Original Article

The polymorphism of the *CARD8* inflammasome-related gene is associated with glutamic-acid-decarboxylase-antibody positivity in patients with type 1 diabetes mellitus

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Background: This study sought to examine the correlation between 2 single-nucleotide polymorphisms (SNPs; rs10403848 and rs2043211) in the caspase recruitment domain-containing protein 8 (*CARD8*) gene and the risks and clinical features of patients with type 1 diabetes mellitus (T1DM) in the Han Chinese population.

Methods: A case-control study involving the Han Chinese population was designed, and individuals diagnosed with classical T1DM and healthy controls were enrolled in this study. MassARRAY genotyped the SNPs of rs10403848 and rs2043211. Logistic regression and chi-square analyses were conducted to compare the allele distributions and genotypes of the T1DM and healthy control participants. A Kruskal-Wallis 1-way analysis of variance was used to perform the genotype-phenotype analysis for the T1DM patients.

Results: In total, 510 participants with classical T1DM and 531 sex-matched healthy control participants participated in this study. The *CARD8* SNP of rs2043211 was significantly associated with the rate of glutamic-acid-decarboxylase-antibody (GADA) positivity among T1DM patients (P=0.021). However, no significant differences in the distributions of alleles or the genotypes of rs10403848 and rs2043211 were observed between the case and control groups, and these 2 SNPs were not associated with T1DM under various inheritance models.

Conclusions: The rs10403848 and rs2043211 polymorphisms of *CARD8* were not associated with susceptibility to T1DM. However, rs2043211 was found to be correlated with GADA positivity in participants with T1DM.

Keywords: Type 1 diabetes mellitus (T1DM); single-nucleotide polymorphism (SNP); caspase recruitment domain-containing protein 8 (*CARD8*)

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Introduction

Type 1 diabetes mellitus (T1DM), which mainly induced by autoreactive T lymphocytes, is an autoimmune disorder characterized by the progressive destruction of pancreatic islet beta-cells (1,2). Autoimmune diabetes accounts for 5–19% of diabetes, and the incidence of autoimmune diabetes is growing at a rate of 2–5% per year worldwide (3,4). Patients with T1DM rely on lifelong exogenous insulin administration, and may experience diabetic complications, such as kidney disease, cardiovascular disease, blindness, and lower extremity amputation, all of which can lead to increased mortality and morbidity (5). The precise underlying pathogenic mechanisms remain obscure; however, both genetic and environmental factors contribute to the onset and development of T1DM. Previous research, including genome-wide association studies, linkage analyses, and candidate gene studies, has identified more than 60 susceptibility loci that explain 80–85% of the heritability of T1DM (6,7). Among these loci, the human leukocyte antigen (HLA) region accounts for approximately 50% of the genetic susceptibility of T1DM.

In addition to adaptive immune responses, innate immunity plays a crucial role in the pathogenesis of T1DM. Accumulated evidence indicates that the inflammasome is involved in the development of autoimmune (8), inflammatory (9), and metabolic (10) diseases. The nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, which is the most well-studied inflammasome, has also been associated with multiple sclerosis (11), inflammatory bowel disease (12), and T1DM (13). Upon recognizing pathogen-associated molecular patterns or danger-associated molecular patterns, this polyprotein complex promotes caspase-1 activation and inflammatory cytokine maturation, including interleukin (IL)-1β and IL-18, which further induce adaptive responses (14). It may be that the overactivation of the NLRP3 inflammasome renders individuals more susceptible to autoimmune diseases.

Caspase recruitment domain-containing protein 8 (*CARD8*), also known as TUCAN/CARDINAL), is a protein with a conserved homology domain belonging to the CARD-containing family. *CARD8* is a component of the NLRP3 inflammasome that attenuates the activation of nuclear factor-κB (NF-κB) and caspase-1. Thus, it may have a role in the downregulation of NLRP3 inflammasome function, which leads to the suppression of innate immune responses and inflammation (15-17). *In vivo* research has indicated that *CARD8* decreases the expression of IL-1β by interacting with wild-type NLRP3 and that the knockdown of *CARD8* could lead to the enhanced expression of IL-1β in human monocyte-derived macrophages (16). Thus, the *CARD8* gene represents a novel therapeutic target for autoimmune disorders, including T1DM.

Variants of this inflammasome-related gene have also been associated with a variety of autoimmune diseases and inflammatory diseases. A meta-analysis indicated that the polymorphism of *CARD8* was significantly associated with a decreased incidence of ileal Crohn's disease (CD) and stenotic or fistulizing CD, which suggests that these CD types have protective effects (18). In addition, the single-nucleotide polymorphism (SNP) of *CARD8* was found to be associated with an increased risk of gout in Chinese and European populations (19,20). Further, a Swedish study of 492 patients and 793 population-based controls showed that the minor allele of rs2043211 was associated with a decreased risk of ankylosing spondylitis (21).

However, association studies between *CARD8* SNPs and T1DM are rather limited. Thus, this study sought to investigate the relationships between 2 selected SNPs of *CARD8* (i.e., rs10403848 and rs2043211) and classical T1DM in the Han Chinese population and further the correlation between *CARD8* polymorphisms and the clinical features of individuals with T1DM.

We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-1126).

Methods

Study subjects

In total, 510 patients with classical T1DM and 531 healthy control patients from The Second Xiangya Hospital of Central South University were enrolled in this study. The 2 groups were matched in terms of sex (male/female; 275/235 *vs.* 273/258; P=0.418). To be eligible to participate in the study, the case-patients had to meet the following inclusion criteria: (I) meet the World Health Organization's diagnostic criteria for diabetes (1999); (II) have had an acute onset; (III) have developed an insulin dependency within 6 months after the diabetes diagnosis; and (IV) be positive for at least 1 islet-autoantibodies; that is, glutamic acid decarboxylase antibody (GADA), protein tyrosine phosphatase antibody (IA-2A), or zinc transporter 8 antibody (ZnT8A). Patients with other autoimmune

Table 1 Primer sequences for rs10403848 and rs2043211

SNP	Primer sequence (5'-3')
rs10403848	
Forward	ACGTTGGATGGACAGTGGCAGTGATATACC
Reverse	ACGTTGGATGGGGAAATGCTCTTGAAGCCT
Extension	cTCTGGAGCAACAATATGAAT
rs2043211	
Forward	ACGTTGGATGGAAGATGATGAGACAGAGGC
Reverse	ACGTTGGATGCCCAGATAGTTGACACTCAG
Extension	AGAGGCAGAGCCATTATTG

SNP, single-nucleotide polymorphism.

diseases or those who also had malignant tumors were excluded from the study. To be eligible to participate in the study, the healthy control patients had to meet the following inclusion criteria: (I) have a fasting plasma glucose (FPG) level of <5.6 mmol/L; and (II) have a postprandial plasma glucose (2h-PPG) level of <7.8 mmol/L based on a 75 g oral glucose tolerance test. Patients with autoimmune diseases, cancers, or a family history of diabetes were excluded from the study.

Data on patients' demographic characteristics were collected. Patients' C-peptide (CP) and glycated hemoglobin (HbA1c) levels were measured using chemiluminescence methods (ADVIA Centaur XP Immunoassay System, Siemens, Germany) and automated liquid chromatography (HLC-723G8, Tosoh, Japan), respectively. Diabetes-associated autoantibodies, including GADA, IA-2A, and ZnT8, were detected by quantitative radioligand binding assays (22,23).

The study was approved by the Ethics Committee of The Second Xiangya Hospital (No. 2017-Research-45), and all the research methods were conducted in compliance with the ethical guidelines of the Declaration of Helsinki (as revised in 2013). After being provided with an explanation, the participants or their guardians indicated that they fully understood the study goals and procedures, and provided written informed consent.

DNA extraction

Peripheral venous whole blood samples of each participant were collected in ethylenediaminetetraacetic acid-anticoagulant tubes and kept at –80 ℃. Deoxyribonucleic acid (DNA) was extracted using a GeneNode Genomic DNA Extraction Kit (Genenode Biotech Co. Ltd., Beijing) according to the manufacturer's protocol.

Candidate SNP selection and genotyping

The selection of *CARD8* SNPs was based on recent literature reports that indicated that certain SNPs are associated with T1DM or other autoimmune disorders. The selected SNPs had a minor allele frequency exceeding 0.05 in the Asian population, and were not in the same linkage region. MassARRAY (MassARRAY System, Agena Bioscience, the United States) was used for the genotyping. Polymerase chain reaction primers were designed using ADS2.0 software from Agena Bioscience; the sequences are set out in *Table 1*.

Statistical analysis

SPSS version 20.0 software was used for the statistical analysis. The quantitative data are presented as mean ± standard deviations if they comply with a normal distribution, or medians and interquartile ranges if they do not. Hardy-Weinberg equilibrium (HWE) in the case and control groups was examined using online software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Four genetic models, comprising dominant, recessive, over-dominant and additive models, were adopted to evaluate differences in distributions. The general data between the 2 groups were compared using the Mann-Whitney U test or the chi-squared test. Logistic regression and the chi-squared test were used to compare the frequencies and distributions of the alleles and genotypes between the 2 groups. A Kruskal-Wallis 1-way analysis of variance was employed for the genotype-phenotype analysis of the patients with T1DM. A P value less than 0.05 was considered significant.

Results

Demographic and clinical features of the participants

A total of 510 cases and 531 controls were included in this study. The anthropometry and biochemical features of the 2 groups are set out in *Table 2*, and have been described previously (24). The 2 groups were matched in terms of sex (P=0.418). The age and body mass index (BMI) of the T1DM group were significantly lower than those of the healthy control group (P<0.001 and P<0.001, respectively).

Table 2 Clinical characteristics of case and control participants

Characteristic	T1DM (n=510)	Controls (n=531)	P value
Sex (male/female)	275/235	273/258	0.418
Age (year)	22 (13 to 34)	42 (32 to 51)	<0.001*
BMI (kg/m^2)	18.70 (16.49–20.70)	22.60 (20.40–24.70)	<0.001*
Duration (months)	5.00 (0.50–21.50)	–	–
FPG (mmol/L)	8.91 (6.11–14.40)	4.87 (4.45–5.30)	<0.001*
PPG (mmol/L)	14.90 (9.90–20.70)	5.60 (4.70–6.40)	<0.001*
FCP (pmol/L)	77.50 (17.79–164.10)	–	–
PCP (pmol/L)	142.21 (39.60–279.20)	–	–
HbA1c (%)	10.00 (7.80–12.80)	–	–

Values in the backets represent mean ± standard deviations if the data followed a normal distribution, or interquartile ranges otherwise. *P<0.05 was considered significant. BMI, body mass index; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; FCP, fasting C-peptide; PCP, postprandial C-peptide; HbA1c, glycated hemoglobin.

Table 3 HWE of *CARD8* gene polymorphisms

SNP	Group	Genotype	Observed value	Expected value	P value
rs10403848	Case	AA	49	51.14	0.661
		AG	225	220.72	
		GG	236	238.14	
	Control	AA	46	55.71	0.054
		AG	252	232.57	
		GG	233	242.71	
rs2043211	Case	AA	115	115.31	0.957
		AT	255	254.39	
		TT	140	140.31	
	Control	AA	127	125.36	0.775
		AT	262	265.29	
		TT	142	140.36	

HWE, Hardy-Weinberg equilibrium; SNP, single-nucleotide polymorphism.

Conversely, FPG and 2-h PPG levels in the T1DM group were significantly higher than those in the control group (P<0.001 and P<0.001, respectively).

Allele and genotype distributions of CARD8 polymorphisms

As *Table 3* shows, the distributions of the genotypes of both SNPs in the case and control groups were in the HWE. In addition, no significant difference in the frequency of allele or genotype distributions for *CARD8* gene SNPs of rs10403848 and rs2043211 was observed between the case and control groups (*Table 4*).

Association analysis of the CARD8 gene in case and control groups

As *Tables 5,6* show, no association was found between *CARD8* rs10403848 or rs2043211 and T1DM-risk under

Table 4 Genotype and allele frequencies of rs10403848 and rs2043211 between the case and control groups

SNP	Cases (n=510), n (%)	Controls (n=531), n (%)	OR (95% CI)	P value
rs10403848				
Genotype				
AA	49 (9.6)	46 (8.7)	1.052 (0.676–1.635)	0.823
AG	225 (44.1)	252 (47.5)	0.882 (0.683–1.138)	0.332
GG	236 (46.3)	233 (43.9)	1 (reference)	
Allele				
A	323 (31.7)	344 (32.4)	0.967 (0.805–1.163)	0.723
G	697 (68.3)	718 (67.6)		
rs2043217				
Genotype				
AA	115 (22.5)	127 (23.9)	0.918 (0.651–1.295)	0.628
AT	255 (50.0)	262 (49.3)	0.987 (0.739–1.320)	0.99
TT	140 (27.5)	142 (26.7)	1 (reference)	
Allele				
A	485 (47.5)	516 (48.6)	0.959 (0.808–1.139)	0.635
T	535 (52.5)	546 (51.4)		

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

the dominant, recessive, additive, or over-dominant models.

Genotype-phenotype analysis of T1DM patients

We also performed an association analysis of the clinical and biochemical characteristics and different genotypes of the 2 SNPs in individuals with T1DM. As *Table 7* shows, no association between any of the investigated characteristics and rs10403848 genotype was detected. However, the rs2043211 polymorphism was significantly associated with the rate of GADA positivity (P=0.021), and patients with the TT genotype had a higher rate of GADA positivity (*Table 8*). The remaining characteristics, including sex, onset age, duration, BMI, FCP, 2 h-PCP, HbA1c, triglycerides (TG); total cholesterol (TC), high density lipoprotein (HDL, low density lipoprotein (LDL), IA-2A, and ZnT8A, were not associated with the rs2043211 genotype.

Discussion

T1DM is an organ-specific autoimmune disorder that mainly affects children and adolescents. Its exact pathogenic mechanisms are unknown; however, it is widely acknowledged that the disease is precipitated by environmental factors in individuals with a genetic risk. In addition to adaptive immune responses, accumulated evidence indicates that innate immunity, including various inflammasomes, such as the NLRP3 inflammasome, is involved in the onset and development of T1DM (14). The NLRP3 complex induces the activation of caspase-1 and the release and maturation of potent proinflammatory cytokines, including IL-1β and IL-18. The inflammasome-related gene *CARD8* has been associated with NLRP3 inflammasome activation; however, the precise mechanisms by which this occurs are unclear (25). Additionally, previous studies have indicated that *CARD8* is a negative regulator of caspase-1-dependent IL-1β generation (17), nucleotide-binding oligomerization domain 2-mediated signaling (26), and NF-κB signaling (15), which are important in the development of T1DM. Additionally, recent research suggests that *CARD8* suppresses the activation of the NLRP3 inflammasome (16). All these findings indicate that *CARD8* might be involved in the pathogenesis of T1DM. Thus, we selected 2 *CARD8* SNPs (i.e., rs10403848 and

Table 5 Genotypes and frequency distributions of rs10403848 and rs2043211 between the case and control groups under different genetic models

SNP	Genetic model	Genotype	Case, n (%)	Control, n (%)	OR (95% CI)	P value
rs10403848	Dominant model	AA + AG	274 (53.7)	298 (56.1)	0.908 (0.711–1.159)	0.438
		GG	236 (46.3)	233 (43.9)	1 (reference)	
	Recessive model	AA	49 (9.6)	46 (8.7)	1.121 (0.735–1.709)	0.597
		AG + GG	461 (90.4)	485 (91.3)	1 (reference)	
	Additive model	AA	49 (9.6)	46 (8.7)	0.965 (0.799–1.167)	0.716
		AG	225 (44.1)	252 (47.5)	–	
		GG	236 (46.3)	233 (43.9)	–	
	Over-dominant model	AG	225 (44.1)	252 (47.5)	0.874 (0.685–1.116)	0.280
		AA + GG	285 (55.9)	279 (52.5)	1 (reference)	
rs2043211	Dominant model	AA + AT	370 (72.5)	389 (73.3)	0.965 (0.734–1.268)	0.797
		TT	140 (27.5)	142 (26.7)	1 (reference)	
	Recessive model	AA	115 (22.5)	127 (23.9)	0.926 (0.694–1.235)	0.601
		AT + TT	395 (77.5)	404 (76.1)	1 (reference)	
	Additive model	AA	115 (22.5)	127 (23.9)	0.959 (0.808–1.139)	0.636
		AT	255 (50.0)	262 (49.3)	–	
		TT	140 (27.5)	142 (26.7)	–	
	Over-dominant model	AT	255 (50.0)	262 (49.3)	1.027 (0.805–1.309)	0.832
		AA + TT	255 (50.0)	269 (50.7)	1 (reference)	

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

Table 6 Minor alleles and frequency distributions of rs10403848 and rs2043211 between the case and control groups under different genetic models

SNP	Minor allele	Dominant model		Recessive model		Additive model		Over-dominant model	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
rs10403848	A	0.908 (0.711–1.159)	0.438	1.121 (0.735–1.709)	0.597	0.965 (0.799–1.167)	0.716	0.874 (0.685–1.116)	0.280
rs2043211	A	0.965 (0.734–1.268)	0.797	0.926 (0.694–1.235)	0.601	0.959 (0.808–1.139)	0.636	1.027 (0.805–1.309)	0.832

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

rs2043211) to examine their potential associations with classical T1DM.

The *CARD8* gene, which is located on chromosome 19 (19q13.33), contains 22 exons, and rs10403848 is located in the last intron. A recent study reported that this newly discovered *CARD8* polymorphism is significantly associated with psoriasis vulgaris, an immunologically mediated chronic inflammatory disease, in the Han population of northeastern China (27). Polymorphism rs2043211, which is a nonsense mutation resulting in a truncated *CARD8* protein, is located in exon 5. A previous *in vitro* study showed that this polymorphic allele is related to increased cell death (28); however, the exact mechanism by which this occurs remains unclear.

Association studies between rs2043211 and autoimmune and autoinflammatory diseases are relatively more common

Table 7 Clinical features of patients with classical T1DM and different genotypes of *CARD8* gene rs10403848

Clinical characteristic	Genotype			P value
	AA	AG	GG	
Sample size	49	225	236	–
Sex (male/female)	23/26	124/101	128/108	0.577
Onset age (year)	20 (13.0–33.0)	19 (11.0–33.0)	20 (11.0–33.0)	0.950
Duration (months)	4.00 (0.59–33.00)	4.00 (0.37–17.50)	5.00 (0.59–24.00)	0.736
BMI (kg/m^2)	19.45 (17.10–21.33)	18.64 (16.53–20.80)	18.63 (16.40–20.31)	0.221
FCP (pmol/L)	90.00 (12.40–190.00)	74.25 (19.85–162.93)	75.79 (14.84–159.99)	0.803
PCP (pmol/L)	141.80 (33.08–254.58)	138.64 (53.15–288.34)	139.50 (29.88–264.18)	0.556
HbA1c (%)	9.30 (8.15–11.65)	10.20 (7.40–12.80)	10.00 (8.00–12.50)	0.856
GADA positivity (%)	85.70	89.80	87.70	0.642
GADA titer (U/mL)	274.54 (64.74–561.04)	320.37 (96.81–756.52)	291.44 (83.71–808.73)	0.443
IA-2A positivity (%)	53.30	46.90	45.50	0.636
IA-2A titer (U/mL)	422.34 (86.23–870.79)	194.29 (42.80–634.82)	176.44 (59.42–647.33)	0.136
ZnT8A positivity (%)	27.90	31.70	30.70	0.886
TG (mmol/L)	1.02 (0.62–1.49)	0.90 (0.67–1.24)	0.96 (0.71–1.65)	0.361
TC (mmol/L)	4.30 (3.72–4.73)	4.06 (3.60–4.79)	4.37 (3.66–5.04)	0.160
HDL (mmol/L)	1.41 (1.11–1.62)	1.23 (1.06–1.65)	1.26 (1.01–1.57)	0.733
LDL (mmol/L)	2.24 (1.86–2.91)	2.32 (1.76–2.79)	2.35 (1.75–2.99)	0.863

Values in the backets represent mean ± standard deviations if the data followed a normal distribution, or interquartile ranges otherwise. *P<0.05 was considered significant. BMI, body mass index; FCP, fasting C-peptide; PCP, postprandial C-peptide; HbA1c, glycated hemoglobin; GADA, glutamic acid decarboxylase antibody; IA-2A, protein tyrosine phosphatase antibody; ZnT8A, zinc transporter 8 antibody; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

than such studies of rs10403848. However, the results obtained have sometimes been inconsistent. For example, in a Korean cohort, a significant association between rs2043211 and ulcerative colitis (UC) was detected, and the allele containing a stop codon was found to be related to an increased level of IL-1β in the serum of patients (29). Similarly, a significant association between polymorphism rs2043211 and CD was also found in a British cohort (30). Conversely, no association between rs2043211 and CD or UC was observed in a study of 3 independent European cohorts (31). These conflicting results might in part be due to the different genetic backgrounds of the participants and gene-environmental interactions. Given the potential function of rs10403848 and rs2043211 in inflammatory and immune responses, we aimed to investigate these 2 SNPs within the context of T1DM. As the peak of onset of autoimmune diabetes is puberty (32), we selected older individuals as control participants to increase the reliability of our results.

Our results indicate that the 2 SNPs were not associated with T1DM susceptibility. However, we found that rs2043211 correlated significantly with the positivity rate of GADA, a diabetes-associated antibody that indicates beta-cell destruction and guides T1DM diagnosis and prognosis in clinical settings. Indeed, patients with the TT genotype of rs2043211 exhibited a higher rate of GADA positivity, which is consistent with previous findings that the production and distribution of islet antibodies indicate heritable risk to some extent (33). It has been shown that HLA-DR4 is positively associated IA-2A, and HLA-DQ2 is negatively associated with IA-2A (33). However, this was the first study to investigate the association between SNPs of the *CARD8* gene and classical T1DM in the Han Chinese population. Our work may be helpful in predicting pancreatic islet function

Table 8 Clinical features of patients with classical T1DM and different *CARD8* gene rs2043211 genotypes

Clinical characteristic	Genotype			P value
	AA	AT	TT	
Sample size	115	255	140	–
Sex (male/female)	60/55	144/111	71/69	0.500
Onset age (year)	17 (11.0–32.0)	19 (11.0–32.0)	20 (11.0–33.0)	0.849
Duration (months)	5.00 (0.50–24.00)	5.00 (0.40–23.00)	3.50 (0.52–13.50)	0.968
BMI (kg/m^2)	18.53 (16.50–20.35)	18.60 (16.40–20.69)	19.17 (16.41–20.99)	0.482
FCP (pmol/L)	72.94 (13.45–159.64)	79.00 (18.40–148.14)	68.40 (18.72–182.08)	0.834
PCP (pmol/L)	144.52 (27.51–270.64)	127.00 (37.08–247.58)	160.29 (49.38–346.61)	0.274
HbA1c (%)	9.80 (8.00–12.40)	10.10 (7.90–12.90)	10.10 (7.40–12.18)	0.498
GADA positivity (%)	83.50	87.40	94.30	0.021*
GADA titer (U/mL)	291.44 (77.80–797.57)	364.24 (95.34–795.32)	245.96 (78.28–649.27)	0.219
IA-2A positivity (%)	45.80	49.80	42.30	0.386
IA-2A titer (U/mL)	132.19 (41.56–527.20)	206.48 (47.57–640.06)	213.97 (68.34–773.46)	0.406
ZnT8A positivity (%)	27.40	31.90	31.90	0.704
TG (mmol/L)	1.08 (0.74–1.75)	0.88 (0.66–1.30)	0.98 (0.68–1.38)	0.098
TC (mmol/L)	4.24 (3.58–4.91)	4.29 (3.64–4.97)	4.32 (3.73–4.78)	0.918
HDL (mmol/L)	1.19 (1.02–1.47)	1.34 (1.06–1.70)	1.27 (1.06–1.64)	0.098
LDL (mmol/L)	2.42 (1.69–3.06)	2.37 (1.76–2.99)	2.16 (1.79–2.77)	0.489

Values in the backets represent mean ± standard deviations if the data followed a normal distribution, or interquartile ranges otherwise. *P<0.05 was considered significant. BMI, body mass index; FCP, fasting C-peptide; PCP, postprandial C-peptide; HbA1c, glycated hemoglobin; GADA, glutamic acid decarboxylase antibody; IA-2A, protein tyrosine phosphatase antibody; ZnT8A, zinc transporter 8 antibody; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

and identifying the underlying genetic component of T1DM, which in turn could assist in efforts to provide individualized medicine to patients with T1DM.

This study had a number of limitations. First, we only investigated 2 polymorphisms of *CARD8*. Thus, more SNPs should be assessed to cover the entire gene in the future. Second, the sample sizes in our study were relatively small, which limited the power of this association study. Third, we conducted an association study and did not attempt to elucidate the mechanisms underlying these 2 SNPs. Patients with the TT genotype of rs2043211 had a higher rate of GADA positivity; however, TT genotype is not correlated with the level of GADA. Patients carrying the TT genotype had the lowest GADA level compared to those of heterozygous and wild homozygous. Thus, further investigations should seek to explain these contradictory outcomes and identify the exact function of the polymorphisms. Finally, because T1DM is genetically heterogeneous among ethnic origins, the findings of our study are only applicable in the Han population.

Conclusions

This case-control study sought to clarify underlying associations between classical T1DM and polymorphisms of the inflammasome-related gene *CARD8* in the Han Chinese population. No significant difference between the risk of T1DM and *CARD8* rs10403848 or rs2043211 was detected; however, rs2043211 was found to be significantly associated with the rate of GADA positivity (P=0.021).

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All participants were recruited, and all samples were collected with the appropriate informed consent of the participants or their guardians. The study was approved by the Ethics Committee of The Second Xiangya Hospital of Central South University (No. 2017-Research-45) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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