The tRNAMet 4435A > G mutation in the mitochondrial haplogroup G2a1 is responsible for maternally inherited hypertension in a Chinese pedigree

Zhongqiu Lu1,2,6, Hong Chen2,6, Yanzi Meng2,6, Yan Wang2,6, Ling Xue2,6, Shaoce Zhi1, Qiaomeng Qiu1, Li Yang3, Jun Qin Mo4 and Min-Xin Guan*,2,3,5

Mutations in mitochondrial DNA (mtDNA) have been associated with hypertension in several pedigrees with maternal inheritance. However, the pathophysiology of maternally inherited hypertension remains poorly understood. We reported here clinical, genetic evaluations and molecular analysis of mtDNA in a three-generation Han Chinese family with essential hypertension. Eight of 17 matrilineal relatives exhibited a wide range of severity in essential hypertension, whereas none of the offspring of the affected father had hypertension. The age-at-onset of hypertension in the maternal kindred varied from 31 to 65 years, with an average of 52 years. Sequence analysis of mtDNA in this pedigree identified the known homoplasmic 4435A > G mutation, which is located at immediately 3' end to the anticodon, corresponding to the conventional position 37 of tRNAMet, and 41 variants belonging to the Asian haplogroup G2a1. In contrast, the 4435A > G mutation occurred among mtDNA haplogroups B5a, D, M7a2 and J. The adenine (A37) at this position of tRNAMet is extraordinarily conserved from bacteria to human mitochondria. This modified A37 was shown to contribute to the high fidelity of codon recognition, structural formation and stabilization of functional tRNAs. However, 41 other mtDNA variants in this pedigree were the known polymorphisms. The occurrence of the 4435A > G mutation in two genetically unrelated families affected by hypertension indicates that this mutation is involved in hypertension. Our present investigations further supported our previous findings that the 4435A > G mutation acted as an inherited risk factor for the development of hypertension. Our findings will be helpful for counseling families of maternally inherited hypertension.

European Journal of Human Genetics (2011) 19, 1181–1186; doi:10.1038/ejhg.2011.111; published online 22 June 2011

Keywords: hypertension; mitochondria; tRNA metabolism; maternal inheritance; risk factor

INTRODUCTION

Hypertension is a major public health problem, affecting approximately 1 billion worldwide.¹ The etiology of hypertension is not well understood because of multi-factorial causes. Hypertension can be caused by single-gene or multi-factorial conditions, resulting from interactions between the environment and inherited risk factors.² In fact, human hypertension is a condition associated with endothelial dysfunction and oxidative stress.³,⁴ Mitochondrial dysfunction has been potentially implicated in both human and experimental hypertension.⁵-⁷ Specifically, abnormal mitochondrial respiration results in oxidative stress, uncoupling of the oxidative pathways for ATP synthesis and subsequent failure of cellular energetic processes.⁸ An inefficient metabolism caused by mitochondrial dysfunctions in skeletal and vascular smooth muscles may lead to the elevation of systolic blood pressure and, therefore, may be involved in the development of hypertension.⁶⁷⁹¹⁰ Maternal transmission of hypertension has been implicated in some pedigrees, suggesting that the mutation(s) in mitochondrial DNA (mtDNA) is one of the molecular bases for this disorder.¹⁰-¹⁶ In particular, the 4295A > G and 4263A > G mutations in the tRNA²Le gene, the 4401A > G mutation in the junction between tRNA⁴Thr and tRNAMet genes, as well as the 4435A > G mutation in the tRNAMet gene were associated with essential hypertension.¹⁴-¹⁷

With the aim of investigating a role of the mitochondrial genome in the pathogenesis of hypertension in the Chinese population, a systematic and extended mutational screening of mtDNA has been initiated in several cohorts of essential hypertension Chinese subjects.¹⁴-¹⁷ In the present study, we performed the clinical, genetic and molecular characterization of another Han Chinese family with suggestive maternally transmitted hypertension. Eight of 17 matrilineal relatives in this family exhibited variable severity and age-at-onset in essential hypertension, while none of the offspring of affected fathers had hypertension. Mutational analysis of the mitochondrial genome in this Chinese family identified the known tRNAMet 4435A > G mutation, which is localized at the 3' end adjacent to the anticodon (position 37) of tRNAMet.¹⁷-¹⁹ The adenine at this position of tRNAMet is extraordinarily conserved from bacteria to human mitochondria. The mitochondrial genome in this Chinese family belonged to the Eastern–Asian haplogroup G2a1,²⁰ while the
4435A>G mutation also occurs in the other mtDNA haplogroups: B5a of a Chinese family with hypertension and D5 of a Chinese family with LHON and a Japanese subject with diabetes. The occurrence of the 4435A>G mutation in these genetically unrelated subjects affected by the hypertension suggests that this mutation is involved in the pathogenesis of hypertension.

SUBJECTS AND METHODS
Subjects
As a part of a genetic screening program for hypertension, a Han Chinese family (Figure 1) was ascertained at the Hypertension Clinic of Wenzhou Medical College. Informed consent, blood samples and clinical evaluations were obtained from all participating family members, under the protocols approved by the ethics committee of Wenzhou Medical College 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.

Clinical evaluation
Members of this Chinese family underwent a physical examination and laboratory assessment of cardiovascular disease risk factors. A physician measured the systolic and diastolic blood pressures of subjects using a mercury column sphygmonanometer and a standard protocol. The first and the fifth Korotkoff sounds were taken as indicative of systolic and diastolic blood pressure, respectively. The average of three such systolic and diastolic blood pressure readings was taken as the examination blood pressure. Hypertension was defined according to the recommendation 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 blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of ≥90 mm Hg.

These subjects then underwent a heart function evaluation by electrocardiography (ECG). Signals from the first 10 s of the conventional ECG recording were analyzed automatically in software to quantify all major intervals, axes and QRS complexes. These intervals were analyzed automatically in software to quantify all major intervals, axes and QRS complexes.

Mutational analysis of mitochondrial genome
Genomic DNA was isolated from whole blood cells of participants using Puregene DNA Isolation Kits (Genta Systems, Minneapolis, MN, USA). The entire mitochondrial genome of the proband II-9 was PCR amplified in 24 overlapping fragments by using sets of the light-strand and heavy-strand oligonucleotide primers, as described elsewhere. 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_012920).

For the quantification of the 4435A>G mutation, the PCR segments (700 bp) were amplified using genomic DNA as template and oligodeoxynucleotides corresponding to mtDNA at positions 3861–4560, and subsequently digested with a restriction enzyme NalI. 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 observe whether the 4435A>G mutation is in homoplasmy in these subjects.

Phylogenetic analysis
A total of 17 vertebrate mtDNA 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, Muc muculaa, Nycticebus couang, Pan paniscus, Pan troglodytes, Pongo pygmaeus, Pongo abelli, Papio hamadryas, Tarsius bancanus, and Xenopus laevis (Genbank). The conservation index (CI) was calculated by comparing the human nucleotide variants with those of other 16 vertebrates. The CI was then defined as the percentage of species from the list of 16 different vertebrates that have the wild-type nucleotide at that position.

Haplogroup analyses
The entire mtDNA sequence of the Chinese proband carrying the 4435A>G mutation was assigned to an Asian mitochondrial haplogroup by using the nomenclature of mitochondrial haplogroups.

RESULTS
Clinical presentation
The proband (II-9) began suffering from hypertension at the age of 54 years. As shown in Table 1, his blood pressure was 190/110 mm Hg by then. He came to the Hypertension Clinic of Wenzhou Medical College for further clinical evaluations at the age of 60 years. After the administration of ACE inhibitor, calcium channel blocker (CCB) and diuretic, his blood pressure was 132/80 mm Hg. As shown in Table 2, laboratory assessment of cardiovascular disease risk factors showed that he had a normal range of the index of liver metabolic function, the blood routine and 24-h urinary sodium. The echocardiogram (ECG) showed that his interventricular septal and posterior ventricular wall thickness (13 mm) increased with normal atrial and ventricular dimension. Physical examination showed that he did not have other clinical abnormalities, including diabetes, visual and hearing impairments, renal and neurological disorders. Therefore, he exhibited a typical essential hypertension. The family is originated from Zhejiang Province in Eastern China. All members of this family were interviewed and/or evaluated to identify both personal and medical histories of hypertension and other clinical abnormalities. As shown in Figure 1 and Table 1, 8 of 17 matrilineal relatives had a wide range of severity in hypertension (with blood pressure >140/90 mm Hg even with treatment for hypertension), whereas only 1 of 8 nonmaternal relatives suffered from hypertension. None of the offspring of affected fathers exhibited hypertension. As shown in Table 1, the age at onset of hypertension in eight affected matrilineal relatives of the maternal kindred varied from 31 to 65 years, with an average of...
Table 1 Summary of clinical data for some members in a Chinese pedigree

| Subjects | Gender | Age of test (years) | Age of onset (years) | Systolic pressure (mm Hg) | Diastolic pressure (mm Hg) | IVST (mm) | LVMI (g/m²) | ECG | CR (μmol/l) | UR (μmol/l) |
|----------|--------|--------------------|----------------------|--------------------------|---------------------------|----------|-------------|-----|-------------|-------------|
| I-2      | F      | 65                 | 65                   | 165                      | 110                       | —        | —           | —   | —           | —           |
| II-2     | F      | 63                 | 60                   | 165                      | 110                       | —        | —           | —   | —           | —           |
| II-3     | M      | 70                 | 65                   | 170                      | 95                        | 9        | 123.77      | SB  | 84          | 5.1         |
| II-4     | F      | 64                 | 31                   | 200                      | 100                       | 10       | 84.58       | MI  | 64          | 6.3         |
| II-6     | F      | 56                 | 46                   | 150                      | 96                        | 9        | 77.45       | N   | 58          | 5.5         |
| II-8     | F      | 53                 | 53                   | 150                      | 100                       | 8        | 85.71       | MI  | 55          | 3.9         |
| II-9     | M      | 60                 | 54                   | 190                      | 110                       | 12       | 72.59       | LVH | 70          | 5.5         |
| III-1    | M      | 57                 | 54                   | 140                      | 90                        | 10       | 97.99       | N   | 75          | 7.1         |
| III-3    | M      | 48                 | —                    | 120                      | 80                        | 8        | 59.74       | N   | 80          | 7.2         |
| III-4    | M      | 46                 | —                    | 130                      | 80                        | 10       | 132.66      | MI  | 66          | 5.8         |
| III-5    | F      | 51                 | 51                   | 140                      | 95                        | 9        | 89.69       | MI  | 48          | 4.6         |
| III-6    | M      | 38                 | —                    | 125                      | 85                        | —        | —           | —   | —           | —           |
| III-7    | M      | 38                 | —                    | 120                      | 75                        | —        | —           | —   | —           | —           |
| III-8    | F      | 41                 | —                    | 130                      | 80                        | 8        | 97.15       | ST-E| 63          | 5.9         |
| III-9    | F      | 30                 | —                    | 115                      | 85                        | —        | —           | —   | —           | —           |
| III-10   | F      | 33                 | —                    | 125                      | 80                        | —        | —           | —   | —           | —           |
| III-11   | M      | 26                 | —                    | 135                      | 75                        | —        | —           | —   | —           | —           |

Abbreviations: F, female; M, male; IVST, interventricular septal thickness; LVMI, left ventricular mass index; N, electrocardiography (ECG) was normal; LVH, ECG showed left ventricular hypertrophy; MI, myocardial ischemia; SB, sinus bradycardia; ST-E, ST segment elevation; CR, creatinine; UR, urea nitrogen.

*These patients had anti-hypertension treatment. This table shows pre-treatment blood pressures.

Table 2 Summary of laboratory examinations for some members of a Chinese family

| Subjects | II-4 | II-6 | II-8 | II-9 | Chinese reference |
|----------|------|------|------|------|-------------------|
| Therapy  | Yes  | Yes  | Yes  | Yes  | —                 |
| Alcohol  | No   | No   | No   | Yes  | —                 |
| Tobacco  | No   | No   | No   | No   | —                 |
| FPG, mmol/l | 5.6  | 4.4  | 4.6  | 4.8  | 3.90–6.10         |
| TC, mmol/l | 5.22 | 4.3  | 5.68 | 3.8  | 2.44–6.17         |
| TG, mmol/l | 1.92 | 1.76 | 1.13 | 1.1  | 0.40–1.70         |
| HDL, mmol/l | 1.34 | 1.91 | 1.74 | 1.13 | 1.16–1.42         |
| LDL, mmol/l | 3.04 | 1.45 | 2.97 | 2.2  | 2.10–3.10         |
| UA, μmol/l | 415  | 268  | 226  | 358  | 214–488 (137–363)* |

Abbreviations: FPG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; UA, uric acid.

*Reference value especially for females.

52 years. However, other nine unaffected matrilineal relatives, who were below 52 years, had a tendency to develop the hypertension. There was no evidence that any member of this family had any other cause to account for hypertension. We further examined the end organ damage on the heart and kidney among nine matrilineal relatives and married-in subject II-3 of this family. As shown in Table 1, four (II-4, II-6, II-8, II-9) matrilineal relatives exhibited myocardial ischemia on the ECG recorded, while subject III-8 suffered from segment elevation. In addition, none of eight matrilineal relatives, except the married-in subject II-3 of this family, exhibited an increased interventricular septal thickness.

mtDNA 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-6 were PCR amplified, and each fragment was purified and subsequently analyzed by direct sequence. As shown in Table 3, comparison of the resultant sequences with the revised Cambridge consensus sequence identified the known hypertension-associated 4435A>G mutation in the tRNA<sup>Met</sup> gene and other 41 known nucleoside changes, belonging to the Eastern–Asian haplogroup G2a1. Other mtDNA variants of the proband II-9, there were 12 polymorphisms in the D-loop region, 2 variants in the 12S rRNA gene, 1 variant in the 16S rRNA gene, the 5601C>T mutation in the tRNA<sup>Ala</sup> gene, 17 silent mutations in protein encoding genes (http://www.mitomap.org or http://www.genpat.uu.se/mtDB). These nucleotide variants with 16 other vertebrates. CIs of these variants were calculated by comparing the human resultant sequences with the revised Cambridge consensus sequence. As shown in Table 3, comparison of the mtDNA sequence variations of the Chinese proband to establish the haplogroup affiliation of his mtDNA. Here, mtDNA analysis revealed that these variants were of the Eastern–Asian haplogroup G2a1. The known 4435A>G mutation in the tRNA<sup>Met</sup> gene, as shown in Figure 2, is located at immediately 3′ end to the anticodon,
Table 3 mtDNA variants in one Han Chinese subject (II-6) with hypertension

| Gene      | Position | Replacement | Conservation H/M/B/X | Previously reported |
|-----------|----------|-------------|----------------------|---------------------|
| D-Loop    | 73       | A to G      | Yes                  |                     |
|           | 152      | T to C      | Yes                  |                     |
|           | 263      | A to G      | Yes                  |                     |
|           | 310      | T to CTC    | Yes                  |                     |
|           | 489      | T to C      | Yes                  |                     |
|           | 16223    | C to T      | Yes                  |                     |
|           | 16227    | A to G      | Yes                  |                     |
|           | 16272    | A to G      | Yes                  |                     |
|           | 16271    | C to T      | Yes                  |                     |
|           | 16319    | G to A      | Yes                  |                     |
|           | 16362    | T to C      | Yes                  |                     |
|           | 16519    | T to C      | Yes                  |                     |
| 12S rRNA  | 709      | G to A      | G/A/A/A              | Yes                 |
|           | 750      | A to G      | A/G/A/A              |                     |
|           | 1438     | A to G      | A/A/A/G              |                     |
| 16S rRNA  | 2706     | A to G      | A/A/G/A              | Yes                 |
| tRNAMet   | 4435     | A to G      | A/A/A/A              |                     |
| ND2       | 4769     | A to G      | A/G/A/A              |                     |
|           | 4833     | A to G (Thr to Ala) | T/I/I/L         |                     |
|           | 5108     | T to C      | Yes                  |                     |
| tRNAAla   | 5601     | C to T      | C/C/C/G              |                     |
| CO1       | 7028     | C to T      | Yes                  |                     |
| CO2       | 7600     | G to A      | Yes                  |                     |
| ATP6      | 8701     | A to G (Thr to Ala) | T/S/L/Q         | Yes                 |
|           | 8860     | A to G (Thr to Ala) | T/A/A/T         | Yes                 |
| CO3       | 9377     | A to G      | Yes                  |                     |
|           | 9540     | T to C      | Yes                  |                     |
|           | 9575     | G to A      | Yes                  |                     |
| ND3       | 10398    | A to G (Thr to Ala) | T/T/T/A        | Yes                 |
|           | 10400    | C to T      | Yes                  |                     |
| ND4       | 10873    | T to C      | Yes                  |                     |
|           | 11719    | G to A      | Yes                  |                     |
| tRNAeu(CUN) | 12280   | A to G      | A/G/A/A              |                     |
| ND5       | 12705    | C to T      | Yes                  |                     |
|           | 13563    | G to A      | Yes                  |                     |
|           | 14034    | T to C      | Yes                  |                     |
| ND6       | 14569    | G to A      | Yes                  |                     |
| CYB       | 14766    | C to T (Thr to Ile) | T/S/T/S         | Yes                 |
|           | 14783    | T to C      | Yes                  |                     |
|           | 15043    | G to A      | Yes                  |                     |
|           | 15301    | G to A      | Yes                  |                     |
|           | 15326    | A to G (Thr to Ala) | T/M/I/I        | Yes                 |

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

**See http://www.mitomap.org and http://www.genpat.uu.se/mtDB.**

Regarding the corresponding to the conventional position 37 of tRNAMet.32 An adenine at this position is an extraordinarily conserved base in every sequenced methionine tRNA from bacteria to human mitochondria.32,33 The nucleotide at the position 37 is more prone to modification than those at other places of tRNA.34 The nucleotide modification at this position has been shown to have a pivotal role in the stabilization of tertiary structure and the biochemical function of tRNA.34 To determine if the 4435A>G mutation is present in homoplasy, the fragments spanning the tRNAMet gene were PCR-amplified and subsequently digested with NlaIII. There was no detectable wild-type DNA in all available matrilineal relatives (data not shown), indicating that the 4435A>G mutation was present in homoplasy in these matrilineal relatives.

**DISCUSSION**

In the present study, we have performed the clinical, genetic and molecular characterization of a Han Chinese family with essential hypertension. 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 affected matrilineal relatives in this family varied from 31 to 65 years, with an average of 52 years. This result was comparable to those of other Chinese families with maternally transmitted hypertension.14,15,16,17 Mutational analysis of mitochondrial genome in this family identified the tRNAMet 4435A>G mutation and other 35 variants belonging to the Eastern–Asian haplogroup G2a1.20 On the other hand, the 4435A>G mutation also occurred in the other mtDNA haplogroups B5a, D, M7a2 and J.17–19,35 This suggested that the 4435A>G mutation occurred sporadically and multiplied through evolution of the mtDNA. The 4435A>G mutation was associated with essential hypertension in a Chinese family,17 and other clinical abnormalities including Leber’s hereditary optic neuropathy18 and type 2 diabetes.19 The occurrence of the 4435A>G mutation in these genetically unrelated subjects affected by the hypertension suggests that this mutation is involved in the pathogenesis of hypertension.

It was anticipated that the 4435A>G mutation resulted in a deficient nucleotide modification at position 37 of tRNAMet, thereby altering the structure and function of tRNAMet. Functional significance of the 4435A>G mutation was supported by the fact that ~40–50% reduction in the levels of tRNAMet was observed in cells carrying the 4435A>G mutation.17,18 As a result, a failure in the tRNAMet metabolism is responsible for the reduced rate of mitochondrial protein synthesis. Consequently, these defects led to an impairment of the mitochondrial respiration chain function, reduction of ATP production and increase of reactive oxygen species production. These mitochondrial dysfunctions may contribute to the development of hypertension.7,10,15,36–38 In particular, the impaired mitochondrial function could contribute to the characteristic age-related increase in blood pressure.39 The homoplasmic form, mild mitochondrial dysfunction, late onset and incomplete penetrance of hypertension observed in this Chinese family carrying the 4435A>G mutation suggest that the mutation is an inherited risk factor necessary for the development of hypertension but may by itself be insufficient to produce a clinical phenotype. Indeed, the incomplete penetrance of other clinical abnormalities arises from homoplasmic mtDNA mutations such as hypertension-associated mtDNA 4401A>G mutation,15 deafness-associated 12S rRNA 1555A>G mutation40 and Leber’s hereditary optic neuropathy-associated ND4 11778G>A mutation.41 These homoplasmic mtDNA mutations only exhibited mild mitochondrial dysfunction.15,16,40,42 The other modifier factors such as nuclear modifier genes, environmental and epigenetic factors, and personal lifestyles39,43 may contribute to the development of hypertension in these subjects carrying the 4435A>G mutation. In particular, the tissue specificity of this mutation is likely attributed to tissue-specific RNA modification or the involvement of nuclear modifier genes. The 4435A>G mutation should be added to the list of inherited risk factors for future molecular diagnosis. Those who are positive for the 4435A>G mutation should be warned that they are at risk for the development of hypertension and therefore pay attention to their personal lifestyles. In conclusion, our data support the...
previous observation that impaired mitochondrial function caused by the 4435A>G tRNA\textsubscript{Met} mutation was associated with essential hypertension. Therefore, our findings will be helpful for counseling families of maternally inherited hypertension.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
This work was supported by National Institutes of Health (NIH) grants RO1DC05230 and RO1DC07696 from the National Institute on Deafness and Other Communication Disorders, a start-up fund from Zhejiang University to M-XG.

1 Guidelines Subcommittee: World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. J Hypertens 1999; 17: 151–183.
2 Lifton RP, Gharavi AG, Geller DS: Molecular mechanisms of human hypertension. Cell 2001; 104: 545–556.
3 Romero JC, Reckelhoff JF: State-of-the-Art lecture. Role of angiotensin and oxidative stress in essential hypertension. Hypertension 1999; 34: 943–949.
4 Redon J, Oliva MR, Tornos C et al: Antioxidant activities and oxidative stress byproducts in human hypertension. Hypertension 2003; 41: 1096–1101.
5 Chan SH, Wu KL, Chang KY, Tai MH, Chan JY: Oxidative impairment of mitochondrial electron transport chain complexes in rostral ventrolateral medulla contributes to neurogenic hypertension. Hypertension 2009; 53: 217–227.
6 Ansell DK, Elliott ST, Kane LA et al: Proteomic analysis of pharmacological preconditioning: novel protein targets converge to mitochondrial metabolism pathways. Circ Res 2006; 99: 706–714.
7 Beral-Mizrachi C, Gates AC, Weng S et al: Vascular respiratory uncoupling increases blood pressure and atherosclerosis. Nature 2005; 435: 502–506.
8 Wallace DC: A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005; 39: 359–407.
9 Winstaff U, Najar SM, Ellingsen O et al: Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science 2005; 307: 418–420.
10 Wilson FH, Hariri A, Farihi A et al: A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. Science 2004; 306: 1190–1194.
11 Watson JR B, Khan MA, Desmond RA, Bergman S: Mitochondrial DNA mutations in black Americans with hypertension-associated end-stage renal disease. Am J Kidney Dis 2001; 38: 529–536.
12 Schwartz F, Duka A, Sun F, Cui J, Manolis A, Gawas H: Mitochondrial genome mutations in hypertensive individuals. Am J Hypertens 2004; 17: 629–635.
13 MITOMAP: A Human Mitochondrial Genome Database. Available at http://www.mitomap.org.

14 Li Z, Liu Y, Yang L, Wang S, Guan MX: Maternally inherited hypertension is associated with the mitochondrial tRNA\textsubscript{Met} A4295G mutation in a Chinese family. Biochem Biophys Res Commun 2008; 367: 906–911.
15 Wang S, Li R, Fetterman A et al: Maternally inherited essential hypertension is associated with the novel 4263A>G mutation in the mitochondrial tRNA\textsubscript{Met} gene in a large Han Chinese family. Circ Res 2011; 108: 862–870.
16 Li R, Liu Y, Li Z, Yang L, Wang S, Guan MX: Failures in mitochondrial tRNA\textsubscript{Met} and tRNA\textsubscript{Gln} metabolism caused by the novel 4401A>G mutation are involved in essential hypertension in a Han Chinese Family. Hypertension 2009; 54: 329–337.
17 Liu Y, Li R, Li Z et al: The mitochondrial transfer RNA\textsubscript{Met} 4435A>G mutation is associated with maternally hypertension in a Chinese pedigree. Hypertension 2009; 53: 1083–1090.
18 Gu J, Li R, Zhou X et al: The novel 4435A>G mutation in the mitochondrial tRNA\textsubscript{Met} may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation. Invest Ophthalmol Vis Sci 2006; 47: 475–483.
19 Guo LJ, Oshida Y, Fuku N et al: Mitochondrial genome polymorphisms associated with type-2 diabetes or obesity. Mitochondrion 2005; 5: 15–33.
20 Tanaka M, Cabrera VM, Gonzalez AM et al: Mitochondrial genome variation in eastern Asia and the peopling of Japan. Genome Res 2004; 14: 1832–1890.
21 Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure: The sixth report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure. Arch Intern Med 1997; 157: 2413–2446.
22 Sokoloff M, Lyon TP: The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am Heart J 1946; 37: 161–186.
23 Okin PM, Roman MJ, Devereux RB, Kligfield P: Electrophysiologic identification of increased left ventricular mass by simple voltage-duration products. Am Coll Cardiol 1995; 25: 417–423.
24 Devereux RB, Casale PN, Kligfield P et al: Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986; 57: 450–458.
25 Rieder MJ, Taylor SL, Tobe YO, Nickerson DA: Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. Nucleic Acids Res 1998; 26: 967–973.
26 Andrews RM, Kubacka I, Chinney PF, Lightowlers RN, Turnbull DM, Howell N: Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 1999; 23: 147.
27 A Human Mitochondrial Genome Database. Available at http://www.genpat.uu.se/mtdb.
28 Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA: Sequence and gene organization of mouse mitochondrial DNA. Cell 1981; 25: 417–423.
29 Gadaleta G, Pepe G, De Candia G, Quagliauro C, Sibisa E, Saccone C: The complete nucleotide sequence of the Rattus norvegicus mitochondrial genome: cryptic signals revealed by comparative analysis between vertebrates. J Mol Evol 1989; 28: 497–516.
30 Roe A, Ma DP, Wilson RK, Wong JT: The complete nucleotide sequence of the Xenopus laevis mitochondrial genome. J Biol Chem 1985; 260: 9759–9774.
31 Wallace DC: A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005; 39: 359–407.
32 Florentz C, Sohm B, Tyroen-Toth P, Putz J, Sissler M: Human mitochondrial tRNAs in health and disease. Curr Mol Life Sci 2003; 60: 1356–1375.
33 Sprinzl M, Horn C, Brown M, Ioudovitch A, Steinberg S. Compilation of tRNA sequences and sequences of tRNA genes. Nucleic Acids Res 1998; 26: 148–153.
34 Björk GR. Biosynthesis and function of modified nucleotides; in D. Söll UL, RajBhandary (eds): tRNA: Structure, Biosynthesis and Function. Washington, DC: ASM Press, 1995, pp 165–206.
35 Herrnstadt C, Elson JL, Fahy E et al. Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the Major African, Asian, and European haplogroups. Am J Hum Genet 2002; 70: 1152–1171.
36 Postnov YV, Orlov SN, Budnikov YY, Doroschuk AD, Postnov AY. Mitochondrial energy conversion disturbance with decrease in ATP production as a source of systemic arterial hypertension. Pathophysiology 2007; 14: 195–204.
37 Lopez-Campistol A, Hao L, Xiang W et al. Mitochondrial dysfunction in the hypertensive rat brain: respiratory complexes exhibit assembly defects in hypertension. Hypertension 2008; 51: 412–4129.
38 Addabbo F, Montagnani M, Goligorsky MS. Mitochondria and reactive oxygen species. Hypertension 2009; 53: 885–892.
39 Vasan RS, Beiser A, Seshadri S et al. Residual lifetime risk for developing hypertension in middle-aged women and men: The Framingham Heart Study. JAMA 2002; 287: 1003–1010.
40 Guan MX. Mitochondrial 12S rRNA mutations associated with aminoglycoside ototoxicity. Mitochondrion 2011; 11: 237–245.
41 Qu J, Zhou X, Zhang J et al. Extremely low penetrance of Leber’s hereditary optic neuropathy (LHON) in eight Han Chinese families carrying the ND4 G11778A mutation. Ophthalmology 2009; 116: 558–564.
42 Hoffhaus G, Johns DR, Hurkoi O, Attardi G, Chomyn A. Respiration and growth defects in transmitochondrial cell lines carrying the 11778 mutation associated with Leber’s hereditary optic neuropathy. J Biol Chem 1996; 22: 13155–13161.
43 Djousse L, Driver JA, Gaziano JM. Relation between modifiable lifestyle factors and lifetime risk of heart failure. JAMA 2009; 302: 394–400.