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Association of *ADIPOQ* variants with type 2 diabetes mellitus susceptibility in ethnic Han Chinese from northeast China

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ABSTRACT

Aims/Introduction: To investigate the association between two single nucleotide polymorphisms (SNPs; rs3774261 and rs822393) in the *ADIPOQ* gene and type 2 diabetes mellitus in Han Chinese from northeast China.

Materials and Methods: The present study comprised 993 type 2 diabetes mellitus patients and 966 unrelated controls from northeastern China. Two SNPs were sequenced using SNPscan. The distribution of genotype frequencies of the two SNPs in *ADIPOQ* between cases and controls, and in subgroups stratified based on body mass index, were compared using logistic regression analysis. Linear regression was used to analyze the association between each SNP and clinical indicators.

Results: The GG genotype of rs3774261 increased the risk of type 2 diabetes mellitus compared with the AA genotype in participants with a body mass index <24 (P = 0.021; odds ratio 1.636, 95% CI 1.708–2.484). Rs822393 was correlated with glycosylated hemoglobin (P = 0.043) in controls. Rs3774261 had an association with diastolic blood pressure (P = 0.017) in controls, and in controls with a body mass index <24; rs3774261 also had an association with both systolic blood pressure (P = 0.025) and diastolic blood pressure (P = 0.043).

Conclusions: The present results confirm the association between *ADIPOQ* variants and type 2 diabetes mellitus in northeastern China. However, additional larger replication studies are required to validate these findings.

INTRODUCTION

The prevalence of diabetes, especially type 2 diabetes mellitus, has increased dramatically in China over the past few decades; from 1994 to 2010, the prevalence increased from 2.5% to 11.6%[1]. This disease is considered a serious medical burden on society. Type 2 diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia as a result of pancreatic β-cell dysfunction and insulin resistance. Furthermore, type 2 diabetes mellitus risk is determined by both genetic and environmental factors. Genetic factors in particular play an important role in type 2 diabetes mellitus[2]. Thus, exploration of the genetic mechanisms of type 2 diabetes mellitus will be critical for the prevention and treatment of type 2 diabetes mellitus in China.

Adiponectin, secreted from adipocytes[3], plays an important role in the development of type 2 diabetes mellitus because of its unique contribution to increasing insulin sensitivity and improving islet β-cell dysfunction and fatty acid beta-oxidation[4–7]. The adiponectin gene (*ADIPOQ*, also known as *APM1*, *ACRP30*, GBP28 and *ACDC*) is located in the chromosomal region at 3q27, and spans approximately 17 kb of deoxyribonucleic acid. This region has been identified as a susceptibility locus for metabolic syndrome and type 2 diabetes mellitus[8,9]. Adiponectin is credited with insulin-sensitizing, anti-inflammatory and anti-atherogenic properties[10]. On the basis of biological function, epidemiological data and positional information from linkage studies, *ADIPOQ* is considered to be an important candidate gene for the development of type 2 diabetes mellitus.

Since 2002, many investigators have explored the association between *ADIPOQ* single nucleotide polymorphisms (SNPs) and

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type 2 diabetes mellitus in different ethnic groups from different regions[11–17]. Several SNPs have been shown to have an association with type 2 diabetes mellitus. However, the two SNPs selected in the present study, namely rs3774261 in intron 2 and rs822393 in intron 1, have only been reported in a few populations[18–20]. Rs822393 might be associated with the development of type 2 diabetes mellitus, and the GG genotype of rs3774261 was associated with risk for type 2 diabetes mellitus in a southern Chinese population[18,19]. Furthermore, a study in southern India also showed that rs8222393 and rs3774261 were significantly associated with type 2 diabetes mellitus in that population[20]. In anatomical, archeological, linguistic and genetic data, the northern Han Chinese population is quite different from the southern Chinese Han population. In particular, the Han Chinese population shows a complicated genetic substructure[21]. A previous study postulated that the significant differences between the northern and southern Han Chinese populations could influence association studies, and thus, these differences should be carefully examined[22]. However, these two SNPs, to the best of our knowledge, have not been investigated in other populations. Therefore, in this report, we designed a case–control study and selected these two SNPs to examine the association between *ADIPOQ* SNPs and type 2 diabetes mellitus in Han Chinese from the northeast region using the SNPscan method.

MATERIALS AND METHODS

Participants and clinical data

A total of 1,959 residents from the northeast region of China were recruited for the present study. There were 993 type 2 diabetes mellitus patients and 966 control patients. Our study was approved by the Harbin Medical University Medical Ethics Committee (2014-research-022), and conforms to the provisions of the Declaration of Helsinki. Type 2 diabetes mellitus patients were selected from endocrine inpatients at the second affiliated hospital of Harbin Medical University, and the controls were enrolled from Health Check Centers or outpatient clinics at the same hospital. All participants were recruited consecutively between October 2010 and September 2013.

The criteria for the diagnosis of type 2 diabetes mellitus was established according to the World Health Organization 1999 guidelines as having the following features: a fasting plasma glucose (FPG) level of ≥7.0 mmol/L (126 mg/dL) and/or a 2-h glucose level of ≥11.1 mmol/L (200 mg/dL) after an oral glucose tolerance test. The diagnosis of diabetes in this group was made no more than 6 months earlier, and the patients were not treated with insulin. We defined the day of diagnosis as the putative day of onset.

For the controls to be eligible, they had to meet the following criteria: (i) no family history of type 2 diabetes mellitus; (ii) FPG <5.10 mmol/L and glycosylated hemoglobin (HbA1c) <6.0%; (iii) no use of drugs that affect glucose or lipid metabolism, and (iv) no systemic diseases.

Exclusion criteria included: (i) other types of diabetes; (ii) other diseases, such as coronary artery disease, chronic renal failure or other endocrine diseases; (iii) acute diabetic complications and other serious metabolic disease that might raise glucose levels, and (iv) duration of type 2 diabetes mellitus over 6 months or treatment with insulin.

Anthropometric measurements including weight, height, waist circumference, hip circumference, systolic blood pressure and diastolic blood pressure were obtained using standard techniques. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). The waist-to-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm). Biochemical analyses were carried out with a B200 Auto Analyzer for FPG, serum cholesterol, serum triglycerides, high-density lipoprotein cholesterol (HDL), low-density lipoprotein (LDL), HbA1c and serum fasting insulin concentration. The homeostasis model assessment (HOMA) was used to assess individual insulin resistance (HOMA-IR), for which HOMA-IR was equal to (FPG mmol/L × fasting insulin pmol/L)/22.5; HOMA of β-cell function (HOMA-β) was used to assess the islet β-cell secretion function, and was equal to fasting insulin × 20/(FPG − 3.5).

Genotyping

For genotyping 4 mL of venous blood was collected and stored at −20°C until further analysis. Genomic deoxyribonucleic acid was extracted from peripheral blood leukocytes using the TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China). SNP genotyping was carried out by utilizing the SNPscan™ kit (catalog no.: G0104k; Gnensky Biotechologies Inc., Shanghai, China). This kit was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc., and it is based on double ligation and multiplex fluorescence polymerase chain reaction. In order to validate the genotyping accuracy of the SNPscan™ Kit, a 5% random sample of cases and controls was sequenced twice at all SNPs by different researchers. In detail, we included 100 pairs of blind duplicates, and the concordance rates were more than 98%.

Statistical analysis

SPSS version 17.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Variables were compared between the cases and controls by Student's independent *t*-test. A chi square goodness of fit test was then utilized to evaluate the Hardy–Weinberg equilibrium in the type 2 diabetes mellitus and control groups. Variables with a *P*-value <0.05 was excluded from further analysis. Continuous data are represented as mean ± standard deviation. The distribution of genotype frequencies in cases and controls was compared using logistic regression analysis. The odds ratio (OR) and 95% confidence interval (CI) of the association between genotype and cases/control status were calculated by univariate logistic regression analysis. Age, sex and BMI were adjusted for as confounding variables. Linear regression was used to analyze the association between the SNPs and

clinical indicators, HOMA-IR and HOMA-β. Nominal significance was considered to be $P < 0.05$. Statistical power was assessed using the QUANTO version 1.2 software (developed by by Jim Gauderman PhD and John Morrison MS, Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA). Considering 11.6% prevalence of the disease, a risk allele frequency of 40.4% and an additive genetic model, we had at least 93% power to detect an OR of 1.25 at the 0.05 level.

RESULTS

Clinical and biochemical characteristics of the study participants

The present study participants comprised type 2 diabetes mellitus patients ($n = 993$) and controls ($n = 966$). The clinical and biochemical characteristics of all participants of this study with *t*-test results are presented in Table 1. Compared with the control participants, significant differences were found in age, weight, BMI, waist circumference, hip circumference, WHR, systolic blood pressure, diastolic blood pressure, fasting glucose, total cholesterol, triglycerides, HDL, fasting insulin, HbA1C, HOMA-β and HOMA-IR (all $P < 0.05$). The distributions of height ($P = 0.075$) and LDL ($P = 0.842$) were not significantly different between cases and controls.

Association of *ADIPOQ* variants with type 2 diabetes mellitus

The genotype distributions of both SNPs were found to be in Hardy–Weinberg equilibrium in both cases and controls. The genotype distributions of rs3774261 and rs822393 in *ADIPOQ* are shown in Table 2. In the analysis of rs3774621, no differences in the frequency distributions of the GG and GA genotypes compared with the AA genotype between the case and control groups ($P > 0.05$) were found. Also, rs822393 was not associated with type 2 diabetes mellitus.

Association of *ADIPOQ* variants with type 2 diabetes mellitus in different BMI stratification groups

Table 3 shows the genotype frequencies of the two SNPs in *ADIPOQ* in the stratified analysis based on BMI. According to the guidelines for the prevention of overweight and obesity in Chinese adults[23], we chose a BMI of 24 as the cut-off point between groups. In the BMI <24 group, the GG genotype of rs3774261 was associated with an increased risk of type 2 diabetes mellitus compared with the AA genotype (95% CI 1.708–2.484, $P = 0.021$). After adjustment for age and sex by logistic regression analysis, this SNP conferred independent risk for the disease (OR 1.640, 95% CI 1.407–2.457, $P = 0.030$). Next we tested the association between rs3774261 and type 2 diabetes including an interaction term of BMI*Rs3774261, we also found the GG genotype is a risk genotype of type 2 diabetes mellitus (OR 2.67, 95% CI 1.08–6.68, $P = 0.034$). After

Table 1 | Baseline characteristics of study participants

Characteristic	Type 2 diabetes ($n = 993$)	Controls ($n = 976$)	*P*-value
Sex (male : female)	612:383	568:399	0.22
Age (years)	46.09 ± 12.56	42.93 ± 11.70	<0.0001
Height (m)	1.68 ± 0.77	1.69 ± 0.08	0.075
Weight (kg)	73.16 ± 13.54	66.61 ± 12.39	<0.0001
BMI (kg/m^2)	25.79 ± 0.114	23.32 ± 2.35	<0.001
Waist circumferences (cm)	93.52 ± 0.427	81.25 ± 10.88	<0.001
Hip circumferences (cm)	99.50 ± 7.44	95.79 ± 7.21	<0.001
WHR	0.94 ± 0.06	0.85 ± 0.07	<0.001
Systolic pressure (mmHg)	130.14 ± 17.51	121.28 ± 15.08	<0.001
Diastolic pressure (mmHg)	84.62 ± 11.18	79.22 ± 9.62	<0.001
Fasting plasma glucose (mmol/L)	10.05 ± 3.40	4.84 ± 0.29	<0.001
Total cholesterol (mmol/L)	5.00 ± 1.29	4.84 ± 1.01	0.019
Triglyceride (mmol/L)	2.38 ± 2.25	1.42 ± 0.95	<0.001
HDL-C (mmol/L)	1.21 ± 0.32	1.47 ± 0.35	<0.001
LDL-C (mmol/L)	2.91 ± 0.96	2.92 ± 0.86	0.842
Fasting insulin (μU/mL)	12.90 ± 7.59	7.87 ± 4.43	<0.001
HbAlc (%)	9.30 ± 2.36	5.12 ± 0.47	<0.001
HOMA-β	59.21 ± 169.47	130.7 ± 150.21	<0.001
HOMA-IR	5.77 ± 4.05	1.70 ± 0.97	<0.001

Data are presented as mean ± standard deviation. BMI, body mass index; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; WHR, waist-to-hip ratio.

Table 2 | Association of *ADIPOQ* gene polymorphisms with type 2 diabetes

SNPs	Genotypes	Cases (*n*)	Controls (*n*)	*P*-value	Crude OR (95% CI)	*P*-value†	Adjust† OR (95% CI)
Rs3774261	A/A	307	298		1		1
	G/A	478	504	0.424	0.921 (0.752–1.127)	0.571	0.939 (0.756–1.167)
	G/G	208	164	0.116	1.231 (0.950–1.596)	0.115	1.250 (0.947–1.649)
Rs822393	C/C	272	275		1		1
	C/T	493	471	0.579	1.508 (0.858–1.305)	0.392	1.103 (0.881–1.382)
	T/T	228	220	0.714	1.048 (0.816–1.345)	0.752	1.044 (0.799–1.364)

†Adjusted for age, sex and body mass index. CI, confidence interval; SNP, single nucleotide polymorphisms; OR, odds ratio.

Table 3 | Association of *ADIPOQ* gene variants with type 2 diabetes stratified according to body mass index

SNPs	BMI <24 (kg/m^2)		*P*-value	Crude OR (95% CI)	*P*-value†	Adjust† OR (95% CI)	BMI ≥24 (kg/m)		*P*-value	Crude OR (95% CI)	*P*-value†	Adjust† OR (95% CI)
	Cases (*n*)	Control(*n*)					Cases (*n*)	Control (*n*)				
Rs3774261												
A/A	77	182		1		1	230	116		1		1
G/A	161	294	0.124	1.294 (0.932–1.798)	0.125	1.300 (0.930–1.818)	317	210	0.059	0.761 (0.574–1.011)	0.052	0.754 (0.567–1.003)
G/G	63	91	0.021	1.636 (1.708–2.484)	0.030	1.640 (1.407–2.457)	145	73	0.992	1.002 (0.700–1.435)	0.916	0.981 (0.683–1.407)
Rs822393												
C/C	73	125		1		1	199	119		1		1
C/T	156	286	0.376	1.166 (0.830–1.637)	0.543	1.114 (0.787–1.575)	337	185	0.562	1.809 (0.816–1.455)	0.607	1.080 (0.807–1.445)
T/T	72	156	0.311	1.231 (0.824–1.839)	0.715	1.080 (0.715–1.631)	156	95	0.917	0.982 (0.698–1.382)	0.804	0.975 (0.679–1.351)

†Adjusted for age and sex. BMI, body mass index; CI, confidence interval, OR, odds ratio; SNPs, single nucleotide polymorphisms.

Table 4 | Association between rs3774261 and type 2 diabetes including an interaction term of BMI*rs3774261 with stratified

Variables	*P*-value	Crude OR (95% CI)	*P*-value†	Adjust† OR (95% CI)
GG	0.0340	2.67 (1.08–6.63)	0.0489	2.24 (1.01–6.84)
BMI* rs3774261GG	0.0807	0.61 (0.35–1.06)	0.0999	0.58 (0.34–1.10)

†Adjusted for age and sex. BMI, body mass index; CI, confidence interval; OR, odds ratio.

adjustment for age and sex by logistic regression analysis, this SNP conferred independent risk for the disease (OR 2.24, 95% CI 1.01–6.84, $P = 0.0489$); there was no interaction between rs3774261GG and BMI ($P > 0.05$), and after adjustment for age and sex by logistic regression analysis there was still no statistical significance ($P > 0.05$; Table 4).

Association between these SNPs and clinical variables

As treatment for diabetes might affect metabolic relationships, only control participants were included in the clinical variable analysis. We assessed the relationship of the two SNPs with clinical parameters using generalized linear regression analysis. As shown in Table 5, there was a correlation between rs3774261 and diastolic blood pressure ($P = 0.017$), as well as between rs822393 and HbA1c ($P = 0.043$). Next, we analyzed the association between SNPs and clinical variables in participants with a BMI <24, also including only control participants. We found that only rs3774261 had an association with systolic blood pressure ($P = 0.025$) and diastolic blood pressure ($P = 0.043$; Table 6).

DISCUSSION

In the current study, we successfully replicated the association between *ADIPOQ* gene variants and type 2 diabetes mellitus, which is in line with other studies[11–20]. Ramya[20] found that the AA genotype of rs3774261 was significantly protective for diabetes with an OR of 0.65 (95% CI 0.50–0.86, $P = 0.002$) in India. In the current study, we only observed that the GG genotype of rs3774261 conferred a 1.636-fold risk towards the development of type 2 diabetes mellitus in participants with a BMI <24, compared with the AA genotype. Similar results in different ethnicities further confirm that *ADIPOQ* is an important candidate gene for type 2 diabetes mellitus. In the BMI <24 group, the present results are consistent with Lan *et al.*[19], who found that the GG genotype of rs3774261 conferred a 1.436-fold risk of type 2 diabetes mellitus in the southern Chinese population. Furthermore, the average BMI of the north region of China is higher than that of the south region[24]. This indicates that the increased risk for type 2 diabetes mellitus in rs3774261 carriers might only exist in subjects with a lower BMI. However, overweight (24 ≤ BMI < 28) and obese (BMI ≥28) subjects have more severe insulin resistance[25]. Thus, we

Table 5 | Associations of the genetic variants with prediabetes-related clinical trials among control participants

SNP	FPG (mmol/L)	*P*-value	HbA1c (%)	*P*-value	HOMA-β	*P*-value	HOMA-IR	*P*-value	Systolic pressure (mmHg)	*P*-value	Diastolic pressure (mmHg)	*P*-value
Rs3774261		0.438		0.525		0.150		0.137		0.066		0.017
AA	4.82 ± 0.31		5.12 ± 0.45		126.02 ± 96.16		1.64 ± 0.98		122.44 ± 12.26		80.30 ± 10.50	
GA	4.85 ± 0.28		5.11 ± 0.49		127.40 ± 109.19		1.71 ± 0.96		121.06 ± 14.41		78.91 ± 9.14	
GG	4.84 ± 0.28		5.15 ± 0.45		149.89 ± 281.49		1.78 ± 0.99		119.83 ± 14.83		78.23 ± 9.29	
Rs822393		0.530		0.043		0.742		0.848		0.573		0.263
CC	4.84 ± 0.29		5.12 ± 0.44		128.67 ± 98.58		1.73 ± 1.03		121.17 ± 13.66		79.24 ± 9.12	
CT	4.82 ± 0.30		5.07 ± 0.49		130.93 ± 116.23		1.68 ± 0.96		121.80 ± 15.89		79.71 ± 9.89	
TT	4.86 ± 0.27		5.22 ± 0.46		133.14 ± 241.36		1.71 ± 0.92		120.29 ± 15.69		79.22 ± 9.62	

Data are presented as mean ± standard deviation. FPG, fasting plasma glucose HbA1c, glycosylated hemoglobin; HOMA-β, homeostasis model assessment of beta-cell function, HOMA-IR, homeostasis model assessment of insulin resistance; SNP, single nucleotide polymorphism.

Table 6 | Associations of the genetic variants with prediabetes-related clinical trials among control participants with body mass index less than 24

SNP	FPG (mmol/L)	*P*-value	HbA1c (%)	*P*-value	HOMA-β	*P*-value	HOMA-IR	*P*-value	Systolic pressure (mmHg)	*P*-value	Diastolic pressure (mmHg)	*P*-value
Rs3774261												
AA	4.81 ± 0.32	0.532	5.07 ± 0.42	0.933	113.36 ± 98.32	0.956	1.41 ± 0.79	0.632	119.93 ± 16.61	0.025	78.07 ± 10.26	0.043
GA	4.81 ± 0.29		5.06 ± 0.50		117.61 ± 112.17		1.42 ± 0.81		117.61 ± 12.21		76.27 ± 8.79	
GG	4.77 ± 0.29		5.08 ± 0.43		114.92 ± 70.37		1.42 ± 0.79		115.77 ± 15.46		75.99 ± 9.20	
Rs822393												
CC	4.83 ± 0.28	0.454	5.07 ± 0.41	0.140	103.82 ± 65.22	0.574	1.45 ± 0.92	0.890	117.28 ± 12.73	0.836	76.387 ± 8.33	0.659
CT	4.79 ± 0.32		5.02 ± 0.48		119.23 ± 128.47		1.38 ± 0.70		118.72 ± 15.90		77.51 ± 9.86	
TT	4.80 ± 0.30		5.16 ± 0.46		109.27 ± 63.11		1.48 ± 0.82		117.51 ± 16.61		76.80 ± 9.37	

Data presented as mean ± standard deviation. FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HOMA-β, homeostasis model assessment of beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; SNP, single nucleotide polymorphism.

hypothesized that insulin resistance might not be the pathogenic mechanism of rs3774261 for type 2 diabetes mellitus risk. In the analysis of clinical variables, we found that rs3774261 had an association with blood pressure in both controls and the control subgroup with a BMI <24 ($P < 0.05$), and the blood pressure of GG-genotype carriers was lower than that of AA-or GA-genotype carriers. A study carried out by Jiang *et al.*[26] reported that insulin resistance might play a role in the development of hypertension in Chinese people. This suggests that rs3774261 could improve insulin sensitivity. It further indicates that insulin resistance might not be involved in the pathogenesis of rs3774261 in the risk type 2 diabetes mellitus in northeastern China. The exact functional mechanism of rs3774261 on type 2 diabetes mellitus risk requires further research.

Rs822393 is located in intron 1 of *ADIPOQ*, a region that could give rise to alternatively spliced messenger ribonucleic acids and affect the stability or processing of messenger ribonucleic acid[27], possibly impacting *ADIPOQ* function. Gene variants in this region might affect gene function to give rise to the risk of being infected with disease. In the present study, we failed to replicate the finding of Ramya *et al.*[20], who found that rs822393 conferred a twofold higher risk towards type 2 diabetes mellitus in the southern Indian population. A possible reason for these inconsistent findings could be genetic heterogeneity, different geographic locations, genetic origins and differences in the adiponectin gene structure owing to 'gene–environment' interactions. We found that rs822393 was associated with HbA1c level ($P = 0.043$) in controls; TT homozygous carriers had a higher HbA1c level than carriers of other genotypes at this locus. Rs822393 might affect HbA1c levels through non-glycemic pathways, such as regulating erythrocyte lifespan or glycemic pathways. Those participants through glycemic pathways could have slightly impaired glucose homeostasis, which might not be severe enough to result in detectable type 2 diabetes mellitus. The present results show that the rs822393-TT genotype might increase susceptibility to type 2 diabetes mellitus. As rs822393 has been associated with type 2 diabetes mellitus only in obese Han Chinese people of the southern region[18], we hypothesize that the association between rs822393 and type 2 diabetes mellitus not be significant in China. Further studies and replication in a larger sample will be required to validate this finding.

One limitation of the present study was that we did not estimate serum adiponectin concentration, as low circulating levels of adiponectin are reported to be associated with insulin resistance, type 2 diabetes mellitus and central obesity[28]. Another limitation was that the controls were collected from hospitals in Harbin, so a certain level of selection bias cannot be ruled out. However, all control individuals in our study were those who came to hospitals for routine health examinations, but not hospitalized patients with specific diseases, probably making the controls more representative of the general population. Thus, we believe any potential selection bias to be minimized.

In conclusion, in the present study, we have confirmed that variants of *ADIPOQ* are associated with type 2 diabetes mellitus in Han Chinese from northeast China. Among the two SNPs that were screened in *ADIPOQ*, rs3774261 was associated with an increased risk for type 2 diabetes mellitus, and rs822393 might increase susceptibility to type 2 diabetes mellitus. Hence, additional larger replication studies are required to validate these novel findings.

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DISCLOSURE

The authors declare no conflict of interest.

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