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ORIGINAL ARTICLE

Relationship between type 2 diabetes mellitus and a novel polymorphism C698T in *C5L2* in the Chinese Han population

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Abstract In a previous study, we reported a novel single nucleotide polymorphism (SNP) 698C>T (P233L) in the gene, *C5L2*. This gene has been demonstrated to encode a functional receptor of acylation-stimulating protein (ASP), a G-protein-coupled receptor (GPCR), that has been shown to influence insulin secretion in cultured pancreatic islet cells in vitro and is a stimulator of triglyceride synthesis and glucose transport in vivo. In this study, we evaluated the relationship between this novel *C5L2* SNP and development of type 2 diabetes mellitus (T2DM) in the Chinese Han population. A case–control study examining Chinese Han T2DM patients ($n = 554$) and healthy controls ($n = 648$) was performed to investigate the role of the 698C>T (P233L) *C5L2* polymorphism. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to determine expression of this SNP. Heterozygote carriers of the 698CT *C5L2* genotype were more frequent among T2DM patients (13.5%) than controls (3.2%; $P < 0.001$). The frequency of 698CT heterozygote carriers was significantly higher in women (12.8%) than in male subjects (5.7%, $P < 0.001$). The odds ratio (OR) of T2DM for 698CT carriers was 4.675 [95% confidence interval (CI) 2.840–7.694]. After adjustment of confounding factors such as age, sex, smoking, drinking, hypertension, and triglyceride (TG), total cholesterol, high-density lipoprotein, and low-density lipoprotein levels, the difference remained significant ($P < 0.001$, OR 5.556, 95% CI 2.444–12.630). Furthermore, the diabetic 698CT carriers displayed an increase in their serum TG level. However, there were no significant differences observed in any of the parameters measured in the control group. We conclude that the 698CT genotype of *C5L2* may be an influencing genetic factor for T2DM in the Chinese Han population. These findings also indicate that heterozygous expression of 698CT *C5L2* may contribute to metabolic abnormalities.

Keywords Acylation-stimulating protein · C5L2 · Han population · Type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease for which the etiology and pathogenesis are multifactorial and arise from the complex interplay of genetic and environmental factors [1–3]. The prevalence of diabetes is high and it is associated with high rates of morbidity and mortality. By the year 2030, an estimated 350 million individuals worldwide will suffer from diabetes [4], with approximately 100 million people with T2DM in China alone [5]. Identifying high-risk groups will likely allow for effective prevention and treatment and is a topic of great interest in diabetic research. A large number of genes have emerged from Asian genome-wide association studies (GWAS) that are likely factors in diabetes susceptibility and development, including those encoding variants of potassium voltage-gated channels, KQT-like subfamily member 1 (*KCNQ1*) [6, 7], protein tyrosine phosphatase, receptor type D (*PTPRD*) [8], serine racemase (*SRR*) [8], and peptidase D (*PEPD*) [9].

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C5L2, a G-protein-coupled receptor (GPCR), also known as GPR77, is a identified functional receptor for acylation-stimulating protein (ASP; also known as C3a des-Arg) [10–12]. ASP is an adipokine that acts as an anabolic hormone in the adipose tissue milieu and binds to C5L2 to stimulate triglyceride synthesis (TGS) and glucose transport [10, 11]. In a previous study [13], we identified a novel single nucleotide polymorphism (SNP), 698C>T that causes an amino acid change from proline to leucine at codon 233. In this study, we sought to examine the relationship between this novel SNP and T2DM in the Chinese Han population.

Methods

Ethics statement

This study was conducted according to the Declaration of Helsinki, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

Study population samples

Participants diagnosed with T2DM were recruited at the First Teaching Hospital of Xinjiang Medical University from 2007 to 2010. The healthy participants were selected from the cardiovascular risk survey (CRS). The CRS has been described previously [14]. In this study, 554 patients with T2DM and 648 healthy control participants were enrolled. All study participants were of Han Chinese ethnicity and were from the same geographic area in Xinjiang. Demographic data and subject characteristics, including hypertension, diabetes mellitus, smoking, alcohol consumption, and serum cholesterol, were collected for all study participants. T2DM was diagnosed according to the 1999 World Health Organization diagnostic criteria [15]. In addition, subjects with a history of T2DM, those receiving active treatment with insulin or an oral anti-diabetic agent were considered to have T2DM. Hyperlipidemia was defined as cholesterol level $\geq$5.2 mmol/l or triglyceride level $\geq$2.26 mmol/l. Hypertension, smoking, alcohol consumption, height, and body weight were defined as previously described [16–18]. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

Biochemical analysis

Serum was isolated from blood samples within 30 min and stored at -80°C until analyzed. The plasma concentration of total cholesterol (TC), triglycerides (TG), glucose, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), creatinine (Cr), and uric acid were evaluated using standard methods in the Central Laboratory of First Affiliated Hospital of Xinjiang Medical University as described previously [16–18].

Sample DNA extraction

Blood samples were collected from all participants using a standard venipuncture technique and EDTA-containing tubes. DNA was extracted from the peripheral blood leukocytes using a whole blood genome extraction kit (Beijing Bioteke Corporation, Beijing, China. http://bioteke.technew.cn) as previously described [14, 16].

Genotyping of 698C>T polymorphism

The genotyping method was described in the previous study [13]. Briefly, genotyping for the 698C>T *C5L2* variant was confirmed using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. The forward primer was 5′-ACT ACG GCG GCT CCT CCA-3′ and the reverse primer was 5′-TGT GAG CGA GGG CAA GGC-3′. The annealing temperature was 63°C. The PCR product (15 μl) was incubated overnight with *Bam*HI (5 U) in a total volume of 25 μl at 37°C; the resulting fragments were separated on a 3.0% agarose gel. Absence of the 698C>T variant created a *Bam*HI site that produced two fragments, a 133 and a 153 bp fragment (Fig. 1). Sequenced genomic DNA was used as a positive control.

Statistical analyses

Statistical analyses were conducted using SPSS version 17.0 (SPSS, Chicago, IL, USA). Continuous data are shown as mean ± SD, and the differences between T2MD patients and control subjects were assessed using independent-sample *t* test. The Hardy–Weinberg equilibrium and differences in enumeration data between T2MD

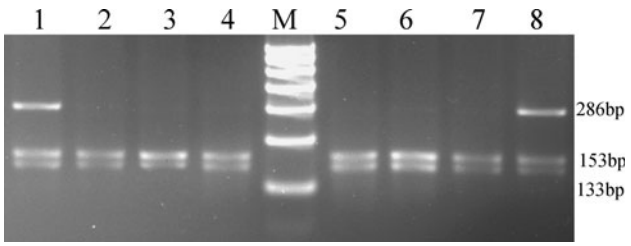

Fig. 1 Restriction fragment length polymorphism analysis for determination of genotype. The CT genotype shows three bands of 286, 153, and 133 bp (1 and 8); The CC genotype shows two bands of 153 and 133 bp (2, 3, 4, 5, 6, and 7, respectively)

patients and control subjects were analyzed using the Chi-square analyses. Allelic frequency distribution was analyzed using 2 × 2 contingency tables using a 5% significance level. Logistic regression analyses were used to assess the contribution of major risk factors.

Results

Participant characteristics

The clinical characteristics of the T2DM patients ($n = 554$) and healthy control subjects ($n = 648$) are shown in Table 1. A number of variables were significantly different between the T2DM patients and the control subjects: hypertension; hyperlipidemia; BMI; SBP; DBP and the serum level of HDL-C, LDL-C, TC and TG (all $P < 0.05$). There was no significant difference in the serum concentration of BUN, smoking, alcohol consumption, age, and sex between the T2DM patients and healthy control subjects (all $P > 0.05$).

Distribution of the 698C>T in T2MD patients and controls

The distribution of the 698C>T SNP was not significantly different between the T2DM group and the control group ($P > 0.05$). The frequency of heterozygous expression of 698C>T *C5L2* was significantly higher in T2DM patients than in control subjects (13.5% vs. 3.2%; $P < 0.001$) (Table 2). The frequency of the T allele in T2DM patients was higher than in control subjects (6.0% vs. 2.0%; $P < 0.001$) (Table 2). The frequency of heterozygote carriers of 698CT *C5L2* was significantly higher in women (12.8%) than men (5.7%) ($\chi^2 = 18.315$, $P < 0.001$). The odds ratio (OR) for carriers of the 698CT genotype for T2DM was 4.675 [95% confidence interval (CI) 2.840–7.694]. After adjusting for confounding factors such as hypertension, hyperlipidemia, smoking, systolic blood pressure, diastolic blood pressure, and HDL-C, LDL-C, TG, TC, and BUN serum levels, the difference remained significant ($P < 0.001$, OR 5.556, 95% CI 2.444–12.630) (Table 3).

Cardiovascular risk factors associated with the CC and CT genotype in T2DM patients and healthy control subjects

We further compared differences in clinical parameters associated with expression of the CC or CT genotype. CT carriers with diabetes had a higher serum level of TG than patients expressing the CC genotype. There was no difference in BMI or LDL, TC, or HDL levels. In addition, in the control subjects, there were no significant differences between the parameters evaluated and the two genotypes (Table 4).

Discussion

Numerous factors such as obesity, hypertension, and hyperlipidemia have been reported to influence the pathogenesis of T2DM, which is thought to be a multifactorial disease similar to essential hypertension and chronic heart disease (CHD). Most recently, the association of gene polymorphisms with T2DM has been a major focus of research.

Recently, we reported a novel polymorphism of *C5L2* and its association with coronary artery disease (CAD) [13]. This nucleotide alteration at codon 233 results in a proline to leucine coding change. A number of genes

Table 1 Characteristics of the participants

Characteristics	Control ($n = 648$)	T2DM ($n = 554$)	χ^2 or t	P value
Age, year, mean (SD)	59.35 (11.28)	58.86 (11.33)	0.748	0.455
Sex, female (%)	220 (33.95)	186 (33.57)	0.019	0.470
Hypertension, *n* (%)	353 (54.48)	336 (60.65)	3.908	0.027
Smoking, *n* (%)	291 (44.90)	267 (48.19)	1.254	0.144
Drinking, *n* (%)	178 (27.47)	164 (29.60)	0.639	0.231
Hyperlipemia, *n* (%)	37 (5.71)	309 (55.78)	510.861	<0.001
BMI, kg/m^2, mean (SD)	25.10 (3.34)	26.15 (3.89)	−4.937	<0.001
SBP, mmHg, mean (SD)	136.03 (25.32)	141.02 (23.19)	−3.195	0.001
DBP, mmHg, mean (SD)	84.11 (16.17)	87.56 (14.91)	−3.45	0.001
TG, mmol/l, mean (SD)	1.26 (0.44)	2.65 (2.19)	−15.176	<0.001
TC, mmol/l, mean (SD)	4.18 (0.99)	4.59 (1.24)	−6.188	<0.001
HDL-C, mmol/l, mean (SD)	1.28 (0.38)	1.19 (0.42)	3.60	<0.001
LDL-C, mmol/l, mean (SD)	2.68 (0.94)	2.81 (0.96)	−1.2.28	0.023
BUN, mmol/l, mean (SD)	5.04 (1.59)	5.13 (1.64)	−0.964	0.335

Table 2 Distribution of genotypes and alleles of C5L2 gene

	Group	*N*	Genotype (*n*, %)		*P*	Allele (Frequency)		*P*
			CC	CT		C	T	
Han population	Control	648	627 (96.76)	21 (3.24)	<0.001	0.98	0.02	<0.001
	T2DM	554	479 (86.46)	75 (13.54)		0.94	0.06	

Table 3 Results of logistic analysis

	B	S.E.	Wald	*P*	OR	95% C.I.
698C>T, *n*	1.715	0.419	16.750	<0.001	5.556	2.444–12.630
Hyperlipemia, *n*	2.872	0.277	107.685	<0.001	17.664	10.269–30.383
TG, mmol/l	1.233	0.185	44.507	<0.001	3.433	2.390–4.933
HDL-C, mmol/l	−1.181	0.296	15.901	<0.001	0.307	0.172–0.549
Constant	−3.870	0.658	34.574	<0.001	0.021	

Table 4 Cardiovascular risk factors between CC and CT genotype in control and diabetes, respectively

	Control			Diabetes		
	CC	CT	*P*	CC	CT	*P*
Smoking, *n*, %	282 (45.12)	9 (42.86)	0.510	241 (50.42)	26 (34.21)	0.006
Hypertension, *n*, %	341 (54.56)	12 (57.14)	0.511	297 (47.52)	39 (51.32)	0.051
SBP, mmHg	135.87 ± 25.17	141.63 ± 30.1	0.371	139.8 ± 22.61	148.03 ± 25.29	0.008
DBP, mmHg	84.02 ± 16.11	87.38 ± 18.19	0.413	86.97 ± 14.57	91.33 ± 16.56	0.028
TC, mmol/l	4.19 ± 0.98	3.832 ± 0.95	0.443	4.549 ± 1.10	4.85 ± 1.85	0.053
TG, mmol/l	1.257 ± 0.445	1.3347 ± 0.395	0.483	2.162 ± 1.77	2.724 ± 2.235	0.041
HDL-C, mmol/l	1.28 ± 0.37	1.21 ± 0.425	0.462	1.181 ± 0.417	1.257 ± 0.463	0.156
LDL-C, mmol/l	2.68 ± 0.944	2.62 ± 0.90	0.073	2.822 ± 0.980	2.748 ± 0.980	0.179
BMI, kg/m^2	25.08 ± 3.370	25.95 ± 2.122	0.251	26.09 ± 4.01	26.55 ± 3.07	0.343

involved in glucose metabolism are associated with increased risk of developing CHD and/or T2DM. For example, Huang et al. [19] reported that a −607 C/A polymorphism in the promoter of the IL-18 gene was associated with an increase in the 2 h post-loading plasma glucose level in Chinese subjects. Karadeniz et al. [20] demonstrated that a 2518G/A polymorphism in the gene encoding monocyte chemoattractant protein-1 (MCP-1) was related to T2DM in patients with nephropathy in Turkey. These recent findings have aided in dissecting the molecular pathophysiology of CHD and T2DM and in identifying novel therapeutic targets [21].

In the current report, we demonstrated that a polymorphism of *C5L2* occurs at higher frequency in Chinese Han patients with T2DM than in control subjects and expression of 698C>T is associated with a higher risk of T2DM (OR 4.675). Logistic regression analyses suggested that, after adjusting for confounding factors, the CT genotype remained a significant risk factor for developing T2DM (OR 5.556), suggesting that *C5L2* is associated with diabetes. Marcil et al. [22] identified a novel variant of *C5L2*, S323I. This variant was associated with familial combined hyperlipidemia (FCHL) in a French–Canadian family. Individuals expressing this variant often displayed numerous features common to a metabolic syndrome, such as insulin resistance [23, 24], hypertension [25], and low HDL cholesterol [26, 27]. Given that a metabolic syndrome may lead to an increase risk of developing T2DM [28], it might be reasonable to assume that FCHL patients are predisposed to T2DM [29].

The results in this study also indicated that the frequency of the heterozygote carriers of the 698CT genotype of *C5L2* was significantly higher in women than in men. It has been reported that the distribution and function of adipose tissue is influenced by sex steroids (estrogen, testosterone, and progesterone) and there are differences in fat distribution between men and women [30]. Muraki et al. [31] discovered that estrogen modulates insulin-stimulated glucose uptake by mature adipocytes via the estrogen receptor alpha. In addition, estrogen can mediate increases in plasma ASP and C3, factors associated with both obesity and insulin resistance [10].

Diabetic CT carriers have increased TG, while non-diabetic, control CT carriers did not display such a

difference. Notably, ASP binds to C5L2 resulting in a net accumulation of adipose TG stores [10, 11, 32], and initiating a cascade of events that includes phosphorylation, β-arrestin-2-GFP translocation, and receptor internalization [15]. Activation of the receptor initiates a variety of signaling pathways that include glucose transporter translocation [10, 32, 33], leading to increased glucose transport. Gain-of-function studies in HEK-293 (HEK-hC5L2) cells stably transfected with human *C5L2* [34] showed that, following ASP stimulation, glucose transport was significantly increased, resulting in net accumulation of insulin sensitivity; this did not occur when non-transfected cells were exposed to ASP. ASP-*C5L2* mediated increases in glucose transport may be a potential underlying mechanism that contributes to the modulation of insulin sensitivity, the survival of pancreatic β cells, and increased fasting glucose [35–37]. ASP may play a role in regulating islet function through the augmentation of glucose-stimulated insulin secretion via a direct action on the β cells [38].

In conclusion, our results demonstrated that, in individuals of Chinese Han ethnicity, T2MD is associated with the 698C>T polymorphism of human *C5L2*. This finding enhances the general knowledge of disease-associated genetic variants. GWAS in different populations evaluating this polymorphism and T2DM risk are required to further confirm this risk association.

Limitations

There were several limitations in our study. First, we did not evaluate inflammatory factors such as CRP, SAA, IL, etc. Also, we did not determine the plasma levels of ASP and C5a. Second, this study was not a longitudinal design, which did not allow for the establishment of a cause–effect relationship between T2DM and this genotype. Finally, this study did not address the functional role of this SNP in T2DM or metabolic dysfunction.

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