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Biochemical and Biophysical Research Communications 368 (2008) 18–22
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The mitochondrial ND1 T3308C mutation in a Chinese family with the secondary hypertension

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Received 25 December 2007
Available online 14 January 2008

Abstract

Mutations in mitochondrial DNA have been associated with hypertension. We report here the clinical, genetic, and molecular characterization of one four-generation Han Chinese family with hypertension. Two matrilineal relatives in this family exhibited the variable degree of a secondary hypertension (renal hypertension) at the age-at-onset of 42 and 56 years old, respectively. Sequence analysis of the complete mitochondrial DNA in this pedigree revealed the presence of the known hypertension-associated ND1 T3308C mutation and 42 other variants, belonging to the Asian haplogroup D4h. The T3308C mutation resulted in the replacement of the first amino acid, translation-initiating methionine with a threonine in ND1. Furthermore, the ND3 T3308C mutation also locates in two nucleotides adjacent to the 3′ end of mitochondrial $tRNA^{Leu(UUR)}$. Thus, this T3308C mutation caused an alteration on the processing of the H-strand polycistronic RNA precursors or the destabilization of ND1 mRNA. The occurrence of the T3308C mutation in these genetically unrelated pedigrees affected by diseases but absence of 242 Chinese controls as well as the mitochondrial dysfunctions detected in cells carrying this mutation indicate that this mutation is involved in the pathogenesis of hypertension. However, the mild biochemical defects, the lower penetrance of hypertension in this Chinese family and the presence of some control populations suggested the involvement of other modifier factors in the pathogenesis of hypertension associated with this ND1 T3308C mutation.

Keywords: Hypertension; Mitochondrial DNA; Mutation; Processing; tRNA; Renal; Modifiers; Chinese

Hypertension is one of the most common human sufferings, affecting approximately 1 billion individuals worldwide and 130 million in China [1]. Hypertension can be classified as either essential (primary) or secondary. Essential hypertension indicates that no specific medical cause can be found to explain a patient's condition. Secondary hypertension indicates that the high blood pressure is a result of (i.e., secondary to) another condition, such as kidney disease or certain tumors (especially of the adrenal gland) [2]. The etiology of hypertension is not well understood due to the multi-factorial causes. Hypertension can be caused by single or multi-factors including hereditary, environmental and personnel factors. Of hereditary factors, the maternal transmissions of hypertension have been implicated in some pedigrees, suggesting that the mutation(s) in mitochondrial DNA (mtDNA) is one of the molecular bases for this disorder [3–7]. Recently, several mtDNA point mutations have been identified to be associated with hypertension. These mutations included the A1555G mutation in the 12S rRNA gene [8], the A3260G and C3303T mutations in the $tRNA^{Leu(UUR)}$ gene [9,10], the A8348G and G8363A mutations in the $tRNA^{Lys}$ gene [11,12], the A4295G and A4300G mutations in the $tRNA^{Ile}$ gene [13–15]. Most recently, the T4291C mutation in $tRNA^{Ile}$ gene has been associated with a cluster of

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0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.12.193

metabolic defects including essential hypertension, hypercholesterolemia and hypomagnesaemia in a large family [16].

To investigate a role of mitochondrial genome in the pathogenesis of hypertension in Chinese population, we have initiated a systematic and extended mutational screening of mtDNA in a large cohort of hypertension subjects in the Geriatric Cardiology Clinic at the Chinese PLA General Hospital, China [17]. In this study, the mutational screening of ND1 gene led to the identification of the known disease-associated T3308C mutation in one Han Chinese pedigree with secondary hypertension due to congenital deformity of the kidney. To assess the contribution that mtDNA variants make toward the phenotypic expression of the T3308C 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 firth 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) [18] and the World Health Organization-International Society of Hypertension [2] as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or greater.

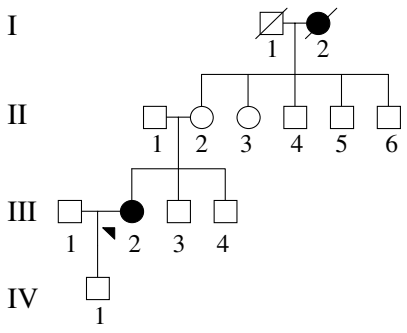

Fig. 1. A four-generation Han Chinese pedigree with hypertension. Affected individuals are indicated by filled symbols. Arrowhead denotes proband.

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 ND1 gene were amplified by PCR using oligodeoxynucleotides corresponding to positions 3149–3169 and 3942–3961 [19]. PCR fragments were purified and subsequently analyzed by direct sequencing analysis. The allelic frequency of the T3308C mutation in 242 Chinese controls was determined as detailed elsewhere [19]. The entire mitochondrial genomes of the proband carrying T3308C 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 [19]. 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) [20].

Results and discussion

The proband (III-2) was a 36 years old woman. She came to the Geriatric Cardiology clinic of Chinese PLA General Hospital because of intermittent lumbago, gross hematuria for 1 month and dizziness for 1 year. Her blood pressures were ranged from 130 to 100 mmHg (diastolic hypertension, stage 1). Her electrocardiography (ECG) showed normal sinus rhythms, T wave flatting or inverse in leads I, avL, V_2–V_6. Sonography, pyelography and magnetic resonance imaging (MRI) examinations exhibited that she had left multiple renal lithiasis, left nephrohydrosis and congenital deformity of the kidney (horseshoe kidney). Physical examination and laboratory assessment of cardiovascular disease risk factors showed no other clinical abnormalities, including diabetes, vision and hearing loss, and neurological disorders. Thus, this subject exhibited a secondary hypertension (renal 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, only her maternal grandmother (I-2) suffered from secondary hypertension at the age of 56 years old, while other members of this family did not have significant clinical abnormalities. There is no evidence that any member of this family had any other known cause to account for hypertension.

To understand role of mitochondrial genome in hypertension, we first performed the mutational analysis of $tRNA^{Leu(UUR)}$ and ND1 genes by PCR amplification and subsequent sequence analysis of the PCR fragments derived from proband III-2 and one unrelated Chinese control. As shown in Fig. 2A, the known T3308C mutation in the ND1 gene was identified in this Chinese subject. In fact, the T3308C mutation was associated with hypertension [7], bilateral striatal necrosis and clinical features resembling the syndrome of mitochondrial encephalomyopathy, lactic adicosis and stroke-like episodes (MELAS) [21,22] as well as tumors [23,24]. This mutation was also implicated to contribute to higher penetrance of hearing loss in a large African family than Japanese and French pedigrees carrying the $tRNA^{Ser(UCN)}$ T7511C mutation [25,26]. The frequency of the T3308C mutation in Chinese control

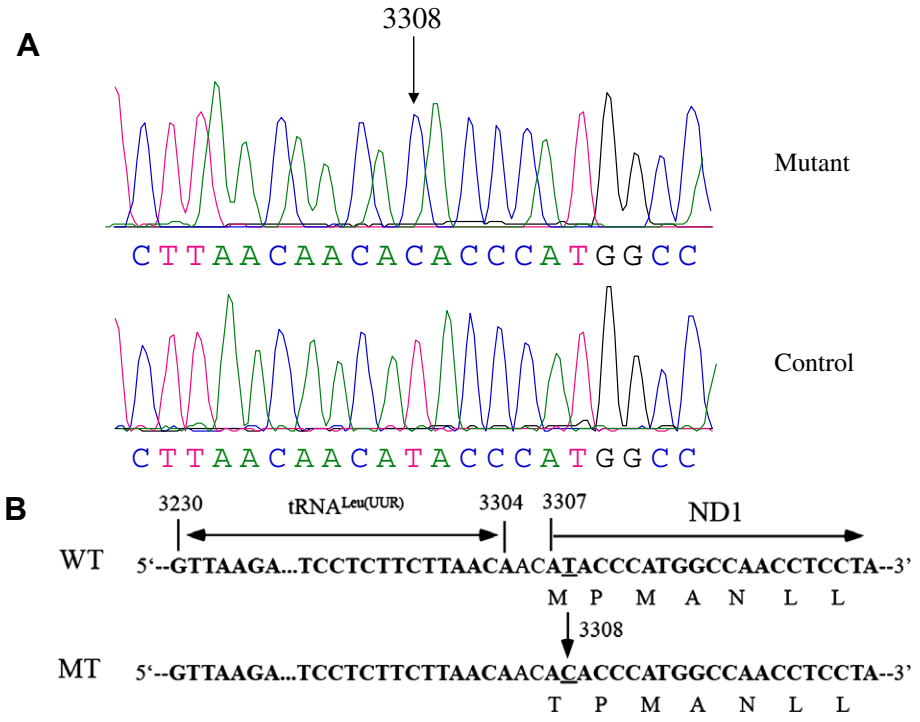

Fig. 2. Identification of the T3308C mutation in the mitochondrial ND1 gene. (A) Partial sequence chromatograms of ND1 gene from affected individual II-2 and a married-in-control II-I. An arrow indicates the location of the base changes at position 3308. (B) A schema of mtDNA sequence at position 3308 and adjacent sequence of ND1 and $tRNA^{Leu(UUR)}$ from wild-type (WT) [20] and mutant (MT). Arrow indicates the position of the T3308C mutation.

population was then examined by sequencing the PCR fragment spanning the ND1 gene. This mutation was absent in 242 Chinese controls. The T3308C mutation, as shown in Fig. 2B, resulted in the replacement of the first amino acid, translation-initiating methionine with a threonine in ND1 [20]. The truncated ND1 polypeptide seems to retain partial function. It is possible that another methionine at position 3 of ND1 could serve as the initiation codon when the first methionine is changed by the T3308C mutation. Furthermore, the ND3 T3308C mutation also locates in two nucleotides adjacent to the 3′ end of the $tRNA^{Leu(UUR)}$. Thus, it was anticipated that the T3308C mutation also affected the processing of the H-strand polycistronic RNA precursors [27]. Indeed, a significant reduction in steady-state levels of both ND1 mRNA and adjacent $tRNA^{Leu(UUR)}$ was observed in the cybrids carrying this T3308C mutation [25]. These defects are likely due to an alteration on the processing of the H-strand polycistronic RNA precursors or the destabilization of ND1 mRNA by this mutation [27].

To determine the role of mitochondrial variants/haplotypes in the phenotypic manifestation of the T3308C mutation, the DNA fragments spanning the entire mtDNA of the proband III-2 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 D4h [28]. Of these nucleoside changes, there were eight polymorphisms in the D-loop region, 2 variants in the 12S rRNA gene, 3 variant in the 16S rRNA gene, the C14747T mutation in $tRNA^{Glu}$ gene, 19 silent mutations and 10 minsense mutations in protein encoding genes [29]. These missense mutations are T3644C (V113A) in ND1 gene, the C5178A (L237M) in the ND2 gene, the A7673G (I30V) in the CO2 gene, the C8414T (L17F) in A8 gene, the A8701G (T59A) and A8860G (T112A) in the A6 gene, the G9300A (A32T) in the CO3 gene, the A10398G (T114A) in the ND3 gene, the G13145A (S270N) in the ND5 gene, and the G15773A (V343M) in the cyto *b* gene. These variants in tRNA, rRNAs and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from other organisms including mouse [30], bovine [31], and *Xenopus laevis* [32]. None of variants in the polypeptides, except V113A in ND1 gene, were highly evolutionarily conserved and implicated to have significantly functional consequence.

In summary, the occurrence of the T3308C mutation in these genetically unrelated pedigrees affected by diseases but absence of 242 Chinese controls as well as the mitochondrial dysfunctions detected in cells carrying this mutation indicate that this mutation is involved in the pathogenesis of hypertension. However, the mild biochemical defects, the lower penetrance of hypertension in this Chinese family and the presence of some control populations [22] suggested the involvement of other modifier factors in the pathogenesis of hypertension, as in the case of

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
	574	C to CCC		No
	16174	C to T		Yes
	16223	C to T		Yes
	16362	T to C		Yes
12S rRNA	750	A to G	A/A/G/-	Yes
	1438	A to G	A/A/A/G	Yes
16S rRNA	2706	A to G	A/G/A/A	Yes
	3010	G to A	G/G/A/A	Yes
	3106	C Del	C/T/T/T	Yes
ND1	3308	T to C (Met to Thr)	M/M/M/M	Yes
	3336	G to A		Yes
	3644	T to C (Val to Ala)	V/V/V/V	Yes
ND2	4769	A to G		Yes
	4883	C to T		Yes
	4895	G to A		Yes
	5048	T to C		Yes
	5178	C to A (Leu to Met)	L/T/T/T	Yes
CO1	7028	C to T		Yes
	7181	C to T		Yes
CO2	7673	A to G (Ile to Val)	I/I/I/A	Yes
A6	8414	C to T (Leu to Phe)	L/F/M/W	Yes
A8	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	9300	G to A (Ala to Thr)	A/T/V/A	Yes
	9540	T to C		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
	11335	T to C		Yes
	11719	G to A		Yes
ND5	12408	T to C		Yes
	12705	C to T		Yes
	13145	G to A (Ser to Asn)	S/N/N/N	Yes
	13667	C to T		No
ND6	14668	C to T		Yes
$tRNA^{Glu}$	14727	T to C	A/T/T/C	Yes
Cyt b	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

[a] Concervation 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.

the deafness-associated 12S rRNA A1555G mutation [33] and the LHON-associated ND4 G11778A mutation [34].

Acknowledgments

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

References

[1] D. Gu, K. Reynolds, X. Wu, J. Chen, X. Duan, P. Muntner, G. Huang, R.F. Reynolds, S. Su, P.K. Whelton, J. He, Prevalence, awareness, treatment, and control of hypertension in china, Hypertension 40 (2002) 920–927.
[2] 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee, J. Hypertens. 17 (1999) 151–183.
[3] 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.
[4] D.C. Wallace, Mitochondrial defects in cardiomyopathy and neuromuscular disease, Am. Heart J. 139 (2000) S70–S85.
[5] M. Hirano, M. Davidson, S. DiMauro, Mitochondria and the heart, Curr. Opin. Cardiol. 16 (2001) 201–210.
[6] 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.
[7] 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.
[8] 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.
[9] 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.
[10] 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.
[11] 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.
[12] 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.
[13] 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.
[14] 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.
[15] 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.
[16] 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.
[17] Z. Li, Y. Liu, L. Yang, S. Wang, M.X. Guan, Maternally inherited hypertension is associated with the mitochondrial $tRNA^{Ile}$ A4295G

mutation in a Chinese family, Biochem. Biophys. Res. Commun. 367 (2008) 906–911.
[18] 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.
[19] 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.
[20] 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.
[21] Y. Campos, M.A. Martin, J.C. Rubio, M.C.G. Olmo, A. Cabello, J. Arenas, Bilateral striatal necrosis and MELAS associated with a new T3308C mutation in the mitochondrial ND1 gene, Biochem. Biophys. Res. Commun. 238 (1997) 323–325.
[22] H. Rocha, C. Flores, Y. Campos, J. Arenas, L. Vilarinho, F.M. Santorelli, A. Torroni, About the 'pathological' role of the mtDNA T3308C mutation, Am. J. Hum. Genet. 65 (1999) 1457–1459.
[23] K. Polyak, Y. Li, H. Zhu, C. Lengauer, J.K. Willson, S.D. Markowitz, M.A. Trush, K.W. Kinzler, B. Vogelstein, Somatic mutations of the mitochondrial genome in human colorectal tumours, Nat. Genet. 20 (1998) 291–293.
[24] J.J. Yeh, K.L. Lunetta, N.J. van Orsouw, F.D. Moore, G.L. Mutter Jr., J. Vijg, P.L. Dahia, C. Eng, Somatic mitochondrial DNA (mtDNA) mutations in papillary thyroid carcinomas and differential mtDNA sequence variants in cases with thyroid tumours, Oncogene 19 (2000) 2060–2066.
[25] X. Li, N. Fischel-Ghodsian, F. Schwartz, Q. Yan, R.A. Friedman, M.X. Guan, Biochemical characterization of the mitochondrial $tRNA^{Ser(UCN)}$ T7511C mutation associated with nonsyndromic deafness, Nucleic Acids Res. 32 (2004) 867–877.
[26] R. Li, K. Ishikawa, J.H. Deng, L. Yang, Y. Tamagawa, Y. Bai, K. Ichimura, M.X. Guan, Maternally inherited nonsyndromic hearing loss is associated with the mitochondrial $tRNA^{Ser(UCN)}$ 7511C mutation in a Japanese family, Biochem. Biophys. Res. Commun. 328 (2005) 32–37.
[27] M.X. Guan, J.A. Enriquez, N. Fischel-Ghodsian, R. Puranam, C.P. Lin, M.A. Marion, G. Attardi, The deafness-associated mtDNA 7445 mutation, which affects $tRNA^{Ser(UCN)}$ precursor processing, has long-range effects on NADH dehydrogenase ND6 subunit gene expression, Mol. Cell. Biol. 18 (1998) 5868–5879.
[28] 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.
[29] 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.
[30] 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.
[31] 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.
[32] 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.
[33] 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.
[34] 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.