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Original Article

CREB-regulated transcription coactivator 3 (*CRTC3*) polymorphism associated with type 2 diabetes and hyperlipidemia in the Taiwanese population

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ABSTRACT

Objective: Type 2 diabetes mellitus (T2D) is a pathologically and genetically heterogeneous disease influenced by genetic and environmental factors. This study aims to investigate the association between T2D and polymorphism(s) in CREB-regulated transcription coactivator 3 (*CRTC3*) in Asian Taiwan.
Materials and methods: In this study, 417 participants with T2D and 197 without T2D were recruited. Anthropometrics, the metabolic profile, blood pressure, fasting plasma glucose, glycosylated hemoglobin (HbA1c), serum triglycerides, serum total cholesterol, low-density lipoprotein, high-density lipoprotein (HDL), and C-peptide were analyzed. TaqMan genotyping was used to identify individual genotypes, and the association of *CRTC3* polymorphism with clinical and biochemical parameters was assessed.
Results: Single nucleotide polymorphism (SNP) rs8033595 showed an association with diabetes ($p = 0.031$) and hyperlipidemia ($p = 0.002$). Odds ratio analysis showed that A carriers (AA or AG) had a protective effect against developing T2D and hyperlipidemia. Moreover, individuals with the GG genotype had higher frequencies of developing T2D and hyperlipidemia comorbidity.
Conclusion: In this study, conducted for the first time in Asian Taiwan, rs8033595 of the *CRTC3* gene was associated with T2D and hyperlipidemia in our study population.

1. Introduction

Type 2 diabetes mellitus (T2D) results from complex interactions between genetic and environmental factors [1–3]. According to the World Health Organization, the risk of developing T2D is approximately 50% hereditary [4,5]. Genetic factors affecting T2D prevalence include increasing age, ethnicity, a family history of diabetes, and genetic variants. Environmental factors such as lifestyle also contribute to the development of T2D, along with other factors, including physical inactivity [6], obesity [7–10], and insulin resistance [11].

Genome-wide association studies (GWASs), a type of population-based association study, have been performed in a systemic search for links between the genotype and phenotype of candidate genes involved in complex diseases. GWASs can be used to detect variations associated with diseases throughout the genome. GWASs have shown six new gene regions related to T2D, and these variants alter body mass index (BMI) and are associated with obesity [12–17]. GWASs provide information that clarifies the understanding of the genetic basis of common and complex diseases and illustrate novel pathways, thus explaining ~10% of disease heritability [18,19]. Common variations in the recognized genes related to T2D identified by GWAS are as follows: *TCF7L2*, *PPARG*, *KCNJ11*, *WFS1*, *HNF1B*, *SLC30A8*, *HHEX*, *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *FTO*, *JAZF1*, *CDC123-CAMK1D*, *TSPAN8-LGR5*, *THADA*, *ADAMTS9*, *NOTCH2*, and *KCNQ1* [20–22]. The identification of the *TCF7L2* gene as a T2D risk gene was unexpected [23]. The *KCNQ1* gene was recently discovered in the Japanese population [24,25]; *PTPRD* and *SRR* were reported in the Taiwanese population by Tsai et al in 2010

Conflicts of interest: none.

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[26]. The linkage and candidate gene approach also revealed several genetic associations with T2D. However, association and linkage studies on T2D showed inconsistent results that were not reproducible when applied to multiple populations [27,28].

The CREB-regulated transcription coactivator 3 (*CRTC3*) gene is a member of the *CRTC* family, which includes *CRTC1*, *CRTC2*, and *CRTC3*. The members are distinguished by their expression profiles and they are conserved in mammals [29–34]. *CRTC3* contributes to lipolysis and fatty acid oxidation influences the accumulation of brown fat cells and burns the fat of white fat cells to maintain body temperature [35,36]. A common human *CRTC3* variant was found to increase transcriptional activity and was associated with adiposity in two distinct Mexican-American cohorts. The results suggest that adipocyte *CRTC3* plays a role in the development of obesity in humans. We therefore supposed that *CRTC3* is a candidate gene associated with T2D. We studied 417 patients with T2D and 197 controls to investigate the association between their anthropometric characteristics and selected single nucleotide polymorphisms (SNPs) of *CRTC3*. We also evaluated the association of the selected SNPs with other symptoms related to metabolic syndrome.

2. Materials and methods

2.1. Clinical sample collection

The T2D group consisted of 417 Taiwanese patients who were recruited from Chang Gung Memorial Hospital in Keelung, Taiwan. All of the recruited patients fulfilled the following criteria: (1) aged 30–75 years; (2) had been diagnosed with diabetes for >5 years; (3) fasting plasma glucose > 6.93 mmol/L (126 mg/dL); and (4) glycosylated hemoglobin (HbA1c) > 6%. In addition, we recruited 197 individuals as the control group from the same hospital. The control participants underwent blood pressure measurement and laboratory tests, including blood biochemistry [fasting plasma glucose, HbA1c, serum triglycerides, serum total cholesterol, low-density lipoprotein, high-density lipoprotein (HDL), and C-peptide] to rule out T2D. The Institutional Review Board of Chang Gung Memorial Hospital approved the study protocol, and informed written consent was obtained from each participant prior to when the study was conducted.

2.2. DNA preparation

Genomic DNA was extracted from each participant's blood sample using the QIAamp DNA Blood kit (Qiagen, Hilden, Germany (operational)), according to the manufacturer's instructions. The extracted genomic DNA was analyzed by agarose gel electrophoresis, quantified by spectrophotometry, and stored at −80°C until use.

2.3. SNP genotyping

Genotyping was performed on the SNPs rs8033595 (*CRTC3* S72N) and rs7179095 (*CRTC3* 3′ UTR) using the Taqman SNP genotyping assay [Applied Biosystems (ABI), Forest City, CA, USA]. The primers and probes for the selected SNPs were included in the ABI Assay-on-Demand Kit. Reactions were performed according to the manufacturer's protocol. The fluorescent probe was detected using the ABI Prism 7900 Real-Time PCR System (ABI Life Technologies, Foster City, California, United States).

2.4. Statistical analysis

The quality of the genotype data was evaluated using Hardy-Weinberg equilibrium (HWE) proportion tests. Single-point association analyses were performed using χ^2 and *t* tests. The data were further evaluated using logistic regression adjusted for other factors in an odds ratio analysis. The logistic regression and odds ratio analysis were performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of the participants

The study consisted of 614 Taiwanese adults (417 T2D patients and 197 control individuals). The mean age of the participants was 57.25 ± 10.1 years (57.2 ± 10.6 and 57.3 ± 9.6 years for the T2D patients and controls, respectively). The mean duration of pathogenesis of T2D was 14.07 ± 7.16 years (range, 1–42 years). A comparison of the results of anthropometric measurements of cases and controls showed that the BMI, weight, waist circumference, hip circumference, waist-to-hip ratio (W-HR), and HDL values were significantly different between the two groups, with no significant differences in the prevalence by gender. The five indicators for metabolic disease are: (1) fasting glucose level; (2) blood pressure; (3) HDL cholesterol level; (4) triglyceride level; and (5) waist circumference. Low levels of HDL cholesterol indicate a risk of hyperlipidemia. In our cohorts, there were significant differences in hyperlipidemia and hypertension between the control participants and the T2D patients, as shown in Table 1.

3.2. Genotype associated with the spectrum of clinical symptoms

The two selected SNPs, rs8033595 and rs7179095, were genotyped and analyzed in our participants. The genotyping frequency distribution of selected SNPs was not derived from HWE. The rs8033595 SNP showed nominal association with the anthropometric indices of T2D in our cohort (Table 2). SNP rs8033595 was also associated with hyperlipidemia in our study participants (Table 3). In addition, odds ratio analysis was performed for diabetes and hyperlipidemia using logistic regression, after adjusting for other significant factors (Table 4). A-carriers, including those with AG + GG, had a lower risk of association with diabetes and hyperlipidemia. Thus, the A allele was protective against the development of diabetes or hyperlipidemia (Table 4). The other

Table 1
Clinical characteristics of the two groups.

		Control	T2D	χ^2	*t* test	*p*
n		197	417			
Age (y)		57.2 ± 10.6	57.3 ± 9.6		0.179	0.86
Sex (M/F)		77/111	204/211		2.169	0.14
BMI (kg/m^2)		23.407 ± 3.475	25.021 ± 4.7107		4.2090	<0.0001
Weight (kg)		61.50 ± 10.73	65.72 ± 12.145		4.1683	<0.0001
Waist (cm)		80.39 ± 9.427	88.8 ± 10.278		9.7148	<0.0001
Hip (cm)		95.06 ± 6.247	98.02 ± 8.502		4.3612	<0.0001
Waist:hip ratio		0.846 ± 0.092	0.901 ± 0.075		7.8701	<0.0001
HDL (mg/dL)		41.45 ± 12.14	45.741 ± 13.63		3.7683	<0.0002
Sex (*n*)	M	82	206	3.249		0.071
	F	115	211			
Hypertension	-	145	60	241.716		<0.0001
	+	42	357			
Hyperlipidemia	-	84	57	77.027		<0.0001
	+	104	357			
Waist[a]	-	145	186	55.317		<0.0001
	+	43	231			

BMI = body mass index; F = female; HDL = high-density lipoprotein; M = male; T2D = type 2 diabetes mellitus.

[a] Defined as waist circumference <90 cm in males, <80 cm in females; + defined as waist circumference ≥90 cm in men, ≥80 cm in women. The total sample count is inconsistent in the control group, because the clinical data for some individuals were unavailable.

Table 2
Genotype analysis of type 2 diabetes mellitus (T2D) patients compared with controls.

SNP	Genotype	*n*		χ^2	*p*
		Control	T2D		
rs8033595	AA	9 (5)	7 (2)	6.973	0.031
	AG	64 (32)	113 (27)		
	GG	124 (63)	297 (71)		
rs7179095	AA	25 (13)	38 (9)	2.768	0.251
	AG	87 (44)	175 (42)		
	GG	85 (43)	204 (49)		

Data are presented as *n* (%).
SNP = single nucleotide polymorphism.

factors, including BMI, weight, and W-HR, were tested for their association with the genotype, but the results were not statistically significant (data not shown).

3.3. The rs8033595 SNP is associated with T2D and hyperlipidemia comorbidity

The association of the selected SNPs with T2D and hyperlipidemia comorbidity was further examined by the χ^2 test. A significant *p* value for rs8033595 revealed that the relationship of the rs8033595 genotype and T2D and hyperlipidemia comorbidity was independent. The results indicated that T2D and hyperlipidemia comorbidity were associated with the rs8033595 genotypes, but not rs7179095 (Table 5).

A high percentage of the individuals with the G/G and A/G genotypes were found to have diabetes and hyperlipidemia comorbidities, indicating that rs8033595 is associated with the symptoms of metabolic syndrome, especially in patients with both T2D and hyperlipidemia (Fig. 1).

4. Discussion

T2D mellitus is the most common endocrine disease, characterized clinically by peripheral insulin resistance, disruption of glucose uptake, and pancreatic islet β-cell failure. We analyzed the association between the *CRTC3* gene and T2D in the Taiwanese population. The results showed that functional SNP rs8033595 (S72N) was associated with both T2D and hyperlipidemia in our study population.

Weight gain is known as an important factor contributing to the development of T2D. Several genes related to obesity have been described, including the following: *IRS1* gives rise to increased levels of bad cholesterol and blood glucose concentration [37]; *BRD2* reduces inflammation in fat cells and prevents β-cell failure in the pancreas, thereby protecting against obesity-induced diabetes [38]; *CMAH* decreases insulin production [39]; *ZFP69* affects blood sugar levels and the malfunction of fat metabolism [40]; and the *CRTC* family is insulin-sensitive under high fat diet conditions and reduces hepatic glucose output. The *CRTC3* gene was recently found to play a role in weight gain, and its polymorphism was found to be associated with obesity [29–36].

The CREB coactivator (*CRTC3*) promotes obesity by attenuating the β-adrenergic receptor signaling pathway, this response having been activated by catecholamine signals. The catecholamine signal pathway is related to the adipose-derived hormone leptin and maintains energy balance by burning fat. It is believed to disrupt adipocytokine signaling, leading to the development of insulin resistance [35]. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC-1α*) is regulated by the CREB proteins in mitochondrial biogenesis in liver cells. The *CRTC3* expression involved in this mechanism is silenced in response to *PGC-1α* induction due to rotenone inhibition, and blocks downstream mitochondrial biogenesis [11,36]. Dysfunction of mitochondria leads to impaired insulin secretion, causing hyperglycemia, hyperinsulinism, and the development of T2D [9,19]. Overexpression of *CRTC3* increases mitochondrial biogenesis, affecting both glucose utilization and fatty acid oxidation. *CRTC3* activates translation through the CRE sites in the SIK/TORC signaling pathway and regulates the

Table 3
Clinical characteristics of participants for genotype testing analysis.

Clinical characteristic	SNP	Genotype	*n*		χ^2	*p*
			Control	Case		
Hypertension			Without	With		
	rs8033595	AA	5 (2)	10 (3)	0.111	0.946
		AG	55 (27)	114 (29)		
		GG	145 (71)	275 (69)		
	rs7179095	AA	27 (13)	36 (9)	2.400	0.301
		AG	85 (40)	176 (44)		
		GG	102 (48)	187 (47)		
Hyperlipidemia			without	With		
	rs8033595	AA	7 (5)	9 (2)	12.491	0.002
		AG	58 (38)	119 (26)		
		GG	88 (58)	333 (72)		
	rs7179095	AA	16 (10)	47 (10)	2.409	0.300
		AG	73 (48)	189 (41)		
		GG	64 (42)	225 (49)		
Waist			without	With		
	rs8033595	AA	10 (3)	6 (2)	1.636	0.441
		AG	104 (31)	73 (27)		
		GG	226 (66)	195 (71)		
	rs7179095	AA	35 (10)	28 (10)	0.466	0.792
		AG	149 (44)	113 (41)		
		GG	156 (46)	133 (49)		

Data are presented as *n* (%).
SNP = single nucleotide polymorphism.

Table 4
Odds ratio (OR) analysis for the A allele of rs8033595 in diabetes and hyperlipidemia.

Phenotype	Genotype	Count		OR (95% CI)	*p*
		Control	Case		
Diabetes[a]		Without	With		
	AA	9 (5)	7 (2)	0.166 (0.039, 0.715)	0.016
	AG	64 (32)	113 (27)	0.751 (0.446, 1.300)	0.318
	GG (ref.)	124 (63)	297 (71)	1	
Hyperlipidemia[b]		Without	With		
	AA	7 (5)	9 (2)	0.459 (0.153, 1.376)	0.164
	AG	58 (38)	119 (26)	0.573 (0.374, 0.879)	0.011
	GG (ref.)	88 (58)	333 (72)	1	

Data are presented as *n* (%), unless otherwise indicated.
CI = confidence interval.
[a] Adjusted by sex, hypertension, hyperlipidemia, and waist size.
[b] Adjusted by sex, hypertension, diabetes, and waist size.

Table 5
Association of *CRTC3* polymorphisms with diabetes/hyperlipidemia comorbidity.[a]

SNP	Genotype	0	1	2	χ^2	*p*
rs8033595	AA	6	40	47	19.353	0.001
	AG	4	42	118		
	GG	6	95	256		
rs7179095	AA	9	46	38	5.925	0.205
	AG	23	68	73		
	GG	31	148	178		

SNP = single nucleotide polymorphism.
[a] Clinical phenotypes were defined as 0 = neither diabetes nor hyperlipidemia, 1 = diabetes or hyperlipidemia, 2 = with both diabetes and hyperlipidemia. The *p* values were generated using 3×3 χ^2 test.

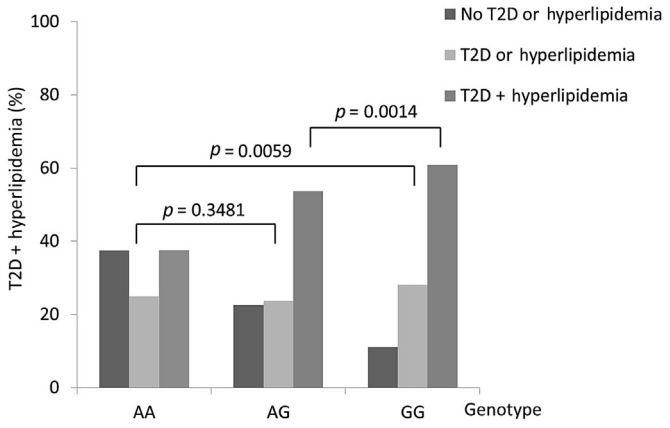

Fig. 1. Three genotypes of single nucleotide polymorphism (SNP) rs8033595 with different frequencies of phenotype distribution. The first phenotype group had neither diabetes nor hyperlipidemia, the second phenotype group had diabetes or hyperlipidemia, and the final phenotype group had both diabetes and hyperlipidemia. The *p* value was generated by the 3×3 χ^2 test according to the number of participants in Table 5.

expression of steroidogenic genes such as *StAR* and *PPARGC1A* [35,36,41,42].

CRTC3 may be a switch to control the number of brown fat cells that control obesity by attenuating β-adrenergic receptor signaling in adipocyte tissues, and maintaining energy balance by reducing adenyl cyclase activity by upregulating the expression of *RGS2*. *CRTC3* should contribute to the development of insulin resistance and T2D. In a previous study, the 72N allele was associated with BMI in Mexican-Americans, but not in non-Hispanic whites [35]. The association between T2D and *CRTC3* is still not clear. We observed a *CRTC3* variant allele, rs8033595 G > A, that encodes a missense variant (serine changed to asparagine), and rs7179095 (A/G) in the 3′ UTR region (in a putative miRNA-4761-5p binding site identified by a miRBase search) in the Human Database of Single Nucleotide Polymorphisms. In our study, the T2D cohort showed that T2D and hyperlipidemia are associated with the rs8033595 SNP, which is one of 2233 SNPs located in the coding region of the forward strand. It is a missense variant (G to A) encoding a protein in which serine is mutated to asparagine (S72N). Our results show that *CRTC3* increases the risk of hyperlipidemia; thus, it provides insight in the context of a physiologically relevant system for lipid metabolism and glucose uptake mechanisms. Individuals with the *CRTC3* risk allele have a higher chance of developing both diabetes and hyperlipidemia than those without this allele.

SNP rs8033595 (S72N) was associated with both T2D and hyperlipidemia in our study population. Its presence indicated a risk of both T2D and hyperlipidemia in individuals homozygous for the G allele and should be evaluated further in prospective studies. This is the first data demonstrating the association of a genetic variant with T2D and hyperlipidemia comorbidity in the Taiwan population. The genetic role of this association as well as that of other *CRTC3* SNPs warrants further investigation, both in Han populations living in different geographic regions and in other Asian populations.

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