neuropathy (LHON). Therefore, the combination of T3394C and G15927A mutations may account for high penetrance of hypertension in this family. Furthermore, mt-tRNA\textsuperscript{Thr} gene was the hot spot for pathogenic mutations associated with hypertension, which gives novel insight into the early diagnosis, detection and management of essential hypertension.

Keywords: mt-tRNA\textsuperscript{Thr}, variants, essential hypertension, Chinese family

Introduction

Hypertension is a major public health problem, affecting approximately 1 billion worldwide [1]. Moreover, hypertension is one of the most important modifiable risk factors for cardiovascular disease and renal disease. To date, the etiology of hypertension is not well understood because it is often a multi-factorial condition. However, it is now generally believed that hypertension could be caused by single-gene or multi-factorial conditions resulting from interactions between the environmental and inherited risk factors [2]. Among these genetic factors, the maternal inheritance of hypertension has been observed in numerous families, indicating that variants in mtDNA are involved in the pathogenesis of hypertension [3, 4]. Recently, several mtDNA point mutations have been identified to be associated with maternally inherited hypertension [5-7]. Among these mutations, mt-tRNA is a hotspot for pathogenic mutations’ locations [8]. However, the relationship between mtDNA mutations and high blood pressure (BP) remains unclear.

To investigate the effect of mtDNA mutations on hypertension pathogenesis, in this study, a systematic mutational analysis was performed of mt-tRNA\textsuperscript{Thr} gene in 500 hypertensive individuals and 300 controls from the People’s Hospital of Xintai City. Moreover, a maternally inherited Chinese family with tRNA\textsuperscript{Thr} G15927A mutation was identified. To see the contribution of mtDNA genetic background to hypertension, the complete mtDNA genes were identified in this family with hypertension.

Materials and methods

Subjects

A total of 500 genetically unrelated Han Chinese subjects with hypertension were ascertained at the People’s Hospital of Xintai City.
Moreover, a total of 300 healthy Han Chinese subjects with gender and age-matched were enrolled from the same area as controls. This study was in compliance with the Declaration of Helsinki. Informed consent, blood samples, and clinical evaluations were obtained from all participants, under protocols approved by the Ethic Committees of the People’s Hospital of Xintai City. All participants were interviewed and evaluated to identify both personal and medical histories of hypertension and other clinical abnormalities. The age of these participants ranged from 35 to 68 years, with the media age of 57 years.

**Measurements of BP**

One physician measured the systolic and diastolic BP of all subjects using a mercury column sphygmomanometer. The first and the fifth Korotkoff sounds were indicative of systolic and diastolic BP, respectively. The average of three measured BP readings was taken as the examination BP. Hypertension was defined according to the guidelines of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC VI) and the World Health Organization-International Society of Hypertension as a systolic BP of 140 mmHg or higher and/or a diastolic BP of 90 mmHg or greater or with a history of hypertension and under antihypertensive drug treatment [9].

**Mutational analysis of mt-tRNA<sup>Thr</sup> gene**

Genomic DNA was isolated using the Puregene DNA Isolation Kits according to the instructions (Gentra Systems, Minneapolis, MN). Sequence analysis of the PCR-amplified DNA fragment spanning mt-tRNA<sup>Thr</sup> gene was performed and the primer sequence for mt-tRNA<sup>Thr</sup> was: forward: 5'-TGAAACTTC GGCTCACTCCT-3', reverse: 5'-GAGTGGTTAATAGGGTGATAG-3'. Following PCR amplification and electrophoresis, the PCR fragment spanning the mt-tRNA<sup>Thr</sup> gene was purified and analyzed using an ABI 3700 automated DNA sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Furthermore, genetic variants were identified in the mt-tRNA<sup>Thr</sup> gene by comparing the sequence data with the Cambridge reference sequence (NC_012920) [10].

**Phylogenetic conservation analysis**

To analysis the phylogeny of mt-tRNA<sup>Thr</sup> mutations, vertebrate mtDNA sequences for interspecific analysis were used and these species included *Elephas maximus*, *Macropus robustus*, *Homo sapiens*, *Hylobates lar*, *Macaca mulatta*, *Pan paniscus*, *Pan troglodytes*, *Capra hircus*, *Lama pacos*, and *Orycteropus afer*. The conservation index (CI) was then calculated by comparing the human nucleotide variants with the other species. Notably, the CI≥75% was considered as functional potential [11].

**Clinical and molecular characterization of a hypertension family with mt-tRNA<sup>Thr</sup> G15927A mutation**

One Han Chinese family with hypertension, as shown in Figure 3, was ascertained in the People’s Hospital of Xintai City. The entire mitochondrial genomes of the affected individuals were PCR in 24-overlapping fragments by use of sets of light-strand and the heavy-strand oligonucleotide primers, as described in a previous study [12]. Double-stranded automatic sequencing was performed using an ABI PRISM 3700 sequencing machine (Applied Biosystems Inc., Foster City, CA, USA). The sequence was compared with the human mitochondrial reference sequence (GenBank Accession No: NC_012920) [10].

**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. p<0.05 was considered to indicate a statistically significant difference.

**Results**

**Screening for the mutations in mt-tRNA<sup>Thr</sup> gene in patients with hypertension**

Screening of mt-tRNA<sup>Thr</sup> mutations was performed in a cohort of 500 Han Chinese patients with hypertension, who were diagnosed as hypertension by the People’s Hospital of Xintai City. PCR amplification was performed of this mt-tRNA gene and then the purified PCR product was sequenced. As shown in Figure 1, after electrophoresis, the size of the PCR product spanning the entire mt-tRNA<sup>Thr</sup> gene was 1126 bp. In addition, sequence analysis of mt-tRNA<sup>Thr</sup> led us to identify three mutations: the G15927A, A15951G and A15924G, as shown
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Evaluation of mt-tRNA<sup>Thr</sup> mutations

To distinguish deleterious mutations from polymorphisms, these mt-tRNA mutations were evaluated using the following three criteria: (1) present in <1% of the controls; (2) CI ≥ 75% [11]; (3) potential structural alterations and functional significance. As shown in Table 1, G15927A, A15951G and A15924G were not presented in control subjects, moreover, the CIs of these mutations were 100% for all, and may cause the alternations in mt-tRNA structures and functions, suggesting that these mutations may be involved in the pathogenesis of hypertension.

Clinical features of the hypertension family with mt-tRNA<sup>Thr</sup> G15927A mutation

The proband (III-9) was a 33-year-old women came from Xintai area of Shandong province, she went to the People’s Hospital of Xintai City for treatment of hypertension, as shown in Table 2, clinical evaluation showed that her BP was 90/150 mmHg. Further comprehensive family history and physical examination suggested that II-1, II-5, II-8, II-12 were hypertensive patients.

As shown in Figure 3, this familiar history was consistent with a maternal inheritance. In particular, the penetrance of hypertension in this family was 54.5%. In addition, there was a wide range in the age at onset of hypertension in this family, varying from 33 to 60 years (Table 2), with an average of 48.6 years old. Furthermore, members from this family showed no other clinical abnormalities, including diabetes, cardiovascular diseases, visual dysfunction, and neurological disorders, suggesting that hypertension was the only phenotype.

Mutational analysis of mitochondrial genome

Maternal transmission of hypertension in this family suggested mitochondrial involvement and led us to analyze the mitochondrial genome.

Figure 1. PCR amplification of mt-tRNA<sup>Thr</sup> gene in hypertension patients, arrow indicates the PCR product, which is 1126 bp.

Figure 2. Secondary structure of mt-tRNA<sup>Thr</sup> gene, arrow indicates the G15927A, A15924G and A15951G mutations.

In Figure 2, of these, the G15927A mutation was identified in two cases (0.4%), the A15951G mutation was found in three cases (0.6%), the A15924G mutation was found in one case (0.2%) (Table 1). All of the nucleotide changes were verified by sequence analysis of both strands and were present at homoplasy. However, these mutations were absent in 300 controls.
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In addition, the T3394C and G15927A mutations were absent in the control subjects, had a statistical significance with the p<0.05.

Indeed, the T3394C mutation was present in homoplasmy only in the maternal lineage of this pedigree. The tyrosine at amino acid position 30 was extremely conserved in ND1 polypeptide among different organisms [17], moreover, the T3394C mutation has been reported to be associated with LHON [18].

Discussion

In the present study, potential pathogenic mt-tRNA\textsuperscript{Thr} mutations were screened in a cohort of 500 hypertensive individuals and 300 control subjects. Using PCR and direct Sanger sequencing (Figure 1), three possible pathogenic mutations were identified: G15927A, A15951G and A15924G (Figure 2, Table 1). Of these, the G15927A mutation affected a highly conserved guanine at position 42 at the anticodon-stem of mt-tRNA\textsuperscript{Thr}, destabilizing the conservative base pairing (28C-42G) [19]. Previous studies suggested that the G15927A mutation caused a significantly reduction of mt-tRNA\textsuperscript{Thr} steady-state level and impaired mitochondrial translation [20]. While the homoplasmic A15951G mutation was located adjacent to 3’ end, at conventional position 71 of mt-tRNA\textsuperscript{Thr}. The adenine (A71) at this position was highly conserved from bacteria to human mitochondria. In fact, the A15951G mutation has been implicated to be important for tRNA identity and pre-tRNA processing [21, 22]. Further functional analysis of cell lines derived from the patients carrying the A15951G mutation showed a marked decreased level of

Table 1. Characterization of three mt-tRNA\textsuperscript{Thr} mutations in hypertensive patients

<table>
<thead>
<tr>
<th>Gene mutation</th>
<th>tRNA domain</th>
<th>Position</th>
<th>Number (n)</th>
<th>Percent (%)</th>
<th>CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G15927A</td>
<td>Anticodon stem</td>
<td>42</td>
<td>2</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>A15951G</td>
<td>Acceptor arm</td>
<td>71</td>
<td>3</td>
<td>0.6</td>
<td>100</td>
</tr>
<tr>
<td>A15924G</td>
<td>Anticodon stem</td>
<td>39</td>
<td>1</td>
<td>0.2</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Summary of clinical data for the matrilineal relatives in this family with hypertension

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age at onset</th>
<th>Age at test</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Systolic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1</td>
<td>Male</td>
<td>55</td>
<td>66</td>
<td>95</td>
<td>140</td>
</tr>
<tr>
<td>II-5</td>
<td>Male</td>
<td>50</td>
<td>60</td>
<td>100</td>
<td>160</td>
</tr>
<tr>
<td>II-8</td>
<td>Female</td>
<td>45</td>
<td>59</td>
<td>95</td>
<td>135</td>
</tr>
<tr>
<td>II-12</td>
<td>Female</td>
<td>60</td>
<td>70</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>III-9</td>
<td>Female</td>
<td>33</td>
<td>36</td>
<td>90</td>
<td>150</td>
</tr>
<tr>
<td>III-8</td>
<td>Female</td>
<td>/</td>
<td>31</td>
<td>70</td>
<td>130</td>
</tr>
</tbody>
</table>

of matrilineal relatives. PCR amplification was performed of fragments spanning the entire mtDNA and sequenced the PCR products. In addition to the well-known G15927A mutation, as shown in Table 3, matrilineal relatives in this family exhibited a set of mtDNA polymorphisms belonging to human mitochondrial haplogroup B5b1 [13]. Of these, there were 5 variants in D-loop gene, 2 known variants in 12S rRNA gene, 1 variant in 16S rRNA gene, 1 mutation in tRNA\textsuperscript{Thr} gene, while other variants were mainly localized at protein-coding genes. These variants in RNAs and polypeptides were further evaluated by conservation analysis using the sequences from mouse [14], bovine [15] and Xenopus laevis [16]. However, none of these variants were evolutionarily conserved except for the T3394C and G15927A mutations (Figure 4).
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Table 3. mtDNA mutations in this family with hypertension

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Replacement</th>
<th>Conservation (H/B/M/X)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CRS&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-loop</td>
<td>73</td>
<td>A to G</td>
<td>A</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>152</td>
<td>T to C</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>Ins C</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>16189</td>
<td>T to C</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>16519</td>
<td>T to C</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>12S rRNA</td>
<td>750</td>
<td>A to G</td>
<td>A/A/A/-</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>1438</td>
<td>A to G</td>
<td>A/A/A/G</td>
<td>A</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>2706</td>
<td>A to G</td>
<td>A/G/A/A</td>
<td>A</td>
</tr>
<tr>
<td>ND1</td>
<td>3394</td>
<td>T to C (Tyr to His)</td>
<td>Y/Y/Y/Y</td>
<td>T</td>
</tr>
<tr>
<td>ND2</td>
<td>4769</td>
<td>A to G</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>CO1</td>
<td>7028</td>
<td>C to T</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>CO3</td>
<td>9540</td>
<td>T to C</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>ND3</td>
<td>10398</td>
<td>A to G (Thr to Ala)</td>
<td>T/T/T/A</td>
<td>A</td>
</tr>
<tr>
<td>ND5</td>
<td>12705</td>
<td>C to T</td>
<td>I/L/L/T</td>
<td>C</td>
</tr>
<tr>
<td>Cytb</td>
<td>14766</td>
<td>C to T (Thr to Ile)</td>
<td>T/S/T/S</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>15301</td>
<td>G to A</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>tRNA&lt;sub&gt;Thr&lt;/sub&gt;</td>
<td>15927</td>
<td>G to A</td>
<td>G/G/G/G</td>
<td>G</td>
</tr>
</tbody>
</table>

<sup>a</sup>Conservation of amino acid for polypeptides or nucleotide for RNAs in human (H), bovine (B), mouse (M), and Xenopus laevis (X).
<sup>b</sup>CRS: Cambridge reference sequence.

Figure 4. Identification of ND1 T3394C mutation using PCR and direct sequencing.

Mt-tRNA<sub>Thr</sub>, when compared with the controls. Therefore, the A15951G was a pathogenic mutation associated with hypertension since it may cause the mitochondrial dysfunction. Moreover, the homoplasmic A15924G occurred at the anticodon stem of mt-tRNA<sub>Thr</sub>, notably, it has been reported to be associated with several diseases such as mitochondrial encephalopathy [23], Parkinson’s disease [24], idiopathic cardiomyopathy [25] and fatal infantile respiratory enzyme deficiency [26]. At the molecular level, the A15924G mutation localized at anticodon stem of mt-tRNA<sub>Thr</sub> (conventional position 39), and disrupted the conservative base pairing (31U-39A). Thus, it can be speculated that the A15924G mutation may alter the secondary structure of mt-tRNA<sub>Thr</sub>, and subsequently resulted in failure of tRNA metabolism. Therefore, G15927A, A15951G and A15924G were the primary mutations associated with hypertension.

Among these cases carrying the mt-tRNA<sub>Thr</sub> mutations, only one patient with the G15927A mutation manifested the maternally inherited pattern of hypertension. As shown in Figure 3, hypertension as a sole clinical phenotype occurred in matrilineal relatives but not in other members in this family. Notably, this family exhibited the high penetrance of hypertension, which was 54.5%.

G to A transition at position 15927 in mt-tRNA<sub>Thr</sub> gene was observed, which was present in all matrilineal relatives in homoplasmic form, but was absent in 300 controls, suggested that this mutation

was the molecular basis for hypertension. In fact, the G15927A mutation disrupted a very conservative base pairing (28C-42G) on the anticodon stem of mt-tRNA<sup>Thr</sup>. An abolished base pair at the same position in mt-tRNA<sup>Thr</sup> by the A4300G mutation associated with hypertrophic cardiomyopathy altered this mt-tRNA metabolism [27]. Thus, alteration of the tertiary structure of the mt-tRNA<sup>Thr</sup> by the G15927A mutation may lead to a failure in this tRNA metabolism. Approximately ~45% reductions in the level of mt-tRNA<sup>Thr</sup> were observed in cells carrying the G15927A mutation [28]. Therefore, G15927A was a primary mutation for the pathogenesis of hypertension. However, five affected individuals in this family exhibited the various severities, age at onset of hypertension, suggesting that the G15927A mutation itself was insufficient to produce the clinical phenotypes; hence, other modified factors such as environmental factors, mitochondrial haplogroup and nuclear genes were involved in hypertension expression [29-31].

Mutational analysis of the entire mitochondrial genome in the matrilineal relatives from this family showed the presence of ND1 T3394C mutation (Figure 4), as well as a set of genetic polymorphisms. In particular, ND1 T3394C mutation caused the substitution of a highly conserved histidine for tyrosine (Y30H) at amino acid position 30. In fact, the tyrosine at position 30 in ND1 is highly conserved among 27 organisms [18, 20]. Indeed, this mutation has been associated with LHON in a Finnish family [32] and four Chinese families [18] and metabolic diseases [33] and one Chinese family with hearing loss [34]. Similarly, the ND1 T3394C was also a pathogenic mutation associated with hypertension. Therefore, the combination of ND1 T3394C and mt-tRNA<sup>Thr</sup> G15927A mutations may contribute to the high penetrance and expressivity of hypertension in this Chinese family.

In summary, our study indicates that mt-tRNA<sup>Thr</sup> is the hot spot for pathogenic mutations associated with hypertension. Moreover, G15927A, A15951G and A15924G mutations will alter the secondary structure and subsequently lead to failure in mt-tRNA<sup>Thr</sup> metabolism, are involved in the pathogenesis of hypertension. Furthermore, the combination of ND1 T3394C and mt-tRNA<sup>Thr</sup> G15927A mutations may be responsible for the high penetrance of hypertension in this Chinese family, our data shaded novel insight into the early diagnosis, detection and management of hypertension.

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Disclosure of conflict of interest

None.

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References


Mt-tRNA<sup>Thr</sup> variants and hypertension


[31] Xu Y, Chen X, Huang H, Liu W. The Mitochondrial tRNAAla T5655C mutation may modulate the
Mt-tRNA<sup>TM</sup> variants and hypertension

phenotypic expression of tRNA<sup>Met</sup> and tRNA<sup>Gln</sup> A4401G mutation in a Han Chinese family with essential hypertension. Int Heart J 2017; 58: 95-99.

