The Effect of SOCS2 Polymorphisms on Type 2 Diabetes Mellitus Susceptibility and Diabetic Complications in the Chinese Han Population

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Research

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Abstract

Propose: We explored the effect of *SOCS2* polymorphisms on the development of type 2 diabetes mellitus (T2DM) and diabetic complications.

Methods: The subjects consisted of 500 T2DM patients and 501 healthy volunteers. Five variants in *SOCS2* were genotyped by Agena MassARRAY system. Logistic regression analysis was utilized to calculate the odds ratio (OR) and 95% confidence intervals (95% CI).

Results: Rs3825199 (OR = 1.44, p = 0.007), rs11107116 (OR = 1.39, p = 0.014) and rs10492321 (OR = 1.48, p = 0.004) had the increased T2DM risk. Moreover, the contribution of *SOCS2* polymorphisms to T2DM risk was associated with age, gender, smoking and drinking and BMI. *SOCS2* variants also had the reduced risk for T2DM patients with diabetic nephropathy, diabetic retinopathy and coronary heart disease.

Conclusions: This study firstly reported that rs3825199, rs11107116 and rs10492321 in *SOCS2* conferred to the increased risk for T2DM occurrence in the Chinese Han population.

Introduction

Type 2 diabetes mellitus (T2DM) is a serious metabolic disorder with chronic hyperglycemia characterized by impaired insulin secretion and resistance [1]. Globally, the International Diabetes Federation (IDF) displayed more than 451 million people with diabetes in 2017 [2]. With the aging population and the westernization of lifestyle, the prevalence of diabetes in China has been rising rapidly from 0.67% in 1980 up to 10.4% in 2013[3]. In China, approximately 11% of the population has diabetes, with a significant proportion remaining undiagnosed [4]. The pathogenesis of T2D is complicated and multifactorial, which is driven by environmental, lifestyle and genetic factors. Age, sex, cigarette smoking, alcohol drinking and overweight have been reported to be risk factors for T2DM [5]. In addition, genetic factors contribute strongly to the etiology and manifestation of T2DM. To date, many risk loci have been recognized to affect T2DM susceptibility [6–8], but numerous loci remain to be detected.

Suppressor of cytokine signaling 2 (SOCS2) protein is a member of the suppressor of cytokine signaling family, which is negative regulators of cytokine and growth factor signaling [9, 10]. SOCS2 protein was reported to interact with the insulin-like growth factor-1 receptor (IGF1R) and decrease its biological actions [11]. SOCS2 was downregulation in diabetes, which might be related to either insulin deficiency or resistance [12]. SOCS2 was involved in hyperglycaemia and glucose intolerance caused by the abnormal regulation of proinsulin processing and insulin secretion in beta cells [13]. The overexpression of SOCS2 possess the protective function in the development of diabetic nephropathy by reducing the expression of inflammatory cytokines and suppressing the activation of JAK/STAT pathway[14]. These physiological studies proposed that SOCS2 might play an important role in diabetes, but the role of genetic polymorphism within *SOCS2* gene for T2DM predisposition has been less studied. Therefore, we chose *SOCS2* gene as a candidate gene to explore the effect of single-nucleotide polymorphisms (SNPs) in *SOCS2* on the development of T2DM.

Here, five SNPs (rs10859525, rs3825199, rs11107116, rs10492321, and rs10859563) in *SOCS2* were genotyped to examine the contribution of genetic variants in *SOCS2* to the risk of T2DM occurrence at single-locus and combined SNPs interface. Our study also investigated whether the relationship of *SOCS2* polymorphisms with T2DM risk persists across age, gender, lifestyle and BMI. Further, the contribution of *SOCS2* polymorphisms to the susceptibility diabetic complications was explored in the Chinese Han population.

Results

Baseline characteristics of subject

A total of 500 T2DM cases (59.87 ± 12.87 years, 358 males and 142 females) and 501 controls (59.85 ± 9.34 years, 358 males and 143 females) were recruited. The distribution in age and sex between the T2D patients and controls was similar (p = 0.973 and p = 0.508, respectively). There were statistical differences between the two groups with respect to biochemical indexes including fasting blood glucose, total cholesterol, LDL-C, HDL-C, urea, serum uric acid (all p < 0.05, Table 1).

Table 1
Characteristics of patients with T2DM and controls

Variable	Cases (n = 500)	Controls (n = 501)	p
Age (year, mean ± SD)	59.87 ± 12.87	59.85 ± 9.34	0.973
> 60 / ≤ 60	240/260	268/233	
Gender Male/Female	358/142	358/143	0.508
BMI (kg/m^2) < 24 / ≥ 24	203/239	130/188	
Unavailable	58	183	
Smoking Yes / No	219/280	98/164	
Unavailable	1	239	
Drinking Yes / No	109/385	103/140	
Unavailable	6	258	
T2DM duration (years) >10 / ≤10	193/307		
Fasting blood glucose (mmol/L)	8.14 ± 3.35	5.65 ± 0.51	**< 0.001**
Total cholesterol (mmol/L)	4.18 ± 2.01	4.93 ± 4.00	**< 0.001**
Triglyceride (mmol/L)	1.91 ± 1.90	1.74 ± 0.97	0.088
LDL-C (mmol/L)	2.46 ± 0.90	2.61 ± 0.76	**0.012**
HDL-C (mmol/L)	1.05 ± 0.72	1.16 ± 0.55	**0.024**
Urea (mmol/L)	6.52 ± 3.26	5.42 ± 2.78	**< 0.001**
Creatinine (μmol/L)	71.20 ± 52.66	68.74 ± 12.87	0.322
GFR (mL/min)	96.62 ± 22.22	96.07 ± 19.78	0.710
Serum uric acid (μmol/L)	333.17 ± 99.15	318.33 ± 76.64	**0.013**
Diabetic complications			
Diabetic nephropathy	146		
Diabetic retinopathy	69		
T2DM with coronary heart disease	126		
T2DM with hypertension	269		
T2DM, type 2 diabetes mellitus; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UCRP, ubiquitin cross-reactive protein.			
p values were calculated by χ^2 test for continuous variables and student's t test for categorical variables.			
Bold indicate that p < 0.05 indicates statistical difference.			

Analysis for association between SOCS2 **variants and T2DM susceptibility**

As shown in Table 2, three SNPs in *SOCS2* (rs3825199, rs11107116 and rs10492321) were associated with the increased risk of T2DM occurrence. The risk genotype of rs3825199-AG, rs11107116-GT and rs10492321-TA were more prevalent in patients than controls exhibiting a higher susceptibility to T2DM: for rs3825199, AG vs AA, OR = 1.44, 95% CI: 1.11–1.88, $p = 0.007$; for rs11107116, GT vs GG, OR = 1.39, 95% CI: 1.07–1.81, $p = 0.014$; for rs10492321, TA vs TT, OR = 1.48, 95% CI: 1.13–1.93, $p = 0.004$, respectively. In addition, the elevated risk association with T2DM was also observed in the dominant model (rs3825199, OR = 1.35, 95% CI: 1.05–1.73, $p = 0.020$; rs11107116, OR = 1.30, 95% CI: 1.01–1.67, $p = 0.038$; and rs10492321, OR = 1.40, 95% CI: 1.09–1.81, $p = 0.009$).

Table 2
Correlation between *SOCS2* variants and T2DM risk

SNPs ID	Models	Genotype	Case	Control	Adjusted by age and gender	
					OR (95%CI)	*p*
rs3825199	Allele	A	646	676	1	
		G	354	326	1.14 (0.94–1.37)	0.176
	Genotype	AA	200	237	1	
		AG	246	202	**1.44 (1.11–1.88)**	**0.007**
		GG	54	62	1.03 (0.68–1.56)	0.881
	Dominant	AG-GG vs AA			**1.35 (1.05–1.73)**	**0.020**
	Recessive	GG vs AA-AG			0.86 (0.58–1.26)	0.437
	Log-additive	AA + AG + GG			1.14 (0.94–1.37)	0.177
rs11107116	Allele	G	653	679	1	
		T	347	323	1.12 (0.93–1.35)	0.243
	Genotype	GG	205	238	1	
		GT	243	203	**1.39 (1.07–1.81)**	**0.014**
		TT	52	60	1.01 (0.66–1.52)	0.978
	Dominant	GT-TT vs GG			**1.30 (1.01–1.67)**	**0.038**
	Recessive	TT vs GG-GT			0.85 (0.58–1.27)	0.430
	Log-additive	GG + GT + TT			1.12 (0.93–1.35)	0.243
rs10492321	Allele	T	617	656	1	
		A	383	346	1.18 (0.98–1.41)	0.080
	Genotype	TT	183	224	1	
		TA	251	208	**1.48 (1.13–1.93)**	**0.004**
		AA	66	69	1.17 (0.79–1.73)	0.428
	Dominant	TA-AA vs TT			**1.40 (1.09–1.81)**	**0.009**
	Recessive	AA vs TT-TA			0.95 (0.66–1.37)	0.791
	Log-additive	TT + TA + AA			1.18 (0.98–1.41)	0.081

SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ means the data is statistically significant.

Stratified analysis for the relationship of SOCS2 variants to T2DM risk

Stratified analyses were performed to explore the relationship between *SOCS2* SNPs and T2DM risk factors, including age, gender, smoking, drinking and BMI. Stratified by gender, rs3825199, rs11107116 and rs10492321 conferred to the increased T2DM risk among males not females under the allele, genotype, dominant and additive models (Suppl_Table 3). Based on age, the study population was stratified into two groups: those older than 60 years and those younger than or equal to

60 years. No significant relation of *SOCS2* variants to T2DM risk in those older than 60 years was observed. While, high risk association was found for rs3825199, rs11107116 and rs10492321 in subjects aged ≤ 60 years (Suppl_Table 3).

In smoker, increased risk of T2DM developing was found for rs10492321 polymorphism (Suppl_Table 4). Among non-smoker, the risk effect of rs3825199, rs11107116 and rs10492321 on T2DM occurrence was observed under the genotype and dominant models. In drinker, rs10859525 was a protective factor for T2DM developing, while rs3825199 increased T2DM susceptibility. In non-drinker, a trend of higher risk of developing T2DM was also found for subjects with the AG/AG-GG genotypes of rs3825199, GT genotype of rs11107116 and TA/TA-AA genotypes of rs10492321 (Suppl_Table 4). Among subjects with BMI > 24 kg/m^2, rs10859563 was associated with the reduced T2DM predisposition. In subjects with BMI ≤ 24 kg/m^2, rs3825199-AG genotype and rs11107116-GT genotype had 1.66- and 1.64- fold increased risk of developing T2DM than their reference genotype (Suppl_Table 5).

Analysis for association between SOCS2 variants and diabetic nephropathy or diabetic retinopathy in T2DM patients

We next investigated the association between *SOCS2* variants and diabetic nephropathy or diabetic retinopathy in T2DM patients (Table 3). We found that rs10859525 (G vs A, OR = 0.68, $p = 0.017$; AA + AG + GG, OR = 0.71, $p = 0.040$) and rs10859563 (C vs G, OR = 0.72, $p = 0.022$; CC vs GG, OR = 0.51, $p = 0.024$; GC-CC vs GG, OR = 0.61, $p = 0.016$; GG + GC + CC, OR = 0.70, $p = 0.013$) had the reduced risk of diabetic nephropathy in T2DM patients. Moreover, the protective effect of rs10859525 on the risk of diabetic retinopathy in T2DM patients was observed under the allele (OR = 0.63, $p = 0.042$), genotype (OR = 0.53, $p = 0.034$), dominant (OR = 0.53, $p = 0.025$) and additive (OR = 0.63, $p = 0.042$) models.

Table 3
Association of *SOCS2* variants with diabetic nephropathy and diabetic retinopathy in T2DM patients

SNP ID	Model	DN vs No DN		DR vs No DR	
		OR (95%CI)	*p*	OR (95%CI)	*p*
rs10859525	G vs A	0.68 (0.50–0.94)	**0.017**	0.63 (0.41–0.99)	**0.042**
	AG vs AA	0.74 (0.49–1.12)	0.152	0.53 (0.29–0.95)	**0.034**
	GG vs AA	0.47 (0.20–1.12)	0.087	0.53 (0.19–1.50)	0.232
	AG-GG vs AA	0.69 (0.46–1.03)	0.069	0.53 (0.31–0.92)	**0.025**
	GG vs AA-AG	0.53 (0.23–1.25)	0.147	0.70 (0.25–1.91)	0.480
	AA + AG + GG	0.71 (0.51–0.99)	**0.040**	0.63 (0.40–0.98)	**0.042**
rs10859563	C vs G	0.72 (0.55–0.96)	**0.022**	1.01 (0.69–1.48)	0.965
	GC vs GG	0.65 (0.42–1.00)	0.051	1.04 (0.56–1.92)	0.899
	CC vs GG	0.51 (0.28–0.91)	**0.024**	1.01 (0.44–2.32)	0.980
	GC-CC vs GG	0.61 (0.40–0.91)	**0.016**	1.03 (0.58–1.86)	0.912
	CC vs GG-GC	0.65 (0.38–1.11)	0.113	0.99 (0.47–2.06)	0.969
	GG + GC + CC	0.70 (0.53–0.93)	**0.013**	1.01 (0.68–1.51)	0.956

T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; DN, diabetic nephropathy; DR, diabetic retinopathy; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ indicates statistical significance.

Association of SOCS2 variants in T2DM patients with coronary heart disease or hypertension versus controls

Additionally, the association of *SOCS2* variants with the combined effect of T2DM and coronary heart disease/hypertension was examined (Table 4). We found that rs3825199 (GG vs AA, OR = 0.35, $p = 0.035$; and GG vs AA-AG, OR = 0.31, $p = 0.014$), rs11107116 (TT vs GG, OR = 0.28, $p = 0.020$; and TT vs GG-GT, OR = 0.25, $p = 0.009$) and rs10859563 (C vs G, OR = 0.70, $p = 0.015$; CC vs GG, OR = 0.48, $p = 0.020$; and GG + GC + CC, OR = 0.70, $p = 0.018$) had the reduced risk for T2DM patients with coronary heart disease compared with healthy controls. However, there was no significant association for T2DM patients with hypertension.

Table 4
Association of *SOCS2* variants in T2DM patients with coronary heart disease or hypertension versus controls

SNP ID	Model	T2DM patients with CHD		T2DM patients with hypertension	
		OR (95%CI)	*p*	OR (95%CI)	*p*
rs3825199	G vs A	0.90 (0.66–1.21)	0.470	1.12 (0.90–1.4)	0.303
	AG vs AA	1.33 (0.88–2.01)	0.182	1.38 (1.00–1.91)	0.050
	GG vs AA	0.35 (0.13–0.93)	**0.035**	1.02 (0.61–1.70)	0.951
	AG-GG vs AA	1.11 (0.74–1.66)	0.617	1.30 (0.96–1.77)	0.095
	GG vs AA-AG	0.31 (0.12–0.79)	**0.014**	0.86 (0.53–1.40)	0.548
	AA + AG + GG	0.88 (0.65–1.20)	0.419	1.12 (0.89–1.40)	0.344
rs11107116	T vs G	0.87 (0.65–1.18)	0.381	1.11 (0.89–1.39)	0.353
	GT vs GG	1.29 (0.85–1.95)	0.225	1.33 (0.96–1.84)	0.083
	TT vs GG	0.28 (0.10–0.82)	**0.020**	1.00 (0.60–1.68)	0.999
	GT-TT vs GG	1.07 (0.72–1.61)	0.738	1.26 (0.93–1.71)	0.144
	TT vs GG-GT	0.25 (0.09–0.71)	**0.009**	0.87 (0.53–1.42)	0.566
	GG + GT + TT	0.85 (0.62–1.16)	0.309	1.10 (0.88–1.38)	0.414
rs10859563	C vs G	0.70 (0.53–0.93)	**0.015**	0.93 (0.75–1.15)	0.489
	GC vs GG	0.75 (0.48–1.16)	0.190	0.82 (0.58–1.16)	0.258
	CC vs GG	0.48 (0.25–0.89)	**0.020**	0.87 (0.57–1.34)	0.533
	GC-CC vs GG	0.66 (0.44–1.00)	0.052	0.84 (0.61–1.15)	0.272
	CC vs GG-GC	0.56 (0.31–1.00)	0.050	0.98 (0.67–1.43)	0.913
	GG + GC + CC	0.70 (0.52–0.94)	**0.018**	0.92 (0.74–1.14)	0.435

T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; CHD, coronary heart disease; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ indicates statistical significance.

The influence of the combined SNPs on the susceptibility to T2DM

Pairwise linkage disequilibrium (LD) and haplotype analyses were conducted for *SOCS2* variants. Figure 1 revealed a LD block in *SOCS2* SNPs (rs3825199, rs11107116 and rs10492321), and the D' values of rs3825199-rs11107116, rs11107116-rs10492321 and rs3825199-rs10492321 were 0.99, 0.99 and 0.99, respectively. The frequencies of haplotypes (GTA, AGA and AGT) and the result of haplotype analysis was showed in Table 5. No significant association between *SOCS2* haplotypes and

T2DM risk in the whole population, whereas GTA and AGT haplotypes conferred to the increased T2DM risk in males (OR = 1.29, 95% CI: 1.03–1.60, $p = 0.026$ and OR = 1.34, 95% CI: 1.08–1.66, $p = 0.008$).

Table 5
Correlation of *SOCS2* haplotypes with T2DM risk in the whole population and males

SNP	Haplotype	Frequency		χ^2	p^a	Adjusted by age and gender	
		Case	Control			OR (95% CI)	p^b
Whole population							
rs3825199|rs11107116|rs10492321	GTA	0.346	0.319	1.60	0.206	1.13 (0.94–1.36)	0.206
rs3825199|rs11107116|rs10492321	AGA	0.028	0.023	0.51	0.474	1.23 (0.70–2.17)	0.468
rs3825199|rs11107116|rs10492321	AGT	0.383	0.349	2.45	0.118	1.15 (0.96–1.38)	0.121
Males							
rs3825199|rs11107116|rs10492321	GTA	0.360	0.305	5.04	**0.025**	**1.29 (1.03–1.60)**	**0.026**
rs3825199|rs11107116|rs10492321	AGA	0.028	0.021	0.73	0.392	1.35 (0.68–2.69)	0.388
rs3825199|rs11107116|rs10492321	AGT	0.398	0.330	7.24	**0.007**	**1.34 (1.08–1.66)**	**0.008**

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ respects the data is statistically significant.

The MDR analysis was employed to assess the influence of SNP-SNP interaction in *SOCS2*. Table 6 displayed the results obtained from MDR analysis for one- to five-locus models. *SOCS2* rs10492321 was the best single factor model (Testing accuracy = 0.533; cross–validation consistency = 6/10). Moreover, the best combination was five-locus model (Testing accuracy = 0.33; cross–validation consistency = 10/10).

Table 6
SNP–SNP interaction models in *SOCS2* for T2DM risk by MDR analysis

Best combination	Training Bal. Acc.	Testing Bal. Acc.	CVC	χ^2	*p*	OR (95% CI)
rs10492321	0.545	0.533	6/10	7.80	**0.0052**	1.42 (1.11–1.83)
rs10859525,rs10492321	0.556	0.526	5/10	11.70	**0.0006**	1.55 (1.20–1.98)
rs10859525,rs10492321,rs10859563	0.577	0.517	5/10	22.02	**< 0.0001**	1.82 (1.42–2.34)
rs10859525,rs11107116,rs10492321,rs10859563	0.587	0.513	5/10	28.46	**< 0.0001**	1.98 (1.54–2.55)
rs10859525,rs3825199,rs11107116,rs10492321,rs10859563	0.589	0.533	10/10	29.81	**< 0.0001**	2.01 (1.56–2.59)

MDR, multifactor dimensionality reduction; Bal. Acc., balanced accuracy; CVC, cross–validation consistency; OR, odds ratio; CI, confidence interval.

p values were calculated using χ^2 tests.

Bold indicate that $p < 0.05$ indicates statistical significance.

Discussion

In our study, we detected the potential effect of *SOCS2* genetic variants on T2DM incidence and found that three SNPs in *SOCS2* (rs3825199, rs11107116 and rs10492321) were associated with increased the risk towards T2DM occurrence in the Chinese Han population. Specially, the contribution of *SOCS2* polymorphisms to T2DM risk might be associated with age, gender, lifestyle (smoking and drinking) and BMI factors. Among T2DM patients, rs10859525 and rs10859563 had the reduced risk of diabetic nephropathy, and rs10859525 had the protective effect on the risk of diabetic retinopathy. Additionally, rs3825199, rs11107116 and rs10859563 had the reduced risk for T2DM patients with coronary heart disease compared with healthy controls. Moreover, GTA and AGT haplotypes had higher T2DM susceptibility among males. The results combined SNPs revealed that rs10492321 was the best single factor model and the best combination was five-locus model. This is the first study reporting the association between *SOCS2* variants and T2DM predisposition in the Chinese Han population.

SOCS2 gene, located in chromosome 12q22, has emerged as the negative regulator on insulin and growth hormone pathways. Several studies reported that *SOCS2* genetic variants were associated with some diseases, including acromegaly, growth hormone deficiency and extreme obesity [15–17]. *SOCS2* gene as risk gene was identified to be associated with molecular networks for T2DM [18]. The increasing evidence reveals that SOCS2 protein plays an important role in T2DM development, involving regulation of the insulin signaling and pancreatic β-cell function [19]. However, little is known about the impact of *SOCS2* genetic variants on T2DM occurrence. Only one study reported that *SOCS2* SNPs were related to the risk of T2DM in the Japanese [20]. No previous studies have reported an association between these SNPs (rs10859525, rs3825199, rs11107116, rs10492321, and rs10859563) and T2DM risk. Here, our findings firstly demonstrated that *SOCS2* rs3825199, rs11107116 and rs10492321 might be risk factors towards increased T2DM predisposition among the Chinese Han population. Our study supported that *SOCS2* variants might contribute to the pathogenesis of T2DM.

T2DM is the result of combined effects of genetic background, gender, aging, lifestyle, obesity and other factors [21]. Age and gender differences in risk, onset and progress of T2DM was found in previous studies [22, 23]. Stratified by gender and age,

rs3825199, rs11107116 and rs10492321 conferred to the increased T2DM risk among males not females, and among the subjects aged at ≤ 60 years. Haplotype analysis revealed that GTA and AGT haplotypes had higher T2DM susceptibility among males. Our finding suggested that the association between *SOCS2* polymorphisms and T2DM susceptibility was gender- and age- specific. In addition, active smoking is reported to be a risk factor for T2DM and moderate alcohol consumption is related to a reduced T2DM risk [24, 25]. Epidemiological studies showed that obesity had the important role on T2DM occurrence, and the incidence of nearly 90% of T2DM patients is associated with being overweight [26, 27]. Our results displayed that rs3825199 and rs11107116 conferred to the higher T2DM susceptibility in non-smokers. *SOCS2* rs10859525 displayed a protective effect for T2DM risk among drinkers, while rs11107116 and rs10492321 had the higher risk for T2DM developing in non-drinkers. Stratified by BMI, rs3825199 and rs11107116 increased T2DM susceptibility in subjects with BMI ≤ 24 kg/m^2, whereas rs10859563 was related to the reduced T2DM risk among subjects with BMI > 24 kg/m^2. These results showed that smoking, alcohol drinking and BMI might contribute to the relationship of *SOCS2* polymorphisms to T2DM risk.

With the increase in incidence of T2DM, a rise in prevalence of secondary comorbidities including diabetic nephropathy and diabetic retinopathy is anticipated[28]. Our results showed that rs10859525 and rs10859563 had the reduced risk of diabetic nephropathy, and rs10859525 had the protective effect on the risk of diabetic retinopathy among T2DM patients. Considering that coronary artery diseases and hypertension are related to the occurrence and development of T2DM [29, 30], we examined the association of *SOCS2* variants with the combined effect of T2DM and coronary heart disease/hypertension. We found that rs3825199, rs11107116 and rs10859563 had the reduced risk for T2DM patients with coronary heart disease compared with healthy controls, but not significantly associated with hypertension. However, our results should be necessary to confirm the results in a larger sample size.

Inevitably, this study has several limitations. First, all subjects were Han Chinese population recruited from the same hospital, and it is may not be generalized to other ethnicities. Second, only five SNPs chosen for exploration in our study cannot represent the genetic polymorphisms in *SOCS2* thoroughly. Third, the sample size for stratification analysis is insufficient, hence we cannot exclude the false positive results. Finally, the functional and mechanistic studies of *SOCS2* polymorphisms on T2DM were not performed. Therefore, future studies with large scale and multicenter are needed to authenticate our finding, and studies for multiple SNPs and functional effect of SNPs on *SOCS2* are also desired.

In summary, this is the first study reported that rs3825199, rs11107116 and rs10492321 in *SOCS2* were conferred to the increased risk towards T2DM occurrence in the Chinese Han population. Specially, the contribution might be associated with age, gender, lifestyle (smoking and drinking) and BMI factors. *SOCS2* polymorphisms also associated with the reduced risk for T2DM patients with diabetic nephropathy, diabetic retinopathy and coronary heart disease. Furthermore, GTA and AGT haplotypes had higher T2DM susceptibility among males, and risk accumulation effect on T2DM incidences was found in SNP-SNP interaction. Our findings may help increase the understanding of *SOCS2* genetic polymorphisms in the pathogenesis T2DM in the Chinese Han population.

Materials And Methods

Study Subjects

The study group consisted of 500 T2DM patients and 501 healthy volunteers from the First Affiliated Hospital of Xi'an Jiaotong University. All enrolled subjects were unrelated Chinese Han ethnicity. T2DM patients were diagnosed as fasting plasma glucose ≥ 7.0 mmol/L according to the WHO diagnostic criteria. Patients with type 1 diabetes, gestational diabetes, malignancy, acute infections, inflammation, other chronic diseases or other endocrine disease, and not receiving any drugs like antidiabetics were excluded. The controls were age and sex matched, no history of diabetes and other chronic diseases. Information on demographics, life style factors and clinical characteristics of the participants was obtained from standardized questionnaires and medical record, including age, sex, body mass index (BMI), smoking, drinking, fasting blood glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol

(HDL-C), urea, creatinine, serum uric acid, glycated hemoglobin, and insulin (Table 1). The protocol of this study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University and conformed to the Declaration of Helsinki. All individuals provided written informed consent prior to sample collection.

SNP Genotyping

Five milliliters of venous blood samples were collected in EDTA-coated tubes and genomic DNA was obtained from peripheral leukocytes by GoldMag DNA isolation Kit (GoldMag Co. Ltd., Xi'an, China). Five SNPs (rs10859525, rs3825199, rs11107116, rs10492321, and rs10859563) in *SOCS2* were selected based on minor allele frequency (MAF) of each SNP > 5%, call rates > 95% and Hardy–Weinberg equilibrium (HWE) $p > 0.05$ (Suppl_Table 1). Genotyping of *SOCS2* polymorphism was determined by Agena MassARRAY system (Agena, San Diego, CA, USA) with incorporated software for primer design (Suppl_Table 2) and data management[31, 32]. The accordance rate of about 5% of the samples selected for replication was 100%.

Statistical Analysis

Differences in the distribution of demographic and clinical characteristics between T2DM patients and the control group were analyzed using the chi-square test or Student t-test, as appropriate. The deviation from HWE for each SNP was determined using goodness-of-fit χ^2 tests in controls. Logistic regression analysis after adjusting for age and gender was utilized to investigate the relationship of SNPs to T2DM predisposition by calculating the odds ratio (OR) and 95% confidence intervals (95% CI) [33]. The influence of combined SNPs on T2DM susceptibility was determined using haplotype analysis and multifactor dimensionality reduction (MDR) analysis. Data analyses were performed using IBM® SPSS version 18.0 (SPSS Inc., Chicago, IL), PLINK version 2.1.7, Haploview 4 version.2 software and MDR version 3.0.2 software. A p-value < 0.05 was considered as the threshold for statistically significant.

Declarations

Acknowledge

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Disclosure Statement

The authors declare that they have no conflict of interest.

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Authors' contributions

The work presented here was carried out in collaboration between all authors. Juan Pan and Rui Tong carried out the molecular genetic studies and drafted the manuscript. Qing Deng, Yanni Tian and Ning Wang designed the methods and experiments, performed the statistical analyses and interpreted the results. Yanqi Peng, Sijia Fei and Wei Zhang designed primers and performed the SNP genotyping experiments. Jiaqi Cui, Chaoying Guo and Juanchuan Yao worked on associated data collection and their interpretation. Cui Wei and Jing Xu conceived of the study, participated in the design and coordination of the study, and funded the study. All authors read and approved the final manuscript.

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34. Legends.

Figures

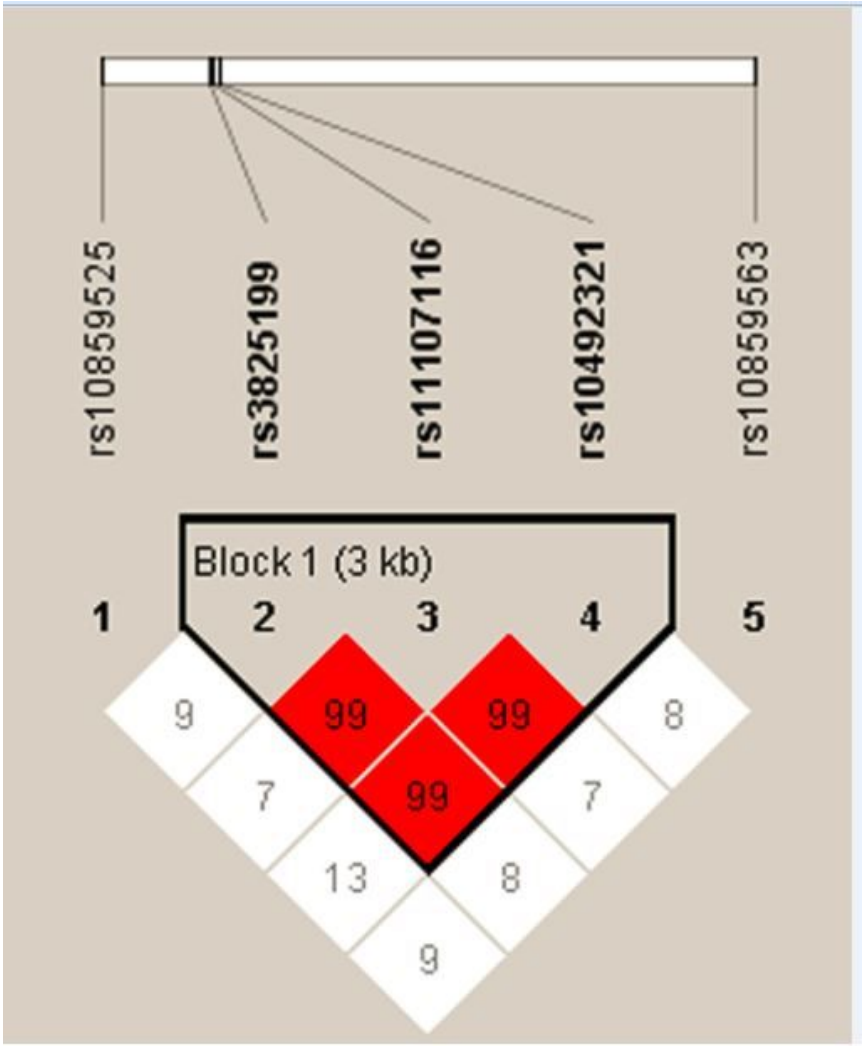

Figure 1

Haplotype block map for the linkage disequilibrium between the genetic variants in SOCS2. The numbers of matrices represent the D' value for the SNP pairs.

Figure 2

The interaction dendrogram for SOCS2 SNP-SNP interaction.

Supplementary Files

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