Int J Clin Exp Med 2016;9(2):4873-4878
www.ijcem.com /ISSN:1940-5901/IJCEM0016999

Case Report

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

Yu Ding[1,3*], Beibei Gao[2,3*], Liang Zhou[2,3], Haiying Xu[2,3], Meiya Li[4], Jinyu Huang[2,3]

[1]Central laboratory, Hangzhou First People's Hospital, Hangzhou, Zhejiang, China; [2]Department of Cardiology, Hangzhou First People's Hospital, Hangzhou, Zhejiang, China; [3]Affiliated Hangzhou Hospital, Nanjing Medical University, Hangzhou, Zhejiang, China; [4]Analytical testing Center, Zhejiang University of Traditional Chinese Medicine, Hangzhou, Zhejiang, China. [*]Equal contributors.

Received September 28, 2015; Accepted December 12, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: We reported here the molecular characterization of a mutation: A8343G in the TψC loop of mitochondrial tRNA^{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^{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^{Lys} associated disorders.

Keywords: mt-tRNA^{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$^{Leu(UUR)}$ 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^{Lys} in a 57-year-old

Table 1. The patient's laboratory test results

Lab tests	Results	Normal range
Alanine Transaminase (ALT)	32.0 U/L	7-40
Aspartate Transaminase (AST)	16.0 U/L	13-35
γ-Glutamyltransferase (γ-GGT)	23.0 U/L	7-45
Total Bilirubin (TBIL)	26.0 μmol/L	1-25
Conjugated Bilirubin (CB)	6.3 μmol/L	0-8
Indirect Bilirubin (IBIL)	19.7 μmol/L	1-20.1
Creatine (Cr)	68.0 μmol/L	1-106
Urine Acid (UA)	203 μmol/L	89-416
Glycosylated serum protein (GSP)	3.01 mmol/L	1-2.01
Glucose (GLU)	16.84 mmol/L	3.6-6.1
Total Triglycerides (TG)	5.31 mmol/L	0.56-1.7
Total Cholesterol (TC)	6.51 mmol/L	3.1-6
ApoA1	1.41 g/L	1.2-1.76
ApoB	1.38 g/L	0.63-1.2
ApoE	9.1 mg/dl	3-7
High Density Lipoprotein (HDL)	1.27 mmol/L	0.9-1.95
Low Density Lipoprotein (LDL)	4.16 mmol/L	1.31-3.37
Lipoprotein (a) (Lp (a))	72.0 mg/dl	1-30
Na^+	134.0 mmol/L	137-147
K^+	3.75 mmol/L	3.5-5.3
Cl^-	99.0 mmol/L	99-110
HbA1c	10%	4-6

woman who manifested type 2 diabetes mellitus, atherosclerosis and essential hypertension.

Methods

Subject

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

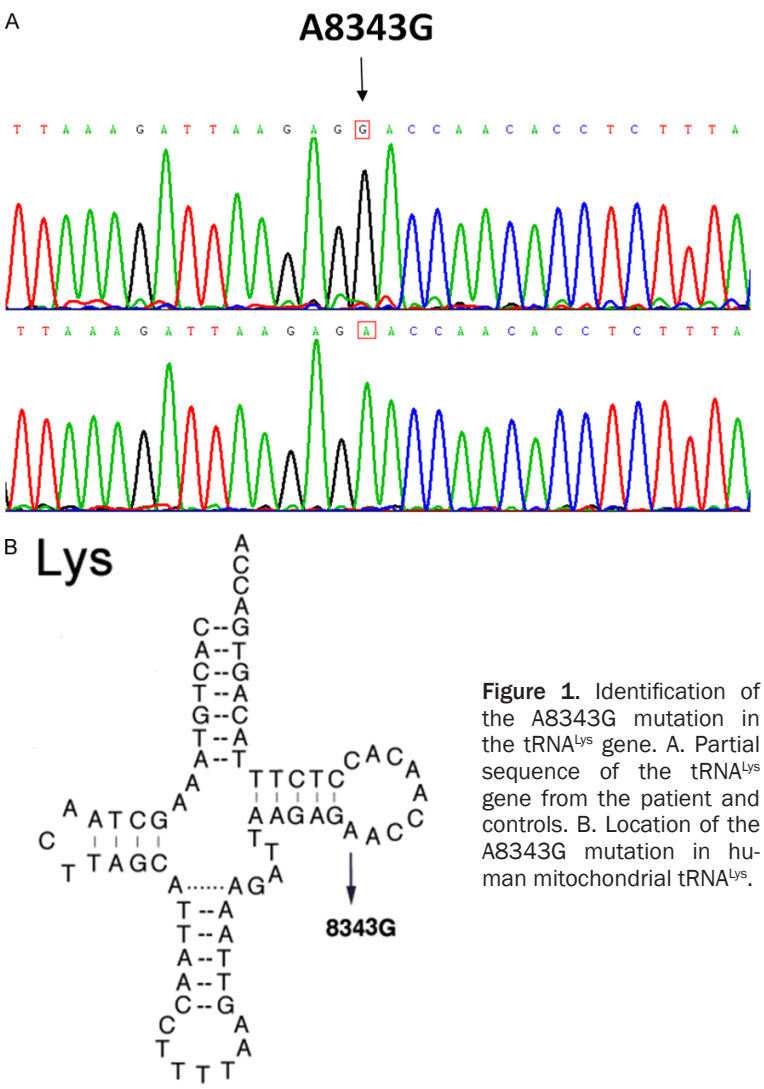

Figure 1. Identification of the A8343G mutation in the tRNA^{Lys} gene. A. Partial sequence of the tRNA^{Lys} gene from the patient and controls. B. Location of the A8343G mutation in human mitochondrial tRNA^{Lys}.

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

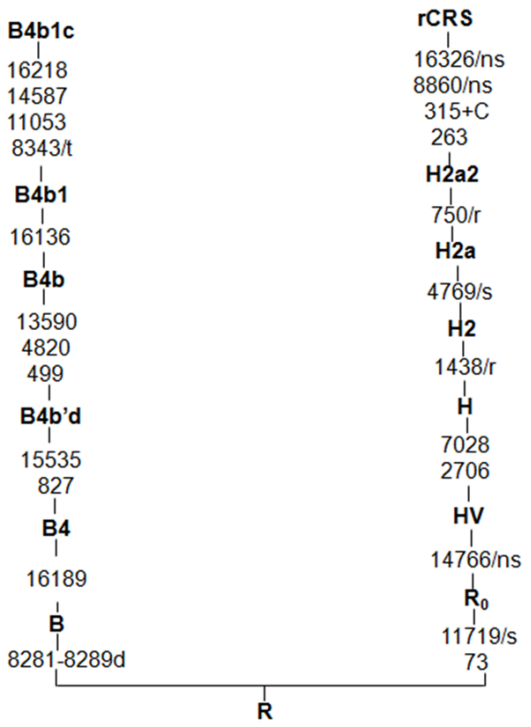

Figure 2. Classification tree of the complete mtDNA sequence of the patient in this study, along with the revised Cambridge reference sequence (rCRS). [14]Suffix "+C" indicates an insertion of cytosine, "d" indicates a deletion, and "/s" and "/ns" denote synonymous and nonsynonymous variants in the mtDNA, respectively. Variations in transfer RNA and ribosomal RNA genes are denoted by "/t" and "/r", respectively.

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 $tRNA^{Lys}$ 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 $tRNA^{Lys}$ 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 $tRNA^{Lys}$. 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.

Acknowledgements

This work was supported by grants from the Ministry of Public Health of Zhejiang Province (No. 2013KYA158), Nanjing Medical University (No. 2010NJMU011) and Hangzhou Bureau of Science and Technology (No. 20120533Q03), Zhejiang Natural Science Foundation (No. Y15H020003) and Beijing LS Cardiovascular Health Foundation (No. LSG151132).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jinyu Huang, Department of Cardiology, Hangzhou First People's Hospital, Nanjing Medical University, Hangzhou, *Zhejiang,* China. Tel: 0086-0571-87065701; Fax: 0086-0571-87065701; E-mail: hjyu41@sohu.com

References

[1] Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nat Rev Genet 2005; 6: 389-402.

[2] Greaves LC, Reeve AK, Taylor RW, Turnbull DM. Mitochondrial DNA and disease. J Pathol 2012; 226: 274-286.

[3] Zeviani M, Bertagnolio B, Uziel G. Neurological presentations of mitochondrial diseases. J Inherit Metab Dis 1996; 19: 504-20.

[4] Lev D, Nissenkorn A, Leshinsky-Silver E, Sadeh M, Zeharia A, Garty BZ, Blieden L, Barash V, Lerman-Sagie T. Clinical presentations of mitochondrial cardiomyopathies. Pediatr Cardiol 2004; 25: 443-50.

[5] Taylor RW, Giordano C, Davidson MM, d'Amati G, Bain H, Hayes CM, Leonard H, Barron MJ, Casali C, Santorelli FM, Hirano M, Lightowlers RN, DiMauro S, Turnbull DM. A homoplasmic mitochondrial transfer ribonucleic acid mutation as a cause of maternally inherited hypertrophic cardiomyopathy. J Am Coll Cardiol 2003; 41: 1786-96.

[6] Hirano M, Davidson M, DiMauro S. Mitochondria and the heart. Curr Opin Cardiol 2001; 16: 201-210.

[7] Abbott JA, Francklyn CS, Robey-Bond SM. Transfer RNA and human disease. Front Genet 2014; 5: 158.

[8] Kobayashi Y, Momoi MY, Tominaga K, Momoi T, Nihei K, Yanagisawa M, Kagawa Y, Ohta S. A point mutation in the mitochondrial tRNA(Leu)(UUR) gene in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes). Biochem Biophys Res Commun 1990; 173: 816-22.

[9] Banegas JR, Segura J, Ruilope LM, Luque M, García-Robles R, Campo C, Rodríguez-Artalejo F, Tamargo J; CLUE Study Group Investigators. Blood pressure control and physician management of hypertension in hospital hypertension units in Spain. Hypertension 2004; 6: 1338-44.

[10] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33 Suppl 1: S62-9.

[11] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15: 539-53.

[12] Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. Circ Res 2008; 102: 401-14.

[13] Rieder MJ, Taylor SL, Tobe VO, 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: 967-73.

[14] Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 1999; 23: 147.

[15] Kong QP, Bandelt HJ, Sun C, Yao YG, Salas A, Achilli A, Wang CY, Zhong L, Zhu CL, Wu SF, Torroni A, Zhang YP. Updating the East Asian mtDNA phylogeny: a prerequisite for the identification of pathogenic mutations. Hum Mol Genet 2006; 15: 2076-86.

[16] Khusnutdinova E, Gilyazova I, Ruiz-Pesini E, Derbeneva O, Khusainova R, Khidiyatova I, Magzhanov R, Wallace DC. A mitochondrial etiology of neurodegenerative diseases: evidence from Parkinson's disease. Ann N Y Acad Sci 2008; 1147: 1-20.

[17] Ballinger SW. Mitochondrial dysfunction in cardiovascular disease. Free Radic Biol Med 2005; 38: 1278-95.

[18] Ding Y, Xia B, Yu J, Leng J, Huang J. Mitochondrial DNA mutations and essential hypertension (Review). Int J Mol Med 2013; 32: 768-74.

[19] Suzuki T, Nagao A, Suzuki T. Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. Annu Rev Genet 2011; 45: 299-329.

[20] Levinger L, Mörl M, Florentz C. Mitochondrial tRNA 3' end metabolism and human disease. Nucleic Acids Res 2004; 32: 5430-41.

[21] Houshmand M, Lindberg C, Moslemi AR, Oldfors A, Holme E. A novel heteroplasmic point mutation in the mitochondrial tRNA(Lys) gene in a sporadic case of mitochondrial encephalomyopathy: de novo mutation and no transmission to the offspring. Hum Mutat 1999; 13: 203-9.

[22] Pütz J, Dupuis B, Sissler M, Florentz C. MamittRNA, a database of mammalian mitochondrial tRNA primary and secondary structures. RNA 2007; 13: 1184-90.

[23] Lu Z, Chen H, Meng Y, Wang Y, Xue L, Zhi S, Qiu Q, Yang L, Mo JQ, Guan MX. The tRNAMet 4435A>G mutation in the mitochondrial haplogroup G2a1 is responsible for maternally inherited hypertension in a Chinese pedigree. Eur J Hum Genet 2011; 19: 1181-6.

[24] Li R, Liu Y, Li Z, Yang L, Wang S, Guan MX. Failures in mitochondrial tRNAMet and tRNA GIn metabolism caused by the novel 4401A>G mutation are involved in essential hyperten-

sion in a Han Chinese Family. Hypertension 2009; 54: 329-37.
[25] Sprinzl M, Vassilenko KS. Compilation of tRNA sequences and sequences of tRNA genes. Nucleic Acids Res 2005; 33: D139-40.
[26] Björk GR. Biosynthesis and function of modified nucleosides. In tRNA: Structure, Biosynthesis and Function, Söll, D. and U.L. Raj Bhandary ads. Washington, DC: ASM Press; 1995.
[27] Rinaldi T, Lande R, Bolotin-Fukuhara M, Frontali L. Additional copies of the mitochondrial Ef-Tu and aspartyl-tRNA synthetase genes can compensate for a mutation affecting the maturation of the mitochondrial tRNAAsp. Curr Genet 1997; 31: 494-6.
[28] Belostotsky R, Frishberg Y, Entelis N. Human mitochondrial tRNA quality control in health and disease: a channelling mechanism? RNA Biol 2012; 9: 33-39.