Original Article

Association of Common Genetic Variants in Mitogen-activated Protein Kinase Kinase Kinase Kinase 4 with Type 2 Diabetes Mellitus in a Chinese Han Population

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Abstract

Background: A study has identified several novel susceptibility variants of the mitogen-activated protein kinase kinase kinase kinase 4 (*MAP4K4*) gene for type 2 diabetes mellitus (T2DM) within the German population. Among the variants, five single nucleotide polymorphisms (SNPs) of MAP4K4 (rs1003376, rs11674694, rs2236935, rs2236936, and rs6543087) showed significant association with T2DM or diabetes-related quantitative traits. We aimed to evaluate whether common SNPs in the *MAP4K4* gene were associated with T2DM in the Chinese population.
Methods: Five candidate SNPs were genotyped in 996 patients newly diagnosed with T2DM and in 976 control subjects, using the SNPscan™ method. All subjects were recruited from the Second Affiliated Hospital, Harbin Medical University from October 2010 to September 2013. We evaluated the T2DM risk conferred by individual SNPs and haplotypes using logistic analysis, and the association between the five SNPs and metabolic traits in the subgroups.
Results: Of the five variants, SNP rs2236935T/C was significantly associated with T2DM in this study population (odds ratio = 1.293; 95% confidence interval: 1.034–1.619, $P = 0.025$). In addition, among the controls, rs1003376 was significantly associated with an increased body mass index ($P = 0.045$) and homeostatic model assessment-insulin resistance ($P = 0.037$).
Conclusions: *MAP4K4* gene is associated with T2DM in a Chinese Han population, and *MAP4K4* gene variants may contribute to the risk toward the development of T2DM.

Key words: Chinese Han Population; Mitogen-activated Protein Kinase Kinase Kinase Kinase 4; Single Nucleotide Polymorphism; Type 2 Diabetes Mellitus

Introduction

A national survey conducted in 2010 showed that the prevalence of diabetes in China adults was 11.6%.[1] Type 2 diabetes mellitus (T2DM) is the most common type of diabetes characterized by hyperglycemia, which is caused by insulin resistance (IR) and β-cell failure. Although the exact etiology is unknown, the complex interactions of multiple environmental/lifestyle risk factors and genetic variants are considered to be involved in the pathogenesis of the disease. Asian people, including Chinese, have been suggested to have a stronger genetic predisposition to T2DM because they are more centrally obese and have a more pronounced dysfunction in early insulin secretion.[2,3] It is, therefore, likely that the Asian population represents a good resource for identifying susceptibility genes of T2DM.

Recently, several studies showed that inflammation was an integral part of the pathogenesis of T2DM.[4-6] Mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) is an upstream mediator of tumor necrosis factor-alpha (TNF-α) action on β cells, and is very important in the pathogenesis of T2DM. MAP4K4 belongs to the sterile 20 family of serine/threonine kinases, and the *MAP4K4* gene locus is on human chromosome 2q11.2-q12. MAP4K4 is expressed in several tissues and cell types and represents a TNF-α-inducible upstream activator of the c-Jun amino-terminal kinase pathway.[7,8] MAP4K4 is a potential mediator of cytokine-induced cellular IR. Silencing *MAP4K4*

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rescues human skeletal muscle cells and adipocytes from TNF-α-induced IR and improves glucose uptake. A study has shown that its silencing in macrophages suppresses systemic inflammation, thereby preventing diabetes.[9]

Sartorius *et al.* found that *MAP4K4* gene polymorphisms (rs1003376, rs11674694, rs2236935, rs2236936, and rs6543087) had a relationship between prediabetes or IR in the German population.[10] All these single nucleotide polymorphisms (SNPs) showed a significant association with T2DM or diabetes-related quantitative traits in the German population. Of the five SNPs, SNP rs11674694 was significantly associated with T2DM, and three SNPs (rs6543087, rs11674694, and rs1003376) had significant associations with 2-h glucose levels. SNPs rs6543087 and rs11674694 were also nominally associated with decreased insulin sensitivity, and another two SNPs (rs2236936 and rs2236935) showed associations with reduced insulin release, driven by effects only in lean subjects as shown in the study of the German population. However, to the best of our knowledge, the contribution of this locus to T2DM susceptibility in the Chinese Han population has not yet been evaluated.

Due to the roles of MAP4K4 in inflammation and IR, we conducted a case–control study to investigate whether common SNPs of the human *MAP4K4* gene are associated with T2DM and diabetes-related traits in the Chinese Han population, using the SNPscan™ method. The T2DM risk conferred by individual SNPs and haplotypes was evaluated. Moreover, the association between the five SNPs and metabolic traits were also investigated in subgroups.

Methods

Study subjects and phenotypic definitions

Using a case–control approach, a total of 2000 unrelated subjects from Chinese Han population were recruited from October 2010 to September 2013, comprising 1000 T2DM patients and 1000 normoglycemic control subjects. Inclusion criteria for this study were as follows: (1) All subject ages were between 20 and 79 years; (2) The diagnoses of T2DM patients were according to the World Health Organization 1999 with specified standards; (3) For elimination of prediabetes (impaired fasting glucose and impaired glucose tolerance), we recruited controls with an fasting plasma glucose (FPG) level of ≤5.1 mmol/L and glycated hemoglobin (HbA1c) ≤6%. All cases were recruited from the Inpatient Department of Affiliated Second Hospital, Harbin Medical University, Harbin, China. The control subjects were enrolled from an annual health check conducted at the same hospital. Exclusion criteria for this study were as follows: (1) the course was more than one year of T2DM, (2) individuals with T1DM, with other metabolic and/or endocrine diseases or with severe liver or renal dysfunction, and (3) individuals taking drugs.

We collected background data on gender, age, family history, and anthropometric measurements, including height, weight, waist and hip circumferences, and blood pressure of all subjects. Peripheral venous blood samples were collected in tubes during the fasting state in all subjects. Quantitative measurements of plasma insulin were carried out using the double-antibody radioimmunoassay. FPG was quantified by the glucose oxidase-peroxidase procedure. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein (LDL) levels were measured by an automatic biochemical analyzer. HbA1c levels were measured using an high performance liquid chromatography system (Bio-Rad DIA-MAT glycosylated hemoglobin analyzer system; Bio-Rad, Hercules, CA, USA).

Homeostasis model assessment-IR (HOMA-IR) was calculated as $(c[\text{glucose}]\ [\text{mmol/L}]_0 \times c[\text{insulin}]\ [\text{mU/L}]_0/22.5)$ with c = concentration, and HOMA-β was calculated as $(20 \times c[\text{insulin}]\ [\text{mU/L}]_0/[c(\text{glucose})\ (\text{mmol/L})]_0 - 3.5)$ with c = concentration.[10]

Sample size was assessed using the PS Power and Sample Size software version 3.0 (Biostatistics, Nashville, USA). Assuming an allele frequency of 0.655 (minor allele frequency) in the control group, sample sizes of 456 in each group would be adequate to detect an odds ratio (*OR*) of 1.5 at a power of 80% and significance level of 5%.

The study protocol was approved by the Ethics Committee of Harbin Medical University, China, and all subjects gave written informed consent.

Genotyping

We collected approximately 4 ml of blood from both T2DM patients and control subjects, then the genomic DNA was extracted from peripheral blood leukocytes by using the TIANamp Genomic DNA Kit (Tiangen Biotech., Beijing, China). Five variants (rs1003376, rs11674694, rs2236935, rs2236936, and rs6543087) were genotyped using the SNPscan™ method (Genesky Biotechnologies Inc., Shanghai, China). The SNPscan technique provides a high-throughput and cost-saving SNP genotyping method based on double ligation and multiplex fluorescence polymerase chain reaction. To verify the genotyping accuracy using the SNPscan™ Kit, 5% random samples from patients and controls were genotyped twice at all SNPs by different individuals. We included 100 pairs of blind duplicates and the concordance rates were >98%.

Statistical analyses

Statistical analyses were performed with SPSS statistical software version 19.0 (SPSS, Chicago, IL, USA). Hardy–Weinberg equilibrium was tested using the Chi-square test. The statistical significance between the two study groups was determined for each variable using an independent-samples *t*-test or Mann–Whitney *U*-test. Data for categorical variables were expressed as numbers (percentages) and data for continuous variables as mean ± standard deviation (SD) or median (P_{25}, P_{75}). Logistic regression analysis was used to compare the allele and the genotype frequencies between the cases and the controls. The above logistic regression procedure

was repeated after adding gender, age, and body mass index (BMI) as covariates. Linear regression analyses were applied to test the associations of each SNP with quantitative traits in T2DM and control subjects. In all subjects, two-side *P* values < 0.05 were considered statistically significant.

Results

Clinical and biochemical characteristics of study subjects

There were four subjects in T2DM group and 24 subjects in controls have not detected the genotypes, so finally the T2DM group includes 996 subjects, and the control group includes 976 subjects.

The clinical characteristics of the participants are shown in Table 1. There were 612 males and 383 females (mean age, 46.1 ± 12.6 years) in the patients and 568 males and 399 females (mean age, 42.9 ± 11.7 years) in the control subjects. When compared with the control subjects, the patients with T2DM had higher BMI, waist and hip circumference, waist-hip ratio, blood pressure, HDL-C, TC and TG, fasting glucose, fasting insulin, HbA1c, and HOMA-β presented with more IR ($P < 0.001$). However, no significant differences were found in gender, height, and LDL-C, between the T2DM and control subjects ($P > 0.05$).

Association of mitogen-activated protein kinase kinase kinase kinase 4 gene variants with type 2 diabetes mellitus

The genotype distributions are summarized in Table 2. All genotyped SNPs were found to be independent as suggested by Hardy–Weinberg equilibrium in the combined case–control sample. Among the five SNPs in the *MAP4K4* gene, logistic regression analyses showed a risk toward the susceptibility of T2DM among the study subjects with an *OR* of 1.293 (95% confidence interval [*CI*]: 1.034–1.619, $P = 0.025$) for the CT genotype of rs2236935 after adjusting for age, gender, and BMI. However in the cases of rs2236936 GC and rs6543087 AT genotypes, the unadjusted *OR* for the GC and AT genotypes were observed to be 1.207 (95% *CI*: 1.001–1.455, $P = 0.048$) and 1.276 (95% *CI*: 1.032–1.579, $P = 0.025$), respectively, and the significance was lost after adjusting for age, sex, and BMI. We failed to replicate the effect for SNP rs11674694, which was previously studied in the German population ($P > 0.05$).

Linkage disequilibrium estimations between mitogen-activated protein kinase kinase kinase kinase 4 single nucleotide polymorphisms and haplotype analyses

Haplotype analysis was performed to evaluate the combined effect of five SNPs using Haploview. The linkage disequilibrium (LD) structure is shown in Figure 1, and LD coefficients (D′ and r^2) among the four variants (rs11674694, rs2236935, rs2236936, and rs6543087) suggested that the degree of LD was relatively high. The haplotype distributions were not significantly different between cases and controls [Table 3].

Associations between single nucleotide polymorphisms in mitogen-activated protein kinase kinase kinase kinase 4 gene and quantitative traits among control subjects

To identify potential mediators that link the MAP4K4 variants with T2DM, we tested the five SNPs for their associations with diabetes-related quantitative traits [Table 4].

Table 1: Baseline characteristics of individuals in the cases and controls

Characteristics	Type 2 diabetes (*n* = 996)	Controls (*n* = 976)	Statistical values	*P*
Gender (male:female)	612:383	568:399	1.62*	0.220
Age (years)	46.1 ± 12.6	42.9 ± 11.7	5.78†	<0.001
Height (m)	1.68 ± 0.08	1.69 ± 0.08	−1.86†	0.063
Weight (kg)	73.13 ± 13.45	66.63 ± 12.39	11.13†	<0.001
BMI (kg/m^2)	25.78 ± 3.58	23.33 ± 3.35	15.70†	<0.001
Waist circumferences (cm)	93.49 ± 10.45	81.27 ± 10.88	25.40†	<0.001
Hip circumferences (cm)	99.48 ± 7.44	95.77 ± 7.21	11.13†	<0.001
WHR	0.94 ± 0.06	0.85 ± 0.07	30.89†	<0.001
Systolic pressure (mmHg)	130.16 ± 17.51	121.30 ± 15.09	12.03†	<0.001
Diastolic pressure (mmHg)	84.64 ± 11.17	79.24 ± 9.64	11.42†	<0.001
Fasting plasma glucose (mmol/L)	10.04 ± 3.40	4.82 ± 0.29	47.40†	<0.001
Total cholesterol (mmol/L)	5.00 ± 1.29	4.88 ± 1.01	2.30†	0.022
Triglyceride (mmol/L)	2.38 ± 2.25	1.42 ± 0.95	12.30†	<0.001
HDL-C (mmol/L)	1.21 ± 0.32	1.47 ± 0.35	−17.14†	<0.001
LDL-C (mmol/L)	2.91 ± 0.96	2.92 ± 0.86	−0.23†	0.815
Fasting insulin (mU/L)	12.90 ± 7.59	7.87 ± 4.43	17.90†	<0.001
HbA1c (%)	9.30 ± 2.36	5.12 ± 0.47	53.90†	<0.001
HOMA-β	38.86 (22.09, 69.85)	103.31 (69.92, 149.01)	−25.87‡	<0.001
HOMA-IR	4.81 (2.77, 7.48)	1.51 (1.01, 2.16)	−29.97‡	<0.001

Data are presented as *n*, mean ± SD or median (P_{25}, P_{75}). *χ^2; †*t*; ‡*Z*. BMI: Body mass index; WHR: Waist-hip ratio; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; HOMA-β: Homeostasis model assessment of beta-cell function; HOMA-IR: Homeostasis model assessment of insulin resistance; SD: Standard deviation; BMI: Body mass index; HbA1c: Glycated hemoglobin.

Table 2: Association analysis of *MAP4K4* candidate SNPs for type 2 diabetes

dbSNP	Cases (*n* (%))	Controls (*n* (%))	*P*	Crude *OR* (95% *CI*)	*P**	Adjust* *OR* (95% *CI*)
rs1003376						
C/C	642 (64.5)	610 (63.1)		1		1
G/C	309 (31.1)	298 (30.8)	0.880	0.985 (0.812–1.196)	0.510	0.933 (0.758–1.148)
G/G	44 (4.4)	59 (6.1)	0.096	1.411 (0.940–2.118)	0.189	0.746 (0.482–1.155)
rs11674694						
C/C	264 (26.5)	268 (27.7)		1		1
C/T	517 (52.0)	468 (48.4)	0.287	1.121 (0.908–1.385)	0.418	1.098 (0.876–1.377)
T/T	214 (21.5)	231 (23.9)	0.633	0.940 (0.731–1.210)	0.935	0.989 (0.755–1.295)
rs2236935						
T/T	725 (72.9)	747 (77.2)		1		1
C/T	261 (26.2)	204 (21.1)	0.010	1.318 (1.069–1.626)	0.025	1.293 (1.034–1.619)
C/C	9 (0.9)	16 (1.7)	0.194	0.580 (0.254–1.320)	0.284	0.622 (0.260–1.484)
rs2236936						
G/G	543 (54.6)	564 (58.3)		1		1
G/C	394 (39.6)	339 (35.1)	0.048	1.207 (1.001–1.455)	0.230	1.130 (0.925–1.381)
C/C	57 (5.7)	64 (6.6)	0.685	0.925 (0.635–1.347)	0.817	0.953 (0.635–1.431)
rs6543087						
T/T	247 (24.8)	273 (28.3)		1		1
A/T	530 (53.3)	459 (47.5)	0.025	1.276 (1.032–1.579)	0.108	1.205 (0.960–1.512)
A/A	218 (21.9)	234 (24.2)	0.820	1.030 (0.800–1.325)	0.938	0.989 (0.755–1.297)

*Adjusted for age, gender, and BMI. *CI*: Confidence interval; *OR*: Odds ratio; SNPs: Single nucleotide polymorphisms; *MAP4K4*: Mitogen-activated protein kinase kinase kinase kinase 4.

Table 3: Haplotype frequencies of *MAP4K4* gene variants in control and patients with type 2 diabetes

Haplotype block (>1% frequency)	Haplotype frequency	χ^2	*P*
TTGT	0.478	0.124	0.683
ACGT	0.234	0.141	0.576
ACCC	0.131	1.038	0.098
ACCT	0.117	2.841	0.691
TCGT	0.040	1.453	0.953

MAP4K4: Mitogen-activated protein kinase kinase kinase kinase 4.

As treatment for diabetes may have affected metabolic relationships, only controls were included in the metabolic traits analyses. We assessed the relationships of the five SNPs in the *MAP4K4* gene with clinical parameters using linear regression analyses. Among the control subjects, the heterozygous AC of rs1003376 was associated with a decreased BMI ($P = 0.045$) and HOMA-IR ($P = 0.037$) value. There were no significant differences in the other four SNPs when compared with the clinical parameters of the control subjects.

Discussion

In this study, we evaluated the association between the five SNPs of the *MAP4K4* gene and T2DM susceptibility. One of the variants, rs2236935, had a significant association with T2DM risk. The positive association of this variant remained significant after the adjustment for age, sex, and BMI. In contrast with our findings, rs2236935 showed the associations with reduced insulin release only in lean subjects among the German population study.[10]

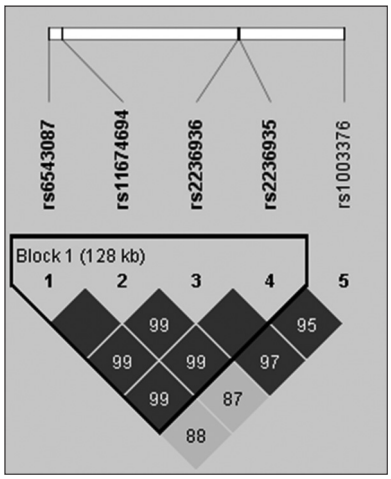

Figure 1: Linkage disequilibrium patterns of five typed single nucleotide polymorphisms in the Chinese population. This plot was generated using the Haploview program with the confidence intervals setting. One block was determined. The rs number (top: from left to right) corresponds to the single nucleotide polymorphism name and the number in each square is D' values or r^2 values of single nucleotide polymorphisms. The measure of linkage disequilibrium (D' or r^2) among all possible pairs of single nucleotide polymorphisms is shown graphically according to the shade of color, whereas white represents very low linkage disequilibrium values and dark represents very high linkage disequilibrium values.

Although the rs11674694 C/T was also reportedly associated with T2DM and IR within the German population,[10] in contrast, it was not significantly associated with T2DM among the Chinese population. However, we found a positive association of rs11674694 C/T with TG levels among the male study subjects (data not shown). Several studies have

Table 4: Associations of the genetic variants with prediabetes-related clinical trials among control subjects

SNP	BMI (kg/m²)	*P*	*B*	FPG (mmol/L)	*P*	*B*	HDL (mmo/L)	*P*	*B*
rs1003376									
CC	23.5 ± 3.3	0.045	−0.356	4.83 ± 0.29	0.745	0.011	1.46 ± 0.35	0.519	0.009
GC	23.1 ± 3.4			4.84 ± 0.28			1.49 ± 0.37		
GG	22.9 ± 3.1			4.84 ± 0.29			1.46 ± 0.31		
rs11674694									
CC	23.1 ± 3.3	0.333	0.146	4.84 ± 0.28	0.855	−0.001	1.50 ± 0.35	0.253	−0.021
CT	23.4 ± 3.4			4.84 ± 0.29			1.47 ± 0.36		
TT	23.4 ± 3.3			4.83 ± 0.29			1.46 ± 0.36		
rs2236935									
TT	23.3 ± 3.4	0.313	0.233	4.84 ± 0.27	0.738	0.007	1.48 ± 0.36	0.146	−0.043
CT	23.4 ± 3.4			4.83 ± 0.30			1.44 ± 0.34		
CC	24.4 ± 3.0			4.93 ± 0.17			1.45 ± 0.25		
rs2236936									
GG	23.2 ± 3.3	0.306	0.178	4.83 ± 0.29	0.474	0.011	1.47 ± 0.36	0.784	0.012
GC	23.5 ± 3.4			4.84 ± 0.29			1.47 ± 0.34		
CC	23.5 ± 3.6			4.86 ± 0.27			1.50 ± 0.34		
rs6543087									
TT	23.4 ± 3.3	0.277	−0.162	4.82 ± 0.31	0.410	0.011	1.45 ± 0.36	0.187	0.021
AT	23.4 ± 3.4			4.85 ± 0.28			1.47 ± 0.34		
AA	23.1 ± 3.3			4.84 ± 0.29			1.49 ± 0.36		

SNP	HbA1c (%)	*P*	*B*	HOMA-β	*P*	*B*	HOMA-IR	*P*	*B*
rs1003376									
CC	5.12 ± 0.48	0.728	0.009	107.59 (70.97, 154.62)	0.924	−0.761	1.55 (1.01, 2.26)	0.037	−0.112
GC	5.11 ± 20.50			99.01 (67.92, 144.00)			1.44 (1.01, 1.98)		
GG	5.16 ± 0.45			100.63 (57.51, 147.46)			1.39 (0.92, 1.91)		
rs11674694									
CC	5.13 ± 0.48	0.924	0.001	105.43 (72.26, 150.13)	0.629	−3.260	1.50 (1.02, 2.02)	0.984	0.009
CT	5.11 ± 0.47			101.33 (68.44, 148.28)			1.51 (1.00, 2.13)		
TT	5.13 ± 0.46			106.54 (70.41, 152.62)			1.51 (0.95, 2.24)		
rs2236935									
TT	5.11 ± 0.48	0.587	0.023	104.69 (68.94, 148.37)	0.383	−9.033	1.51 (1.01, 2.12)	0.985	−0.009
CT	5.13 ± 0.46			102.38 (70.32, 157.22)			1.49 (0.98, 2.25)		
CC	5.14 ± 0.40			103.71 (84.75, 126.08)			1.69 (1.27, 2.14)		
rs2236936									
GG	5.11 ± 0.48	0.557	0.011	103.13 (68.96, 148.85)	0.378	−6.901	1.50 (0.97, 2.11)	0.684	0.017
GC	5.12 ± 0.47			103.17 (69.21, 150.93)			1.49 (1.02, 2.17)		
CC	5.15 ± 0.41			107.13 (81.00, 139.67)			1.68 (1.03, 2.37)		
rs6543087									
TT	5.13 ± 0.47	0.897	−0.003	111.11 (71.60, 168.00)	0.975	−0.208	1.55 (0.99, 2.3)	0.380	−0.038
AT	5.11 ± 0.47			99.36 (66.67, 143.57)			1.46 (0.99, 2.06)		
AA	5.13 ± 0.48			106.64 (72.49, 150.97)			1.50 (1.04, 2.12)		

The results of linear regression analysis after adjusting for age, gender, and BMI were shown. Data are presented as mean ± SD or median (P_{25}, P_{75}). SNPs: Single-nucleotide polymorphisms; BMI: Body mass index; FPG: Fasting plasma glucose; HDL-C: High-density lipoprotein cholesterol; HbA1c: Glycated hemoglobin; HOMA-β: Homeostasis model assessment of beta-cell function; HOMA-IR: Homeostasis model assessment of insulin resistance; SD: Standard deviation; BMI: Body mass index.

reported that ectopic deposition of TGs in lipid droplets is strongly associated with IR and plays an important role in the pathogenesis of T2DM.[11-14] Those participants with high TG levels may, therefore, have slight IR, which may not be severe enough to result in detectable impaired glucose homeostasis and T2DM.

In this study, SNP rs1003376 had a negative correlation with BMI and HOMA-IR among the controls. That suggested that individuals with SNP rs1003376 may have a lower susceptibility to IR, which may have a protective effect on T2DM. Furthermore, within the nonobese subjects, SNP rs1003376 had a negative correlation with HOMA-IR (data not shown), which was inconsistent with the previous study among the German population.[10] Such an association within rs1003376 and HOMA-IR among nonobese people may suggest that those participants with lower HOMA-IR values may have protection to the disease due to reduced IR. Therefore, the SNP rs1003376 may be implicated in the

pathogenesis of T2DM mainly through reduced IR to lower the risk for T2DM among nonobese individuals. However, this study indicated that rs1003376 had a positive correlation with HbA1c values among obese people, which suggested that the genetic variations rs1003376 in *MAP4K4* may be involved in prediabetes susceptibility via impacting islet β-cell function and individual IR in the studied obese subjects.

A previous study found that TNF-α treatment increases *MAP4K4* gene expression in rat pancreatic β cells.[15] Silencing the *MAP4K4* gene can protect against peripheral IR induced by TNF-α in adipose tissue and skeletal muscle.[15] An increased BMI represents a substantial risk factor for T2D,[16] and may be a major reason that rs1003376 appears to be a protecting factor against T2DM in nonobese subjects, but a risk factor in obese subjects. Therefore, the same gene locus may control the risk to develop T2DM through different mechanisms.

Our study provides unique contributions, including the study of individuals from homogeneous populations of the same ethnicity. However, several inherent limitations should be noted. First, because controls were collected from Hospitals in Harbin, some level of selection bias could not be eliminated. However, all control individuals in our research were those who came to hospitals for routine health examinations and were not hospitalized patients with specific diseases. Therefore, the controls were more representative of the general population, and the potential selection bias was believed to be minimized. Another limitation of this study is that we lacked the systemic inflammatory parameters such as plasma TNF-α and interleukin-6 concentrations for these subjects, so we could not determine the relationship between *MAP4K4* gene variants with inflammatory cytokines. According to previous studies, this may have an effect on IR.[10,15] In the future, additional well-designed investigations with larger sample sizes are needed to confirm our findings.

In conclusion, our results present new evidences for the associations between T2DM and the *MAP4K4* gene in the Chinese Han population. Additional well-designed investigations with larger sample sizes are needed to confirm our findings and to fully delineate the role of *MAP4K4* in the pathogenesis of T2DM.

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Conflicts of interest

There are no conflicts of interest.

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