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Original Article

Association of rs3814570 and rs7094463 polymorphisms in TCF7L2 gene with susceptibility to type 2 diabetes mellitus in a Chinese Uygur population

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Abstract: Genetic polymorphisms of transcription factor 7-like 2 (TCF7L2) on chromosome 10q were reported to be associated with type 2 diabetes mellitus (T2DM) in different populations. The aim of the present study was to investigate the association between the rs3814570 and rs7094463 polymorphisms of the TCF7L2 gene and T2DM in a Chinese Uygur population. A total of 1858 people including 941 T2DM patients and 917 controls were selected for the present study. All T2DM patients and controls were genotyped for the same two single nucleotide polymorphisms (SNPs) (rs3814570, and rs7094463) by using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The rs3814570 polymorphism was found to be associated with T2DM in genotypes, dominant model, recessive model, additive model, and allele frequency (all $P<0.05$), after adjustment of the major confounding factors, the significant difference was retained (all $P<0.05$). General linear model analysis showed rs3814570 polymorphism was associated with glucose levels by analyses of a dominant model ($P = 0.031$). However, the difference did not remain statistically significant after multivariate adjustment ($P = 0.059$). The CC genotype of rs3814570 had significantly higher plasma glucose levels (7.30±3.39 mmol/L vs 6.65±3.17 mmol/L) when compared with the CT + TT genotype. In addition, the frequency of the C-G haplotype was significantly higher in the T2DM patients than in the controls ($P = 0.0123$), and the frequency of the T-G haplotype was significantly lower in the T2DM patients than in the control subjects ($P = 0.0003$). The distribution of rs7094463 genotypes, dominant model, recessive model, additive model and allele frequency showed no significant difference between the T2DM patients and control subjects ($P>0.05$, respectively). Our results indicated that the rs3814570 polymorphism of the TCF7L2 gene was associated with T2DM in the Uygur populations of China. The TT genotype of 3814570 in the TCF7L2 gene might be protective genetic markers of T2DM, the CC genotype of 3814570 might be genetic risk markers of T2DM in the Uygur population in China.

Keywords: TCF7L2, genetic polymorphism, type 2 diabetes mellitus, case control study

Introduction

Type 2 diabetes mellitus (T2DM) is a group of metabolic disorders characterized by the incapability of pancreatic beta cells to increase insulin secretion in order to compensate for insulin resistance in peripheral tissues [1]. T2DM is a multifactorial disease, environmental factors and lifestyle changes are considered to play an important role in the epidemic of T2DM, but the inherent susceptibility to the T2DM is widely attributed to complex genetic factors. Therefore, the identification of genetic polymorphisms influencing T2DM is a main focus of research in order to improve the understanding of the precise mechanisms underlying the pathogenesis of T2DM. To date, genome-wide association studies have confirmed a considerable number of genes are associated with T2DM risk [2-4]. In this context, Grant et al.

[5] identified a microsatellite marker, DG10-S478, within intron 3 of transcription factor 7-like 2 (TCF7L2) that was strongly associated with T2DM in samples of 1,185 case and 931 control subjects from Icelandic, 228 case and 539 control subjects from Denmark, 361 case and 530 control subjects from the U.S. Five single nucleotide polymorphisms (SNPs) (rs12255372, rs7903146, rs7901695, rs11196205, and rs7895340) also showed strong evidence of an association with T2DM in these three case-control sample groups. Since then, this association has been replicated in various populations [6-15]. Up to now, TCF7L2 has been considered one of the most important known genetic risk factors for T2DM [16-18]. The TCF7L2 gene is a member of the T-cell factor (TCF)/lymphoid enhancing factor family of high mobility group box-containing transcription factors involved in the Wnt signaling pathway. This pathway is an important component to the regulation of cell proliferation and differentiation [19]. It is important to point out that T2DM and its complications have great economic impact on individuals, families, and health systems in China. The polymorphisms in TCF7L2 gene have been most studied in Chinese Han population, their association with T2DM in Chinese Han population is still controversial [20, 21]. However, the relationship between TCF7L2 gene and T2DM in Chinese Uygur population remains unclear. Therefore, in the present study, we aimed to investigate the association between the rs3814570 and rs7094463 polymorphisms of the TCF7L2 gene and T2DM in Chinese Uygur population.

Materials and methods

Ethical approval of the study protocol

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China), and was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all participants, and explicitly provided permission for DNA analyses as well as collection of relevant clinical data.

Subjects

All patients with T2DM and control subjects were recruited from The First Affiliated Hospital of Xinjiang Medical University from January 2012 to December 2013. There were 941 T2DM patients (591 men and 350 women) and 917 age-matched healthy individuals (588 men and 329 women) served as control subjects. All T2DM patients and controls included in this study were Uygur Chinese from the same geographic area in Xinjiang. T2DM was defined according to the WHO criteria as fasting plasma glucose >7.0 mmol/l and/or 2 h OGTT ≥11.0 mmol/l. Data and information regarding traditional T2DM risk factors including hypertension, coronary artery disease (CAD), and hyperlipidemia were collected from all study participants. The diagnosis of hypertension was established if patients were on antihypertensive medication or if the mean of 3 measurements of systolic blood pressure (SBP) was >140 mm Hg or diastolic blood pressure (DBP) >90 mm Hg, respectively. CAD was defined as the presence of at least one significant coronary artery stenosis of more than 50% luminal diameter on coronary angiography. Hyperlipidemia was defined as a total plasma cholesterol >6.22 mmol or plasma triglycerides >2.26 mmol and/or the current use of lipid-lowering drugs with an established diagnosis of hyperlipidemia. T2DM patients and control subjects with impaired renal function, malignancy, connective tissue disease, or chronic inflammatory disease were excluded.

Blood collection and DNA extraction

Blood samples were taken from all participants. The blood samples were drawn into a 5 ml ethylene diamine tetraacetic acid (EDTA) tube and centrifuged at 4000×g for 5 min to separate the plasma content. Genomic DNA was extracted from the peripheral leukocytes using standard phenol-chloroform method. The DNA samples were stored at -80°C for future analysis. Before genetic analysis, the final concentration of the DNA was diluted to 50 ng/μL.

Genotyping of the TCF7L2 gene

There are 13076 SNPs for the human TCF7L2 gene listed in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP). Using Haplo view 4.2 software and International Hap Map Project website phase I & II database (http://www.hapmap.org), we obtained two tag SNPs (rs3814570, rs7094463) by using minor allele fre-

quency (MAF) ≥0.05 and linkage disequilibrium patterns with r^2 ≥0.8 as a cut off. Genotyping in the present case-control study was confirmed by the MALDI-TOF Mass Spectrometry. Genotyping procedures were performed according to the manufacturer s iPLEX Application Guide (Sequenom, San Diego, California, USA). The MALDI-TOF Mass Spectrometer was used for data acquisition from the SpectroCHIP. The amplification PCR primer pairs and the extend primers were designed using MassARRAY Assay Design software version 3.1. The results were analyzed using the MassARRAY Typer software version 4.0. The accuracy of the genotyping was determined by assessing the genotype concordance between duplicate samples. We obtained a 100% concordance between the genotyped duplicate samples for the single nucleotide polymorphism (SNP). The genotyping success rate was 100%.

Biochemical analyses

The serum concentrations of glucose, total cholesterol (TC), triglyceride (TG), highdensity lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN), creatinine (Cr) and uric acid (UA) were measured using standard methods in the Central Laboratory of First Affiliated Hospital of Xinjiang Medical University.

Statistical analysis

Data analysis was carried out using the computer software Statistical Package for Social Sciences SPSS 17.0 for Windows (SPSS Institute, Chicago, USA). Continuous variables were presented as mean ± standard deviation and compared using the Student t test between groups. Categorical variables were presented as proportions and compared with the chi-square test. Chi-square tests were used to assess whether genotypes were in Hardy-Weinberg equilibrium (HWE) and to compare genotype and allele frequencies between T2DM cases and control subjects. Logistic regression analyses with effect ratios (odds ratio [OR] and 95% CI [confidence interval]) were used to assess the contribution of the major risk factors. General linear model (GLM) analysis was performed to test for associations between SNP genotypes and serum glucose after adjustment for confounding variables. Based on the genotype data of the genetic variations, we performed linkage disequilibrium (LD) analysis and haplotype-based case-control analysis, using the software SHEs is (http://analysis.bio-x.cn/SHEsisMain.htm). The pair wise linkage disequilibrium analysis was performed using SNP pairs. We used D' values of >0.5 to assign SNP locations to one haplotype block. Single nucleotide polymorphisms with an r^2 value of <0.5 were selected as tagged. In the haplotype-based case-control analysis, haplotypes with a frequency of <0.03 were excluded. The frequency distribution of the haplotypes was calculated by performing a permutation test using the bootstrap method. A 2-sided P<0.05 was considered to indicate statistical significance.

Results

Table 1 shows demographic and clinical characteristics of the study participants. There was no significant difference in age between patients with T2DM and control subjects, which indicated that the study was an age-matched case-control study. For total subjects, BMI, SBP, the serum concentration of glucose, TC, BUN were significantly higher in subjects with T2DM than in the controls (P<0.05), and the serum concentration of HDL, UA were significantly lower for patients with T2DM than for control participants (P<0.05). There was no significant difference in the following variables between the T2DM patients and the control participants in total subjects: DBP, the serum concentration of TG, LDL and creatinine (all P>0.05). For men, the serum concentration of glucose, TC, BUN were significantly higher in subjects with T2DM than in the controls (P<0.05), and DBP, the serum concentration of HDL, UA were significantly lower for patients with T2DM than for control participants (P< 0.05). There was no significant difference in the following variables between the T2DM patients and the control participants in men subjects: BMI, SBP, the serum concentration of TG, LDL and creatinine (all P>0.05). For women, SBP, the serum concentration of glucose, TC were significantly higher in subjects with T2DM than in the controls (P<0.05), and the serum concentration of HDL was significantly lower for patients with T2DM than for control participants (P<0.05). There was no significant difference in the following variables between the T2DM patients and the control participants in women subjects: BMI, DBP, the serum concentration of TG, LDL, UA, creatinine and BUN (all P>0.05).

Table 1. Demographic and clinical characteristics of study participants

	Total			Men			Women		
	T2DM	Control	*p* value	T2DM	Control	*p* value	T2DM	Control	*p* value
Number (n)	941	917		591	588		350	329	
Age (years)	51.19±9.77	50.41±9.82	0.083	51.11±9.96	50.49±9.93	0.283	51.34±9.49	50.15±9.64	0.100
BMI (kg/m^2)	27.67±5.03	27.07±3.94	0.037*	27.79±4.94	27.08±3.58	0.064	27.49±5.16	27.04±4.54	0.419
SBP (mmHg)	126.87±18.98	122.51±17.44	0.006*	125.06±16.23	123.85±18.69	0.523	129.76±22.41	120.28±15.03	<0.001*
DBP (mmHg)	79.35±11.92	81.28±14.00	0.079	78.82±10.74	82.91±14.98	0.002*	80.19±13.56	78.46±11.72	0.376
Glu (mmol/L)	9.52±3.44	5.00±0.90	<0.001*	9.53±3.36	4.98±0.82	<0.001*	9.49±3.58	5.05±1.04	<0.001*
TG (mmol/L)	2.42±2.15	2.50±2.15	0.437	2.65±2.45	2.69±2.31	0.750	2.02±1.41	2.14±1.76	0.363
TC (mmol/L)	4.66±1.36	4.27±1.68	<0.001*	4.62±1.44	4.25±1.68	<0.001*	4.71±1.22	4.33±1.67	0.001*
HDL (mmol/L)	0.95±0.34	1.24±0.32	<0.001*	0.91±0.28	1.18±0.30	<0.001*	1.03±0.40	1.36±0.32	<0.001*
LDL (mmol/L)	2.87±1.49	2.98±0.79	0.064	2.87±1.76	3.00±0.79	0.119	2.87±0.84	2.94±0.80	0.317
UA (μmol/L)	268.98±84.36	284.04±70.28	<0.001*	287.33±85.20	308.22±60.30	<0.001*	238.01±73.26	240.64±66.00	0.636
Cr (μmol/L)	68.43±44.36	70.89±18.20	0.141	74.64±45.35	76.10±13.92	0.484	57.90±40.59	61.64±21.03	0.155
BUN (mmol/L)	5.20±2.32	4.95±1.44	0.008*	5.42±2.36	5.09±1.36	0.006*	4.83±2.19	4.69±1.55	0.375

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as percentages. T2DM: Type 2 diabetes mellitus; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; Glu: Glucose; TG: Triglyceride; TC: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; UA: Uric acid; Cr: Creatinine; BUN: Blood urea nitrogen. The *p* value of the continuous variables was calculated by the Independent t-test. The *p* value of the categorical variables was calculated by X^2 test.
*$P<0.05$.

Table 2. Genotype and allele distributions in patients with T2DM and control participants

Variants	Total			Men			Women		
	T2DM n (%)	Control n (%)	*p* value	T2DM n (%)	Control n (%)	*p* value	T2DM n (%)	Control n (%)	*p* value
rs3814570									
Genotyping									
CC	899 (95.5)	837 (91.3)		561 (94.9)	535 (91.0)		338 (96.6)	302 (91.8)	
CT	11 (1.2)	30 (3.3)		7 (1.2)	19 (3.2)		4 (1.1)	11 (3.3)	
TT	31 (3.3)	50 (5.5)	0.001*	23 (3.9)	34 (5.8)	0.016*	8 (2.3)	16 (4.9)	0.026*
Dominant model									
CC	899 (95.5)	837 (91.3)		561 (94.9)	535 (91.0)		338 (96.6)	302 (91.8)	
CT + TT	42 (4.5)	80 (8.7)	<0.001*	30 (5.1)	53 (9.0)	0.008*	12 (3.4)	27 (8.2)	0.007*
Recessive model									
TT	31 (3.3)	50 (5.5)		23 (3.9)	34 (5.8)		8 (2.3)	16 (4.9)	
CC + CT	910 (96.7)	867 (94.5)	0.023*	568 (96.1)	554 (94.2)	0.130	342 (97.7)	313 (95.1)	0.069
Additive model									
CC	899 (95.5)	837 (91.3)		561 (94.9)	535 (91.0)		338 (96.6)	302 (91.8)	
CT	11 (1.2)	30 (3.3)		7 (1.2)	19 (3.2)		4 (1.1)	11 (3.3)	
TT	31 (3.3)	50 (5.5)	0.002*	23 (3.9)	34 (5.8)	0.027*	8 (2.3)	16 (4.9)	0.018*
Allele									
C	1809 (96.1)	1704 (92.9)		1129 (95.5)	1089 (92.6)		680 (97.1)	615 (93.5)	
T	73 (3.9)	130 (7.1)	<0.001*	53 (4.5)	87 (7.4)	0.003*	20 (2.9)	43 (6.5)	0.001*
rs7094463									
Genotyping									
GG	302 (32.1)	272 (29.7)		195 (33.0)	177 (30.1)		107 (30.6)	95 (28.9)	
GA	449 (47.7)	449 (49.0)		281 (47.5)	276 (46.9)		168 (48.0)	173 (52.6)	
AA	190 (20.2)	196 (21.4)	0.509	115 (19.5)	135 (23.0)	0.285	75 (21.4)	61 (18.5)	0.454
Dominant model									
GG	302 (32.1)	272 (29.7)		195 (33.0)	177 (30.1)		107 (30.6)	95 (28.9)	
GA + AA	639 (67.9)	645 (70.3)	0.257	396 (67.0)	411 (69.9)	0.285	243 (69.4)	234 (71.7)	0.629
Recessive model									
AA	190 (20.2)	196 (21.4)		155 (19.5)	135 (23.0)		75 (21.4)	61 (18.5)	
GG + GA	751 (79.8)	721 (78.6)	0.53	476 (80.5)	453 (77.0)	0.141	275 (78.6)	268 (81.5)	0.347
Additive model									
GG	302 (32.1)	272 (29.7)		195 (33.0)	177 (30.1)		107 (30.6)	95 (28.9)	
GA	449 (47.7)	449 (49.0)		281 (47.5)	276 (46.9)		168 (48.0)	173 (52.6)	
AA	190 (20.2)	196 (21.4)	0.274	115 (19.5)	135 (23.0)	0.127	75 (21.4)	61 (18.5)	0.824
Allele									
G	1053 (56.0)	993 (54.1)		671 (56.8)	630 (53.6)		382 (54.6)	363 (55.2)	
A	829 (44.0)	841 (45.9)	0.268	511 (43.2)	546 (46.4)	0.119	318 (45.4)	295 (44.8)	0.825

T2DM: Type 2 diabetes mellitus; N: Number of participants; SNP: Single nucleotide polymorphism; *P<0.05.

Table 2 shows the distribution of genotypes and alleles of rs3814570 and rs7094463 for the TCF7L2 gene. The genotype distributions of each SNP were in good agreement with the predicted Hardy-Weinberg equilibrium values (data not shown). For total participants, distribution of rs3814570 genotypes, dominant model (CC vs CT + TT), recessive model (TT vs CC + CT), additive model (CC vs CT vs TT) and allele frequency showed significant difference between T2DM and control subjects (P = 0.001, P<0.001, P = 0.023, P = 0.002, P<0.001). For men, distribution of rs3814570 genotypes, dominant model (CC vs CT + TT), additive model (CC vs CT vs TT) and allele frequency showed significant difference between T2DM and control subjects (P = 0.016, P = 0.008, P = 0.027, P = 0.003). For women, distribution of rs3814570 genotypes, dominant model (CC vs CT + TT), additive model (CC vs CT vs TT) and allele frequency showed significant difference between T2DM and control subjects (P = 0.026, P = 0.007, P = 0.018, P = 0.001). For total, men and women subjects, T allele of rs3814570 was significantly higher in controls than in subjects with T2DM (total: 7.1% vs 3.9%; men:

Table 3. Multiple logistic regression analysis for T2DM patients and control subjects (rs3814570)

	Total			Men			Women		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Dominant model (CC vs CT + TT)	0.475	0.298-0.757	0.002*	0.500	0.286-0.874	0.015*	0.474	0.200-1.122	0.089
TG	0.866	0.816-0.920	<0.001*	0.866	0.808-0.927	<0.001*	0.935	0.799-1.094	0.400
TC	1.514	1.344-1.704	<0.001*	1.468	1.280-1.684	<0.001*	1.658	1.291-2.130	<0.001*
HDL	0.029	0.019-0.045	<0.001*	0.017	0.009-0.031	<0.001*	0.035	0.018-0.069	<0.001*
LDL	0.745	0.621-0.894	0.002*	0.808	0.651-1.002	0.052	0.646	0.442-0.942	0.023*

T2DM: Type 2 diabetes mellitus; TG: Triglyceride; TC: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; *P<0.05.

Table 4. Multiple logistic regression analysis for T2DM patients and control subjects (rs7094463)

	Total			Men			Women		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Dominant model (GG vs GA + AA)	0.954	0.751-1.212	0.699	0.985	0.730-1.330	0.923	0.883	0.589-1.324	0.548
TG	0.867	0.817-0.921	<0.001*	0.868	0.810-0.929	<0.001*	0.934	0.799-1.092	0.393
TC	1.516	1.347-1.707	<0.001*	1.469	1.281-1.686	<0.001*	1.667	1.299-2.139	<0.001*
HDL	0.029	0.019-0.045	<0.001*	0.017	0.009-0.031	<0.001*	0.033	0.017-0.066	<0.001*
LDL	0.746	0.621-0.895	0.002*	0.811	0.654-1.005	0.056	0.642	0.440-0.936	0.021*

T2DM: Type 2 diabetes mellitus; TG: Triglyceride; TC: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; *P<0.05.

7.4% vs 4.5%; women: 6.5% vs 2.9%), the dominant model (CC vs CT + TT) of rs3814570 was significantly higher in subjects with T2DM than in controls (total: 95.5% vs 91.3%; men: 94.9% vs 91.0%; women: 96.6% vs 91.8%). For total participants, the recessive model (TT vs CC + CT) of rs3814570 was significantly higher in controls than in subjects with T2DM (5.5% vs 3.3%). For total, men, and women subjects, the distribution of rs7094463 genotypes, dominant model (GG vs GA + AA), recessive model (AA vs GG + GA), additive model (GG vs GA vs AA) and allele frequency did not show a significant difference between the T2DM patients and control subjects (P>0.05, respectively).

Table 3 shows multivariable logistic regression analysis combining genotypes with following variables: the serum concentration of TG, TC, HDL, LDL which were the major confounding factors for T2DM. For total participant and men, after multivariate adjustment, rs3814570 remain significantly associated with T2DM in the dominant model (total: OR = 0.475, 95% confidence interval [CI]: 0.298-0.757, P = 0.002; men: OR = 0.500, 95% confidence interval [CI]: 0.286-0.874, P = 0.015), in the recessive model (total: OR = 0.443, 95% confidence interval [CI]: 0.252-0.777, P = 0.005; men: OR = 0.479, 95% confidence interval [CI]: 0.249-0.919, P = 0.027; data not shown for the recessive model) and in the additive model (total: OR = 0.650, 95% confidence interval [CI]: 0.497-0.850, P = 0.002; men: OR = 0.677, 95% confidence interval [CI]: 0.495-0.927, P = 0.015; data not shown for the additive model). For women, after multivariate adjustment, rs3814570 did not remain significantly associated with T2DM in the dominant model (OR = 0.474, 95% confidence interval [CI]: 0.200-1.122, P = 0.089), in the recessive model (OR = 0.440, 95% confidence interval [CI]: 0.141-1.370, P = 0.157; data not shown for the recessive model) and in the additive model (OR = 0.637, 95% confidence interval [CI]: 0.375-1.080, P = 0.094; data not shown for the additive model). For total, men, and women subjects (**Table 4**), after multivariate adjustment, rs7094463 did not remain significantly associated with T2DM in the dominant model, in the recessive model (data not shown for the recessive model) and in the additive model (data not shown for the additive model).

Table 5 shows that rs3814570 was associated with glucose levels by analyses of a dominate model (P = 0.031). However, the difference did not remain significant after adjustment for the serum concentration of TG, TC, HDL, LDL levels (P = 0.059). In addition, rs7094463 did not show any association with glucose levels before and after adjustment of confounders. To investigate further the functional role of the rs3814570 polymorphism, we

Table 5. Glucose levels and TCF7L2 genotypes

SNP genotypes	Unadjusted model		Adjusted model§	
	Glucose (mmol/L) Mean ± SD	*p* value	Glucose (mmol/L) Mean ± SE	*p* value
rs3814570				
Additive model				
CC (n = 1736)	7.30±3.39		7.21±0.08	
CT (n = 41)	6.27±2.53		6.63±0.52	
TT (n = 81)	6.85±3.45	0.082	6.61±0.37	0.167
Dominant model				
CC (n = 1736)	7.30±3.39		7.21±0.08	
CT + TT (n = 122)	6.65±3.17	0.031*	6.62±0.30	0.059
Recessive model				
TT (n = 81)	6.85±3.45		6.61±0.37	
CC + CT (n = 1777)	7.28±3.37	0.269	7.20±0.08	0.124
rs7094463				
Additive model				
GG (n = 574)	7.48±3.46		7.27±0.14	
GA (n = 898)	7.12±3.25		7.12±0.11	
AA (n = 386)	7.28±3.54	0.149	7.19±0.17	0.681
Dominant model				
GG (n = 574)	7.48±3.46		7.27±0.14	
GA + AA (n = 1284)	7.17±3.34	0.073	7.13±0.09	0.439
Recessive model				
AA (n = 386)	7.28±3.54		7.19±0.17	
GG + GA (n = 1472)	7.26±3.34	0.920	7.17±0.09	0.903

§Analysis of covariance adjusted for TG, TC, HDL and LDL levels; SNP: Single nucleotide polymorphism; *$P<0.05$.

compared the concentrations of glucose between carriers with the CT + TT genotype and carriers with the CC genotype of the rs3814570. Glucose concentrations were significantly higher in subjects with the CC genotype than in participants with the CT + TT genotype in Uygur subjects (7.30±3.39 mmol/L vs 6.65±3.17 mmol/L, $P = 0.031$) (**Figure 1A**). These results indicated that the T allele is associated with lower glucose levels than the C allele in the study participants. However, these differences were not observed in the rs7094463 (**Figure 1B**).

Table 6 shows the result of haplotype analysis. In the haplotype-based case-control analysis of the Uygur population, two strong linkage disequilibrium (LD) patterns were observed between rs3814570 and rs7094463 (D' = 0.835, $r^2 = 0.033$), the rs3814570 and 7094463 are located in one haplotype block, because the D' values were beyond 0.5 and the r^2 values were below 0.5. Therefore, the rs3814570 and 709446 were used to construct the haplotypes. For total participants and men, the frequency of the C-G haplotype was significantly higher for the T2DM patients as compared to the control subjects (total: $P = 0.012$; men: $P = 0.006$). For total, men and women subjects, the frequency of the T-G haplotype was significantly lower for T2DM patients than for control participants (total: $P < 0.001$; MEN: $P = 0.006$; women: $P = 0.014$).

Discussion

In the present study, we found that rs3814570 polymorphism of TCF7L2 gene was associated with T2DM in the Uygur population. After a multivariate adjustment, there was still a significant difference between rs3814570 polymorphism of TCF7L2 gene and T2DM. Our study is the first case-control study to investigate the association between rs3814570 polymorphism of TCF7L2 gene and T2DM in the Uygur populations in Xinjiang, China.

TCF7L2 gene is considered one of the most important candidate genes for T2DM, playing a critical role in blood glucose homeostasis and beta cell function [15]. TCF7L2 gene which spans about 215.9 Kb on human chromosome 10q25 is a high mobility group box containing transcription factor involved in the Wnt signaling pathway, playing an important role in cell development and regulatory mechanisms. Wnt activity is important for lipid and glucose metabolism, pancreatic beta cell proliferation and function and for production of incretin hormone GLP-1 [22]. Wnt signaling is also important for glucagon like peptide-1 (GLP-1) secre-

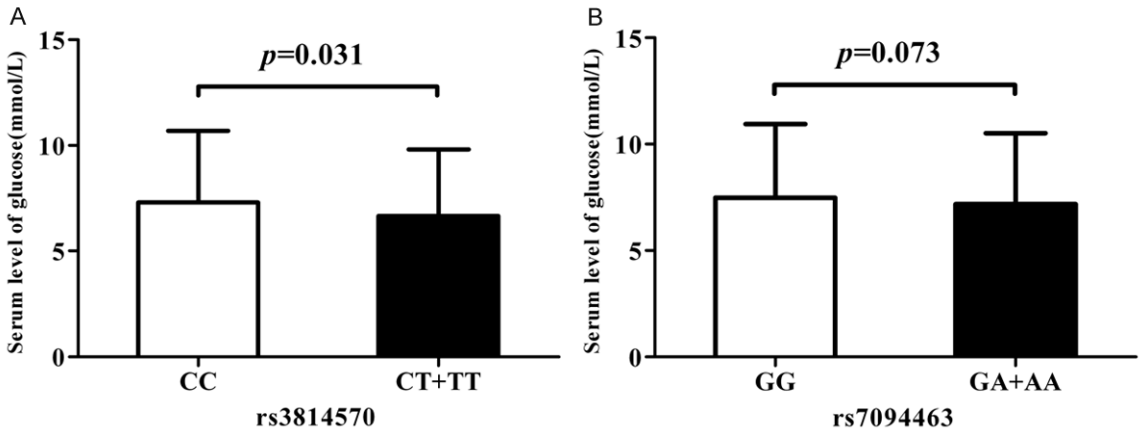

Figure 1. Association of glucose levels with rs3814570 and rs7094463 genotypes in study participants. A. Mean plasma levels of glucose in subjects according to rs3814570 genotypes. CC vs CT + TT: 7.30±3.39 vs 6.65±3.17, *P* = 0.031. B. Mean plasma levels of glucose in subjects according to rs7094463 genotypes. GG vs GA + AA: 7.48±3.46 vs 7.17±3.34, *P* = 0.073.

Table 6. Haplotype analysis in patients with T2DM and in control subjects

Haplotype	rs3814570	rs7094463	Frequency in total			Frequency in men			Frequency in women		
			T2DM	Control	*p* value	T2DM	Control	*p* value	T2DM	Control	*p* value
H1	C	A	0.439	0.451	0.366	0.432	0.461	0.139	0.451	0.433	0.642
H2	C	G	0.522	0.478	0.012*	0.523	0.465	0.006*	0.520	0.501	0.633
H3	T	G	0.038	0.064	<0.001*	0.045	0.071	0.006*	0.026	0.050	0.014*

T2DM: Type 2 diabetes mellitus; Haplotype with frequencies >0.03 were estimated using SHEs is software; The *p* value was calculated by permutation test using the bootstrap method; *$P<0.05$.

tion by the intestinal endocrine L-cells [23]. Therefore, alteration in Wnt signaling pathway could contribute to reduced secretion of GLP-1, which could have impact on insulin secretion. The GLP-1, in concert with insulin, plays a key role in blood glucose homeostasis. It has been estimated that TCF7L2 gene variants seem to confer susceptibility to T2DM indirectly by altering GLP-1 levels [24]. Munoz et al. [25] showed that the TCF7L2 gene is an important factor regulating insulin secretion, which could explain its association with T2DM. Damcott et al. [19] demonstrated that polymorphisms in TCF7L2 increase the risk for T2DM and the polymorphisms likely influence both insulin secretion and insulin sensitivity. Lyssenko et al. [26] reported that the increased risk of T2DM conferred by polymorphisms in TCF7L2 involves the enteroinsular axis, enhanced expression of the gene in islets, and impaired insulin secretion. Florez et al. [27] found that variants in TCF7L2 may be associated with an increased risk of T2DM among persons with impaired glucose tolerance. The risk genotypes in TCF7L2 are associated with impaired beta cell function. Pilgaard et al. [28] suggested that the T allele of rs7903146 in TCF7L2 is associated with impaired insulinotropic action of incretin hormones, reduced 24 h profiles of plasma insulin and glucagon, and increased hepatic glucose production. However, the precise molecular mechanism underlying the association of TCF7L2 polymorphisms with T2DM remains to be elucidated.

The identification of TCF7L2 gene influencing individual susceptibility to T2DM could provide a better understanding of the precise molecular mechanisms underlying pathogenesis and may provide new information for diagnostic treatment and prevention. In 2006, Grant et al. [5] found the TCF7L2 gene was significantly associated with T2DM in an Icelandic case-control cohort. The results have been replicated in Caucasian case-control cohorts from Denmark and the U.S. In addition, five SNPs (rs12255372, rs7903146, rs7901695, rs11196205, and rs7895340) within the same large haplotype block that were correlated with DG10S478 were also associated

with T2DM. Since the original publication, a number of research studies have demonstrated the association between TCF7L2 and T2DM in European, Asian and American cohorts. In the study of Groves et al. [6], their data clearly establish TCF7L2 as a gene of particular importance to the development of T2DM in the U.K. Subjects. Lehman et al. [7] found that polymorphisms in the TCF7L2 genomic region may affect risk for T2DM in Mexican Americans. Mayans et al. [8] indicated that variants in the TCF7L2 gene significantly contribute to T2DM susceptibility in northern Sweden. Sale et al. [9] reported replication of association between T2DM and three SNPs (rs12255372, rs7901695, rs7903146) in the case-control samples from African-American populations. Van et al. [10] provide evidence that TCF7L2 is a major determinant of T2DM risk in Dutch populations and population-attributable risk from this factor in the Dutch T2DM population is 10%. Both the T allele of rs7903146 (OR = 1.41, $P = 4.4\times10^{-5}$) and the T allele of rs12255372 (OR = 1.29, $P = 3\times10^{-3}$) were significantly over-represented in cases. Cauchi et al. [11] strongly confirm that polymorphisms of the TCF7L2 gene contribute to the risk of T2DM in French populations and this transcription factor plays an important role in glucose homeostasis. The risk alleles of rs12255372 and rs7903146 were significantly increase T2DM risk. Barra et al. [12] suggests that TCF7L2 is a common susceptibility gene for type 2 diabetes in the Brazilian subjects. Horikoshi et al. [13] found that the T allele of the rs7903146 of TCF7L2 was significantly associated with T2DM risk in the Japanese population. Acharya et al. [14] revealed that the risk alleles frequency of the rs12255372 and rs4506565 were found to be significantly higher in the T2DM cases than the normal controls, suggesting that they may confer risk for T2DM among the Saudi population. Jyothi et al. [15] demonstrated strong association between risk alleles of variants rs12255372 and rs7903146 and T2DM in the Indian population. Chang et al. [20] found a novel association of the genetic variant rs290487 in the TCF7L2 gene with type 2 diabetes in a Chinese Han population. However, the rs7903146 and rs12255372 of TCF7L2 gene did not show any significant associations with type 2 diabetes T2DM. Ng et al. [21] showed that rs11196205 was associated with T2DM, whereas the association for rs7903146 was not significant in the Chinese Han population. Interestingly, another SNP rs11196218 conferred independent risk for T2DM. Regarding the Japanese population and Indian population, these reports found that rs7903146 was associated with T2DM, which is in agreement with the previous reports in samples from European populations. However, contradictory results were reported for Chinese Han population [20, 21], these two reports suggested that the rs11196218 and rs290487 polymorphisms of the TCF7L2 gene were associated with T2DM in Chinese Han population. The apparent difference in the association of these SNPs in Asians could be attributable to the Chinese Han population might carry a different risk allele in T2DM susceptibility gene TCF7L2, the associations of the rs11196218 and rs290487 polymorphisms might be specific to Chinese Han population. In addition, the environment, diet, and lifestyles greatly differ which might explain the inconsistent results.

In our study, we found that rs3814570 polymorphism of TCF7L2 gene was associated with risk of T2DM in the Uygur population. There was a significant difference in the genotype distribution of rs3814570 between T2DM patients and control subjects. For total, men and women subjects, the T allele frequency of rs3814570 was significantly higher in controls than in subjects with T2DM. Thus, the risk of T2DM was decreased with regard to the T allele of rs3814570 in the Uygur population. This result indicated that T allele of rs3814570 was a protective factor for T2DM. Similarly, for total, men and women subjects, the dominant model (CC vs CT + TT) of rs3814570 was significantly higher in subjects with T2DM than in controls. For total participants, the recessive model (TT vs CC + CT) of rs3814570 was significantly higher in controls than in subjects with T2DM. Furthermore, this difference was retained after adjusting for TG, TC, HDL, LDL using multiple logistic regression analyses. Thus, the risk of T2DM was decreased with the presence of the TT genotype of rs3814570 in the Uygur population. This finding indicated that the TT genotype of rs3814570 in the TCF7L2 gene might be protective genetic markers of T2DM. For total, men, and women subjects, the distribution of rs7094463

genotypes, dominant model (GG vs GA + AA), recessive model (AA vs GG + GA), additive model (GG vs GA vs AA) and allele frequency did not show a significant difference between the T2DM patients and control subjects (P>0.05, respectively). In our study, strong linkage disequilibrium (LD) patterns were observed between rs3814570 and rs7094463 (D' = 0.835, r^2 = 0.033). Therefore, we hypothesized that haplotype analysis would be useful for the assessment of association between haplotypes and T2DM. For total participants and men, we found a susceptible haplotype C-G. For total, men and women subjects, we found a protective haplotype T-G, and these haplotypic analysis results were consistent with genotypic analysis results of rs3814570, which showed that the T allele could be a risk genetic marker and the C allele could be a protective genetic marker of T2DM. Our study shows that rs3814570 was significantly associated with plasma glucose levels by analyses of a dominant model. Such association did not remain significant after multivariate adjustment. Further, individuals with the CT + TT genotype of rs3814570 had significantly lower plasma glucose levels when compared with CC genotype. To our knowledge, the high levels of glucose concentration in plasma increase the risk of T2DM, and thus assumed CC genotype might be a risk genetic marker for T2DM. Together, these results might provide convincing evidence for assuming people who carry CC genotype may have higher probabilities of suffering from T2DM than people who carry CT + TT genotype of rs3814570 of TCF7L2 gene.

We have found that rs3814570 of TCF7L2 are associated with susceptibility to T2DM in the Chinese Uygur population. To the best of our knowledge, rs3814570 polymorphism of the TCF7L2 gene has not been previously investigated in the Chinese Han population. Therefore, further studies with larger sample size should be conducted to confirm our findings in several other populations. Furthermore, additional studies are needed to understand the differences in the contribution of the rs3814570 in TCF7L2 gene to T2DM among different populations.

There are still several limitations must be mentioned in the present study. Firstly, the representativeness of the study sample might not be sufficient enough due to the source of T2DM patients and control subjects were limited to the First Affiliate Hospital of Xinjiang Medical University. Secondly, because of the limited time, we are only able to perform a retrospective study. Therefore, a prospective cohort study has to be conducted over a reasonably long time span to get evidence with higher quality.

In conclusion, we found that the rs3814570 polymorphism of the TCF7L2 gene was associated with T2DM in the Uygur populations of western China. The TT genotype of 3814570 in the TCF7L2 gene might be protective genetic markers of T2DM, the CC genotype of 3814570 might be genetic risk markers of T2DM in the Uygur population in China. However, no significant associations were found between the rs7094463 polymorphism of the TCF7L2 gene and T2DM in the Chinese Uygur population. Our preliminary analysis indicates that the rs3814570 polymorphism may modulate T2DM risk by affecting plasma glucose levels. Further studies are needed to confirm our findings in different population.

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Disclosure of conflict of interest

None.

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