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Maternally inherited hypertension is associated with the mitochondrial $tRNA^{Ile}$ A4295G mutation in a Chinese family

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Abstract

Mutations in mitochondrial DNA have been associated with cardiovascular disease. We report here the clinical, genetic, and molecular characterization of one three-generation Han Chinese family with maternally transmitted hypertension. All matrilineal relatives in this family exhibited the variable degree of hypertension at the age at onset of 36 to 56 years old. Sequence analysis of the complete mitochondrial DNA in this pedigree revealed the presence of the known hypertension-associated $tRNA^{Ile}$ A4295G mutation and 33 other variants, belonging to the Asian haplogroup D4j. The A4295G mutation, which is extraordinarily conserved from bacteria to human mitochondria, is located at immediately 3′ end to the anticodon, corresponding to conventional position 37 of $tRNA^{Ile}$. The occurrence of the A4295G mutation in several genetically unrelated pedigrees affected by cardiovascular disease but the absence of 242 Chinese controls strongly indicates that this mutation is involved in the pathogenesis of cardiovascular disease. Of other variants, the $tRNA^{Glu}$ A14693G and ND1 G11696A mutations were implicated to be associated with other mitochondrial disorders. The A14693G mutation, which is a highly conserved nucleoside at the TψC-loop of $tRNA^{Glu}$, has been implicated to be important for tRNA structure and function. Furthermore, the ND4 G11696A mutation was associated with Leber's hereditary optic neuropathy. Therefore, the combination of the A4295G mutation in the $tRNA^{Ile}$ gene with the ND4 G11696A mutation and $tRNA^{Glu}$ A14693G mutation may contribute to the high penetrance of hypertension in this Chinese family.
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Cardiovascular disease is one of the most common complex disorders. Cardiovascular disease can be caused by a single-gene or multi-factorial 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 [1–5]. Recently, several mtDNA point mutations have been identified to be associated with cardiovascular disease. These mutations included the A1555G mutation in the 12S rRNA gene [6], the A3260G and C3303T mutations in the $tRNA^{Leu(UUR)}$ gene [7,8], the A8348G and G8363A mutations in the $tRNA^{Lys}$ gene [9,10], the A4295G and A4300G mutations in the $tRNA^{Ile}$ gene [11–13]. Most recently, the T4291C mutation in $tRNA^{Ile}$ gene has been associated with a cluster of metabolic defects including essential hypertension, hypercholesterolemia, and hypomagnesaemia in a large family [14].

With an effort to understand a role of mitochondrial genome in pathogenesis of cardiovascular disease in Chinese population, we have initiated a systematic and

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extended mutational screening of mtDNA in a large cohort of hypertension subjects in the Geriatric Cardiology Clinic at the Chinese PLA General Hospital, China. In this investigation, the mutational screening of $tRNA^{Ile}$ gene led to the identification of the known hypertension-associated A4295G mutation in one Han Chinese pedigree. To assess the contribution that mtDNA variants make toward the phenotypic expression of the A4295G mutation, we performed a PCR amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in this family.

Materials and methods

Subjects. As a part of genetic screening program for hypertension, a Han Chinese family (Fig. 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 participating family members, under protocols approved by Ethic 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 individuals from Chinese ancestry.

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 pressure, respectively. The average of three such systolic and diastolic blood pressure reading 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) [15] and the World Health Organization-International Society of Hypertension [16] as a systolic blood pressure of 140 mm Hg or higher and/or a diastolic blood pressure of 90 mm Hg or greater.

Mutational analysis of mitochondrial genome. Genomic DNA was isolated from whole blood of participants using Puregene DNA Isolation Kits (Gentra Systems, Minneapolis, MN). First, subject's DNA fragments spanning the entire mitochondrial $tRNA^{Ile}$ gene were amplified by PCR using oligodeoxynucleotides corresponding to positions 3396–3415 and 4635–4654 [17]. PCR fragments were purified and subsequently analyzed by direct sequencing analysis. The allelic frequency of the A4295G mutation in 242 Chinese controls was determined as detailed elsewhere [11]. The entire mitochondrial genomes of the proband carrying A4295G mutation were PCR amplified in 24 overlapping fragments by use of sets of the light-strand and the heavy strand oligonucleotide primers, as described elsewhere [17]. 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 updated consensus Cambridge sequence (GenBank Accession No. NC_001807) [18].

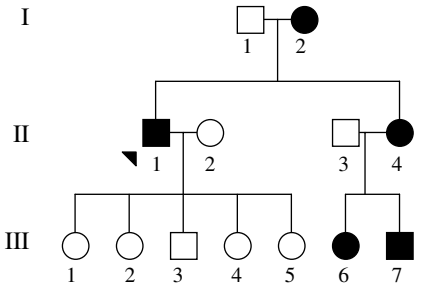

Fig. 1. The Chinese pedigree with hypertension. Affected individuals are indicated by filled symbols. Arrowhead denotes proband.

Results

Clinical presentation

The proband (II-1) began suffering from hypertension at the age of 36 years old. He came to the Geriatric Cardiology clinic of Chinese PLA General Hospital for further clinical evaluations at the age of 51 years old. His blood pressure was 210/120 mm Hg. 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 essential hypertension. The family is originated from Hebei Province in Northern China, and the majority of family members live in the same area. As shown in Fig. 1, this familial history is consistent with a maternal inheritance. None of the offspring of affected fathers has a hypertension, while all matrilineal relatives exhibited hypertension as the sole clinical symptom. In

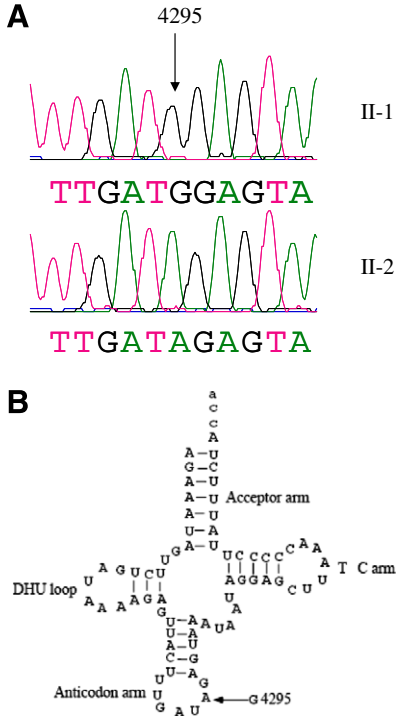

Fig. 2. Identification of the A4295G mutation in the mitochondrial $tRNA^{Ile}$ gene. (A) Partial sequence chromatograms of $tRNA^{Ile}$ gene from affected individual II-1 and a married-in-control II-2. An arrow indicates the location of the base changes at position 42,95. (B) The location of the A2955G mutation in the mitochondrial $tRNA^{Ile}$. Cloverleaf structure of human mitochondrial is derived from Florentz et al. [20]. Arrow indicates the position of the A2955G mutation.

addition, the age at onset of hypertension in this family varies from 30 years to 56 years. There is no evidence that any member of this family had any other known cause to account for hypertension.

Mitochondrial DNA analysis

The maternal transmission of hypertension in this family suggested the mitochondrial involvement and led us to analyze the mitochondrial genome of matrilineal relatives. First, we performed the mutational analysis of $tRNA^{Ile}$ gene by PCR amplification and subsequent sequence analysis of the PCR fragments derived from proband II-1 and one unrelated Chinese control. As shown in Fig. 2A, the known A4295G mutation in the $tRNA^{Ile}$ gene was identified in this Chinese subject. The frequency of this mutation in Chinese control population was then examined by sequencing the PCR fragment spanning the $tRNA^{Ile}$ gene. This mutation was absent in 242 Chinese controls. The A4295G mutation, as shown in Fig. 2B, is located at immediately 3′ end to the anticodon, corresponding to conventional position 37 of $tRNA^{Ile}$ [19,20]. Phylogenetic analysis, as shown in Fig. 3, revealed that an adenine at this position is an extraordinarily conserved base in every sequenced isoleucine tRNA from bacteria to human mitochondria [19,20]. Interestingly, the nucleotide at the position 37 is more prone to modification that those at other places of tRNA [21]. In fact, 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 [21].

To determine the role of mitochondrial variants/haplotypes in the phenotypic manifestation of the A4295G mutation, the DNA fragments spanning the entire mtDNA of the proband II-1 were PCR amplified. Each fragment was purified and subsequently analyzed by direct sequence. As shown in Table 1, the comparison of the resultant sequences with the Cambridge consensus sequence identified a number of nucleoside changes, belonging to the Eastern Asian haplogroup D4j [22]. Of these nucleoside changes, there were seven polymorphisms in the D-loop region, two variants in the 12S rRNA gene, two variants in the 16S rRNA gene, the A14693G mutation in $tRNA^{Glu}$ gene, 12 silent mutations and 7 minsense mutations in protein encoding genes [23]. These variants in tRNA, rRNAs, and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from other organisms including mouse [24], bovine [25] and *Xenopus laevis* [26]. None of variants in the polypeptides were highly evolutionarily conserved and implicated to have significantly functional consequence. However, the ND4 G11696A mutation was implicated to be associated with Leber's hereditary optic neuropathy (LHON) [27]. Furthermore, the A14693G mutation occurs at the extremely conserved nucleotide (conventional position 54) of the TψC-loop of $tRNA^{Glu}$ [19,20]. The A14693G mutation was implicated to be associated with mitochondrial disorders [28,29] and to influence the phenotypic expression of the deafness-asso-

Species	Acc-stem		D-stem	\	D-stem		Ac-stem	Anticd-loop		Ac-stem		T-stem	T-loop	T-stem	Acc-stem	
	1	8	10		22	26	27	32	37	39	44	49		61	66	73
Artibeus Jamaicensis	AGAAATA	TG	TCTG	ATAA	AAGA	G	TTACT	TTGATAG		AGTAA	ATTAT	AGGGG	CTAAATT	CCCCT	TATTTCT	A
Bos taurus	AGAAATA	TG	TCTG	ACAA	AAGA	G	TTACT	TTGATAG		AGTAA	ATAAT	AGAGG	TTCAAAC	CCTCT	TATTTCT	A
Felis catus	AGAAATA	TG	TCTG	ACAA	AAGA	G	TTACT	TTGATAG		AGTAA	AACAT	AGAGG	TTTAAAC	CCTCT	TATTTCT	A
Gorilla gorilla	AGAAATA	TG	TCTG	ATAA	AAGA	G	TTACT	TTGATAG		AGTAA	ATAAT	AGAGG	TTTAAAC	CCCCT	TATTTCT	A
Hippopotamus amphibius	AGAAATA	TG	TCTG	ACAA	AAGA	A	TTACT	TTGATAG		AGTAA	ATAAT	AGAGG	TTCAAGC	CCTCT	TATTTCT	A
Homo sapiens	AGAAATA	TG	TCTG	ATAA	AGAA	G	TTACT	TTCATAG		AGTAA	ATAAT	AGGAG	CTTAAAC	CCCCT	TATTTCT	A
Macropus robustus	AGTAAGG	TC	AGCT	AAATA	AGCT	A	TCGGG	CCCATAC		CCCGA	AAAT	GTTGG	TTTACAT	CCTTC	CCATACT	A
Mus musculus	AGAAATA	TG	TCTG	ATAA	AAGA	A	TTACT	TTGATAG		AGTAA	ATTAT	AGAGG	TTCAAGC	CCTCT	TATTTCT	A
Oryctolagus cuniculus	AGAAATA	TG	TCTG	ATAA	AAGA	G	TTACT	TTGATAG		AGTAA	ATAAT	AGAGG	ATCTTAGC	CCTCT	TATTTCT	A
Pan troglodytes	AGAAATA	TG	TCTG	ATAA	AAGA	A	TTACT	TTGATAG		AGTAA	ATAAT	AGGAG	TTCAAAT	CCCCT	TATTTCT	A
Papio hamadryas	AGAAATA	TG	TCTG	ACAA	AAGA	G	TTACT	TTGATAG		AGTAA	ACAAT	AGAGG	CCCCAAT	CCTCT	TATTTCT	A
Rattus norvegicus	AGAAATA	TG	TCTG	ACAA	AAGA	G	TTACT	TTGATAG		AGTAA	ATAAT	AGAGG	TTTAAAT	CCTCT	TATTTCT	A
Rhinoceros unicornis	AGAAATA	TG	TCTG	ACAA	AAGA	G	TTACT	TTGATAG		AGTAA	ATAAT	AGAGG	TTTAAAC	CCTCT	TATTTCT	A
Sus scrofa	AGAAATA	TG	TCTG	ACAA	AAGA	G	TTACT	TTGATAG		AGTAA	AACAT	AGACG	TTCAAAC	CCTCT	TATTTCT	A

Fig. 3. Alignment of $tRNA^{Ile}$ genes from different species. Arrow indicates the position of the A37 at the anticodon loop of tRNA, correspond to the A4295G mutation.

Table 1
mtDNA variants in one Chinese subject with hypertension

Gene	Position	Replacement	Conservation[a] H/B/M/X	Previously reported[b]
D-loop	73	A to G		Yes
	263	A to G		Yes
	310	T to CTC		Yes
	489	T to C		Yes
	16,051	A to G		Yes
	16,223	C to T		Yes
	16,362	T to C		Yes
12S	750	A to G	A/A/G/-	Yes
rRNA	1438	A to G	A/A/A/G	Yes
16S	2706	A to G	A/G/A/A	Yes
rRNA	3010	G to A	G/G/A/A	Yes
ND1	4048	G to A (Asp to Asn)	D/N/Y/M	Yes
$tRNA^{Ile}$	4295	A to G	A/A/A/A	Yes
ND2	4769	A to G		Yes
	4883	C to T		Yes
	5178	C to A (Leu to Met)	L/T/T/T	Yes
CO1	5993	C to T		Yes
	7028	C to T		Yes
A6	8414	C to T (Leu to Phe)	L/F/M/W	Yes
A8	8860	A to G (Thr to Ala)	T/A/A/T	Yes
ND3	10,398	A to G (Thr to Ala)	T/T/T/A	Yes
	10,400	C to T		Yes
ND4	10,873	T to C		Yes
	11,509	C to T		Yes
	11,617	T to C (Ile to Met)	I/I/I/I	Yes
	11,696	G to A (Val to Ile)	V/T/T/M	Yes
	11,719	G to A		Yes
ND5	12,501	G to A		Yes
	12,705	C to T		Yes
ND6	14,668	C to T		Yes
$tRNA^{Glu}$	14,693	A to G	A/A/A/A	No
Cyt b	14,783	T to C		Yes
	15,301	G to A		Yes
	15,326	A to G (Thr to Ala)	T/M/I/I	Yes

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

ciated 12S rRNA A1555G mutation [30] and the LHON-associated ND1G3460A mutation [31].

Discussion

In the present study, we have performed the clinical, genetic, and molecular characterization of a Chinese family with hypertension. The hypertension as a sole clinical phenotype was only present in all matrilineal relatives of this three-generation pedigree, suggesting that the mtDNA mutation(s) is the molecular basis for this disorder. The mutational analysis of mitochondrial genome identified the A4295G mutation in the $tRNA^{Ile}$ gene in matrilineal relatives of this Chinese family. Indeed, this mutation was initially identified in a family with hypertrophic cardiomyopathy [11] and subsequently in a Finnish family with occipital stroke [12]. In fact, the occurrence of the A4295G mutation in these genetically unrelated pedigrees affected by cardiovascular disease but absence of 242 Chinese controls strongly indicates that this mutation is involved in the pathogenesis of cardiovascular disease.

The A4295G mutation is localized at 3′ end adjacent to the anticodon (position 37) of $tRNA^{Ile}$ [19,20]. In fact, the adenine at this position of $tRNA^{Ile}$ is extraordinarily conserved from bacteria to human mitochondria [20]. Almost all of A37 in tRNAs are modified, such as thiolation and methylation [21]. Indeed, this modified nucleotide contributes to the high fidelity of codon recognition, the structural formation and stabilization of functional tRNAs [32]. 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 $tRNA^{Lys}$ [33]. Also, it has been shown that the modification of A37 stabilizes the 3′ stacking features of the anticodon, thereby improve its interaction with codon [34]. The deficient modification of A37 decreased the activity of the corresponding tRNA [35] and increased +1 frameshifts for $tRNA^{Phe}$ [36], while the A-to-G substitution at position 37 led to a tenfold reduction in the section of tRNAs at the A-site [37]. Most recently, ~50% reduction in the level of $tRNA^{Met}$ was observed in cells carrying the $tRNA^{Met}$ A4435G mutation [38]. The lower level of $tRNA^{Met}$ in cells carrying the $tRNA^{Met}$ A4435G mutation most probably results from a defect in nucleotide modification at position 37 of $tRNA^{Met}$. Therefore, the A4295G mutation may lead to a failure in mitochondrial tRNA metabolism and impairment of mitochondrial translation, thereby causing mitochondrial dysfunctions [11].

Furthermore, the mitochondrial variants/haplotypes have been shown to influence the phenotypic manifestation of the primary mtDNA mutations. In particular, the ND4 G11696A was associated with LHON in the Chinese families with extremely low penetrance of vision loss [28] and was implicated to act in synergy with the primary LHON-associated ND4 G11778A mutation [39]. In addition, the $tRNA^{Glu}$ A14693G mutation occurs at the extremely conserved nucleotide (conventional position 54) of the TψC-loop of $tRNA^{Glu}$ [19,20]. The A14693G mutation was implicated to be associated with mitochondrial disorders [28,29]. The U-to-C mutation at position 14693 occurs at the first base (conventional position 54) of the TψC-loop of $tRNA^{Glu}$. In fact, nucleotides at position of 54 of the TψC-loop of tRNA are often modified, thereby contributing to the structural formation and stabilization of functional tRNAs [21]. Thus, the alteration of structure of these tRNAs by the A14693G mutation may lead to a failure in tRNA metabolism. Most recently, we suggested that the A14693G may play a modifying role in the phenotypic manifestation of the deafness-associated 12S rRNA A1555G mutation [30] and the LHON-associated ND1 G3460A mutation [31] in the Chinese families. Therefore, the combination of the A4295G mutation in the $tRNA^{Ile}$ gene with the ND4 G11696A mutation and $tRNA^{Glu}$ A14693G mutation may contribute to high penetrance of hypertension in this Chinese family.

Acknowledgments

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References

[1] A.P. Brandao, A.A. Brandao, E.M. Araujo, R.C. Oliveira, Familial aggregation of arterial blood pressure and possible genetic influence, Hypertension 19 (1992) II214–II217.
[2] D.C. Wallace, Mitochondrial defects in cardiomyopathy and neuromuscular disease, Am. Heart J. 139 (2000) S70–S85.
[3] M. Hirano, M. Davidson, S. DiMauro, Mitochondria and the heart, Curr. Opin. Cardiol. 16 (2001) 201–210.
[4] B. Watson Jr., M.A. Khan, R.A. Desmond, S. Bergman, Mitochondrial DNA mutations in black Americans with hypertension-associated end-stage renal disease, Am. J. Kidney Dis. 38 (2001) 529–536.
[5] F. Schwartz, A. Duka, F. Sun, J. Cui, A. Manolis, H. Gavras, Mitochondrial genome mutations in hypertensive individuals, Am. J. Hypertens. 17 (2004) 629–635.
[6] F.M. Santorelli, K. Tanji, P. Manta, C. Casali, S. Krishna, A.P. Hays, D.M. Mancini, S. DiMauro, M. Hirano, Maternally inherited cardiomyopathy: an atypical presentation of the mtDNA 12S rRNA gene A1555G mutation, Am. J. Hum. Genet. 64 (1999) 295–300.
[7] M. Zeviani, C. Gellera, C. Antozzi, M. Rimoldi, L. Morandi, F. Villani, V. Tiranti, S. DiDonato, Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA $tRNA^{Leu(UUR)}$, Lancet 338 (1991) 143–147.
[8] G. Silvestri, F.M. Santorelli, S. Shanske, C.B. Whitley, L.A. Schimmenti, S.A. Smith, S. DiMauro, A new mtDNA mutation in the $tRNA^{Leu(UUR)}$ gene associated with maternally inherited cardiomyopathy, Hum. Mut. 3 (1994) 37–43.
[9] F. Terasaki, M. Tanaka, K. Kawamura, Y. Kanzaki, M. Okabe, T. Hayashi, H. Shimomura, T. Ito, M. Suwa, J.S. Gong, J. Zhang, Y. Kitaura, A case of cardiomyopathy showing progression from the hypertrophic to the dilated form: association of Mt8348A→G mutation in the mitochondrial $tRNA^{Lys}$ gene with severe ultrastructural alterations of mitochondria in cardiomyocytes, Jpn. Circ. J. 65 (2001) 691–694.
[10] F.M. Santorelli, S.C. Mak, M. El-Schahawi, C. Casali, S. Shanske, T.Z. Baram, R.E. Madrid, S. DiMauro, Maternally inherited cardiomyopathy and hearing loss associated with a novel mutation in the mitochondrial $tRNA^{Lys}$ gene (G8363A), Am. J. Hum. Genet. 58 (1996) 933–939.
[11] F. Merante, T. Myint, I. Tein, L. Benson, B.H. Robinson, An additional mitochondrial tRNA(Ile) point mutation (A-to-G at nucleotide 4295) causing hypertrophic cardiomyopathy, Hum. Mut. 8 (1996) 216–222.
[12] S. Finnila, I.E. Hassinen, K. Majamaa, Phylogenetic analysis of mitochondrial DNA in patients with an occipital stroke. Evaluation of mutations by using sequence data on the entire coding region, Mut. Res. 458 (2001) 31–39.
[13] R.W. Taylor, C. Giordano, M.M. Davidson, G. d'Amati, H. Bain, C.M. Hayes, H. Leonard, M.J. Barron, C. Casali, F.M. Santorelli, M. Hirano, R.N. Lightowlers, S. DiMauro, D.M. Turnbull, A homoplasmic mitochondrial transfer ribonucleic acid mutation as a cause of maternally inherited hypertrophic cardiomyopathy, J. Am. Coll. Cardiol. 41 (2003) 1786–1796.
[14] F.H. Wilson, A. Hariri, A. Farhi, H. Zhao, K.F. Petersen, H.R. Toka, C. Nelson-Williams, K.M. Raja, M. Kashgarian, G.I. Shulman, S.J. Scheinman, R.P. Lifton, A cluster of metabolic defects caused by mutation in a mitochondrial tRNA, Science 306 (2004) 1190–1194.
[15] The sixth report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, Arch. Intern. Med. 157 (1997) 2413–2446.
[16] 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. J. Hypertens. 17 (1999) 151–183.
[17] M.J. Rieder, S.L. Taylor, V.O. Tobe, D.A. Nickerson, Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome, Nucleic Acids Res. 26 (1981) 967–973.
[18] S. Anderson, A.T. Bankier, B.G. Barrell, M.H.L. deBruijn, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Rose, F. Sanger, P.H. Schreier, A.J.H. Smith, R. Staden, I. Young, Sequence and organization of the human mitochondrial genome, Nature 290 (1981) 457–465.
[19] M. Sprinzl, C. Horn, M. Brown, A. Ioudovitch, S. Steinberg, Compilation of tRNA sequences and sequences of tRNA genes, Nucleic Acids Res. 26 (1998) 148–153.
[20] C. Florentz, B. Sohm, P. Tryoen-Toth, J. Putz, M. Sissler, Human mitochondrial tRNAs in health and disease, Cell. Mol. Life Sci. 60 (2003) 1356–1375.
[21] G.R. Björk, tRNA: structure, biosynthesis and function, in: D. Söll, U.L. RajBhandary (Eds.), tRNA: Structure, Biosynthesis and Function, ASM Press, Washington, DC, 1995, pp. 165–206.
[22] M. Tanaka, V.M. Cabrera, A.M. Gonzalez, J.M. Larruga, T. Takeyasu, N. Fuku, L.J. Guo, R. Hirose, Y. Fujita, M. Kurata, K. Shinoda, K. Umetsu, Y. Yamada, Y. Oshida, Y. Sato, N. Hattori, Y. Mizuno, Y. Arai, N. Hirose, S. Ohta, O. Ogawa, Y. Tanaka, R. Kawamori, M. Shamoto-Nagai, W. Maruyama, H. Shimokata, R. Suzuki, H. Shimodaira, Mitochondrial genome variation in eastern Asia and the peopling of Japan, Genome Res. (2004) 1832–1850.
[23] M.C. Brandon, M.T. Lott, K.C. Nguyen, S. Spolim, S.B. Navathe, P. Baldi, D.C. Wallace, MITOMAP: a human mitochondrial genome database—2004 update, Nucleic Acids Res. 33 (2005) D611–D613.
[24] M.J. Bibb, R.A. Van Etten, C.T. Wright, M.W. Walberg, D.A. Clayton, Sequence and gene organization of mouse mitochondrial DNA, Cell 26 (1981) 167–180.
[25] G. Gadaleta, G. Pepe, G. De Candia, C. Quagliariello, E. Sbisa, C. Saccone, The complete nucleotide sequence of the *Rattus norvegicus* mitochondrial genome: cryptic signals revealed by comparative analysis between vertebrates, J. Mol. Evol. 28 (1989) 497–516.
[26] A. Roe, D.P. Ma, R.K. Wilson, J.F. Wong, The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome, J. Biol. Chem. 260 (1985) 9759–9774.
[27] X. Zhou, Q. Wei, L. Yang, Y. Tong, F. Zhao, C. Lu, Y. Qian, Y. Sun, F. Lu, J. Qu, M.X. Guan, Leber's hereditary optic neuropathy is associated with the mitochondrial ND4 G11696A mutation in five Chinese families, Biochem. Biophys. Res. Commun. 340 (2006) 69–75.
[28] D. Sternberg, E. Chatzoglou, P. Laforet, G. Fayet, C. Jardel, P. Blondy, M. Fardeau, S. Amselem, B. Eymard, A. Lombes, Mitochondrial DNA transfer RNA gene sequence variations in patients with mitochondrial disorders, Brain 124 (2001) 984–994.
[29] C.Y. Tzen, P. Thajeb, T.Y. Wu, S.C. Chen, MELAS with point mutations involving tRNALeu (A3243G) and tRNAGlu (A14693G), Muscle Nerve 28 (2003) 575–581.
[30] W.Y. Young, L. Zhao, Y. Qian, R. Li, J. Chen, P. Dai, S. Zhai, D. Han, M.X. Guan, Variants in mitochondrial $tRNA^{Glu}$, $tRNA^{Arg}$, and $tRNA^{Thr}$ may influence the phenotypic manifestation of deafness-associated 12S rRNA A1555G mutation in three Han Chinese families with hearing loss, Am. J. Med. Genet. A 140 (2006) 2188–2197.
[31] Y. Tong, Y. Mao, X. Zhou, L. Yang, J. Zhang, W. Cai, F. Zhao, X. Wang, F. Lu, J. Qu, M.X. Guan, The mitochondrial $tRNA^{Glu}$ A14693G mutation may influence the phenotypic manifestation of ND1 G3460A mutation in a Chinese family with Leber's hereditary

optic neuropathy, Biochem. Biophys. Res. Commun. 357 (2007) 524–530.

[32] S. Yokoyama, S. Nishimura, Modified nucleosides and codon recognition, in: D. Söll, U.L. RajBhandary (Eds.), tRNA: Structure, Biosynthesis and Function, ASM Press, Washington, DC, 1995, pp. 207–224.

[33] M. Sundaram, P.C. Durant, D.R. Davis, Hypermodified nucleosides in the anticodon of $tRNA^{(Lys)}$ stabilize a canonical U-turn structure, Biochemistry 39 (2000) 12575–12584.

[34] J. Li, B. Esberg, J.F. Curran, G.R. Bjork, Three modified nucleosides present in the anticodon stem and loop influence the in vivo aa-tRNA selection in a tRNA-dependent manner, J. Mol. Biol. 271 (1997) 209–221.

[35] M. Buck, F. Griffiths, Iron mediated methylthiolation of tRNA as a regulator of operon expression in *Escherichia coli*, Nucleic Acids Res. 10 (1982) 2024–2609.

[36] J. Urbonavicius, Q. Qian, J.M. Durand, T.G. Hagervall, G.R. Bjork, Improvement of reading frame maintenance is a common function for several tRNA modifications, EMBO J. 20 (2001) 4863–4873.

[37] M. Yarus, S.W. Cline, P. Wier, L. Breeden, R.C. Thompson, Actions of the anticodon arm in translation on the phenotypes of RNA mutants, J. Mol. Biol. 192 (1986) 235–255.

[38] J. Qu, R. Li, X. Zhou, Y. Tong, F. Lu, Y. Qian, Y. Hu, J.Q. Mo, C.E. West, M.X. Guan, The novel A4435G mutation in the mitochondrial tRNAMet may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation, Invest. Ophthalmol. Vis. Sci. 47 (2006) 475–483.

[39] J. Qu, R. Li, X. Zhou, Y. Tong, L. Yang, J. Chen, F. Zhao, C. Lu, Y. Qian, F. Lu, M.X. Guan, Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family, Mitochondrion 7 (2007) 140–146.