Case Report
Sporadic type 2 diabetes mellitus, atherosclerosis and essential hypertension associated with the mitochondrial tRNA\textsubscript{Lys} A8343G mutation

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Abstract: We reported here the molecular characterization of a mutation: A8343G in the TψC loop of mitochondrial tRNA\textsubscript{Lys} gene of a 57-year-old woman who manifested type 2 diabetes mellitus, arteriosclerosis and essential hypertension. No other family members were affected, suggested that our patient was a sporadic case. Sequence analysis for the entire mitochondrial genome showed the presence of a homoplasmic A8343G mutation and a set of polymorphisms belonging to human mitochondrial haplogroup B4b1c. The adenine (A) at position 8343 was very important in the structural formation and stabilization of functional tRNAs, which was evolutionary conserved in mitochondria of various organisms and also contributed to the high fidelity of the TψC loop of tRNA. Thus, the structural alternation of tRNA by the A8343G mutation may lead to a failure in tRNA metabolism and impair mitochondrial protein synthesis. Therefore, the tRNA\textsubscript{Lys} A8343G mutation may have a potential modifier role in type 2 diabetes mellitus, arteriosclerosis and essential hypertension. Our finding broadened the phenotypic and molecular spectrum of mitochondrial tRNA\textsubscript{Lys} associated disorders.

Keywords: mt-tRNA\textsubscript{Lys}, A8343G mutation, cardiovascular diseases, pathogenicity

Introduction
Mitochondria are vital energy-producing organelles in eukaryotic cells that are primarily responsible for generating ATP by oxidative phosphorylation. Their efficient functioning is determined by both the nuclear genome and the maternally inherited 16.6-kb mitochondrial genome [1]. Therefore, mutations in either mitochondrial or nuclear DNA of the cell may cause pathological loss of mitochondrial function and lead to mitochondrial disease [2]. The most severely affected organs are those with high oxidative metabolism, such as the brain, heart, kidney and endocrine glands [3]. Mitochondrial defects are increasingly recognized to play important roles in the pathogenesis of cardiovascular diseases [4]. Sporadic or inherited mutations in mitochondrial DNA (mtDNA), specifically in mitochondrial transfer RNA (tRNA) genes, have been associated with inherited cardiovascular diseases [5, 6], presumably because of the essential role of tRNAs in the synthesis of proteins involved in energy metabolism [7].

The first report linking a mutation in mitochondrial tRNA (mt-tRNA) to human disease was published in 1990, when mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) was associated with a mutation in the mt-tRNA\textsubscript{Leu(UUR)} gene [8]. Since then, multiple mutations in individual tRNA genes have been associated with multiple diseases, and individual diseases have been found to be caused by mutations in one of several tRNAs. In an attempt to identify mtDNA mutations associated with cardiovascular disease, we performed a systematic and extensive screening for mutations in the mitochondrial genome. Here, we report a homoplasmic mutation in the TψC loop of tRNA\textsubscript{Lys} in a 57-year-old
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A 57-year-old Chinese woman with type 2 diabetes mellitus, atherosclerosis and essential hypertension was referred to Hangzhou First People’s Hospital. Informed consent and blood samples were obtained and a clinical evaluation was performed under protocols approved by the Ethics Committee of Hangzhou First People’s Hospital. The patient underwent a thorough physical examination, a laboratory assessment of cardiovascular risk factors and routine electrocardiography. Moreover, 150 control subjects with an average age of 55 years were selected from a panel of unaffected individuals of Han Chinese ancestry in the same region. Hypertension was defined according to recommendations of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and the World Health Organization-International Society of Hypertension as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher [9]. The diagnosis of diabetes mellitus was based on the criteria of the American Diabetes Association: fasting plasma glucose level of 7 mmol/dL or higher, oral glucose tolerance level of 1.11 mmol/dL (200 mg/dL) or higher, or glycated hemoglobin (HbA1c) concentration of 6.5% or more [10-12].

Biochemical assessment

Serum triacylglycerol, total cholesterol, low-density lipoprotein, high-density lipoprotein, glucose, liver profile and kidney function parameters were determined using routine automated assay methods following an overnight fast.

Molecular genetic analysis

Total DNA was isolated from peripheral blood leukocytes of the patient using Universal Genomic DNA Extraction Kit version 3.0 (Takara Bio Inc., Shiga, Japan). The entire mtDNA was amplified in 24 overlapping fragments as previously described [13]. After PCR amplification, the fragments were purified and subsequently analyzed by direct sequencing in an ABI 3730 automatic DNA sequencer using the BigDye Terminator Cycle Sequencing Kit (Sigma, St. Louis, MO, USA). Sample sequences were compared with the revised Cambridge Reference Sequence from MITOMAP, a human mitochondrial genome database (http://www.mitomap.org/MITOMAP) [14]. Phylogenetic trees, including the MITOMAP phylogeny and the recently updated East Asian mtDNA phylogeny [15], were used to determine the haplogroup.

Results

Clinical features

The patient’s general practitioner reported that she had been diagnosed with type 2 diabetes mellitus, atherosclerosis and hypertension at the ages of 45 and 48, respectively. After the carefully genetic counseling, we noticed that...
she was an orphan, and was adopted by the local Social Welfare Institute. She had been prescribed oral metformin, a calcium channel blocker and an angiotensin receptor blocker. The patient first visited our hospital for clinical evaluation in 2009 because of intermittent dizziness and headache. When she arrived at our hospital, she had been experiencing recurrent headache for the past 10 days. She was short of stature, lean and hypertensive (blood pressure: 190/110 mmHg). Laboratory tests showed normal liver function, high cholesterol and high serum glucose levels. Physical examination and routine electrocardiography showed no other clinical abnormalities associated with mtDNA mutations (including myopathy, vision and hearing impairments and neurological disorders). The biochemical characteristics of the patient are provided in Table 1.

**mtDNA analysis**

To screen for mutations in the mitochondrial genome, we purified and completely sequenced the mtDNA obtained from the peripheral blood leukocytes. Consequently, a homoplasmic A to G transition at position 8343 in the mt-tRNA\(^{Lys}\) gene (MT-TK) was identified (Figure 1A), and this mutation was absent in the 150 healthy controls. To further assess whether the mitochondrial genetic background contributed to the clinical manifestation of the A8343G mutation, the entire mtDNA genome sequence was analyzed using a phylogenetic method. Sequence variations in the mtDNA were displayed in a phylogenetic tree (Figure 2). Haplogroup analysis showed the Chinese origin of the subject, who belonged to mitochondrial haplogroup B4b1c [15]. These sequence variations included three located in the control region (C16218T, T16136C and T16189C), five located in the coding region (G4820A in MT-ND1, A11053G in MT-ND4, G13590A in MT-ND5, A14587G in MT-ND6 and C15535T in MT-CYB) and a 9-bp deletion between MT-CO2 and MT-TK corresponding to positions 8271-8279. All of these sequence variations had been previously reported and had no potential pathogenicity, except for the A8343G mutation in the tRNA\(^{Lys}\) gene, which had been regarded as a possible Parkinson’s disease risk factor [16].

**Discussion**

This is the first report of an association between the homoplasmic A8343G mutation in tRNA\(^{Lys}\) and type 2 diabetes mellitus, atherosclerosis and essential hypertension. Accumulating evidence suggests that mitochondrial damage...
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and dysfunction are involved in cardiovascular diseases and that mtDNA mutations, especially mt-tRNA mutations, play important roles in the pathogenesis of essential hypertension [17, 18]. Mt-tRNA genes are all single-copy genes; therefore, any mutation of a tRNA gene that affects the activity of the trNA will lead to instability of the mitochondrial translation system [19, 20]. The tRNA<sub>Lys</sub> gene is one of the most mutable genes in the mitochondrial genome [21]; to date, 16 different pathogenic mutations and 8 polymorphisms in this gene have been identified as causes of various clinical symptoms (http://mamit-tRNA.u-strasbg.fr) [22].

This patient had no family history (her husband and her son were healthy), suggesting that the A8343G mutation itself was insufficient to produce the clinical phenotype and that other risk factors, such as nuclear modified genes, epigenetic modifications and environmental factors, may contribute to the clinical expression of essential hypertension, as in the case of the A4435G and A4401G mutations [23, 24].

The mitochondrial genome in this patient harbored the homoplasmic A8343G mutation as well as a set of mtDNA variants belonging to the Eastern Asian haplogroup B4b1c (Figure 2) [15]. As shown in Figure 1B, the A8343G mutation occurred at an evolutionarily conserved nucleotide of the tRNA<sub>Lys</sub> gene [25]. This mutation was absent among 150 unrelated Han Chinese control subjects. The A8343G mutation has been implicated as a risk factor for Parkinson’s disease [16]. Interestingly, the A to G mutation at position 8343 affects the first base (conventional position 54) of the TψC loop of tRNA<sub>Lys</sub>. Nucleotides at position 54 of the TψC loop are often post-transcriptionally modified, thereby contributing to the structure and stability of functional tRNA [26]. The A8343G mutation may decrease tRNA aminoacylation as well as binding affinity with the mitochondrial elongation factor Tu, which forms a ternary complex with aminoacyl-tRNA and GTP prior to delivery of the charged tRNA to the A-site of the ribosome during the elongation phase of protein synthesis [27, 28]. Thus, the A8343G mutation may result in a failure of tRNA metabolism and consequently lead to a reduced rate of mitochondrial protein synthesis. These defects may be responsible for reduced activity of the mitochondrial respiratory chain. Subsequently, the A8343G mutation may result in reduced ATP production and increased reactive oxygen species production. Thus, mitochondrial dysfunction may be involved in the pathogenesis of type 2 diabetes mellitus, atherosclerosis and essential hypertension in this patient.

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Disclosure of conflict of interest

None.
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