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**Original Article**

# The Relationship between Mitochondrial DNA T3394C Point Mutation and Patients with Type 2 Diabetes Mellitus in the Bai Ethnic Minority of Northwest Yunnan, China

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**Abstract:**
Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder commonly accompanied by insulin resistance and pancreatic β-cell dysfunction, with increasing evidence implicating mitochondrial DNA (mtDNA) mutations in its pathogenesis. This study aimed to elucidate the potential association between the T3394C point mutation in the mitochondrial NADH dehydrogenase subunit 1 (ND1) gene and susceptibility to T2DM among individuals of the Bai ethnic minority. A total of 200 unrelated Bai participants diagnosed with T2DM and 216 ethnically matched controls with normal glucose tolerance and no family history of diabetes were recruited from Dali, Northwest Yunnan, China. The presence of the T3394C mutation was screened using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis, and statistical evaluation was conducted with SPSS software (version 26.0). The T3394C mutation in the mitochondrial ND1 gene was detected in 5 individuals within the T2DM group (2.50%) and in only 1 control subject (0.46%), representing a statistically significant difference between the two groups ($P<0.05$). Furthermore, this mutation exhibited a clear pattern of maternal inheritance among affected individuals. These findings suggest that the T3394C point mutation in the mitochondrial ND1 gene may be implicated in the development of T2DM in elderly individuals of the Bai ethnic minority, thereby providing novel insights into the mitochondrial genetic basis of diabetes susceptibility in this population.

**Key words**：mitochondrial ND1 gene; type 2 diabetes mellitus; T3394C mutation; Bai ethnic minority; Yunnan

## 1. Introduction

Human mitochondrial DNA (mtDNA) is a 16569bp circular double-stranded DNA molecule, encoding a total of 37 genes, including 13 genes for respiratory chain complex subunits, 2 rRNA genes (12S rRNA and 16S rRNA) and 22 tRNA genes, all of which are necessary for adenosine triphosphate (ATP) production through oxidative phosphorylation and have the characteristics of maternal inheritance.[1-3] tRNALeu(UUR) is a "mutation hotspot" for mitochondrial diabetes, especially the A→G mutation at position 3243. In 1997, the American Diabetes Association (ADA) classified this type of diabetes as diabetes with genetic defects in pancreatic β-cell function.

Mitochondria are known as the "powerhouses" of cells, located at the intersection of key cellular pathways such as energy substrate metabolism, reactive oxygen species (ROS) production, and cell death, and are the main sites for ATP synthesis through oxidative phosphorylation in cells.[4] Variations in mt-DNA can impair mitochondrial function, leading to reduced ATP production, increased ROS production, and altered cellular metabolism.[5] The main feature of type 2 diabetes mellitus (T2DM) is insulin resistance, and multiple lines of evidence indicate that mitochondria play a key role.[6-9] Since mitochondria play a central role in multiple cellular reactions and signaling pathways, mitochondrial dysfunction will affect the occurrence and progression of T2DM.[10] A growing body of evidence suggests that deletions, insertions, or point mutations in mtDNA are associated with diabetes mellitus (DM). [11-12] Mutations in the gene encoding mitochondrial nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 1 (ND1) may lead to a decrease in the activity of mitochondrial respiratory chain complex I enzyme, a reduction in intracellular ATP synthesis, thereby affecting the synthesis and secretion of insulin by pancreatic β-cells, and promoting the occurrence of diabetes.[13-16] T2DM is a prevalent chronic disease affecting more than 400 million people worldwide, driven by genetic and environmental factors. Over 90% of diabetic outpatients have T2DM, which is more common in obese middle-aged and elderly people. [17] Domestic and foreign studies have shown that in populations of different ethnic groups and regions, point mutations in many different sites of the mitochondrial genome may be related to the occurrence of T2DM in the elderly.[18-22] In most cases, mitochondrial diabetes is associated with the m.A3243G mutation in mtRNALeu(UUR). It has been reported that the A to G transition at position 3243 of mtDNA is the most common mutation in mitochondrial diabetes worldwide, with a prevalence ranging from 0.1% to 10%.[22] However, whether the T3394C point mutation in the ND1 gene of mtDNA is related to the occurrence of T2DM in the Bai ethnic minority in China has not been reported.

## 2 Materials and Methods

### 2.1 Ethical Approval and Study Design

The study described herein was granted approval by the Medical Ethics Committee of the Dali University Affiliated Hospital Consortium (approval number: 2024-YXLL18). The research protocol adheres to the ethical principles outlined in the revised Helsinki Declaration (2013) from Brazil. Informed consent was obtained from all individual participants involved in the study, with written documentation of consent being secured prior to their inclusion in the research. [15]

A post hoc power analysis was conducted using G*Power 3.0.10 software (Henrich Heine, University of Dusseldorf, Germany), confirming that the sample size (n=208) was sufficient to detect a medium effect size (w=0.3) with a power of 80% and α=0.05.

### 2.2 Research Objects

200 diabetic patients of local Bai ethnicity in Dali who were treated in the First People's Hospital of Dali and The First Affiliated Hospital of Dali University from January 2024 to September 2025 were selected as the diabetes group, including 98 males and 102 females, aged 20-81 years, with an average age of (58.85±12.90) years. The control group consisted of 216 Bai people with normal glucose tolerance, no family history of diabetes, no acute cardiovascular and cerebrovascular diseases, acute stress conditions, or endocrine system diseases, including 101 males and 115 females, aged 20-85 years, with an average age of (56.06±11.90) years. There were no significant differences in gender, age, and body mass index (BMI) between the two groups, which were comparable. The above research individuals had no blood relationship, traced back to 3 generations were all individuals of the same ethnicity, and had lived in Dali Bai Autonomous Prefecture, Yunnan Province for more than 3 generations. Peripheral venous blood was routinely drawn for genomic DNA extraction.

### 2.3. Inclusion criteria

The inclusion criteria are as follows: (1) Age between 20 and 85 years old; (2) Born and residing in Yunnan for a long time; (3) Within three generations, they belong to the pure Bai ethnic minority and have no close blood relationship; (4) Sign the informed consent form; (5) Meets WHO diagnostic criteria.

### 2.4. Exclusion and Dropout Criteria

The exclusion and dropout criteria are as follows: (1) Cannot cooperate to complete the questionnaire survey; (2) Proactively requesting to withdraw from the research; (3) Incomplete data sets and cannot be fully supplemented; (4) Exclude pregnancy, acute infection, tumors, trauma, heart and liver diseases, and those taking hydroxymethylglutarate monoacyl CoA reductase inhibitors, angiotensin-converting enzyme inhibitors, and thiazolidinedione drugs.

### 2.5 Instruments and Reagents

2.5.1 Instruments: 2720 Thermal Cycler PCR amplifier (Applied Biosystems, USA); Gel imaging system (BIO-RAD, USA); DYY-6C electrophoresis apparatus (Beijing LiuYi Instrument Factory); Biological safety cabinet (Suzhou Antai Air Technology Co., Ltd.); Automatic sterilizer (Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory); DYCZ-24D protein electrophoresis tank (Beijing LiuYi Instrument Factory); HH-4 constant temperature water bath (Shanghai Guohua Electric Appliance Co., Ltd.); Micropipettes (Eppendorf, Germany).

2.5.2 Reagents: Mammalian whole blood genomic DNA extraction kit was purchased from Beijing Tiangen Biotechnology Co., Ltd.; Restriction endonuclease *Hae*III was purchased from NEB, USA; Taq DNA polymerase was purchased from Takara, Dalian; DNA Marker (90602C) was purchased from Mianyang Tianenze Genetic Engineering Co., Ltd., Sichuan; DuRed nucleic acid dye was purchased from Beijing Quanshijin Co., Ltd.; Agarose (Biowest Agarose) was imported and subpackaged from Spain; Ethanol, isopropanol and other chemical reagents were domestic analytical pure.

### 2.6 Methods

#### 2.6.1 Primer Design and Synthesis

Primers were designed with reference to the human mtDNA Cambridge sequence (GenBank: J01415) and synthesized by Kunming Shuoqing Biotechnology Co., Ltd. (PAGE grade purification, purity 99%). The upstream primer (Forward) sequence was 5'-GGATCAGGACATCCCGAT-3', and the downstream primer (Reverse) sequence was 5'-GGTTTTAGGGGCTCTTTGG-3'.

2.6.2 Genomic DNA Extraction

Take 2 ml of peripheral venous blood and add 0.2 ml of EDTA-K2 for anticoagulation. Total DNA was extracted from peripheral blood using a

mammalian whole blood genomic DNA extraction kit according to the instructions, and the concentration and purity of DNA were determined by a NanoDrop nucleic acid/protein analyzer.

2.6.3 Polymerase Chain Reaction Amplification-Restriction Fragment Length Polymorphism Analysis (PCR-RFLP)

The PCR reaction system was: 1 μL of DNA template (50 ng/μL), 2.5 μL of 10×PCR buffer, 1 μL of dNTP, 0.2 μL of Taq DNA polymerase (5U/μL), 1 μL each of upstream and downstream primers, and double-distilled water was added to make the total volume 25 μL. The PCR reaction conditions were: pre-denaturation at 95°C for 5 min, then entering the cycle. Each cycle was denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 50 s, a total of 35 cycles, and finally extension at 72°C for 5 min, stored at 4°C. 10 μL of PCR product was added to 2 μL of 6×loading buffer, mixed, loaded on 1.5% agarose, electrophoresed at a constant voltage of 120V with 1×TAE for 25 min, and the presence of PCR product was observed by a gel imaging system. *Hae*III restriction endonuclease digested the PCR product at 37°C for 30 min, and the digested product was electrophoresed on 15% non-denaturing polyacrylamide gel, stained with nucleic acid dye, and developed and observed by a gel imaging system.

2.6.4 DNA Sequencing

The PCR products of 5 diabetic patients with suspected mutations and 2 diabetic patients without mutations were sent to Kunming Shuoqing Biotechnology Co., Ltd. for DNA purification and sequencing. The sequencing results were analyzed using Seqman in the DNAstar software package, and MegAlign was used for alignment analysis with the standard sequence to search for mutation sites.

2.6.5 Collection of Clinical Data of Patients with T2DM and Control Groups

The gender, age, age of onset, course of disease, family history, body mass index (BMI), total protein (TP), albumin (ALB), globulin (GLB), albumin/globulin (ALB/GLB) , blood urea nitrogen (BUN), serum creatinine (Scr), serum uric acid (SUA), riglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), 2-hour postprandial blood glucose (P2BG), glycosylated hemoglobin (HbA1c), serum C-peptide (C-peptide) and treatment methods (diet, oral hypoglycemic drugs or insulin therapy) of individuals in the diabetes group and normal control group were recorded [23].

### 2.7. Statistical Analysis

All data were statistically analysed using Statistical Product and Service Solutions (SPSS, Inc., Armonk, NY, USA) v26.0 statistical software. Measurement data were expressed as mean ± standard deviation (□x±s) and *t*-test was used. Count data were tested by $\chi 2$ test. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered extremely significant.

## 3 Results

### 3.1 General Clinical Characteristics of the Study Subjects

There were no significant differences in gender, age, and BMI between the T2DM and control groups, which were comparable ($P>0.05$). However, other biochemical variables, such as TP, ALB, GLB, ALB/GLB, BUN, FBG, TG, TC, HDL-C, P2BG, HbA1c, C-peptide and FBG, differed between these two groups ($P<0.05$) (Table 1).

**Table 1. Comparison of clinical data between T2DM group and control group**

| Variables/Group | T2DM Group (n=200) | Control group (n=216) | *P* |
|---|---|---|---|
| Gender (Male/Female) | 98/102 | 101/115 | 0.648 |

| | | | |
|---|---|---|---|
| Age (years) | 58.85±12.90 | 56.06±11.90 | 0.163 |
| Age of onset (years) | 51.40±9.05 | - | - |
| Course of disease (years) | 7.45±6.70 | - | - |
| BMI (kg/m²) | 22.24±3.38 | 22.92±3.29 | 0.287 |
| TP (g/L) | 68.26±7.17 | 75.45±4.49 | <0.001*** |
| ALB (g/L) | 40.40± 5.62 | 45.64± 2.65 | <0.001*** |
| GLB (g/L) | 27.88±4.84 | 29.81±3.86 | <0.001*** |
| ALB/GLB | 1.51± 0.41 | 1.49± 0.31 | 0.022* |
| BUN (mmol/L) | 5.82±2.55 | 5.30± 1.33 | <0.001*** |
| Scr (μmol/L) | 69.01± 26.00 | 70.02± 26.01 | 0.124 |
| SUA (μmol/L) | 341.28±97.80 | 343.34±95.46 | 0.349 |
| TG (mmol/L) | 1.98±2.05 | 1.50±1.13 | <0.001*** |
| TC (mmol/L) | 4.79±1.51 | 4.53±0.94 | <0.001*** |
| LDL-C (mmol/L) | 2.50±0.90 | 2.43±0.78 | 0.764 |
| HDL-C (mmol/L) | 1.27±0.57 | 1.49±0.37 | <0.001*** |
| P2BG (mmol/L) | 15.75±4.64 | 10.79±4.89 | <0.001*** |
| C-peptide (nmol/L) | 21.61+2.96 | 22.92±3.29 | <0.001*** |
| HBA1C (%) | 10.42±3.39 | 5.65±1.15 | <0.001*** |
| FBG (mmol/L) | 8.75±3.34 | 4.79±0.65 | <0.001*** |

Note: *$P$<0.05, **$P$<0.01, ***$P$<0.001

### 3.2 PCR Amplification of Mitochondrial DNA Fragments

The genomic DNA of samples in the diabetes group and control group was amplified by PCR using the synthesized primers. The expected target fragment size was 266bp. 1.5% agarose gel electrophoresis results showed that the amplification effect was good, which was consistent with the expected fragment size, and there were no non-specific bands (Figure 1).

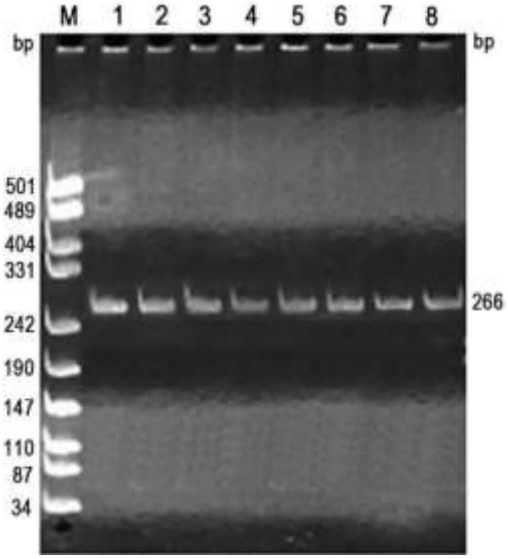


**Figure 1. PCR amplification results of mtDNA ND1 gene fragment.**
**M：DNA marker (90602C); 1-8The PCR amplified of mtDNA ND1 fragment (266bp).**

### 3.3 PCR-RFLP Detection of Mitochondrial tRNALeu (UUR) Gene T3394C Mutation

Through PCR-RFLP analysis (*Hae*III/3394), the wild type can be hydrolyzed by *Hae*III

endonuclease to obtain two restriction fragments of 169bp and 97bp. When T→C mutation occurs at 3394 (tyrosine→histidine), the newly added restriction site cuts 97bp into two fragments of 80bp and 17bp. Therefore, individuals with T3394C mutation can finally obtain 169bp, 80bp, and 17bp fragments. 15% polyacrylamide gel was prepared, and electrophoresis was performed at 150V constant voltage for about 150 min. After electrophoresis, nucleic acid dye staining was performed, and photographed by a gel imaging system. The experimental results showed that 5 cases of T3394C mutation were detected in 200 patients in the T2DM group, including 3 cases of mtDNA heteroplasmic mutation and 2 cases of mtDNA homoplasmic mutation. There was 1 case of T3394C mutation in 218 normal control groups, which was a mtDNA heteroplasmic mutation (Figure 2).

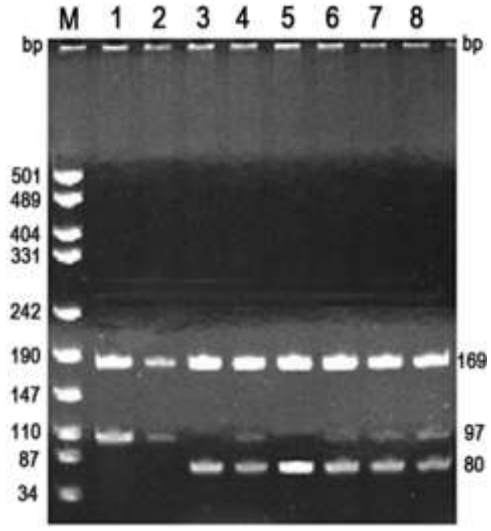


**Figure 2. PCR-RFLP detection of mitochondrial tRNA$^{Leu\ (UUR)}$ gene T3394C mutation. M：DNA Maker (90602C); 1-2: T3394C mutation negative specimen; 4-7: Case group T3394C mutation positive specimen; 8: Normal control group T3394C mutation positive specimen.**

### 3.4 Analysis of Mitochondrial DNA T3394C Mutation

A total of 5 cases of T3394C mutation were detected in 200 patients in the T2DM group, with a mutation rate of 2.50%, and 1 case of mutation in 216 normal control groups, with a mutation rate of 0.46%. There was a significant difference in the T3394C mutation rate between the two groups ($P$<0.05).

### 3.5 Age Distribution of Patients with T3394C Point Mutation

In the T2DM group, five patients with T3394C point mutation and T2DM were all over 45 years old. In the control group, only one man with T3394C point mutation, was also over 45 years old (Table 2).

**Table 2 Table of Age Distribution of T3394C Mutation**

| Group | Total cases | <45岁 | | ≥45岁 | | $P$ |
|---|---|---|---|---|---|---|
| | | Number of cases | Number of mutation cases | Number of cases | Number of mutation cases | |
| T2DM group | 200 | 98 | 0 | 102 | 5 | 0.030 |
| Control group | 216 | 113 | 0 | 113 | 1 | 1.000 |

### 3.5 Relationship between Mutation and Family History

Obvious DM family history ($\chi 2$=1.041, $P$<0.05) and maternal genetic tendency ($\chi 2$=3.840, $P$<0.01) were found in the two groups (Table 3).

**Table 3. Relationship between mitochondrial ND1 gene mutation and T2DM family history**

| | n | Genetic mutations (+) (case) | Incidence of mutations (%) |
|---|---|---|---|
| No family history | 105 | 1 | 0.95 |
| Family history | 95 | 4 | 4.21* |
| Maternal DM | 42 | 4 | 9.52** |
| Paternal DM | 17 | 0 | 0.00 |
| Compatriots suffer from DM | 36 | 3 | 8.33** |

Note:*$P$<0.05, **$P$<0.01

### 3.5. Comparison of Clinical Data of Patients in T2DM Group

Based on the sequencing results, the clinical data of the diabetes group was divided into two groups: those with the T3394C point mutation and those without the T3394C point mutation. The comparison of clinical data between these two groups is shown in Table 4. The HbA1c and FBG levels in the mutation group were significantly higher than those in the non-mutation group ($P$<0.05), while there was no statistically significant difference in other clinical data between the two groups ($P$>0.05) (Table 4).

**Table 4. Comparison of clinical data between T2DM patients with and without T3394C mutation**

| Variables/Group | Mutation Group | No mutation group | *P* |
|---|---|---|---|
| Number of cases | 5 | 195 | - |
| Gender (Male/Female) | 2/3 | 96/99 | 0.684 |
| Age (years) | 59.50±11.90 | 58.20±13.80 | 0.355 |
| Age of onset (years) | 52.50±5.30 | 50.30±12.80 | 0.953 |
| Course of disease (years) | 8.20±7.30 | 6.70±6.10 | 0.245 |
| BMI (kg/m²) | 21.61±2.96 | 22.86±3.80 | 0.147 |
| TP (g/L) | 68.36±6.98 | 68.16±7.01 | 0.330 |
| ALB (g/L) | 41.21± 5.32 | 39.59± 5.38 | 0.216 |
| GLB (g/L) | 27.65±4.84 | 28.11±4.90 | 0.139 |
| ALB/GLB | 1.52± 0.41 | 1.50± 0.40 | 0.382 |
| BUN (mmol/L) | 5.85±2.35 | 5.79±2.40 | 0.117 |
| Scr (μmol/L) | 69.23± 25.46 | 68.79± 26.40 | 0.122 |
| SUA (μmol/L) | 342.10±95.76 | 340.46±96.80 | 0.193 |
| TG (mmol/L) | 2.11±2.05 | 1.85±3.09 | 0.701 |
| TC (mmol/L) | 5.02±1.80 | 4.56±1.21 | 0.252 |
| LDL-C (mmol/L) | 2.65±0.90 | 2.34±0.67 | 0.133 |
| HDL-C (mmol/L) | 1.38±0.57 | 1.15±0.34 | 0.070 |
| P2BG (mmol/L) | 15.71±4.66 | 15.79±4.61 | 0.942 |
| C-peptide (nmol/L) | 0.90±0.12 | 0.89±0.09 | 0.256 |

| | | | |
|---|---|---|---|
| HBA1C (%) | 11.84±4.63 | 9.00±2.14 | 0.006** |
| FBG (mmol/L) | 9.61±3.68 | 7.89±3.00 | 0.041* |

Note:*$P$<0.05, **$P$<0.01

### 3.6 DNA Sequencing Results

The PCR products of all samples were purified and directly sequenced, and the T3394C mutation in 5 cases of suspected mutant diabetic patients and 2 cases of non-mutant diabetic patients was verified by forward sequencing, which proved that the T3394C mutation results of all samples in the case group and control group were reliable (Figure 3).

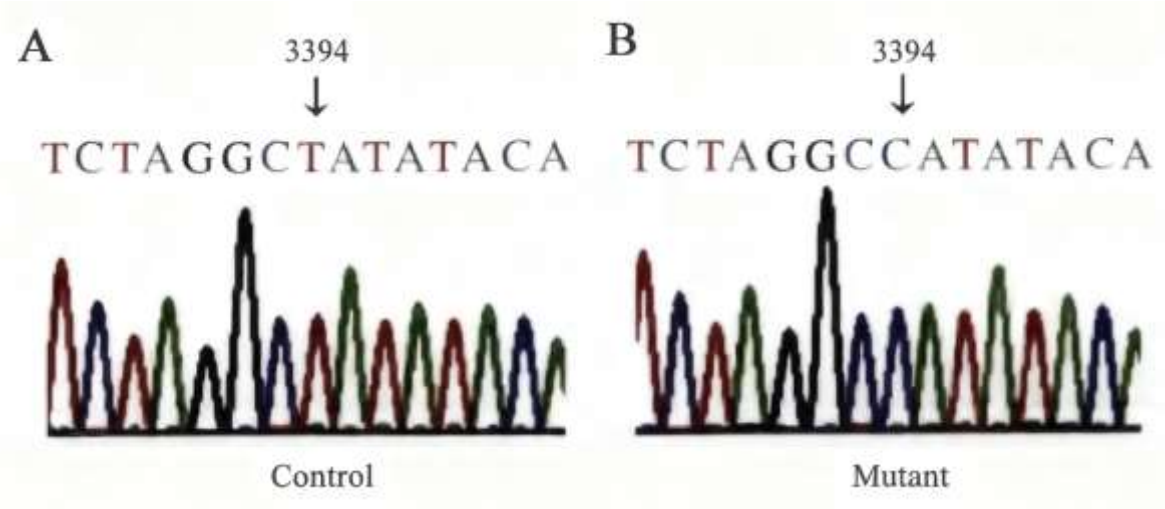


**Figure 3. Identification of tRNALeu(UUR) T3394C mutations by direct DNA sequencing. A: normal sample; the mtDNA3394 indicated by the arrow is T; B: T3394C mutant sample; the mtDNA3394 indicated by the arrow is C.**

## 4. Discussion

MtDNA is directly exposed to free radicals generated during oxidative phosphorylation, and at the same time, due to the lack of histone protection and the relative deficiency of DNA repair enzymes, mtDNA is more vulnerable to damage than nuclear DNA, with a mutation rate 10-20 times higher than that of nuclear DNA, especially in the elderly population. The prevalence of diabetes in people over 60 years old exceeds 10%, and more than 50% of the total diabetic population are elderly diabetic patients. With the in-depth study of human mitochondrial diseases, more than 50 mitochondrial gene mutation sites related to DM have been identified. [23] The most common mutations are point mutations in mtDNA, all of which are missense mutations and exhibit maternal inheritance. [24] ND1 gene T3394C mutation is a missense mutation, which can cause a neutral tyrosine in the mitochondrial respiratory chain ND1 subunit to be replaced by hydrophilic histidine. This mutation can change the spatial configuration of ND1, reduce NADH dehydrogenase activity, reduce ATP synthesis, and insufficient energy supply for pancreatic β-cells, thereby affecting insulin secretion and participating in the occurrence of T2DM.[13]

In 1996, Hirai et al. first reported the existence of mtDNA T3394C mutation in Japanese patients with T2DM, with a mutation rate of 4.90% in patients and 1.30% in normal people. [25] According to the new diagnostic and typing standards of DM of the American Diabetes

Association (ADA) in 1997, mitochondrial diabetes mellitus (MDM), as a new subtype of DM, was officially included in the classification of "other special types of DM", which belongs to a β-cell genetic defect disease. The incidence of ND1 gene T3394C mutation in T2DM population is 3.00-6.00%, and 0.00-2.06% in normal control population.[26] The results of this study showed that the mutation rate of T→C at position 3394 of mtDNA ND1 gene in the Bai ethnic minority with T2DM in Dali was 2.50%, and the mutation rate in the normal control group was only 0.46%. The difference between the two groups was statistically significant, so it is believed that the T3394C mutation of mitochondrial ND1 gene is related to the pathogenesis of T2DM in the Bai ethnic minority in Dali.

Previous studies have shown the presence of mtDNA mutations during the aging process, leading some scholars to view aging as a form of “Age related mitochondrial diseases”, and confirm that mitochondrial mutations and deletions accumulate with age. [27, 28] It can also be seen from this study that the mutation rate of T3394C increases with age. The mutation rate of T3394C in T2DM patients under 45 years old is 0.00%, while the mutation rate in patients over 45 years old is 4.90%. Statistical analysis shows that the mutation rate in patients over 45 years old is significantly higher than that in patients under 45 years old (P<0.05). It is speculated that age factor may play a role in the increase of mutation rate, but this trend does not exist in the normal control group, indicating that age is not the main factor that causes this phenomenon. It also leads to the accumulation of mitochondrial DNA variation under the joint action of other genetic factors, environment, nuclear genes, other mutant genes, etc. When its mutation reaches a certain threshold, diabetes will occur.

Statistical analysis of clinical data showed that the HbA1C and FBG levels in the T3394C mutation group were significantly higher than those in the non T3394C mutation group, and the difference between the two groups was statistically significant (P<0.05); However, there was no statistically significant difference in gender, age, onset age, disease duration, TG, TC, LDL-C, HDL-C, P2BG, BMI and other indicators between the two groups (P>0.05).The increase in blood glucose may have an inducing effect on the occurrence of mutations. However, the age of onset of diabetic patients in the mitochondrial ND1 gene T3394C mutation positive group was older than that in the mutation negative group, with extremely significant difference between the two groups, and the mutation positive patients had obvious maternal genetic characteristics.

At present, the research on the mitochondrial genetic structure of the Bai ethnic minority in Dali, Yunnan and its relationship with related diseases is not particularly clear. This experiment initially clarified the correlation between the T3394C mutation of mitochondrial gene ND1 and patients with T2DM in this population, which has certain guiding significance for the treatment guidance and disease prediction of T2DM in elderly Bai ethnic minority in Dali. Studies have shown that the T3394C point mutation of mitochondrial ND1 gene may be a pathogenic factor of T2DM in the Bai ethnic minority in Dali, Yunnan, which induces the occurrence of diabetes under the combined action of multiple factors, and has the characteristics of maternal inheritance. However, The sample size for this study is relatively small, which may result in sampling errors. The limited sample size necessitates further studies with larger cohorts.[29]

As we known, T2DM has a genetic predisposition, and its pathogenesis is highly complex. The application of modern molecular biology and genetics in diabetes research has deepened our understanding of the disease. Pathogenic mutations at certain sites in mitochondrial gene mutation-related diabetes may be influenced by genetic and environmental

factors, heterogeneity, tissue specificity, and other factors, resulting in complex characteristics of mitochondrial genetic diseases. Research on various mitochondrial gene mutations associated with T2DM can aid in further clinical diagnosis and subtyping, as well as provide practical value for guiding eugenics and prenatal care [27]. Furthermore, understanding the pathogenesis of mitochondrial gene mutation-related DM may help identify drug targets for diabetes treatment and provide a basis for future gene therapy in clinical DM patients.

## Conclusions

In conclusion, our research found that the point mutation T3394C in the ND1 gene of mitochondrial DNA may be associated with the incidence of T2DM in the Bai ethnic minority. Due to differences in genetic background and lifestyle, the mutation rates of mtDNA vary significantly among different ethnic groups in China. It is worth mentioning that, mitochondrial T3394C point mutations in T2DM patients with family history showed obvious maternal inheritance characteristics. Our findings provide additional information for the prevention and treatment of T2DM in the local region. The sample size of this study was small, which may have a certain impact on the statistical results. In the future, larger, multi-center studies are needed to validate these preliminary results and better understand the prevalence of these mutations in the Bai population. This, together with the search for other mitochondrial gene mutations, should allow to fully determine the prevalence of MDM and its specific molecular background in the Bai ethnic minority of Yunnan, Southwest China.

## Disclosure Statement

The authors declare that they have no confficts of interest regarding the publication of this paper.

## Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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## References

1. Sharma, P.; Sampath, H. Mitochondrial DNA Integrity: Role in Health and Disease. *Cells.* **2019**, 8, 100. DOI: 10.3390/cells8020100.
2. Cooley, A. M. Mitochondrial DNA Analysis. *Methods. Mol. Biol.* **2023**, 2685, 331-349. DOI: 10.1007/978-1-0716-3295-6_20.
3. Shah, A.; Bush, C. O.; Perry, R. J. Genetic un derpinnnings of type 2 diabetes. *Adv. Genet.* **2025**,113,54-75. DOI:10.1016/bs.adgen.2024.12.001.
4. Pinti, M. V.; Fink, G. K.; Hathaway, Q. A.; D urr, A. J.; Kunovac, A.; Hollander, J. M. Mito chondrial dysfunction in type 2 diabetes mellit us: an organ-based analysis. *Am. J. Physiol. Endocrinol. Metab.* **2019**, 316, E268-E285. DOI: 10.1152/ajpendo.00314.2018.
5. K, S, P. K.; Jyothi, M. N.; Prashant, A. Mitoc hondrial DNA variants in the pathogenesis an d metabolic alterations of diabetes mellitus. *Mol. Genet. Metab. Rep.* **2024**, 28, 42, 101183. DOI: 10.1016/j.ymgmr.2024.101183.
6. Lefort, N.; Glancy, B.; Bowen, B.; Willis, W. T.; Bailowitz, Z.; De, Filippis, E. A.; Brophy,

C.; Meyer, C.; Højlund, K.; Yi, Z.; et al. Increased reactive oxygen species production and lower abundance of complex I subunits and carnitine palmitoyltransferase 1B protein despite normal mitochondrial respiration in insulin-resistant human skeletal muscle. *Diabetes.* **2010**, 59, 2444-2452. DOI: 10.2337/db10-0174.

7. Sleigh, A.; Raymond-Barker, P.; Thackray, K.; Porter, D.; Hatunic, M.; Vottero, A.; Burren, C.; Mitchell, C.; McIntyre, M.; Brage, S.; et al. Mitochondrial dysfunction in patients with primary congenital insulin resistance. *J. Clin. Invest.* **2011**, 121, 2457-2461. DOI: 10.1172/JCI46405.
8. Skovsø, S.; Panzhinskiy, E.; Kolic, J.; Cen, H. H.; Dionne, D. A.; Dai, X. Q.; Sharma, R. B.; Elghazi, L.; Ellis, C. E.; Faulkner, K.; et al. Beta-cell specific Insr deletion promotes insulin hypersecretion and improves glucose tolerance prior to global insulin resistance. *Nat Commun.* **2022**, 13, 735. DOI: 10.1038/s41467-022-28039-8.
9. Cortés-Rojo, C.; Vargas-Vargas, M. A.; Olmos-Orizaba, B. E.; Rodríguez-Orozco, A. R.; Calderón-Cortés, E. Interplay between NADH oxidation by complex I, glutathione redox state and sirtuin-3, and its role in the development of insulin resistance. *Biochim Biophys Acta Mol Basis Dis.* **2020**, 1866, 165801. DOI: 10.1016/j.bbadis.2020.165801.
10. Duong, A.; Evstratova, A.; Sivitilli, A.; Hernandez, J. J.; Gosio, J.; Wahedi, A.; Sondheimer, N.; Wrana, J. L.; Beaulieu, J. M.; Attisano, L.; et al. Characterization of mitochondrial health from human peripheral blood mononuclear cells to cerebral organoids derived from induced pluripotent stem cells. *Sci. Rep.* **2021**, 11, 4523. DOI: 10.1038/s41598-021-84071-6.
11. Ballinger, S. W.; Shoffner, J. M.; Hedaya, E. V.; Trounce, I.; Polak, M. A.; Koontz, D. A.; Wallace, D. C. Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nat Genet.* **1992**, 1, 11-15. DOI: 10.1038/ng0492-11.
12. You, X.; Huang, X.; Bi, L.; Li, R.; Zheng, L.; Xin, C. Clinical and molecular features of two diabetes families carrying mitochondrial ND1 T3394C mutation. *Ir. J. Med. Sci.* **2022**, 191, 749-758. DOI: 10.1007/s11845-021-02620-4.
13. Al-Ghamdi, B. A.; Al-Shamrani, J. M.; El-Shehawi, A. M.; Al-Johani, I.; Al-Otaibi, B. G. Role of mitochondrial DNA in diabetes Mellitus Type I and Type II. *Saudi J Biol Sci.* **2022**, 29, 103434. DOI: 10.1016/j.sjbs.2022.103434.
14. Vezza, T.; Díaz-Pozo, P.; Canet, F.; de Marañón, A. M.; Abad-Jiménez, Z.; García-Gargallo, C.; Roldan, I.; Solá, E.; Bañuls, C.; López-Domènech, S.; et al. The Role of Mitochondrial Dynamic Dysfunction in Age-Associated Type 2 Diabetes. *World J Mens Health.* **2022**, 40, 399-411. DOI: 10.5534/wjmh.210146.
15. Fang, H.; Hu, N.; Zhao, Q.; Wang, B.; Zhou, H.; Fu, Q.; Shen, L.; Chen, X.; Shen, F.; Lyu, J. mtDNA Haplogroup N9a Increases the Risk of Type 2 Diabetes by Altering Mitochondrial Function and Intracellular Mitochondrial Signals. *Diabetes.* **2018**, 67, 1441-1453. DOI: 10.2337/db17-0974.
16. Mugabo, Y.; Zhao, C.; Tan, J. J.; Ghosh, A.; Campbell, S. A.; Fadzeyeva, E.; Paré, F.; Pan, S. S.; Galipeau, M.; Ast, J.; et al. 14-3-3ζ Constrains insulin secretion by regulating mitochondrial function in pancreatic β cells. *JCI Insight.* **2022**, 7, e156378. DOI: 10.1172/jci.insight.156378
17. Młynarska, E.; Czarnik, W.; Dzieża, N.; Jędraszak, W.; Majchrowicz, G.; Prusinowski, F.; Stabrawa, M.; Rysz, J.; Franczyk, B. Type 2 Diabetes Mellitus: New Pathogenetic Mechanisms, Treatment and the Most Important Complications. *Int. J. Mol. Sci.* **2025**, 26, 1094. DOI: 10.3390/ijms26031094.
18. Jiang, W.; Li, R.; Zhang, Y.; Wang, P.; Wu, T.; Lin, J.; Yu, J.; Gu, M. Mitochondrial DNA Mutations Associated with Type 2 Diabetes Mellitus in Chinese Uyghur Population. *Sci. Rep.* **2017**, 7, 16989. DOI: 10.1038/s41598-017-17086-7.

19. Tan, F.; Cheng, X.; Chen, S.; Chen, Z.; Wang, Y.; Shen, Y. Heterogeneity of mitochondrial DNA in black and white hair of patients with t ype 2 diabetes. *Nan Fang Yi Ke Da Xue Xue B ao*. **2012**, ;32, 85-88.
20. Wang, S.; Wu, S.; Zheng, T.; Yang, Z.; Ma, X .; Jia, W.; Xiang, K. Mitochondrial DNA muta tions in diabetes mellitus patients in Chinese Han population. *Gene.* **2013**, 1, 531, 472-475. DOI: 10.1016/j.gene.2013.09.019.
21. Ding, Y.; Zhang, S.; Guo, Q.; Leng, J. Mitoch ondrial Diabetes Is Associated with the ND4 G11696A Mutation. *Biomolecules.* **2023**, 13, 907. DOI: 10.3390/biom13060907.
22. Maassen, J. A.; 'T Hart, L. M.; Van Essen, E.; Heine, R. J.; Nijpels, G.; Jahangir Tafrechi, R S.; Raap, A. K.; Janssen G. M.; Lemkes H. H. Mitochondrial diabetes: molecular mechanism s and clinical presentation. *Diabetes*. **2004,** 53 , S103-S109. DOI: 10.2337/diabetes.53.2007. s103.
23. Fang, H.; Li, X.; Wang, S.; Zhang, M.; Zhang, V. W.; Xu, C. A non-invasive method for scree ning mitochondrial diabetes. *Front Genet.* **2025**, 16,1536331. DOI: 10.3389/fgene.2025.1536331
24. Lin, L.; Zhang, D.; Jin, Q.; Teng, Y.; Yao, X.; Z hao, T.; Xu, X.; Jin, Y. Mutational Analysis of Mitochondrial tRNA Genes in 200 Patients with Type 2 Diabetes Mellitus. *Int. J. Gen. Med.* **202 1**, 14, 5719-5735. DOI: 10.2147/IJGM.S330973 .
25. Hirai, M.; Suzuki, S.; Onoda, M.; Hinokio, Y. ; Ai, L.; Hirai, A.; Ohtomo, M.; Komatsu, K.; Kasuga, S.; Satoh, Y.; et al. Mitochondrial D NA 3394 mutation in the NADH dehydrogena se subunit 1 associated with non-insulin-depe ndent diabetes mellitus. *Biochem Biophys Res Commun.* **1996**, 219, 951-955. DOI: 10.1006/ bbrc.1996.0324.
26. Duraisamy, P.; Elango, S.; Vishwanandha, V. P.; Balamurugan, R. Prevalence of mitochond rial tRNA gene mutations and their associatio n with specific clinical phenotypes in patients with type 2 diabetes mellitus of Coimbatore. *Genet Test Mol Biomarkers.* **2010**, 14, 49-55. DOI: 10.1089/gtmb.2009.0024.
27. Wang, S.; Wu, S.; Zheng T.; Yang Z.; Ma X.; Ji a, W.; Xiang K. Mitochondrial DNA mutations in diabetes mellitus patients in Chinese Han pop ulation. *Gene* **2013**, 531, 427-475. DOI: 10.101 6/j.gene.2013.09.019..
28. You, X.; Huang, X.; Bi, L.; Li, R.; Zheng, L.; X in, C. Clinical and molecular features of two dia betes families carrying mitochondrial ND1 T33 94C mutation. *Ir. J. Med. Sci.* **2022**, 191, 749-7 58. DOI: 10.1007/s11845-021-02620-4.
29. Hansrote, S.; Croul, S.; Selak, M.; Kalman, B.; Schwartzman, R. J. External ophthalmoplegia w ith severe progressive multiorgan involvement a ssociated with the mtDNA A3243G mutation. *J. Neurol. Sci.* **2002**, 197, 63-67. DOI: 10.1016/s0 022-510x(02)00048-5.