Mitochondrial transfer RNAMet 4435A>G mutation is associated with maternally inherited hypertension in a Chinese pedigree.

Yuqi Liu, Chinese People’s Liberation Army General Hospital
Ronghua Li, Emory University
Zongbin Li, Chinese People’s Liberation Army General Hospital
Xin-Jian Wang, Cincinnati Children’s Hospital Medical Center
Li Yang, Cincinnati Children’s Hospital Medical Center
Shiwen Wang, Chinese People’s Liberation Army General Hospital
Min-Xin Guan, University of Cincinnati College of Medicine

Journal Title: Hypertension
Volume: Volume 53, Number 6
Publisher: American Heart Association | 2009-06, Pages 1083-1090
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1161/HYPERTENSIONAHA.109.128702
Permanent URL: https://pid.emory.edu/ark:/25593/rr5rt

Final published version:
http://dx.doi.org/10.1161/HYPERTENSIONAHA.109.128702

Accessed December 6, 2018 7:37 PM EST
Mitochondrial Transfer RNA\textsuperscript{Met} 4435A>G Mutation Is Associated With Maternally Inherited Hypertension in a Chinese Pedigree

Yuqi Liu, Ronghua Li, Zongbin Li, Xin-Jian Wang, Li Yang, Shiwen Wang, and Min-Xin Guan

Institute of Geriatric Cardiology (Y.L., Z.L., S.W.), Chinese People’s Liberation Army General Hospital, Beijing, China; Division of Human Genetics (R.L., X.-J.W., L.Y., M.-X.G.), Cincinnati Children’s Hospital Medical Center, Ohio; and the Department of Pediatrics (M.-X.G.), University of Cincinnati College of Medicine, Ohio

Abstract

Mitochondrial DNA mutations have been associated with cardiovascular disease. We report here the clinical, genetic, and molecular characterization of 1 Han Chinese family with suggestively maternally transmitted hypertension. Matrilineal relatives in this family exhibited the variable degree of hypertension at the age at onset of 44 to 55 years old. Sequence analysis of entire mitochondrial DNA in this pedigree identified the known homoplasmic 4435A>G mutation, which is located immediately at the 3 prime end to the anticodon, corresponding with the conventional position 37 of tRNA\textsuperscript{Met}, and 35 other variants belonging to the Asian haplogroup B5a. The adenine (A37) at this position of tRNA\textsuperscript{Met} is extraordinarily conserved from bacteria to human mitochondria. This modified A37 was shown to contribute to the high fidelity of codon recognition, the structural formation, and stabilization of functional tRNAs. In fact, a 40% reduction in the levels of tRNA\textsuperscript{Met} was observed in cells carrying the 4435A>G mutation. As a result, a failure in mitochondrial tRNA metabolism, caused by the 4435A>G mutation, led to ≈30% reduction in the rate of mitochondrial translation. However, the homoplasmic form, mild biochemical defect, and late onset of hypertension in subjects carrying the 4435A>G mutation suggest that the 4435A>G mutation itself is insufficient to produce a clinical phenotype. The other modifier factors, such as nuclear modifier genes, environmental, and personal factors may also contribute to the development of hypertension in the subjects carrying this mutation. Our findings imply that the 4435A>G mutation may act as an inherited risk factor for the development of hypertension in this Chinese pedigree.

Keywords

hypertension; mitochondria; mutation; tRNA metabolism; maternal inheritance; risk factor; Chinese

Cardiovascular disease is the leading cause of death in America and the world. Cardiovascular disease includes high blood pressure, coronary heart disease, heart failure, and stroke. In particular, hypertension affects ≈1 billion individuals worldwide and 130
The etiology of cardiovascular disease is not well understood because of the multifactorial causes. Cardiovascular disease can be caused by single-gene or multifactorial conditions, resulting from interactions between environment and inherited risk factors. Of hereditary factors, the maternal transmissions of cardiovascular disease have been implicated in some pedigrees, suggesting that the mutation(s) in mitochondrial DNA (mtDNA) is one of the molecular bases for this disorder. Recently, several mtDNA point mutations have been identified to be associated with cardiovascular disease. These mutations included the 1555A>G mutation in the 12S rRNA gene, the 3260A>G and 3303C>T mutations in the tRNA_{Leu(UUR)} gene, the 8348A>G and 8363A>G mutations in the tRNA_{Lys} gene, and the 4295A>G, 4300A>G, and 4317A>G mutations in the tRNA{Ile} gene. Most recently, the 4291T>C mutation in the tRNA{Ile} gene has been associated with a cluster of metabolic defects, including essential hypertension, hypercholesterolemia, and hypomagnesemia in a large family.

However, the molecular pathogenesis of hypertension in the Chinese population remains poorly understood. To understand a role of mitochondrial genomes in the pathogenesis of cardiovascular diseases in the Chinese population, we have initiated a systematic and extended mutational screening of mtDNA in a large cohort of hypertension subjects at the Chinese People’s Liberation Army (PLA) General Hospital Geriatric Cardiology Clinic. In the present study, we performed the clinical, genetic, and molecular characterizations of another Han Chinese family with suggestive maternally transmitted hypertension. Mutational analysis of a mitochondrial genome in this Chinese family has identified the known tRNA{Met} 4435A>G mutation, which is localized at the 3 prime end adjacent to the anticodon (position 37) of tRNA{Met}. In fact, 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 B5a. To investigate the pathogenic mechanism of the 4435A>G mutation in this Chinese family, these lymphoblastoid cell lines derived from an affected matrilineal relative carrying the 4435A>G mutation and from a Chinese control individual belonging to the same mitochondrial haplogroup lacking the mutation were assessed for the effects of the mtDNA mutation on the mitochondrial tRNA metabolism, including tRNA{Met}, tRNA{Gly}, tRNA_{Leu(UUR)}, and tRNA_{Leu(CUN)}, and the rate of mitochondrial protein synthesis.

### Materials and Methods

#### Subjects

As a part of a genetic screening program for hypertension, a Han Chinese family (Figure 1) was ascertained at the Institute of Geriatric Cardiology of Chinese PLA General Hospital. Informed consent, blood samples, and clinical evaluations were obtained from all of the participating family members, under protocols approved by the ethics committee of Chinese PLA General Hospital 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. The 242 control DNA samples were obtained from a panel of unaffected Han Chinese individuals from the same area.

#### Measurements of Blood Pressure

Members of this Chinese family underwent a physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography. A physician measured the systolic and diastolic blood pressures of subjects using a mercury column sphygmomanometer and a standard protocol. The first and the fifth Korotkoff sounds were taken as indicative of systolic and diastolic blood pressures, respectively. The average of 3
such systolic and diastolic blood pressure readings was taken as the examination blood pressure. Hypertension was defined according to the recommendation of the Sixth Joint National Committee on the 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.

Mutational Analysis of Mitochondrial Genome

Genomic DNA was isolated from the whole blood of participants using Puregene DNA Isolation kits (Gentra Systems). The entire mitochondrial genomes of the proband II-1 and an unaffected Han Chinese control subject A25 were PCR amplified in 24 overlapping fragments by use of sets of the light-strand and the heavy-strand oligonucleotide primers, as described elsewhere. Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. The resultant sequence data were compared with the revised consensus Cambridge sequence (GenBank accession No. NC_001807).

For the quantification of the 4435A>G mutation, the PCR segments (700 bp) were amplified using genomic DNA as a template and oligodeoxynucleotides corresponding with mtDNA at positions 3861 to 4560 and subsequently digested with a restriction enzyme NlaIII. In fact, the 4435A>G mutation creates a novel site for this enzyme. 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 determine whether the 4435A>G mutation is in the homoplasy in these subjects.

Mitochondrial tRNA Analysis

Lymphoblastoid cell lines were immortalized by transformation with the Epstein-Barr virus, as described elsewhere. Cell lines derived from one of the probed II-1s carrying the 4435A>G mutation and 1 Han Chinese control A25 belonging to the same mtDNA haplogroup lacking this mutation were grown in RPMI 1640 (Invitrogen), supplemented with 10% FBS. Total mitochondrial RNA were obtained using a TOTALLY RNA kit (Ambion) from mitochondria isolated from lymphoblastoid cell lines (≈4.0×10⁸ cells), as described previously. Two μg of total mitochondrial RNA were electrophoresed through a 10% polyacrylamide/7 mol/L urea gel in Tris-borate-EDTA buffer (after heating the sample at 65°C for 10 minutes) and then electroblotted onto a positively charged nylon membrane (Roche) for the hybridization analysis with oligodeoxynucleotide probes. For the detection of tRNA Met, tRNA Gly, tRNA-Leu(UUR), and tRNA-Leu(CUN), the following nonradioactive digoxigenin (DIG)-labeled oligodeoxynucleotides specific for each RNA were used: 5′-TAGTACGGGAAGGGTATAACC-3′ (tRNA Met); 5′-TGTTAAGAA GAGGAATTGAA-3′ (tRNA-Leu(UUR)); 5′-TACTCTTTTTTGACTG TGTC-3′ (tRNA Gly); and 5′-TACTTTTATTTGGAGTTGCACC-3′ (tRNA-Leu(CUN)). DIG-labeled oligodeoxynucleotides were generated by using the DIG oligonucleotide Tailing kit (Roche). The hybridization was carried out as detailed elsewhere. Quantification of density in each band was made as detailed previously.

Analysis of Mitochondrial Protein Synthesis

Pulse labeling of the cell lines for 30 minutes with [³⁵S]methionine-[³⁵S]cysteine in methionine-free DMEM in the presence of emetine, electrophoretic analysis of the translation products, and quantification of radioactivity in the whole-electrophoretic patterns or in individual well-resolved bands was carried out as detailed previously.
Results
Clinical Presentation
The proband (II-1) began suffering from hypertension at the age of 44 years old. His blood pressure was 200/100 mm Hg by then. He came to the Chinese PLA General Hospital Geriatric Cardiology Clinic for further clinical evaluations at the age of 64 years old. His blood pressure was 180/100 mm Hg. After the administration of an angiotensin-converting enzyme inhibitor, calcium channel blocker, and diuretic, his blood pressure has ranged from 130/80 to 160/100 mm Hg. Laboratory assessment of cardiovascular disease risk factors showed that he had a normal range of the index of liver and kidney metabolic function, the blood routine, and 24-hour urinary sodium but hypercholesterolemia (9.2 mmol/L). As shown in Table 1, the echocardiogram showed that he had an increase of the interventricular septal and posterior ventricular wall thickness (13 mm) with normal atrial and ventricular dimension. Physical examination showed that he did not have other clinical abnormalities, including diabetes mellitus, vision and hearing impairments, and renal and neurological disorders. Therefore, he exhibited a typical essential hypertension. The family is originated from Beijing in Northern China. As shown in Figure 1, this familial history is suggestive of a maternal inheritance. None of the offspring of the affected father had hypertension. His mother (I-1) was diagnosed as having hypertension at 55 years old, with a blood pressure of ≤160/100 mm Hg, and his younger brother (II-3) suffered from hypertension at the age of 54 years old, with a blood pressure of 150/98 mm Hg. However, other members of this family had normal blood pressure. Comprehensive family medical histories of these individuals showed no other clinical abnormalities, including diabetes mellitus, vision and hearing impairments, and renal and neurological disorders.

Mitochondrial DNA 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-1 were PCR amplified, and each fragment was purified and subsequently analyzed by direct sequence. As shown in Table 2, the comparison of the resultant sequences with the revised Cambridge consensus sequence identified 36 nucleoside changes, belonging to the Eastern Asian haplogroup B5a. Furthermore, the sequences of entire mtDNA in a Han Chinese control subject A25 belonging to the haplogroup B5a were determined. Of these nucleoside changes in mtDNA of the proband II-1, there were 14 polymorphisms in the D-loop region, 4 variants in the 12S rRNA gene, 1 variant in the 16S rRNA gene, the 4435A>G mutation in the tRNA<sub>Met</sub> gene, the previously identified CO2/tRNA<sub>Lys</sub> intergenic 9-bp deletion corresponding with mtDNA at positions 8271 to 8279, and the 8 known silent mutations and 7 missense mutations in protein encoding genes (http://www.mitomap.org or http://www.genpat.uu.se/mtDB). These missense mutations are the 8141G>A (186A>T) in the CO2 gene, the 8584G>A (20A>T) and 8860A>G (112T>A) in the A6 gene, the 10398A>G (114T>A) in the ND3 gene, and the 14766C>T (7T>I), 15236A>G (164I>V), and 15326A>G (194T>A) in the Cyth gene. These variants in rRNAs and polypeptides were further evaluated by phylogenetic analyses of these variants and sequences from other organisms, including the mouse, bovine, and Xenopus laevis. None of the variants in the polypeptides and rRNAs were highly evolutionarily conserved and implicated to have a significantly functional consequence. In addition, there were 32 variants in the mitochondrial genome in a Chinese control subject, A25. As shown in Table 2, both proband II-1 and control subject A25 shared 25 known mtDNA variants belonging to the haplo-group B5a. On the hand, the control subject A25 lacked the 4435A>G mutation in the tRNA<sub>Met</sub> gene, the 1393G>A variant in the 12S rRNA, and the 8141G>A variant in the CO2 gene.
The known 4435A>G mutation in the tRNA\textsuperscript{Met} gene, as shown in Figure 2, is located immediately at the 3 prime end to the anticodon, corresponding with conventional position 37 of the tRNA\textsuperscript{Met}.\textsuperscript{35} In fact, an adenine at this position is an extraordinarily conserved base in every sequenced methionine tRNA from bacteria to human mitochondria.\textsuperscript{35,36} Interestingly, the nucleotide at position 37 is more prone to modification that those at other places of tRNA.\textsuperscript{37} The nucleotide modification at this position has been shown to play a pivotal role in the stabilization of tertiary structure and the biochemical function of tRNA.\textsuperscript{37} To determine whether the 4435A>G mutation is present in homoplasmy, the fragments spanning the tRNA\textsuperscript{Met} gene were PCR amplified and subsequently digested with \textit{Nla}III. There was no detectable wild-type DNA in 3 matrilineal relatives (data not shown), indicating that the 4435A>G mutation was present in homoplasmy in these matrilineal relatives. In addition, this mutation was absent in 242 Chinese controls.

**Mitochondrial tRNA Analysis**

To further examine whether the 4435A>G mutation alters the tRNA\textsuperscript{Met} metabolism, the steady-state level of the tRNA\textsuperscript{Met} was determined by isolating total mitochondrial RNA from lymphoblastoid cell lines, separating them by a 10% polyacrylamide/7 mol/L urea gel, electrophoretically separating the tRNA\textsuperscript{Met} and hybridizing with a nonradioactive DIG-labeled oligodeoxynucleotide probe specific for tRNA\textsuperscript{Met}. After stripping the blots, the DIG-labeled probes, including tRNA\textsuperscript{Leu(UUR)}, tRNA\textsuperscript{Gly}, and tRNA\textsuperscript{Leu(CUN)}, were hybridized with the same blots for normalization purposes.

As shown in Figure 3A, the amounts of tRNA\textsuperscript{Met} in the mutant cell line derived from the proband II-1 carrying the 4435A>G mutations were markedly decreased, as compared with those in the cell line derived from a Chinese control A25 belonging to the same haplogroup but lacking those mtDNA mutations. For comparison, the average levels of tRNA\textsuperscript{Met} in the control or mutant cell lines were normalized to the average levels in the same cell line for the tRNA\textsuperscript{Leu(UUR)}, tRNA\textsuperscript{Gly}, and tRNA\textsuperscript{Leu(CUN)}, respectively. As shown in Figure 3B, the average levels of tRNA\textsuperscript{Met} in the mutant cell line derived from II-1 ranged among ≈55% of the control after normalization to tRNA\textsuperscript{Leu(UUR)}, ≈67% of controls after normalization to tRNA\textsuperscript{Gly}, and ≈57% of controls after normalization to tRNA\textsuperscript{Leu(CUN)}.

**Mitochondrial Protein Synthesis Defect**

To examine whether a defect in mitochondrial translation occurred in lymphoblastoid cell lines carrying the 4435A>G mutation, cells from 1 lymphoblastoid cell line derived from the proband carrying the 4435A>G mutation and a Chinese control A25 were labeled for 30 minutes with [\textsuperscript{35}S]methionine-[\textsuperscript{35}S]cysteine in methionine-free regular DMEM in the presence of 100 μg/mL of emetine to inhibit cytosolic protein synthesis.\textsuperscript{29} Figure 4A shows typical electrophoretic patterns of the mitochondrial translation products of the mutant and control cell lines. Patterns of the mtDNA-encoded polypeptides of the cells carrying the 4435A>G mutation were qualitatively identical, in terms of electrophoretic mobility of the various polypeptides, to those of the control cells and of 143B.TK\textsuperscript{−} cells. However, the cell line carrying the 4435A>G mutation showed a clear tendency toward a decrease in the total rate of labeling of the mitochondrial translation products, relative to those of control cell line. Figure 4B shows a quantification of the results of a large number of labeling experiments and electrophoretic runs, which were carried out by densitometric analysis of appropriate exposures of the fluorograms and normalization to data obtained for the 143B.TK\textsuperscript{−} sample. In fact, the overall rate of labeling of the mitochondrial translation products in the cell line derived from the proband carrying the 4435A>G mutation was decreased ≈30% relative to the mean value measured in the control cell line.
Discussion

In the present study, we have performed the clinical, genetic, and molecular characterizations of a Han Chinese family with essential hypertension. The hypertension as a sole clinical phenotype was only present in all of the matrilineal relatives of this 4-generation pedigree. Clinical and genetic evaluations revealed the variable severity and age at onset in hypertension. In particular, the age at onset was 44.0, 54.0, and 55.0 years old in 3 affected matrilineal relatives, with an average of 51 years old. The suggestively maternal transmission of hypertension in this family suggested that the mtDNA mutation(s) is the molecular basis for this disorder. Mutational analysis of the mitochondrial genome in this family identified the tRNA\text{Met} 4435A>G mutation and other 35 variants belonging to the Eastern Asian haplogroup B5a.\textsuperscript{19} On other hand, the 4435A>G mutation was also identified in the haplogroup D5 of a Chinese family\textsuperscript{18} and a Japanese subject,\textsuperscript{38} in the haplogroup D of an Asian individual,\textsuperscript{39} and in the haplogroup M7a2 of 2 Japanese subjects,\textsuperscript{38} as well as in the haplogroup J of an European subject.\textsuperscript{39} This suggested that the 4435A>G mutations occurred sporadically and multiplied through evolution of the mtDNA. This mutation was present only in matrilineal relatives of this family in the homoplasmic form but not in other members of this family or in 242 Han Chinese controls. Indeed, our previous study indicated that the 4435A>G mutation may modulate the phenotypic manifestation of Leber’s hereditary optic neuropathy–associated ND4 11778G>A mutation in a Chinese family,\textsuperscript{18} whereas the 4435A>G mutation was also associated with type 2 diabetes mellitus in 3 Japanese patients.\textsuperscript{38}

The 4435A>G mutation is located at the immediate 3 prime end to the anticodon, corresponding with conventional position 37 of the tRNA\text{Met}.\textsuperscript{35} In fact, the adenine at the 37 position of tRNA\text{Met} is extraordinarily conserved among 150 different species (http://w3appli.u-strasbg.fr/mamit-trna/tables.asp?amino acid = 19).\textsuperscript{36} Almost all of the A37 in tRNAs are modified, eg, thiolation and methylation.\textsuperscript{37} Indeed, this modified nucleotide contributes to the high fidelity of codon recognition, as well as the structural formation and stabilization of functional tRNAs.\textsuperscript{40} In Escherichia coli, nucleotide modifications at positions 37 and 34 are responsible for the stabilization of the canonical loop structure in the anticodon domain of tRNA\text{Lys}.\textsuperscript{41} Also, it has been shown that the modification of A37 stabilizes the 3 prime stacking features of the anticodon, thereby improving its interaction with the codon.\textsuperscript{42} The deficient modification of A37 decreased the activity of the corresponding tRNA\textsuperscript{43} and increased +1 frameshifts for tRNA\text{Phe},\textsuperscript{44} whereas the A-to-G substitution at position 37 led to a 10-fold reduction in the section of tRNAs at the aminocytic-trNA binding site.\textsuperscript{45} Furthermore, the 4295A>G mutation at the 37 position of tRNA\text{Ile} has been associated with hypertrophic cardiomyopathy in white pedigrees\textsuperscript{12,46} and hypertension in a Chinese pedigree.\textsuperscript{16} Most recently, the 4291T>C mutation at the anticodon region of mitochondrial tRNA\text{Ile} has been associated with hypertension, hypercholesterolemia, and hypomagnesemia.\textsuperscript{15}

In the current study, compared with a control cell lacking the mutation, ≈40% reduction in the levels of tRNA\text{Met} was observed in cells carrying the 4435A>G mutation. The lower levels of tRNA\text{Met} in cells carrying the 4435A>G mutation most probably result from a defect in nucleotide modification at position 37 of tRNA\text{Met}. As a result, a shortage of the tRNA\text{Met} is responsible for the reduced rate of mitochondrial protein synthesis. Subsequently, these defects led to an impairment of the function of the mitochondrial respiration chain, reduction of ATP production, and increase of reactive oxygen species production. These mitochondrial dysfunctions likely contribute to the development of hypertension.\textsuperscript{47,48} However, the levels of total tRNA\text{Met} in mutant cells are above a proposed threshold, which is 30% of the control level of tRNA, to support a normal rate of mitochondrial translation,\textsuperscript{26,27} indicating that the 4435A>G mutation itself is insufficient to
produce a clinical phenotype, as in the cases of deafness-associated 12S rRNA 1555A>G mutation\(^ {49}\) and Leber’s hereditary optic neuropathy-associated ND4 11778G>A mutation.\(^ {50}\) The other modifier factors, eg, nuclear modifier genes, environmental factors, and personal lifestyles, also contribute to the development of hypertension in these subjects carrying the 4435A>G mutation. Therefore, the 4435A>G mutation may act as an inherited risk factor for the development of hypertension in this Chinese pedigree.

**Perspectives**

The genetic and biochemical evidence of the present study indicate that the mitochondrial tRNA\(^ {\text{Met}}\) 4435A>G mutation is likely associated with essential hypertension. The tissue specificity of this pathogenic mtDNA mutation is likely attributed to the tissue-specific posttranscriptional modification or the contribution of nuclear modifier genes. The 4435A>G mutation should be added to the list of inherited risk factors for future molecular diagnosis. Thus, our finding provides new insights into the molecular mechanism, management, and treatment of maternally inherited hypertension. Future research should further explore the emerging link among hypertension, mitochondrial dysfunction, and their causative-effect relationship.

**Acknowledgments**

**Sources of Funding**

This work was supported by National Institutes of Health grants RO1DC05230 and RO1DC07696 from the National Institute on Deafness and Other Communication Disorders (to M.-X.G.) and National Key Basic Research and Development Project 973 Fund 2007CB07403 (to S.W.).

**References**


27. Guan MX, Enriquez JA, Fischel-Ghodsi N, Puranam R, Lin CP, Marion MA, Attardi G. The deafness-associated mtDNA 7445 mutation, which affects tRNA_Ser (UCN) precursor processing.


44. Urbonavicius J, Qian Q, Durand JM, Hagervall TG, Bjork GR. Improvement of reading frame maintenance is a common function for several tRNA modifications. EMBO J 2001;20:4863–4873. [PubMed: 11532950]


Figure 1.
The Chinese pedigree with hypertension. Affected individuals are indicated by filled symbols. Arrowhead denotes proband.
Figure 2.
Figure 3.
Northern blot analysis of mitochondrial tRNA. A, Equal amounts (2 μg) of total mitochondrial RNA from various cell lines were electrophoresed through a denaturing polyacrylamide gel, electroblotted, and hybridized with DIG-labeled oligonucleotide probes specific for the tRNA^{Met}. The blots were then stripped and rehybridized with DIG-labeled tRNA^{Leu(UUR)}, tRNA^{Gly}, and tRNA^{Leu(CUN)}, respectively. B, Quantification of mitochondrial tRNA levels. Average relative tRNA^{Met} content per cell, normalized to the average content per cell of tRNA^{Gly}, tRNA^{Leu(UUR)}, or tRNA^{Leu(CUN)} in control and mutant cell lines. The values for the latter are expressed as percentages of the average values for the control cell lines. The calculations were based on 3 independent determinations of tRNA^{Met} content.
content in each cell line and 3 determinations of the content of each reference RNA marker in each cell line. Error bars indicate 2 SEMs.
Figure 4.
Electrophoretic patterns of the mitochondrial translation products of lymphoblastoid cell lines and of 143B.TK− cells labeled for 30 minutes with [35S]methionine in the presence of 100 μg/mL of emetine. Samples containing equal amounts of protein (30 μg) were run in SDS/polyacrylamide gradient gels. COI, COII, and COIII, subunits I, II, and III of cytochrome c oxidase; ND1, ND2, ND3, ND4, ND4L, ND5, and ND6, subunits 1, 2, 3, 4, 4L, 5, and 6 of the respiratory chain reduced nicotinamide-adenine dinucleotide dehydrogenase; A6 and A8, subunits 6 and 8 of the H+ATPase; and CYTb, apocytochrome b. B, Quantification of the rates of labeling of the mitochondrial translation products, after a 30-minute [35S]methionine pulse, in lymphoblastoid cell lines. The rates of mitochondrial protein labeling, determined as detailed in Materials and Methods, are expressed as percentages of the value for 143B.TK− in each gel, with error bars representing 2 SEMs. A total of 3 independent labeling experiments and 3 electrophoretic analyses of each labeled preparation were carried out on lymphoblastoid cell lines. The vertical arrows refer to 2 SEs.
Table 1

Summary of Echocardiogram of the Proband at the Various Ages

<table>
<thead>
<tr>
<th>Variables</th>
<th>64</th>
<th>66</th>
<th>70</th>
<th>72</th>
<th>Chinese Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVST, mm</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>17</td>
<td>8 to 11</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>10</td>
<td>11</td>
<td>14</td>
<td>8</td>
<td>8 to 11</td>
</tr>
<tr>
<td>AID, mm</td>
<td>32</td>
<td>34</td>
<td>34</td>
<td>38</td>
<td>26±3</td>
</tr>
<tr>
<td>LAID, mm</td>
<td>34</td>
<td>37</td>
<td>43</td>
<td>43</td>
<td>&lt;40</td>
</tr>
<tr>
<td>LEDID, mm</td>
<td>53</td>
<td>49</td>
<td>58</td>
<td>50</td>
<td>46±4</td>
</tr>
<tr>
<td>LESID, mm</td>
<td>32</td>
<td>33</td>
<td>36</td>
<td>33</td>
<td>30±4</td>
</tr>
<tr>
<td>EF, %</td>
<td>69</td>
<td>61</td>
<td>67</td>
<td>62</td>
<td>50 to 70</td>
</tr>
</tbody>
</table>

IVST indicates interventricular septal thickness; LVPW, left ventricular posterior wall; AID, atrial inner dimension; LAID, left atrial inner dimension; LEDID, left ventricular end-systolic inner dimension; LESID, left ventricular end-diastolic inner dimension; EF, ejection fraction.
Table 2

mtDNA Variants in 1 Han Chinese Subject (II-1) With Hypertension and 1 Han Chinese Control Subject

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Replacement</th>
<th>Conservation (H/M/B/X)</th>
<th>rCRS‡</th>
<th>II-1</th>
<th>A25</th>
<th>Previously Reported‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-loop</td>
<td>73</td>
<td>A to G</td>
<td></td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>210§</td>
<td>A to G</td>
<td></td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>235</td>
<td>A to G</td>
<td></td>
<td>A</td>
<td>G</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>A to G</td>
<td></td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>294</td>
<td>T to C</td>
<td></td>
<td>T</td>
<td>C</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>T to CTC</td>
<td></td>
<td>T</td>
<td>CTC</td>
<td>CTC</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16093</td>
<td>T to C</td>
<td></td>
<td>T</td>
<td>C</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16140§</td>
<td>T to C</td>
<td></td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16183</td>
<td>A to C</td>
<td></td>
<td>A</td>
<td>C</td>
<td>C</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16189</td>
<td>T to C</td>
<td></td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16190</td>
<td>C to CC</td>
<td></td>
<td>C</td>
<td>CC</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16260</td>
<td>C to T</td>
<td></td>
<td>C</td>
<td>T</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16266§</td>
<td>C to A</td>
<td></td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16291</td>
<td>C to T</td>
<td></td>
<td>C</td>
<td>T</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16319</td>
<td>G to A</td>
<td></td>
<td>G</td>
<td>A</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>16519</td>
<td>T to C</td>
<td></td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>Yes</td>
</tr>
<tr>
<td>12S rRNA</td>
<td>709§</td>
<td>G to A</td>
<td>G/A/A/A/A</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>A to G</td>
<td>A/G/A/A</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>1393</td>
<td>G to A</td>
<td>G/T/A/A</td>
<td>G</td>
<td>A</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>1438</td>
<td>A to G</td>
<td>A/A/A/G</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>2706</td>
<td>A to G</td>
<td>A/A/G/A</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td>ND1</td>
<td>3537§</td>
<td>A to G</td>
<td>A/G</td>
<td>A</td>
<td>G</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>tRNA^{Met}</td>
<td>4435</td>
<td>A to G</td>
<td>A/A/A/A</td>
<td>A</td>
<td>G</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>ND2</td>
<td>4769</td>
<td>A to G</td>
<td>A/G/A/A</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td>CO1</td>
<td>6970</td>
<td>C to T</td>
<td>C/T/A/A</td>
<td>C</td>
<td>T</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>7001</td>
<td>A to G</td>
<td>A/G/A/A</td>
<td>A</td>
<td>G</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>7028</td>
<td>C to T</td>
<td>C/T/A/A</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>Yes</td>
</tr>
<tr>
<td>Gene</td>
<td>Position</td>
<td>Replacement</td>
<td>Conservation (H/M/B/X)</td>
<td>rCRS†</td>
<td>II-1</td>
<td>A25</td>
<td>Previously Reported‡</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>-------------</td>
<td>-----------------------</td>
<td>-------</td>
<td>------</td>
<td>-----</td>
<td>---------------------</td>
</tr>
<tr>
<td>CO2</td>
<td>7852</td>
<td>G to A</td>
<td>G</td>
<td>A</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8065</td>
<td>G to A</td>
<td>G</td>
<td>A</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO2</td>
<td>8141</td>
<td>G to A (Ala to Thr)</td>
<td>A/S/S/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC7</td>
<td>8271 to 79$§$</td>
<td>9 bp del</td>
<td>9 bp del</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>8584$§$</td>
<td>G to A (Ala to Thr)</td>
<td>A/V/V/I</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>8860</td>
<td>A to G (Thr to Ala)</td>
<td>T/A/A/T</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td>CO3</td>
<td>9950$§$</td>
<td>T to C</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td></td>
<td>Yes</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>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td>Nd4L</td>
<td>10754</td>
<td>A to G</td>
<td>A</td>
<td>G</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ND4</td>
<td>11719</td>
<td>G to A</td>
<td>G</td>
<td>A</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytb</td>
<td>14766</td>
<td>C to T (Thr to Ile)</td>
<td>T/I/S/S</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>14989</td>
<td>C to T</td>
<td>C</td>
<td>T</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15229</td>
<td>T to C</td>
<td>T</td>
<td>C</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15235</td>
<td>A to G</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15236</td>
<td>A to G (Ile to Val)</td>
<td>I/I/I/I</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>15326</td>
<td>A to G (Thr to Ala)</td>
<td>T/I/M/I</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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

† rCRS indicates revised Cambridge reference sequence.23


§ Sequence variations used to establish the haplogroup affiliation of each mtDNA are shown.