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THE MITOCHONDRIAL tRNA^{Gly} T10003C MUTATION MAY NOT BE ASSOCIATED WITH DIABETES MELLITUS

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ABSTRACT

Mitochondrial DNA (mtDNA) mutations have long been proposed to play important roles in the pathogenesis of diabetes mellitus (DM). A large proportion of these mutations are localized at the *mt-tRNA* genes. Owing to its high mutation rate, a growing number of mt-tRNA mutations have been reported; however some of them are neutral genetic polymorphisms and will not result in the alteration of the mitochondrial function responsible for DM. In this study, we reassessed a recent reported "pathogenic" mutation, tRNA^{Gly} T10003C, in a clinical manifestation of DM. We first performed the conservation assessment of this mutation between different species. Moreover, the bioinformatics analysis was used to predict the secondary structure of mt-tRNA^{Gly} in wild type version and the mutant carrying the T10003C mutation. We also screened the presence of the T10003C mutation in 500 unrelated DM patients and 300 healthy controls. We noticed that the T10003C mutation was not very conserved and did not cause the secondary structure change of mt-tRNA^{Gly}. Moreover, this mutation was absent in the 500 unrelated DM patients and controls, suggesting that this mutation may be a rare event in the human population. In conclusion, the current study showed no association between the T10003C mutation and DM in humans.

Keywords: Association; Diabetes mellitus (DM); mitochondrial tRNA^{Gly} (mt-tRNA^{Gly}); T10003C mutation.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a highly prevalent disease worldwide. The T2DM is featured with relative higher level of blood glucose than normal subjects, moreover it manifested insulin resistance in several conditions [1]. The etiology of T2DM is not well understood, but it is now generally believed that this disease may be related to the genetic, personal and environmental factors. Among these, genetic polymorphisms in nuclear genes have been reported to be associated with T2DM [2]. However, the maternally inherited pattern of T2DM may also be frequent [3], highlighting the importance of mitochondrial disfunction in T2DM. In fact, approximately 85.0% of mitochondrial diabetes cases are associated with the A3243G mutation in the *tRNA$^{Leu(UUR)}$* gene [4], moreover, some other point mutations, such as T3264C and T3271C in the *tRNA$^{Leu(UUR)}$* gene, are reported in patients with DM [5,6]. These mutations impaired the mitochondrial protein synthesis, subsequently affecting the oxidative phosphorylation (OXPHOS) complexes, thus causing the mitochondrial dysfunction responsible for DM [7]. As a result, the *mt-tRNA* genes have become novel targets for investigation the relationship between mitochondrial dysfunction and T2DM.

However, it come to our attention that the genotype-phenotype relationship between DM and *mt-tRNA* gene mutations was still controversial. At the same time, many T2DM associated mt-tRNA variants have been reported on PubMed. We have noticed that most of them were common genetic variations becosause they did not meet the pathogenicity scoring system proposed by Yarham et al. [8], such as the association between *tRNA^{Phe}* C628T mutation and hearing loss [9]. Distinguishing the polymorphisms and disease associated *mtDNA* mutations is important, both for clinical physician and genetic scientists.

In the current investigation, we evaluated the association between a recent reported mt-tRNA^{Gly} T10003C mutation and DM. First of all, we performed a systematic database search for the frequency of this mutation; second, we also reassessed the conservation index (CI)

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of the T10003C mutation, and finally, we performed a case-control study to screen this mutation in 500 unrelated DM patients and 300 healthy individuals.

MATERIALS AND METHODS

Database Searches. With the purpose of comparing different reports regarding the T10003C mutation, we performed a systematic review for this mutation using the Google scholar, PubMed Central and Human MITOMAP database. The literature search was carried out using these combined keywords: "mitochondrial T10003C mutation" or "mitochondrial T10003C variant." We excluded studies if the crucial data were not reported in original paper.

Evolutionary Conservation Analysis. To understand the potential pathogenic role of the T10003C mutation, we analyzed the CI of this mutation. Briefly, 10 vertebrate *mt-tRNA^{Gly}* gene sequences were selected for this analysis. The conservation index (CI) was then calculated by comparing the human nucleotide variants with nine other vertebrates [10]. We regarded the CI as the percentage of species from the list of 10 different primates that have the wild-type at the corresponding position. The CI >75.0% was proposed to have a functional potential.

Haplogroup Analysis. We used the Phylotree (www.phylotree.org) to determine the haplogroup status of the T10003C mutation [11].

Prediction of the Secondary Structure of tRNA^{Gly} With and Without the T10003C Mutation. We used the RNA Fold Webserver program (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) to predict the secondary structures of the wild-type version of mt-tRNA^{Gly} and the mutant carrying the T10003C mutation [12].

Mutational Analysis of the T10003C Mutation. Five hundred unrelated DM patients including 250 male and 250 females were recruited from the Department of Endocrinology and Metabolism, People's Hospital of Zhengzhou University, Zhengzhou, Zhengzhou, Henan Province, People's Republic of China. The age ranged from 50 to 80 years with the median at 61. In addition, 300 healthy, age- and gender-matched controls, obtained from the same area were also enrolled in this study. This study was approved by the Ethics Committee of the People's Hospital of Zhengzhou University.

The clinical diagnosis of DM was according to the American Diabetes Association criteria: the level of fasting plasma glucose of ≥7.0 mmol/dL, or the level of the oral glucose tolerance ≥1.11 mmol/dL, or Hb A_{1c} concentration ≥6.5% [13]. Determination of the T10003C mutation was performed by polymerase chain reaction (PCR) amplification and sequencing analysis of the targeted region spanning the *mt-tRNA^{Gly}* gene. The following primers were used: forward 5'-TCT CCA TCT ATT GAT GAG GGT CT-3'; reverse 5'-AAT TAG GCT GTG GGT GGT TG-3'; after PCR amplification, the fragment was purified and subsequently analyzed by direct sequencing in an ABI PRISM® 3700 automatic DNA sequencer using the BigDye Terminator Cycle sequencing reaction kit (Applied Biosystems Inc., Foster City, CA, USA). The data was then compared with the reversed consensus Cambridge sequence (GenBank Accession No. NC_12920) [14].

Statistical Analyses. We used the Statistical Package for the Social Sciences (SPSS), version 17.0 software to analyze the statistical significance (SPSS Inc., Chicago, IL, USA). Fisher's exact test was performed to evaluate the differences in categorical variables, and a value of $p < 0.05$ was regarded as statistically significant.

RESULTS

Relationship Between the T10003C Mutation and Diabetes Mellitus. As a result, two potential articles concerning the association between the T10003C mutation and DM have been identified. After carefully reading the complete manuscripts, we found that one of them described a Chinese family with T2DM [15], while another paper, which met our inclusion criteria, reported a Chinese family with T2DM and deafness [16]. However, after carefully checking these reported families, it looked as if they were from the same family.

Evolutionary Conservation Analysis of the T10003C Mutation. With the purpose of understanding the molecular basis of the T10003C mutation, we performed the evolutionary conservation analysis for this mutation between different species. As shown in Figure 1, the T10003C mutation occurred in the D-stem of tRNA^{Gly} (position 13). Nucleotide at that position was not very conserved (CI = 50.0%), suggesting that the T to C transition at 10003 position may not be involved in the pathogenesis of DM.

Phylogenetic Analysis of the T10003C Mutation. We further performed a haplogroup analysis for the T10003C mutation, based on the Phylotree. We noticed that the T10003C mutation belonged to the East Asian mitochondrial haplogroup M11b [11].

The T10003C Mutation did not Alter the Structure of tRNA^{Gly}. To test whether T10003C mutation caused the tRNA^{Gly} structure alteration, the sequence of the wild-type and the mutant carrying the T10003C mutation were predicted using RNA Fold software. As shown in Figure 2, the T10003C mutation did not change the secondary

Organism	Acc-stem		D-stem	D-loop	D-stem		Ac-stem	Anticd-loop	Ac-stem	V-region	T-stem	T-loop	T-stem	Acc-stem	
	1	8	10 13	15	22	26	27	32	39	44	49	58	61	66	73
Homo sapiens	ACTCTTT	TA	GTAT	AAATA	GTAC	C	GTTAA	CTTCCAA	TTAAC	TAGT	TTTGA	CAACAT	TCAAA	AAAGAGT	A
Procavia capensis	ATTCTTT	TA	GTAC	AAACCA	GTAC	A	CCCGA	CTTCCAA	TCAGG	AAAT	TTCAG	ACTAAT	CTGAA	AAAGAAT	A
Dugong dugon	ACTCTTT	TA	GTAC	CAAATA	GTAC	G	ACTGA	CTTCCAA	TCAGT	AAGC	CTTGG	TCAAAT	CCAAG	AAAGAGT	A
Tamandua tetradactyla	ACCCTTT	TA	GTAA	AAATAA	GTAC	A	GCTGA	CTTCCAA	TTAGC	AAGT	TCCAG	ACAAAC	CTGGA	AAAGGGT	A
Mus musculus	ACTCCCT	TA	GTAT	AATTA	ATAT	A	ACTGA	CTTCCAA	TTAGT	AGAT	TCTGA	ATAAAC	CCAGA	AGAGAGT	A
Manis tetradactyla	ATTTTCT	GA	GTAC	ATGCA	GTAC	A	GTTAA	CTTCCAA	TTAAC	AAAC	TCTGG	TAAAAT	CCAGA	AGAAAAT	A
Ursus americanus	GCTTCTT	TA	GTAC	CGATCA	GTAC	A	ATTGA	CTTCCAA	TCAAT	CAGC	TCTGG	TGCAAT	CCAGA	AGGAAGT	A
Lepus europaeus	ACTCTTT	TA	GTAT	TAACTA	GTAC	A	TCTGA	CTTCCAA	TCAGT	TAGT	TTTGG	TATAAAT	CCAAA	AAAGAGT	A
Myoxus glis	ACTCCCT	TA	GTAT	AATCA	GTAC	A	ACTGA	CTTCCAA	TCAGT	TAGT	TTCAG	GTTTAAT	CTGAA	AGGGAGT	A
Orycteropus afer	ACCCTCT	TA	ATAT	AACTAA	ATAT	A	ACTGA	CTTCCAA	TCAGT	AAAT	CCTGG	AAAACC	CCAGG	AGAGAGT	A

Figure 1. Sequence alignment of the *tRNA*^{Gly} gene from different species; the arrow indicates position 13 corresponding to the T10003C mutation.

structure of tRNA^{Gly}, indicating that this mutation had little effect on tRNA^{Gly} folding, reinforcing the idea that this mutation was not pathogenic.

Screening of the T10003C Mutation in 500 Unrelated DM Patients. To investigate the allelic frequency of the T10003C mutation in the general population, we used a PCR-Sanger sequence to detect this mutation in 500 unrelated DM patients and 300 healthy controls. However, we failed to identify any mutations in the *tRNA*^{Gly} gene, suggesting that this mutation may be a rare event in the human population.

DISCUSSION

In this study, we investigated the potential pathogenic role of T2DM associated tRNA^{Gly} T10003C mutation. Mitochondria are the powerful machine in cells whose primary role is to generate adenosine triphosphate (ATP), through OXPHOS. More recently, the role of the mitochondrial dysfunction in the pathogenesis of DM has been studied extensively. Alterations in mitochondrial function in human β cells resulted in the impaired glucose-stimulated insulin secretion. Mutations in mtDNA,

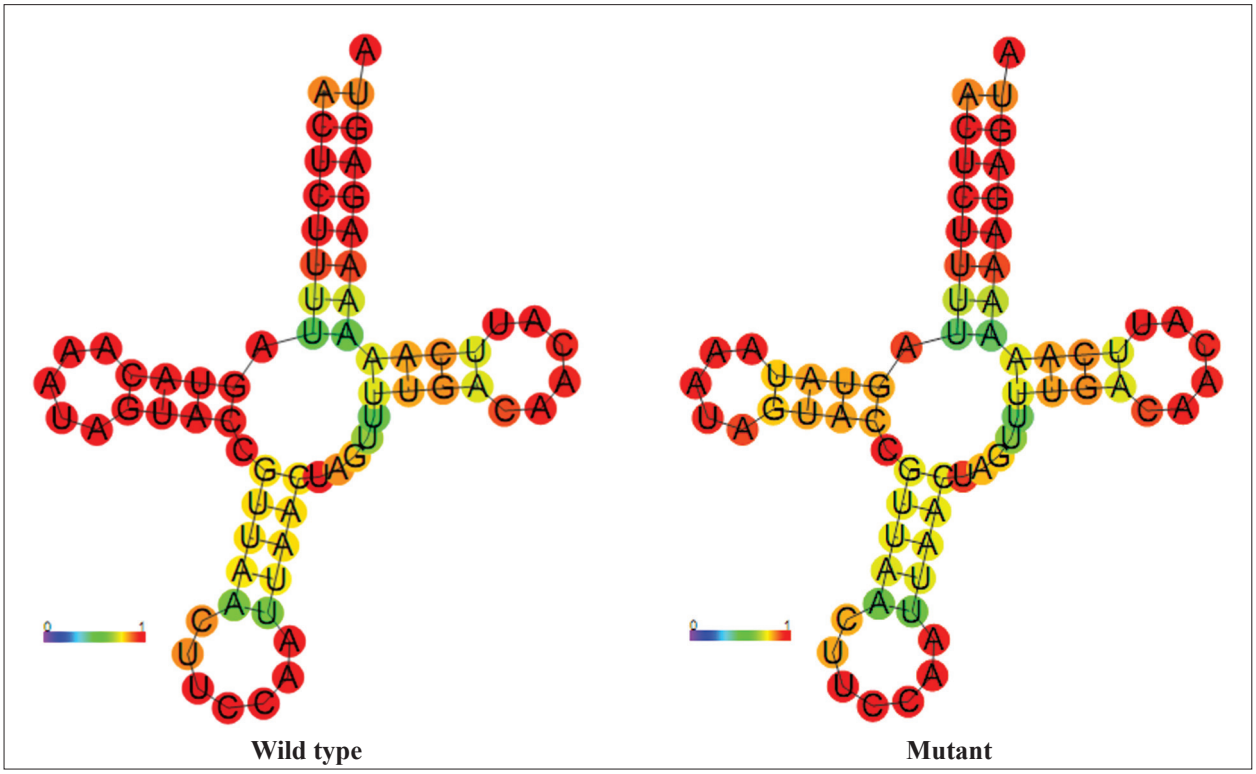

Figure 2. Prediction of the secondary structure of the *tRNA*^{Gly} gene with and without the T10003C mutation.

especially *mt-tRNA* genes, found to be associated with DM. In particular, one of the most common pathogenic mtDNA mutation was A3243G in the $tRNA^{Leu(UUR)}$ gene. This mutation was reported to decrease the steady-state level of the $tRNA^{Leu(UUR)}$ and resulted with the impairment of amino-acylation ability [17], and subsequently, mitochondrial protein synthesis failed [18]. However, there is a number of mt-tRNA variations that were wrongly classified as a "pathogenic" mutation, such as the C628T variant [9,19].

With this regard, this study reassessed the possible association between the T10003C mutation and DM. Database searches for the presence of this mutation led us to identify two potential records that were mentioned in the results sections [15,16]. Mutational analysis of the proband from the maternally inherited DM identified a set of polymorphisms; some of these were obviously pathogenic in the human population, for example, the G15924A mutation in the $tRNA^{Thr}$ gene, was reported to be a fatal infantile respiratory enzyme deficiency- associated pathogenic mutation [20]. Moreover, the 12S rRNA T1095C mutation was a deafness associated primary mutation [21], whereas the A6 A8701G mutation was found to be associated with cardiomyopathy in the Han Chinese population [22]. Therefore, it seemed that beside the T10003C mutation, other mutations may also contribute to DM in this Chinese family.

At the molecular level, the T10003C mutation localized at the D-stem of the $tRNA^{Gly}$ gene (position 13), was not very conserved among different species (Figure 1). Furthermore, to explore the structure to function relations, we used the RNA Fold Webserver program to predict the optimal secondary structure of $tRNA^{Gly}$ through free energy minimization. As shown in Figure 2, it was quite obvious that T10003C mutation failed to cause the alternation of $tRNA^{Gly}$ structure, moreover, we did not detect the T10003C mutation in 500 unrelated DM patients and 300 control subjects, suggesting that it may be a genetic polymorphism rather than a pathogenic mutation.

In conclusion, there was no direct evidence to support the association between the T10003C mutation and DM. Further studies including larger samples and functional analyses are needed to verify this conclusion.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Šeruga M, Makuc J, Završnik M, Cilenšek I, Ekart R, Petrovič D. Polymorphism of angiotensin-converting enzyme (rs4340) and diabetic nephropathy in Caucasians with type 2 diabetes mellitus. Balkan J Med Genet. 2017; 19(2): 29-34.
2. Popović D, Nikolajević Starčević J, Šantl Letonja M, Makuc J, Cokan Vujkovac A, Reschner H, *et al.* PECAM-1 gene polymorphism (rs668) and subclinical markers of carotid atherosclerosis in patients with type 2 diabetes mellitus. Balkan J Med Genet. 2016; 19(1): 63-70.
3. Lu J, Wang D, Li R, Li W, Ji J, Zhao J, *et al.* Maternally transmitted diabetes mellitus associated with the mitochondrial tRNA(Leu(UUR)) A3243G mutation in a four-generation Han Chinese family. Biochem Biophys Res Commun. 2006; 348(1): 115-119.
4. Naing A, Kenchaiah M, Krishnan B, Mir F, Charnley A, Egan C, *et al.* Maternally inherited diabetes and deafness (MIDD): Diagnosis and management. J Diabetes Complications. 2014; 28(4): 542-546.
5. Tsukuda K, Suzuki Y, Kameoka K, Osawa N, Goto Y, Katagiri H, *et al.* Screening of patients with maternally transmitted diabetes for mitochondrial gene mutations in the tRNA[Leu(UUR)] region. Diabet Med. 1997; 14(12): 1032-1037.
6. Suzuki Y, Suzuki S, Hinokio Y, Chiba M, Atsumi Y, Hosokawa K, *et al.* Diabetes associated with a novel 3264 mitochondrial tRNA(Leu)(UUR) mutation. Diabetes Care. 1997; 20(7): 1138-1140.
7. Leonard JV, Schapira AH. Mitochondrial respiratory chain disorders I: Mitochondrial DNA defects. Lancet. 2000; 355(9200): 299-304.
8. DiMauro S, Schon EA. Mitochondrial DNA mutations in human disease. Am J Med Genet. 2001; 106(1): 18-26.
9. Zhu Q, Zhou Y, Jin X, Lin X. The role of mitochondrial tRNAPhe C628T variant in deafness expression. Mitochondrial DNA. 2015; 26(1): 2-6.
10. Helm M, Brulé H, Friede D, Giegé R, Pütz D, Florentz C. Search for characteristic structural features of mammalian mitochondrial tRNAs. RNA. 2000; 6(10): 1356-1379.
11. van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 2009; 30(2): E386-394.

12. Gruber AR, Lorenz R, Bernhart SH, Neuböck R, Hofacker IL. The Vienna RNA Website. Nucleic Acids Res. 2008; 36(Web Server issue): W70-W74.
13. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2011; 34(Suppl 1): S62-S71.
14. Andrews RM, Kubacka I, Chinnery PF, Light-owlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet. 1999; 23(2):147.
15. Li W, Wen C, Li W, Wang H, Guan X, Zhang W, *et al.* The tRNA(Gly) T10003C mutation in mitochondrial haplogroup M11b in a Chinese family with diabetes decreases the steady-state level of tRNA(Gly), increases aberrant reactive oxygen species production, and reduces mitochondrial membrane potential. Mol Cell Biochem. 2015; 408(1-2): 171-179.
16. Liu H, Li R, Li W, Wang M, Ji J, Zheng J, *et al.* Maternally inherited diabetes is associated with a homoplasmic T10003C mutation in the mitochondrial tRNA(Gly) gene. Mitochondrion. 2015; 21: 49-57.
17. Wittenhagen LM, Kelley SO. Dimerization of a pathogenic human mitochondrial tRNA. Nat Struct Biol. 2002; 9(8): 586-590.
18. Janssen GM, Maassen JA, van Den Ouweland JM. The diabetes-associated 3243 mutation in the mitochondrial tRNA(Leu(UUR)) gene causes severe mitochondrial dysfunction without a strong decrease in protein synthesis rate. J Biol Chem. 1999; 274(42): 29744-29748.
19. Yarham JW, Al-Dosary M, Blakely EL, Alston CL, Taylor RW, Elson JL, *et al.* A comparative analysis approach to determining the pathogenicity of mitochondrial tRNA mutations. Hum Mutat. 2011; 32(11): 1319-1325.
20. Yoon KL, Aprille JR, Ernst SG. Mitochondrial tRNA(thr) mutation in fatal infantile respiratory enzyme deficiency. Biochem Biophys Res Commun. 1991; 173(6): 1112-1115.
21. Zhao L, Young WY, Li R, Wang Q, Qian Y, Guan MX. Clinical evaluation and sequence analysis of the complete mitochondrial genome of three Chinese patients with hearing impairment associated with the 12S rRNA T1095C mutation. Biochem Biophys Res Commun. 2004; 325(4): 1503-1508.
22. Zhu Y, Gu X, Xu C. A Mitochondrial DNA A8701G mutation associated with maternally inherited hypertension and dilated cardiomyopathy in a Chinese pedigree of a consanguineous marriage. Chin Med J (Engl). 2016; 129(3): 259-266.