THE MITOCHONDRIAL TRNA^{GLY} T10003C MUTATION MAY NOT BE ASSOCIATED WITH DIABETES MELLITUS

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ABSTRACT

Mitochondrial genome mutations have been recognized as more and more important factor in the pathogenesis of type 2 diabetes mellitus (T2DM). A large proportion of these mutations are localized at 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 therefore, will not result in the alternation of mitochondrial function which is responsible for DM. In this study, we reassessed a recent reported "pathogenic" mutation: $tRNA^{Gly}$ T10003C, in clinical manifestation of DM. We first performed the evolutionary conservation assessment for the T10003C mutation between various species. Moreover, the bioinformatics analysis was used to predict the changes in tRNA structure between the wild type and the mutant T10003C variant. 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 and 300 controls, suggesting that this mutation may be a rare event in human population. In conclusion, the current study showed no association between T10003C mutation and DM.

Keywords: mitochondrial $tRNA^{Gly}$; T10003C mutation; diabetes mellitus; association

INTRODUCTION

T2DM is a highly prevalent disease worldwide. 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 is related to both genetic and environmental factors, and their interaction. 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 dysfunction in T2DM. In fact, approximately 85% of mitochondrial diabetes cases are associated with the A3243G mutation in $tRNA^{Leu(UUR)}$ gene [4], moreover, some other point mutations, such as T3264C and T3271C in $tRNA^{Leu(UUR)}$ gene, are reported in patients with DM [5,6]. These mutations impaired the mitochondrial protein synthesis, subsequently affected the oxidative phosphorylation (OXPHOS); thus, a defect in respiratory chain will alter the mitochondrial function, and contributed to the progress of T2DM [7]. As a result, the mt-tRNA genes have become novel targets for investigation the relationship between mitochondrial dysfunction and T2DM.

However, it came to our attention that whether an mt-tRNA variant caused the T2DM or not was still controversial. At the same time, a lot of T2DM-associated mt-tRNA variants have been reported from Pubmed Central, we noticed that most of them were common genetic variations because 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]. Detecting the disease-associated mitochondrial

DNA (mtDNA) pathogenic mutations seems important for both 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 database searches for the frequency of this mutation; second, we reassessed the conservation index (CI) of the T10003C mutation, finally, we performed a case-control study to screen this mutation in a cohort of 500 unrelated DM patients and 300 healthy individuals.

MATERIALS AND METHODS

Database Searches. With the purpose of comparing different reports regarding the mt-tRNA^{Gly} T10003C mutation, we performed a systematic review for the presence of this mutation in Google Scholar, Pubmed Central and Human MITOMAP database. We used these combined keywords: "mitochondrial T10003C mutation" or "mitochondrial T10003C variant". If the paper was not original study, we excluded it.

Evolutionary Conservation Assessment. 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 CI was then calculated [10]. The CI>75% was proposed to have functional potential.

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

Mt-tRNA^{Gly} structure prediction. We applied 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 tRNA^{Gly} and the mutant carrying the T10003C mutation [12].

Mutational Analysis for the T10003C Mutation. A total of 500 unrelated DM patients (250 male and 250 females), enrolled from Endocrinology and Metabolism Department, People's Hospital of Zhengzhou University, participated to this case-control study. The age ranged from 50 to 80 years with the median at 61. In addition, 300 healthy controls with the age and gender matched obtained from the same area were also enrolled in this study. This study was approved by the Ethics Committee of Zhengzhou University.

The clinical diagnosis of DM was according to the American Diabetes Association (ADA), which was as follows: the level of fasting plasma glucose ≥7mmol/dL, or the level of oral glucose tolerance ≥ 1.11mmol/dL, or HbA1c ≥6.5% (13). For detecting the T10003C mutation, we used PCR and sequencing analysis of the target region spanning the tRNA^{Gly} gene. The primers information were: forward: 5'-TCTCCATCTATTGATGAGGGTCT-3'; reversed: 5'- AATTAGGCTGTGGG TGGTTG-3', after PCR amplification, the fragment was purified and sequenced, we then used DNA Star software to detect the mutation [14].

Statistical analysis. We used the SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) to determine the statistical significance. The Fisher's exact test was performed to evaluate the differences in categorical variables, $P<0.05$ was regarded as statistical significance.

RESULTS

Relationship between the T10003C Mutation and T2DM. As a result, two potential articles concerning the association between the T10003C mutation and T2DM 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 check for these reported families, it looked as if they were from the same family.

Evolutionary Conservation Analysis for the T10003C Mutation. To see whether T10003C mutation played a role in T2DM, the evolutionary conservation analysis was performed. As shown in **Figure 1**, the T10003C mutation occurred at D-stem of $tRNA^{Gly}$ (position 13), nucleotide at that position was not very conserved (CI=50%), suggesting that the T to C transition at 10003 position may not be involved in the pathogenesis of T2DM.

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 $tRNA^{Gly}$ gene from different species, arrow indicates the position 13, corresponding to the T10003C mutation.

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

The T10003C Mutation did not Alter the Structure of tRNA^{Gly}. To test if the T10003C mutation caused the tRNA^{Gly} structure alternation, we predicted the wild type version of tRNA^{Gly} and the mutant carrying this mutation using RNA Fold Webserver Programme. As shown in **Figure 2**, the T10003C mutation did not change the structure of tRNA^{Gly}, indicating that this mutation had little effect on tRNA^{Gly} folding.

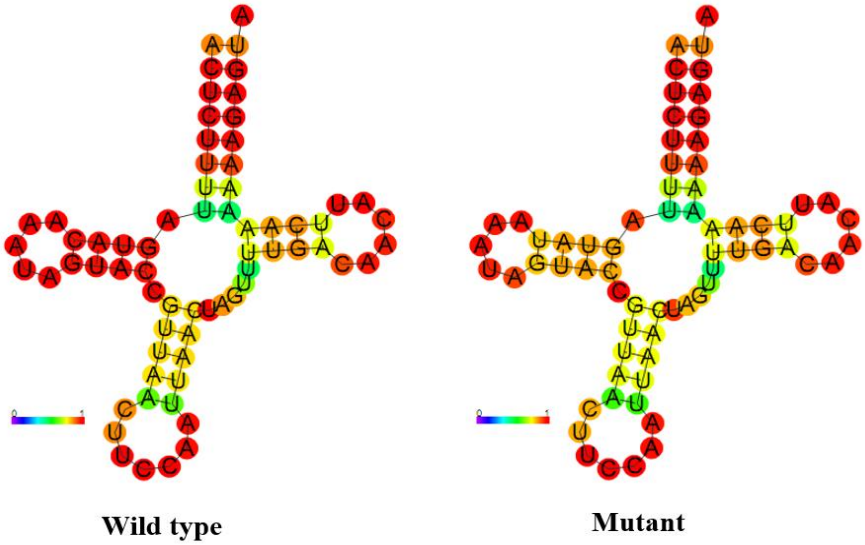

Figure 2. Prediction of the secondary structure of tRNA^{Gly} gene with and without the T10003C mutation.

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

DISCUSSION

The present study investigated the potential pathogenic role of DM-associated $tRNA^{Gly}$ T10003C mutation. Mitochondria are the powerful machine in cells whose primary role is to generate ATP through OXPHOS. More recently, the role of mitochondrial dysfunction in the pathogenesis of DM has been studied extensively. Alternations in mitochondrial function in human β-cells caused the impairment of glucose-stimulated insulin secretion. MtDNA mutations, especially mt-tRNA mutations were found to be related to 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 $tRNA^{Leu(UUR)}$ and resulted the impairment of aminoacylation ability [17], subsequently, mitochondrial protein synthesis failed [18]. Nevertheless, there is a number of mt-tRNA variations were wrongly classified as "pathogenic", a case in point was mt-$tRNA^{Phe}$ 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 result section [15,16]. Mutational analysis of proband from the maternally inherited DM identified a set of polymorphisms; some of them were obviously pathogenic in human population, for example, the $tRNA^{Thr}$ G15924A was reported to be a fatal infantile respiratory enzyme deficiency-associated pathogenic mutation [20]. Moreover, the 12S rRNA T1095C mutation was deafness-associated primary mutation [21], whereas the A6 A8701G mutation was found to be associated cardiomyopathy in Han Chinese

population [22]. Therefore, it seemed that beside the T10003C mutation, other mutations may also contribute to the DM in this Chinese family.

By molecular level, the T10003C mutation is localized at the D-stem of $tRNA^{Gly}$ gene (position 13), which is not very conserved from different species (**Figure 1**). Furthermore, to explore the structure to function relations, we used RNA Fold Webserver program to predict 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 large samples and functional analysis were needed to verify this conclusion.

Conflict of Interest. None.

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