

Analysis of PTEN expression and promoter methylation in Uyghur patients with mild type 2 diabetes mellitus

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Abstract

Phosphatase and tension homolog deleted on chromosome 10 (*PTEN*) was considered as a promising target in type 2 diabetes mellitus (T2DM) because of its negative effects on insulin resistance. Alteration in DNA methylation is thought to play a role in the pathogenesis of T2DM. The aim of the present study was to quantitatively evaluate the promoter methylation of *PTEN* in Uyghur patients with mild T2DM. We evaluated methylation levels in 21 CpG sites from -2515bp to -2186bp relative to the translation initiation site in 55 cases of T2DM and 50 cases of normal glucose tolerance (NGT) using the MassARRAY spectrometry. In addition, *PTEN* mRNA and protein levels were measured by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and western blotting to determine whether DNA methylation alterations were responsible for *PTEN* expression. Compared with NGT groups, the *PTEN* mRNA expression was significantly higher in Uyghur patients with mild T2DM groups. We also showed that *PTEN* protein expression was upregulated in Uyghur patients with mild T2DM groups, but the level of protein kinase B (*AKT*) was downregulated. *PTEN* methylation in T2DM patients was significantly lower than that in NGT groups. In addition, 2 CpG units demonstrated a significant difference between the NGT and Uyghur patients with mild T2DM groups. Furthermore, there was a negative association between promoter methylation and *PTEN* expression. Together, these findings suggest that epigenetic inactivation of *PTEN* plays an important role in Uyghur patients with mild T2DM. The aberrant methylation of CpG sites within the *PTEN* promoter may serve as a potential candidate biomarker for T2DM in the Uyghur population.

Abbreviations: *AKT* = protein kinase B, BMI = body mass index, CGI = CpG island, FINS = fasting plasma insulin, FPG = fasting plasma glucose, *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase, HbA1c = hemoglobin A1c, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, NGT = normal glucose tolerance, *PTEN* = phosphatase and tension homolog deleted on chromosome 10, qRT-PCR = quantitative reverse transcriptase polymerase chain reaction, T2DM = type 2 diabetes mellitus.

Keywords: DNA methylation, *PTEN*, Type 2 diabetes mellitus

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex polygenic disease that commonly results from defects in insulin secretion and/or the diminished sensitivity of target tissues to insulin.^[1] T2DM is also

a leading cause of death worldwide. Therefore, understanding the molecular pathogenesis of T2DM is important for clinicians who manage patients with T2DM.

As the largest ethnic population, the majority of Uyghurs live in the Xinjiang Uyghur Autonomous Region (XUAR) of Western China. The prevalence of T2DM is significantly higher in Uyghurs than in other ethnicities with T2DM.^[2] The differences in the prevalence of diabetes might be due to differences in dietary habit. The Uyghur population commonly consumes large amounts of beef, mutton, and butter, which increases the risk of developing diabetes. More recently, emerging evidence points to an important role for epigenetic mechanisms in the development of T2DM.^[3] Our previous study demonstrated that the miR-375 promoter is hypomethylated in Kazak patients with T2DM, which may be involved in the pathogenesis of T2DM.^[4] However, whether aberrant methylation patterns occur in Uyghur T2DM patients remains poorly understood.

Phosphatase and tension homolog deleted on chromosome 10 (*PTEN*) is a tumor suppressor, which is involved in basic cellular functions such as adhesion, migration, proliferation, and cell survival.^[5] Although *PTEN* was first identified as a candidate tumor suppressor gene, recent studies have suggested that *PTEN* antagonizes the action of phosphatidylinositol 3-kinase (PI3K), a key kinase upstream of protein kinase B (*AKT*) in the insulin signaling cascade and plays an important role in glucose metabolism.^[6,7] Genome-wide linkage studies in Mexican American populations have identified a susceptibility locus for

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T2DM on chromosome 10q.^[8] *PTEN* may be a candidate gene for T2DM susceptibility.^[9] However, it is important to note that only 25% of cancer patients demonstrate a correlation between the loss of the *PTEN* protein and the mRNA level,^[10] which emphasizes the importance of *PTEN* regulation at the post-transcriptional and post-translational levels. In the cancer area, the epigenetic regulation of the *PTEN* within or near CpG islands (CGIs) in the corresponding promoter regions can result in the aberrant expression of *PTEN*. Our laboratory has previously shown that the hypermethylation of the promoter of the *PTEN* is a common event in soft tissue sarcomas, which may play a role in the oncogenesis of soft tissue sarcomas.^[11] These findings strengthen the functional role of epigenetic *PTEN* inactivation, but its exact role in T2DM is unclear.

Therefore, the aim of the present study was to quantitatively evaluate the promoter methylation of *PTEN* in Uyghur patients with mild T2DM patients using MassARRAY spectrometry. Moreover, our investigation sought to determine whether aberrant *PTEN* methylation patterns occur in Uyghur patients with mild T2DM patients and to determine whether the observed aberrant expression of *PTEN* is caused by the aberrant methylation of the gene. Furthermore, we attempted to determine whether any of these alterations have potential value as biomarkers of T2DM in the Uyghur population.

2. Materials and methods

2.1. Ethics statement

Written informed consent was obtained from all participating patients before enrollment in the study. This study was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Shihezi University School of Medicine and was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

2.2. Study participants

Fifty patients with newly diagnosed mild T2DM (hemoglobin A1c (HbA1c) = $6.76 \pm 0.15\%$, no symptoms) from the Uyghur population and another 50 healthy controls from the same population with normal glucose tolerance (NGT) were enrolled from the Department of Endocrinology and Metabolism at the First Affiliated Hospital of the Shihezi University School of Medicine between 2013 and 2014. Glucose tolerance was measured by oral glucose tolerance test (OGTT), and 2006 WHO criteria were applied. Any individuals who may have had *PTEN*-related diseases (Cowden, et al), an infectious disease before or during the recruitment, active inflammatory disease, previous treatment with antidiabetic agents, a previous diagnosis of DM, HbA1c $\geq 7.0\%$ were excluded from this study. Calculate Homeostasis model assessment-insulin resistance, HOMA-IR = fasting plasma glucose (FPG) \times fasting plasma insulin (FINS)/22.5.

2.3. Nucleic Acid Isolation

The total RNA was extracted from the peripheral blood mononuclear cells (PBMCs) of T2DM patients using Trizol, and the RNA obtained was treated with RNase-free DNAase I (Invitrogen Life Technologies, Carlsbad, CA). Genomic DNA was isolated from blood cells using the DNeasy Blood and Tissue Kit (Qiagen, Germany), according to the manufacturer's instructions. The nucleic acid samples were quantified by measuring the absorption at 260 nm.

2.4. Quantitative reverse transcriptase-PCR (qRT-PCR)

qRT-PCR was performed using a SYBR-green fluorescence quantification system in a Step-1 real-time PCR 96-well plate (Applied Biosystems, Warrington, UK). Each cDNA sample (2.5 μ L) was used as a template for the PCR amplification mixture containing forward and reverse primers (900 mM each). The PCR primer sets were designed to amplify the transcript from the *PTEN*, according to information in GeneBank. The sequences of primers used for *PTEN* were 5'-CGAACTGGTGTAATGATATGT-3' (forward) and 5'-CATGAACCTGTCTTCCCCGT 3' (reverse). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an internal control to confirm the success of the RT reaction. The primers for *GAPDH* amplification were as follows: forward primer, 5'-CACTGGCGTCTTACCAC-CATG-3', and reverse primer, 5'-GCTTACCACCTTCTT-GATGTCA-3'.

2.5. Western blotting

Blood peripheral mononuclear cells were isolated using Ficoll PM 400 Lymphocyte Separation Buffer and homogenized in RIPA buffer (50 mM Trisbase, 1 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% Triton X100, and 1% sodium deoxycholate). Proteins were separated on a 12% SDS-PAGE gel and analyzed by immunoblotting, with *GAPDH* used as a gel-loading control. The primary antibodies used were rabbit anti-human *PTEN* polyclonal antibody (1:1000 dilution, ab32199, Abcam, Cambridge, UK), anti-rabbit p-Akt antibody (1:1500, ab38449, Abcam, Cambridge, UK) and mouse anti-human anti-*GAPDH* monoclonal antibody (1:2000 dilution, ab9485, Abcam, Cambridge, UK). A goat anti-rabbit secondary antibody (HRP-conjugate, ab181658, Abcam, Cambridge, UK) was used at a 1:10,000 dilution. The blots were detected using the ECL chemiluminescence reagent. Band analyses were performed using the Image Lab software version 4.1 (Bio-Rad Laboratories, Hercules, CA). Experiments were repeated at least 3 times.

2.6. MassARRAY spectrometry methylation analysis

The sequence of the CpG island (CGI) was identified using the UCSC genome browser (<http://genome.ucsc.edu/>), (chr10:89621773-89624128% GC = 58.1 and Obs/Exp CpG = 0.86). The target of the promoter region was 1 amplicon (Fig. 1 and Fig. 2), as previously reported.^[12-14] We designed 1 primer set for the methylation analysis of the *PTEN* promoter region by EpiDesigner software (<http://epidesigner.com>; Table 1). For the reverse primer, an additional T7 promoter tag was added for in vivo transcription, and a 10-mer tag was added to the forward primer to adjust for the melting temperature differences. The primers used in the present study specifically detected the promoter sequence of the *PTEN* gene rather than that of the *PTEN* pseudogene (Fig. 2).^[15]

To quantitatively evaluate the promoter methylation of *PTEN* in Uyghur T2DM patients, the high-throughput MassARRAY platform (Sequenom, San Diego, CA) was used as previously described.^[16] Briefly, primers for the *PTEN* CGI were used to amplify bisulfite-treated DNA, and the PCR products were spotted on a 384-pad SpectroCHIP (Sequenom, San Diego, CA), followed by spectral acquisition on a MassARRAY Analyzer. The methylation data of individual units (1 to 3 CpG sites per unit) were generated by the EpiTyper v1.0.5 software (Sequenom, San Diego, CA).

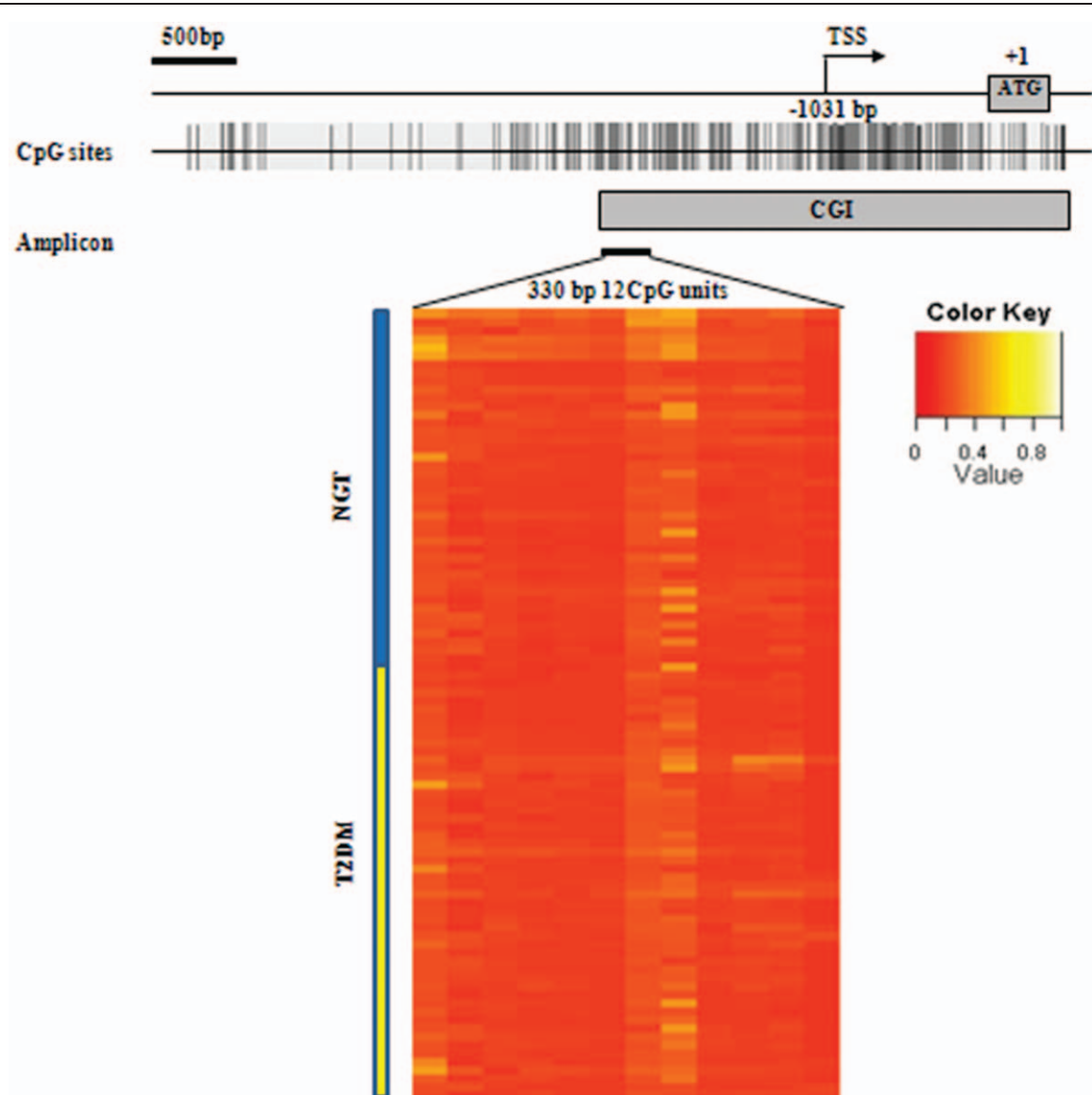


Figure 1. Genomic map of *PTEN* CGI and the DNA methylation status at 1 amplicon of *PTEN*. MassARRAY analysis was used to quantify the DNA methylation of *PTEN* in T2DM and NGT samples. Arrow indicates the transcriptional start site (TSS) at -1031 bp. ATG, the translation start codon, at +1 indicates the translation initiation site. Vertical bars, CpG sites. Gray filled boxes, CGI; black filled bars, MassARRAY regions studied: amplicon characteristics are shown beneath the black bars; bp indicates base pair. Each row represents a sample. Top, 45 normal glucose tolerance samples (NGT), and bottom, 49 cases of T2DM samples. Each column represents a CpG unit, which is a single CpG site or a combination of CpG sites. Color coding reflects the degree of methylation, with yellow being 100% and red being 0%.

2.7. Statistical analysis

Student *t* test and analysis of variance (ANOVA) were used to detect differences in the mean values of the variables. Fisher exact test was used to analyze differences in the rate of each variable. Shapiro–Wilk test was applied to test for normal distribution. All analyses included Bonferroni-corrected multiple comparison follow-ups. $P < .05$ was considered significant. The distances between CpG methylation sites and transcription start sites were calculated using the RMySQL package and the SQL database version of the UCSC genome browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>). Two-dimensional clustering was determined using the heatmap.2 function in the gregmisc package. Classical multidimensional scaling was performed using the cmdscale function, and visualization was performed using the

scatter plot3d function in the same package. Power calculations were performed using the Power and Sample Size Calculation 3.0 software. Correlation analyses were performed using linear regression with a 2-tailed Pearson's test, where the *P* value is indicative of a non-zero slope, which is described according to the 95% confidence interval (CI). Tests for statistical significance were performed with the standard function in the R statistical environment.

3. Results

3.1. Characteristics of the participants

For the 100 participants included in our analysis, the mean age at diabetes diagnosis was 54 years (range, 37 to 75 years) for the



Figure 2. CpG methylation status in *PTEN* promoter. The translation start site at +1 (start codon, ATG, arrow) and the TSS at -1031 (arrow) are shown. DNA sequence is numbered from the translation start site, and CpG dinucleotides are numbered. Sequence of *PTEN* amplicon is indicated by double underlines and spans positions -2190 to -2542 with respect to the translation start site within the core promoter region (red). The single underline represents *PTEN* pseudogene sequence on chromosome 9, showing the region of high homology to *PTEN*. Genomic sequence showing *PTEN* exon 1 (yellow). CpG sites 1–21 were undetectable by MassARRAY analysis in the 2 groups.

50 T2DM participants and 52 years (range, 35 to 76 years) for the 50 NGT participants. The clinical characteristics of the 2 groups demonstrated a significant difference in FBG, 2 h post-glucose load blood glucose (2h OGTT), FINS, 2 h plasma insulin

after glucose overload (PINS), body mass index (BMI), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and HbA1c (see Table 2). Gender distribution, age, triglyceride (TG), systolic

Table 1

Sequences of MassARRAY primers and positions relative to the translational start codon for the assays used to analyze the DNA methylation of *PTEN*.

	Primer	Sequence (5'→3')	Position
Amplicon	PTEN-F	aggaagagagTGA	-2,515 to -2,186
		GTTTTAGGTTTTA	
		GTTTTTTGGTTTGT	
		3	
PTEN-R	cagtaatacgcactcac		
	tatagggagaaggctTAAAAAA		
	CTTCCAAATCCCACTCC		
	3'		

blood pressure (SBP), and diastolic blood pressure (DBP) in the T2DM group were not significantly different from the NGT group.

3.2. The transcription levels of *PTEN* in Uyghur T2DM patients

To determine whether there was a significant difference in the transcription levels of *PTEN* between the Uyghur T2DM and NGT groups, the transcription levels of *PTEN* were determined by qRT-PCR. Figure 3A demonstrates that the relative transcription level of *PTEN* in the NGT samples was 1, and the relative transcription level of *PTEN* in the Uyghur T2DM samples was 3.5 (min = 0.000211 and max = 0.000879, SD: 0.0001765, *t* test, *P* < .05). The relationship between the transcription level of *PTEN* and the clinical characteristics were also evaluated (data not shown). However, there was no significant association between the transcription level of *PTEN* and clinical characteristics.

Table 2

Clinical characteristics of subjects with NGT and Uyghur patients with mild T2DM.

Characteristic	Mild T2DM	NGT
Gender (male/female)	20/30	26/24
Age (years)	55.62 ± 9.55	54.15 ± 8.68
BMI (kg/m ²)	29.75 ± 4.85	25.45 ± 2.83*
SBP (mmHg)	131.15 ± 1.32	130.20 ± 1.36
DBP (mmHg)	79.08 ± 1.13	79.70 ± 1.15
FPG (mmol/L)	7.20 ± 0.22	5.12 ± 0.35**
2hOGTT (mmol/L)	11.34 ± 0.27	6.36 ± 0.32**
FINS (μU/mL)	9.25 ± 4.72	5.72 ± 2.60*
PINS (μU/mL)	58.35 ± 20.37	33.56 ± 18.22**
TC (mmol/L)	5.43 ± 1.18	5.17 ± 1.50
TG (mmol/L)	1.74 ± 0.23	1.50 ± 0.52
LDL-C (mmol/L)	3.23 ± 0.24	2.25 ± 0.53*
HDL-C (mmol/L)	1.15 ± 0.32	1.23 ± 0.34
HOMA-IR	3.62 ± 1.34	1.26 ± 0.79**
HbA1c (%)	6.76 ± 0.15	4.91 ± 0.50**

Data are the mean ± SD. 2hOGTT=2hour post-glucose load blood glucose, BMI=body mass index, DBP=diastolic blood pressure, FINS=fasting plasma insulin, FPG=fasting plasma glucose, HbA1c=hemoglobin A1c, HDL-C=High-density lipoprotein cholesterol, HOMA-IR=homeostasis model of assessment/insulin resistance, LDL-C=low-density lipoprotein cholesterol, NGT=normal glucose tolerance, PINS=2 hour plasma insulin after glucose overload, SBP=systolic blood pressure, T2DM=type 2 diabetes mellitus, TC=total cholesterol, TG=triglyceride; *t* test.

* *P* < .01.

** *P* < .001 compared with the NGT group.

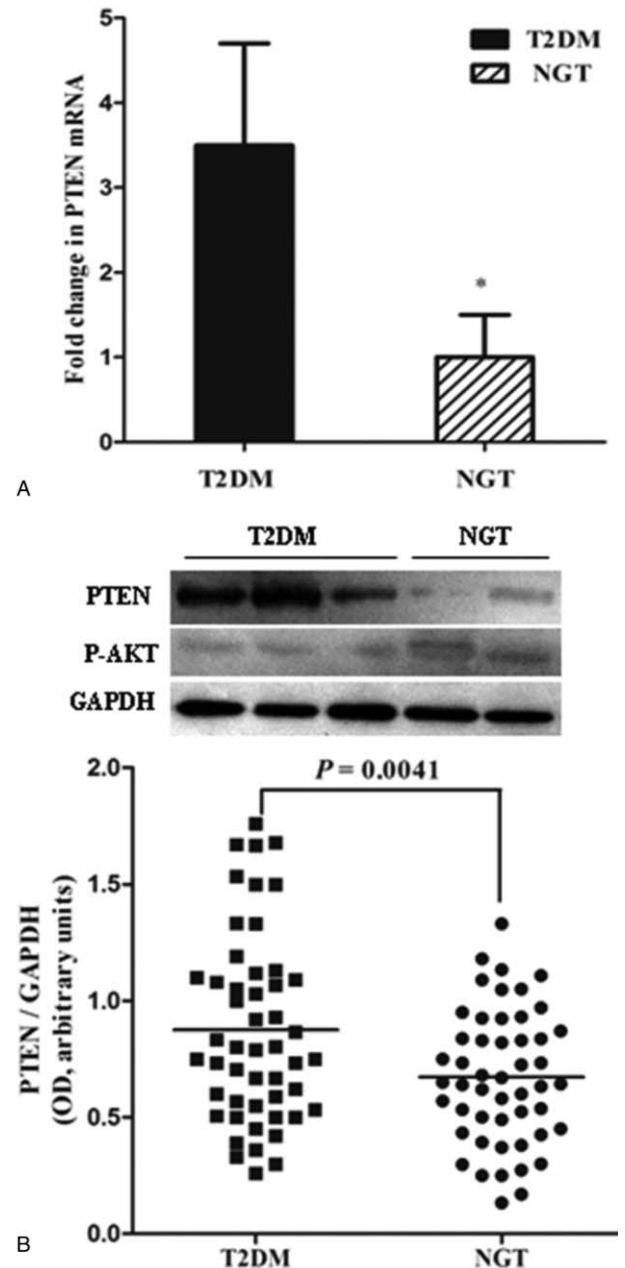


Figure 3. Upregulation of the *PTEN* gene in T2DM patients. (A) The transcription levels of *PTEN* in Uyghur T2DM and NGT samples were detected using qRT-PCR. Results were normalized to *GAPDH* mRNA levels. The values represent the average transcription level with SD of 3 independent experiments, and the significance was determined by Student *t* tests between the indicated groups (*t* test, * *P* < .05, Bonferroni corrected for multiple comparisons). (B) Representative western blot for *PTEN* and *AKT* expression in PBMCs taken from subjects with or without T2DM. Dot plot represents *PTEN* expression normalized to *GAPDH* expression. *P* values refer to differences between the groups as determined by *t* test.

3.3. *PTEN* expression

To determine whether the overexpression of *PTEN* occurred in the Uyghur T2DM patients, a Western blot analysis was carried out. As shown in Figure 3B, the protein expression levels of *PTEN* in the PBMCs of patients with mild T2DM were significantly higher than the PBMCs of the NGT subjects (NGT: 0.67 ± 0.29, T2DM: 0.88 ± 0.40, *t* test, *P* = .0041). The

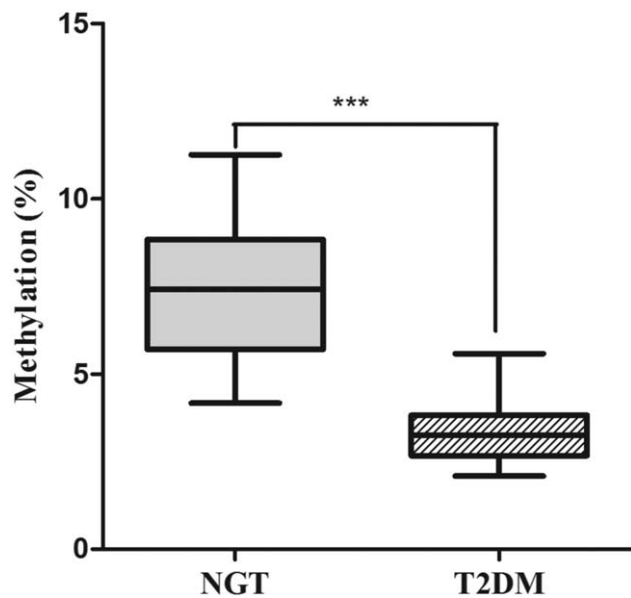


Figure 4. The overall methylation levels are displayed within the amplicon as box plots for T2DM patients compared with NGT subjects. The mean methylation levels of the 27 CpG residues in 50 samples from each population were determined by MassARRAY, and the data are presented as the mean \pm standard deviation (SD). (*t* test, *** $P < .0001$, Bonferroni corrected for multiple comparisons).

expression level of *AKT* protein in mild T2DM was significantly downregulated (*t* test, $P = .013$).

3.4. Aberrant promoter methylation of *PTEN*

We performed a quantitative high throughput analysis of DNA methylation within *PTEN* using the MassARRAY system: $-2,515$ bp to $-2,186$ bp relative to the translation initiation site (Fig. 1, Fig. 2, and Table 1). The methylation status of the *PTEN* promoters was studied in all of the samples collected from NGT ($n = 50$) and T2DM groups ($n = 50$). A 330-bp region of the *PTEN* promoter containing 21 CpG sites, which could be divided into 15 CpG units, was examined using the MassARRAY system. Among these units, 3 CpG units (6 CpG sites) did not yield successful measurements. The final data set consisted of 12 CpG units from 94 samples. A 95% confidence interval was calculated for each subject at each CpG site to exclude outliers from each participants of methylation, after which the mean methylation overall and for each CpG site was calculated. The average DNA methylation frequency ranged from 2.08% to 5.58% in the Uyghur T2DM samples and from 4.17% to 11.25% in the NGT samples. The aberrant methylation of *PTEN* in the Uyghur T2DM samples was significantly lower than those in the NGT samples, with averages of 3.27% and 7.28%, respectively (*t* test, $P < .0001$, Fig. 4). Furthermore, there was no significant association between *PTEN* methylation and the clinical characteristics of the patients (data not shown). These data demonstrate the occurrence of the aberrant promoter methylation of *PTEN* in the T2DM samples. In addition, we used an unsupervised 2-way hierarchical clustering of the CpG unit methylation of the Uyghur T2DM and NGT samples (Fig. 1). The clearly different methylation patterns make it possible to identify them. These data demonstrate the occurrence of the aberrant promoter methylation of *PTEN* in Uyghur T2DM patients.

3.5. Methylation levels at individual CpG sites along the *PTEN* promoter region

We next examined the methylation status of individual CpG sites within the promoter region. There was some variability in the methylation of the individual CpG sites for the different samples. The methylation level of most CpG unit in the Uyghur T2DM samples was numerically lower than that of the NGT samples; CpG_9 and CpG_21 were significantly different between these groups (Fig. 5, 1-way ANOVA, $P < .05$).

3.6. Correlation between the promoter methylation and transcription levels of *PTEN*

To further explore the role of methylation, we analyzed the correlation analysis between the methylation levels of the *PTEN* promoter and the transcription levels of *PTEN*. In the Uyghur T2DM and NGT groups, we identified an inverse negative correlation of the DNA methylation levels and the transcription levels of *PTEN* (95% CI for the slope: -0.00009339 to -0.00002474 , $P = .001$, $r = -0.45$) (Fig. 6A).

3.7. Correlation between *PTEN* expression and promoter methylation

In addition to finding evidence of the relationship of promoter methylation and *PTEN* expression, we performed a correlation analysis of *PTEN* levels and promoter methylation using a linear regression. The linear regression analysis revealed a significant correlation of *PTEN/GADPH* (2-tailed Pearson test, 95% CI for the slope: -0.1897 to -0.03355 , $P = .006$, $r = -0.38$) (Fig. 6B).

4. Discussion

In the past decade, great efforts have been made to unravel the pathogenesis of T2DM. The recent data highlight *PTEN* as a candidate gene for T2DM susceptibility. Previous studies have identified 4 *PTEN* polymorphisms in Caucasian patients with T2DM, but they were not associated with the disease.^[17] In another study, Ishihara H et al suggested that the change of C to G at position—9 of *PTEN* is associated with the insulin resistance of T2DM, possibly due to a potentiated hydrolysis of the *PI3k* product, and illustrated the importance of *PTEN* activity in diseases other than cancer.^[9] However, although genetic alterations might underlie the loss of *PTEN* in the pathogenesis of T2DM, the regulation mechanism of *PTEN* in T2DM remains to be clarified.

Epigenetic modifications of DNA, such as methylation, have been suggested to play a significant role in the pathogenesis of T2DM.^[18] Recent studies have demonstrated that DNA methylation contributed to the downregulation of *PTEN* during tumorigenesis.^[19,20] Previous study suggested that *PTEN* promoter methylation may be a mechanism for *PTEN* downregulation or silencing in metabolic syndrome.^[21] However, the methylation of the *PTEN* promoter region has yet to be elucidated in Uyghur T2DM patients. In the present study, we hypothesized that aberrant *PTEN* methylation patterns occur in Uyghur T2DM patients and the aberrant methylation affects *PTEN* expression at the transcriptional and protein levels.

For the first time, we employed MassARRAY spectrometry to evaluate methylation patterns at multiple CpG sites within the promoter region of *PTEN* in T2DM. The core promoter of *PTEN*, located at positions -2013 to -2549 , was capable of governing the maximum promoter activity.^[22] So we turned our

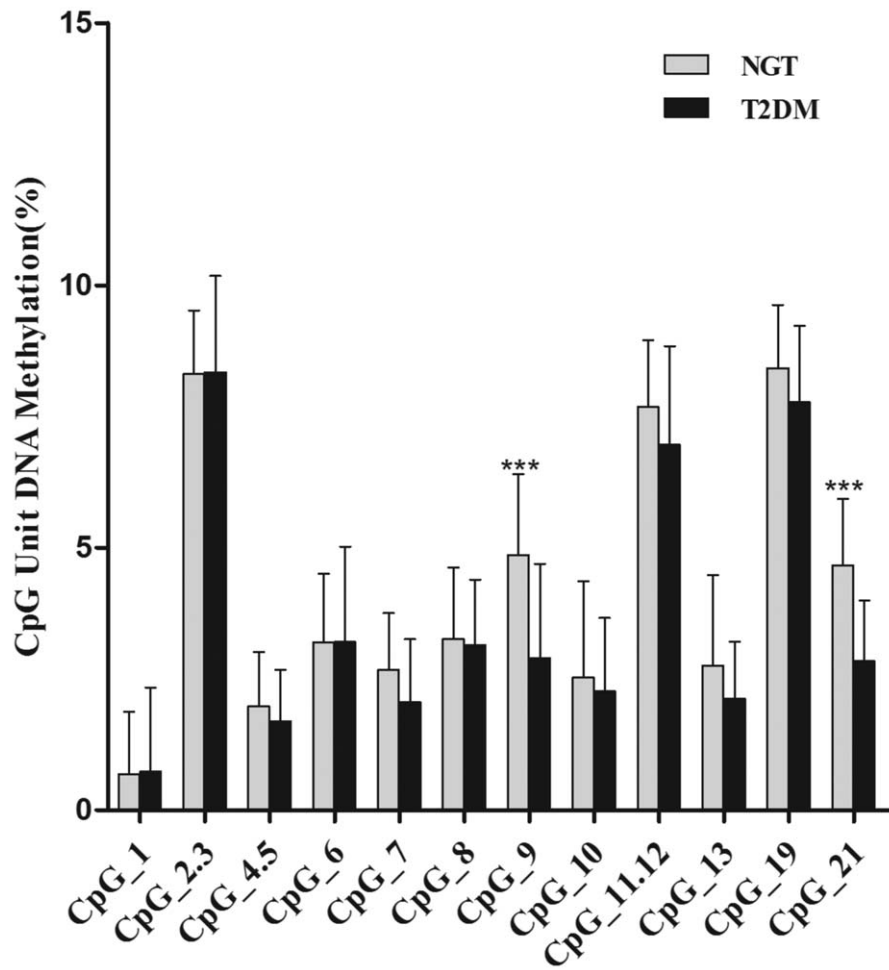


Figure 5. Comparison of *PTEN* methylation among T2DM and NGT samples. The average methylation of the CpG units of the amplicon is presented for the T2DM and NGT samples. T2DM carriers compared with NGT subjects: 1-way ANOVA, * $P < .05$, Bonferroni corrected for multiple comparisons. The data are presented as the mean \pm standard deviation (SD).

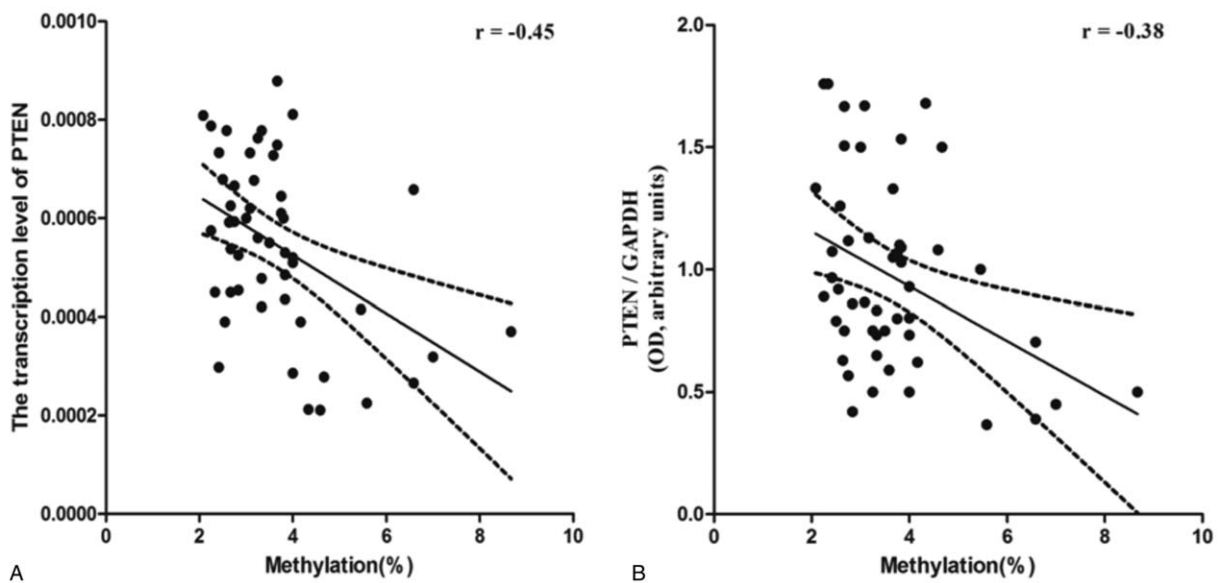


Figure 6. (A) Correlation analysis of the transcription levels of *PTEN* and promoter methylation (95% CIs for the slope: -0.00009339 to -0.00002474 , $P = .001$, $r = -0.45$) by linear regression with a 2-tailed Pearson test. (B) Correlation analysis of *PTEN* expression and promoter methylation (2-tailed Pearson test, 95% CIs for the slope: -0.1897 to -0.03355 , $P = .006$, $r = -0.38$).

attention to the upstream region, located 2.190 to 2.542 kilobases from the translation start site, ATG. We found that the aberrant methylation of *PTEN* was significantly lower in Uyghur patients with mild T2DM than in the NGT subjects. Our findings suggest that the promoter hypomethylation of *PTEN* is a common event in T2DM patients, which may play a role in the pathogenesis of T2DM in the Uyghur population.

We further evaluated the aberrant methylation status of CpG units. Previous studies have suggested that quantitative cytosine methylation profiling can be used to identify molecular markers in tumors.^[23,24] These studies have revealed specific hypermethylated CpG sites that are useful in the diagnosis of cancers. In this study, we demonstrated significant differences in the frequency of methylation at individual CpG units between the Uyghur T2DM and NGT groups. The methylation level of most CpG units in the Uyghur T2DM patients was numerically lower than those in the NGT subjects. CpG₉ and CpG₂₁ were significantly different between these groups. This result revealed that the aberrant methylation of the CpG sites within the *PTEN* promoter region may serve as a potential candidate biomarker for T2DM in the Uyghur population.

PTEN deregulation is implicated in many human diseases, including diabetes. Recently, in mice lacking 1 copy of *PTEN*, the phosphorylation and activation of the pathway are increased, resulting in increased insulin sensitivity.^[25] The tissue-specific knockout of *PTEN* in mice has been shown to affect liver, muscle, adipose tissue, and pancreatic beta cells to improve glucose tolerance.^[26–30] Moreover, the upregulation of *PTEN* may result in more severe insulin resistance by blocking the intracellular insulin signaling pathway.^[31] Mocanu et al reported an increased *PTEN* level in the Goto Kakizaki rat heart model and suggested that diabetes may be associated with increased *PTEN* levels.^[32] A previous study demonstrated that the increased expression of *PTEN* in the adipose and muscles of T2DM rats may play an important role in the insulin resistance of T2DM.^[33] Zhu et al suggested that *PTEN* regulates renal extracellular matrix production via activated Akt and increased CTGF in diabetes mellitus.^[34] Similarly, it has been previously shown that *PTEN* is over expressed in patients with gestational diabetes mellitus.^[35] We took T2DM PBMCs as study subject and found that *PTEN* expression was significantly increased and the expression level of AKT proteins was significantly decreased in the Uyghur T2DM patients, supporting findings that suggest that *PTEN* plays an important role in glucose metabolism.

An important limitation of our study is that DNA methylation, mRNA, and protein levels could be a function of differences in cellular heterogeneity between the blood samples.^[36] However, it should be noted that these confounding factors are not confined to blood, but rather apply to any tissue that contains a mixture of cell types.^[37] Thus, to overcome these limitations, novel epigenetic approaches using cell-sorting or laser capture micro-dissected tissues should be adopted. Another limitation in our study is the lack of other epidemiology information, such as alcohol use, smoking status, and eating habits, which may help us to assess the interaction between *PTEN* and other environmental factors in T2DM.

In summary, these findings suggest that the hypomethylation of *PTEN* promoter is a common event in Uyghur patients with mild T2DM, which may play a role in the pathogenesis of T2DM in the Uyghur population. It was suggested that the activation of *PTEN* may be due to hypomethylation in the promoter of *PTEN*. The aberrant methylation of CpG sites within *PTEN* promoter

may serve as a potential candidate biomarker for T2DM in the Uyghur population.

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