

Research Paper

Distribution of Cytotoxic T Lymphocyte-Associated Antigen-4 Promoter Polymorphisms in Taiwanese Patients with Type 2 Diabetes Mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) is associated with chronic inflammation, suggesting the metabolic abnormalities are originated from or exacerbated by cytokine overproduction. Cytokines and counter-regulatory molecules are crucial in keeping the balance of immune responses and, therefore, are potential candidates involved in T2DM etiology, development and complications. Our previous reports identify several significant associations between the genotypes of cytokine genes and T2DM and/or the clinical lipid parameters, which strongly suggest the participation of immune-regulatory molecules in lipid metabolism. The aim of this study is to determine the distribution of gene encoding cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), a T-cell negative regulator, in T2DM patients and health subjects. Genomic DNA was extracted from 287 Taiwanese T2DM patients and 278 ethnic- and age-matched healthy subjects, and two *CTLA-4* polymorphisms (-318 C/T and +49 A/G) were analyzed by polymerase chain reaction-restriction fragment length polymorphism. Intriguingly, *CTLA-4* -318 genotype was associated with circulatory triglycerides in T2DM subjects ($P=0.019$) although no significant association between *CTLA-4* -318 ($P=0.119$) and +49 ($P=0.2$) genotypes with T2DM was identified. In addition, *CTLA-4* +49 genotype was significantly associated with the ratio between total cholesterol and high-density lipoprotein ($P=0.004$) in control subjects. Our results suggest that *CTLA-4* may be involved in lipid metabolism and affect T2DM disease progression and/or the development of diabetic complications although this gene does not represent a major risk factor for T2DM.

Key words: type 2 diabetes mellitus; cytotoxic T lymphocyte-associated antigen-4; genetic polymorphisms

Introduction

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes characterized by abnormally high blood glucose. People with risk factors such as dyslipidemia, impaired glucose

tolerance, and hypertension (so-called metabolic syndrome) are susceptible to develop T2DM. The global prevalence of diabetes is estimated to reach 4.4% with more than 350 million affected people in year 2030 [1]. The prevalence of T2DM varies among different ethnic populations, with the highest rate found in Pima Indians (as high as ~50%) [2]. In Taiwan, more than 98% of diabetic patients are characterized as T2DM [3], affecting more than 1 million individuals.

The etiology of T2DM is still an enigma although insulin resistance is the major characteristics. Genetic and environmental factors are the study focus of T2DM etiology, and linkage studies have localized some of the genes that influence the development of this disorder. Cytokines and counter-regulatory molecules are crucial in the regulation of immune responses and, therefore, are potential candidates involved in T2DM etiology. Evidence regarding cytokine gene polymorphisms among T2DM patients is increasing, however, with conflicting and inconclusive observations [4].

Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), a negative regulator of T cell activation and proliferation, plays important roles in regulating immune surveillance and responses [5]. Abundant evidence has documented the association of several single nucleotide polymorphisms (SNPs) of the CTLA-4 gene and autoimmune diseases such as type 1 diabetes mellitus [6-10]. Nevertheless, the putative association between CTLA-4 and the more prevalent T2DM does not cause much attention. Only very limited reports with contradictory conclusions regarding the study of CTLA-4 and T2DM are documented [11-14]. The discrepancy indicates that unique genetic characteristics may be involved in T2DM pathogenesis among different ethnic population [15]. Therefore, it is tempting to identify whether the CTLA-4 SNPs would contribute to T2DM pathogenesis and/or clinical manifestations in Taiwan. To inspect this hypothesis, the frequencies of the CTLA-4 SNPs (-318C/T and +49A/G) among patients with T2DM as well as the association of these polymorphisms with patients' biochemical features were examined in Taiwanese population.

Materials & Methods

Study subjects

Whole blood samples of 278 Taiwanese patients with T2DM were obtained from the Divisions of Endocrinology and Metabolism and the Department of Pathology and Laboratory Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei. For reference, control population consisted of 287 unrelated, ethnic-

and age-matched non-diabetic healthy subjects were recruited from Shin Kong Wu Ho-Su Memorial Hospital, Taipei, by selecting those with normal blood glucose. The diagnosis of T2DM was based on the clinical characteristics, magnitude of residual insulin or C-peptide secretory responses. Information about patients' clinical biochemical manifestations were listed in Table 1. Informed consent from each study subject was obtained after the nature of study was fully explained. The study was approved by the Ethics Review Committee of Shin Kong Wu Ho-Su Memorial Hospital.

Table 1. Demographic and biochemical data of study subjects in this study

	Control (n=287) [†]	T2DM (n=278) [†]	P [†]
HbA1c (%)	5.54±0.32	8.18±2.24	<0.001
Fasting glucose (70-110 mg/dL)*	87.52±7.95	148.3±59.96	<0.001
ALT (<40 U/L)*	38.36±23.26	32.1±27.93	<0.001
HDL-C (>35 mg/dL)*	52.38±13.53	45.76±15.87	<0.001
LDL-C (<160 mg/dL)*	118.55±29.26	100.34±32.50	<0.001
Triglycerides (20-200 mg/dL)*	118.49±67.38	214.25±486.09	<0.001
Cholesterol (125-240 mg/dL)*	193.98±32.85	185.09±63.09	<0.001
CHO/HDL	2.78±2.00	1.97±0.82	<0.001
Creatinine (0.6-1.4 mg/dL)*	0.80±0.18	0.91±0.41	0.016

Data were presented as mean ± standard deviation.

*Numbers in parenthesis indicated the normal reference range of each biochemical test.

[†]Mann-Whitney test.

Biochemical analysis

Serum samples from the study subjects were isolated and subjected to analysis by automated biochemical analyzers, including fasting glucose, cholesterol (CHO), triglycerides (TGs), high density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine, alanine aminotransferase (ALT) [by AU680/AU5800, Beckman Coulter, USA] and glycosylated hemoglobin HbA1c [by HLC-723 G8, Tosoh, Japan]. CHO/HDL was calculated and documented as ratio by levels of CHO over HDL.

CTLA-4 genotyping

CTLA-4 genotyping were conducted as described [16]. Genomic DNA was extracted from peripheral blood mononuclear cells using GenoMaker commercial kit (GenePure Technology, Taiwan). Amplification of +49 and -318 fragments was performed in a volume of 30 µL containing 100 ng of template DNA, 10 mmol/L Tris-HCl (pH9.0), 50 mM KCl, 3 mM MgCl₂, 0.01% gelatin and 0.1% Triton X-100, 50 µM dNTP, 0.5 µM of each primer (as listed in Table 2) and 1 unit GeneTaq DNA polymerase (GenePure Technology, Taiwan). The parameters for thermocycling were as followings: an initial denaturation at 95°C for 5 minutes, followed by 30

cycles of denaturation at 95°C for 1 min; annealing at 55°C for 1 min; extension at 72°C for 1 min; and followed by final extension at 72°C for 5 minutes. Identification of the 2 alleles at +49 and -318 polymorphic sites was performed by incubating PCR product with *Bbv* I and *Mse* I, respectively, followed by electrophoresis.

Table 2. Primer sequences used for the detection of genetic polymorphisms.

Genotypes	Primer sequence	Restriction enzyme	PCR product (bps)
<i>CTLA-4</i>			
-318C>T	5'- AATGAATTGGACTGGATGG -3'	<i>Mse</i> I	247
	5'-TTACGAGAAAGGAAGCCGTG -3'		
+49A>G	5'-CCACGGCTTCCTTTCGTA-3' 5'AGTCTCACTCACCTTGCAG-3'	<i>Bbv</i> I	329

Statistical analysis

Data analysis started with descriptive statistics, including mean and standard deviation for continuous variables, and frequency for categorical variables. If necessary, natural logarithm transformation was used to enhance normality for blood biochemistry parameters with skewed distribution. Mann-Whitney test was applied for comparisons of each of the blood biochemistry parameters between diabetic and controls subjects, and Chi-square test for comparing frequencies of different genotypes and alleles between groups. Moreover, student's *t*-test and non-parametric Kruskal Wallis test were applied to compare means of respective blood biochemistry parameters among subjects with different *CTLA-4* genotypes, respectively. The statistical software of SPSS software was applied for the analyses. Statistical differences were defined as $P < 0.05$ for all statistical tests.

Results

Distribution of *CTLA-4* genotypes among the study subjects

The present study aimed at investigating the distribution of *CTLA-4* genetic polymorphisms among the healthy subjects and T2DM patients. Detail information about the clinical biochemical manifestations among the study subjects were listed in Table 1. Significant differences of all the biochemical data were identified between the control and T2DM subjects.

Table 3 showed the distribution of *CTLA-4* genotyping results among the study subjects. In non-diabetic control individuals, 215 (74.9%), 67

(23.3%) and 5 (1.7%) carried *CTLA-4* -318 C/C, heterologous C/T and T/T genotype, respectively. In T2DM patients, 227 (81.7%), 49 (17.9%) and 2 (0.7%) carried C/C, C/T and T/T genotype, respectively. The prevalence of -318*C and -318*T allele in control individuals was 86.6% and 13.4%, respectively; and the corresponding frequencies in T2DM patients was 90.3% and 9.7%. No significant difference of *CTLA-4* -138 genotypic distribution between subject groups was identified ($P=0.119$), while marginal significance was observed in the *CTLA-4* -138 allelic distribution ($P=0.063$).

In terms of the *CTLA-4* +49 polymorphisms (Table 3), 36 (12.5%), 150 (52.3%) and 101 (35.3%) control subjects carried A/A, heterologous A/G and G/G genotype, respectively. In patients with T2DM, 33 (11.9%), 127 (45.7%) and 118 (42.4%) carried A/A, A/G and G/G genotype, respectively. The prevalence of +49*A and +49*G alleles in control individuals was 38.7% and 61.3%, respectively; and that in T2DM counterpart was 34.7% and 65.3%. Neither the *CTLA-4* +49 genotypic ($P=0.2$) nor allelic ($P=0.175$) distribution between subject groups showed significant differences.

Table 3. Frequencies of genotypic polymorphisms in T2DM patients and control subjects.

	Control n (%)	T2DM n (%)	P† value
<i>CTLA-4</i> -318C/T			
C/C	215 (74.9)	227 (81.7)	0.119
C/T	67 (23.3)	49 (17.9)	
T/T	5 (1.7)	2 (0.7)	
C allele	497 (86.6)	502 (90.3)	0.063
T allele	77 (13.4)	54 (9.7)	
<i>CTLA-4</i> +49A/G			
A/A	36 (12.5)	33 (11.9)	0.2
A/G	150 (52.3)	127 (45.7)	
G/G	101 (35.2)	118 (42.4)	
A allele	222 (38.7)	193 (34.7)	0.175
G allele	352 (61.3)	363 (65.3)	

†Number in the parentheses indicated the percentage.

†Chi-square test.

Association of *CTLA-4* genotypes with triglycerides and the ratio of total cholesterol to high-density lipoprotein cholesterol

The correlation between study subjects' biochemical manifestations with their genotypes was subsequently analyzed. For *CTLA-4* -318 polymorphisms (Table 4), significant association between the genotypes with TGs was identified in T2DM patients ($P=0.019$). In addition, a tendency reaching significant association between *CTLA-4* -318 genotypes with fasting glucose ($P=0.069$) and CHO/HDL ($P=0.067$) was observed in control subjects. As for +49 SNPs

(Table 5), significant association between genotypes with the ratio of total CHO to HDL-C (CHO/HDL) was identified in healthy subjects ($P=0.004$). Besides, marginal significance between genotypes and ALT was noticed ($P=0.063$). Nevertheless, no significant association between +49 genotypes and the biochemical parameters in the T2DM patients was found.

Table 4. Associations between *CTLA-4* -318 genotypes and clinical parameters

Parameter	Genotype			P†
	C/C	C/T	T/T	
Control				
HbA1c	5.52±0.33	5.62±0.27	5.48±0.29	0.070
Fasting glucose	87.15±8.19	88.98±7.17	83.80±5.07	0.069
ALT	37.46±20.08	40.83±31.9	44.00±11.85	0.687
HDL-C	52.27±12.78	52.39±12.78	56.80±21.88	0.917
LDL-C	116.76±29.32	125.27±28.17	105.20±31.32	0.149
Triglycerides	118.73±68.32	118.06±64.49	113.80±79.12	0.873
Cholesterol	192.15±31.5	200.49±36.36	185.40±35.40	0.455
CHO/HDL	2.86±2.19	2.45±1.16	3.72±1.86	0.067
Creatinine	0.80±0.18	0.80±0.19	0.86±0.22	0.815
T2DM				
HbA1c	8.21±2.27	8.09±2.16	7.50±0.56	0.919
Fasting glucose	148.74±61.07	146.41±55.95	144.00±48.08	0.85
ALT	32.48±29.81	29.81±16.28	40.00±42.43	0.836
HDL-C	45.12±13.96	48.74±22.86	45.00±5.66	0.337
LDL-C	100.19±32.30	101.96±34.07	77.50±0.71	0.459
Triglycerides	230.72±535.16	137.76±93.21	227.5±92.63	0.019
Cholesterol	186.63±66.60	175.96±42.31	235.00±70.72	0.231
CHO/HDL	2.00±0.86	1.82±0.52	3.05±0.92	0.093
Creatinine	0.92±0.40	0.90±0.43	0.64±0.08	0.331

*The data were presented as the mean ± SEM

†Nonparametric statistics: Kruskal Wallis test

Table 5. Associations between *CTLA-4* +49 genotypes and clinical parameters

Parameter	Genotype			P†
	A/A	A/G	G/G	
Control				
HbA1c	5.50±0.39	5.57±0.30	5.52±0.32	0.375
Fasting glucose	89.19±5.67	87.47±7.44	86.99±9.26	0.609
ALT	29.83±15.74	39.23±25.68	40.13±21.18	0.063
HDL-C	53.99±15.11	51.29±12.97	53.42±13.75	0.402
LDL-C	127.39±34.14	118.82±28.68	114.96±27.81	0.082
Triglycerides	108.19±49.70	123.47±71.89	114.75±65.81	0.543
Cholesterol	202.11±40.23	194.27±31.60	190.64±31.55	0.313
CHO/HDL	2.25±1.17	2.82±2.52	2.90±1.19	0.004
Creatinine	0.77±0.17	0.81±0.19	0.80±0.17	0.733
T2DM				
HbA1c	8.33±2.26	8.12±2.40	8.21±2.06	0.441
Fasting glucose	147.42±58.61	149.88±59.13	146.83±61.65	0.881
ALT	28.67±27.04	32.90±27.52	32.12±28.79	0.309
HDL-C	43.70±11.29	45.26±13.68	46.87±18.92	0.871
LDL-C	97.42±31.91	101.35±35.27	100.08±29.65	0.775
Triglycerides	229.18±316.78	166.89±134.34	261.45±714.23	0.426
Cholesterol	187.97±54.48	180.95±69.77	188.78±57.7	0.139
CHO/HDL	2.09±0.99	1.90±0.72	2.01±0.86	0.394
Creatinine	0.86±0.28	0.88±0.36	0.96±0.48	0.759

*The data were presented as the mean ± SEM

†Nonparametric statistics: Kruskal Wallis test

Combining the results regarding the significant association between *CTLA-4* -318 SNPs with triglyceride in T2DM patients and *CTLA-4* +49 SNPs with CHO/HDL in control subjects, it suggests that *CTLA-4* may play certain role in regulating lipid metabolism.

Discussion

T2DM is associated with chronic inflammation. Diabetic patients with long duration are susceptible to develop multiple complications. Pickup *et al.* first discovered that blood concentrations of acute-phase response markers in circulation of type 2 diabetic patients are increased [3]. Accumulating evidence demonstrates that diabetes is associated with enhanced cytokine production, suggesting the metabolic abnormalities are originated from or exacerbated by cytokine overproduction [17-20]. Cytokines and counter-regulatory molecules are crucial in keeping the balance of immune responses and, therefore, are potential candidates involved in T2DM etiology, development and complications. IL-1, IL-6 and TNF- α are known to trigger the elevation of inflammatory markers, which demonstrates that control of T cell proliferation and activation might be one of the important factors in T2DM pathogenesis. Moreover, our previous reports reveal several significant associations between genotypes of the cytokine genes and T2DM and/or the clinical lipid profiles, including (1) *IL-4* and *IL-10* with T2DM [15,21]; (2) *IL-4*, *IL-4R α* and *TNF- α* with HDL-C [15,22,23]; and (3) *TNF- α* with fasting glucose levels [22]. Collectively, these results strongly suggest that the immune-regulatory molecules are involved in diabetic development and lipid metabolism.

CTLA-4 is a transmembrane glycoprotein which acts as a co-stimulatory molecule in regulating T cell activation [24-27]. More than 100 SNPs in the gene encoding *CTLA-4* are implicated in the regulation of its expression and/or activity. Among the *CTLA-4* SNPs, +49A/G and -318C/T are implicated in several diseases, such as Graves' disease [28,29], type 1 diabetes [13,28,30,31], systemic lupus erythematosus [32], Hashimoto thyroiditis [33], Addison's disease [34], T2DM [14] and malignancies [35-37]. Wang XB *et al.* reported that *CTLA-4* -318*T allele may contribute to *CTLA-4* upregulation [38]. Another study suggested that patients carrying +49*G allele and thus having higher soluble *CTLA-4* levels had a lower incidence of acute rejection in liver transplantation [39]. However, the association between *CTLA-4* SNPs and promoter activity is still controversial since Ligiers *et al.* reported that individuals carrying *CTLA-4* -318*T allele and homozygous +49 A/A genotype have higher levels of *CTLA-4* mRNA and protein [27]. The

possible association between *CTLA-4* SNPs and cytokine levels remains inconclusive. Han *et al.* [40] reported *CTLA-4* +49GG genotype is associated with lower TNF- α and IFN- γ . On the contrary, another study documented that type 1 diabetic patients carrying +49 G allele have higher levels of TNF- α and IFN- γ [41].

By combining our findings in regard to the associations between immune-regulatory molecules with T2DM and/or lipid profiles, we speculate that *CTLA-4*, the negative regulator of immune response, may be one of the internal factors which regulate lipid metabolism and thus participate in diabetic onset and/or progression. Therefore, the distribution of *CTLA-4* genotypes in diabetic patients and the corresponding associations between the genotypes with diabetic manifestations were investigated. Our results demonstrate that *CTLA-4* SNPs are associated with clinical biochemical parameters. For the *CTLA-4* -318 SNPs, lower serum TGs level was observed in T2DM individual carrying C/T genotype ($P=0.019$, Table 4). Marginal significance is also identified between -318 genotypes with fasting glucose ($P=0.069$) and CHO/HDL ($P=0.067$) in control subjects (Table 5). Our data also showed the significant association between *CTLA-4* +49 genotypes with CHO/HDL ($P=0.004$, Table 5) in control subjects. These results reveal the correlation between *CTLA-4* SNPs and lipid parameters, suggesting *CTLA-4* may be involved in lipid metabolism and affect T2DM disease progression.

The finding that *CTLA-4* -318 SNPs are associated with TGs supports the study from Rau *et al.* [14], in which the author concluded that *CTLA-4* genotype is correlated with diabetic progression such as earlier start of insulin treatment and the development of microangiopathic lesions, although this gene does not represent a major risk factor for T2DM. Despite elevated TGs are often associated with insulin resistance and T2DM, TGs and cardiovascular risks are tightly associated [42]. Excess amounts of circulatory TGs worsen microvascular and macrovascular diseases, increase glomerular injury and accelerate the progression of nephropathy in diabetic patients [43]. Therefore, TGs are considered as a significant and independent risk factor for diabetic patients to develop cardiovascular diseases and angiopathies. Collectively, *CTLA-4* may be a prognostic factor for patients in the development of microangiopathy based on its correlation with lipid metabolism, rather than contribute directly to T2DM onset. In addition, evidence has suggested the implication of *CTLA-4* in energy metabolism. Patsoukis *et al.* [44] reported that *CTLA-4* is involved in the metabolic profile of non-activated T cells

through inhibiting glycolysis. They provide the clue that *CTLA-4* mediated lipid metabolism through regulating the rate-limiting enzyme of fatty acid oxidation, carnitine palmitoyltransferase (CPT1A), and the major triacylglycerol hydroxylase adipocyte triglyceride lipase (ATGL). Although it may be premature to make solid conclusion regarding the role of *CTLA-4* in lipid metabolism, it is possible that *CTLA-4* SNPs is an internal factor which regulates lipid metabolism by mediating CPT1A and ATGL.

Several *CTLA-4* genetic variants other than -318 C/T and +49 A/G, such as -1722C/T, -1661A/G, CT60 (rs3087243), CT61 (rs51157131), J031 (rs11571302) and J027-1 (rs11571297), are reported [32,45-50]. Except for -318 C/T and +49 A/G, the functions and/or effects of these SNPs are less characterized. At the best of our knowledge, among these SNPs, only one study documented the distribution of -1722 T/C genotypes between the control and T2DM subjects, with no significant difference identified [51].

Another interesting finding is the marginal significance between *CTLA-4* +49 SNPs and ALT in the control subjects. Our results demonstrate that individuals carrying +49*G allele have higher ALT. Despite no significant association between +49 genotypic frequencies and ALT in T2DM population was found, our results support the study from Kanno *et al.* [52] that primary biliary cirrhosis patients with *CTLA-4* +49 G/G genotype had higher ALT. Besides, HBV patients with *CTLA-4* +49*A allele show lower circulatory ALT [53]. Taken the above results together, it suggests that *CTLA-4* SNPs may be implicated in serum ALT level and play certain roles in hepatic metabolism and diseases.

Abundant evidence indicates that *CTLA-4* SNPs are implicated in autoimmune diseases and malignancy. Only very limited reports with contradictory conclusions regarding the study of *CTLA-4* and T2DM are documented. Gribben *et al.* [54] demonstrated that *CTLA-4* may be a candidate gene to confer T2DM susceptibility by mediating antigen-specific apoptosis and progressive pancreatic β -cell failure of T2DM. Nevertheless, our study echoes the conclusions from several studies that *CTLA-4* does not represent a major risk factor for T2DM [12-14,55,56]. The discrepancy is speculated to be resulted from ethnic difference and sample size (as discussed below).

Ethnic difference may be one of factors leading to the conflicting results of gene polymorphisms, as the distribution of genetic polymorphisms in a certain gene is diverse among study subjects with different racial origins. Hence, it is intriguing to consider the distribution of the variants in *CTLA-4* +49 and -318 SNPs among different ethnic populations. For

addressing this issue, the frequencies of *CTLA-4* SNPs among different ethnic populations are compared (Table 6). The prevalence of *CTLA-4* +49 A/A genotype varies among the populations, with the highest rate found in Middle East area (Turkish and Iranian Kurdish, about 62~67%) [12,57], followed by Estonian (about 30%) [13], and the lowest rate in Germany [14], Iranian [51], Chinese [55] and Taiwanese (~15%). While no significant association was documented between T2DM and *CTLA-4* +49 SNPs, *CTLA-4* is identified as a susceptible gene of T1DM in Iranian Kurdish [56] and Chinese [55], and T2DM in Iranian [51]. Interestingly, while relatively similar *CTLA-4* -318 genotypic/allelic distribution are reported among Turkish, Iranian and Taiwanese populations, significant association between this SNPs and T2DM is only identified in Iranian [12,51]. This discrepancy reflected that differential genetic characteristics and possible distinct etiological/environmental factors may be involved among ethnic populations.

Table 6. Distribution of *CTLA-4* polymorphisms among different ethnic populations

CTLA4 +49 A/G Population		Genotype			Allele		Reference
		A/A	A/G	G/G	G	A	
Turkish							
72	T2DM	59.7%	33.3%	6.9%	23.6%	76.4%	[12]
169	Control	66.9%	26.6%	6.5%	19.8%	80.2%	
Estonian							
70	T1DM	27.1%	41.4%	31.5%	-	-	[13]
305	T2DM	31.1%	50.0%	18.9%	-	-	
252	Control	30.4%	53.6%	16.0%	-	-	
Germany							
300	T2DM	11%	47%	42%	65%	35%	[14]
466	Control	15%	46%	39%	62%	38%	
Chinese							
402	T1DM	6.4%	48.3%	45.3%	69.4%	30.6%	[52]
330	T2DM	9.4%	51.8%	38.8%	64.7%	35.3%	
482	Control	14.9%	50.0%	35.1%	60.1%	39.9%	
Iranian Kurdish							
60	T1DM	41.7%	53.3%	5%	32.5%	67.5%	[53]
56	T2DM	62.5%	32.1%	5%	21.4%	78.6%	
107	Control	62.6%	33.6%	3.7%	20.6%	79.4%	
Iranian							
110	T2DM	8.1%	37.8%	54.1%	73%	27%	[51]
100	Control	20%	39%	41%	60.5%	39.5%	
Taiwanese							
278	T2DM	11.9%	45.7%	42.4%	65.3%	34.7%	This study
287	Control	12.5%	52.3%	35.2%	61.3%	38.7%	
CTLA4 -318 C/T Population		Genotype			Allele		Reference
		C/C	C/T	T/T	C	T	
Turkish							
72	T2DM	76.4%	19.4%	4.2%	86.1%	13.9%	[12]
169	Control	68.6%	25.4%	5.9%	81.4%	18.6%	
Iranian							
110	T2DM	67.6%	23.4%	9%	79.3%	20.7%	[51]
100	Control	88%	10%	2%	93%	7%	
Taiwanese							
278	T2DM	81.7%	17.6%	0.7%	90.3%	9.7%	This study
287	Control	74.9%	23.3%	1.7%	86.6%	13.4%	

Sample size is another factor influencing the experimental results and leading to the possible discrepancy. As a matter of fact, we had recruited 996 study subjects in total (520 controls and 476 patients) and successfully investigated the genotypes of all the recruited subjects, hoping to draw a conclusion with higher statistical power by enlarging the sample size. Supplementary Table S1 showed the distribution of *CTLA-4* genotyping among these 996 study subjects. Significant difference of *CTLA-4* +49 genotypic ($P=0.014$) and allelic ($P=0.016$) frequencies between diabetic and control groups was identified, while neither the genotypic ($P=0.720$) nor allelic ($P=0.870$) distribution of *CTLA-4* -318 between subject groups showed significant differences. Nevertheless, due to the availability of completed information regarding blood chemistry parameters listed in Table 1, we can only analyze the association between the genotypes/alleles and clinical parameters among 287 control and 278 patients.

In summary, this study discloses the significant association between *CTLA-4* promoter genotypic distribution and lipid metabolism-related parameters although no significant association between *CTLA-4* genotypes and T2DM was observed. Our results suggest that *CTLA-4* may be involved in lipid metabolism and affect T2DM disease progression and/or the development of diabetic complications although this gene does not represent a major risk factor for T2DM.

Supplementary Material

Table S1. <http://www.medsci.org/v15p0395s1.pdf>

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Abbreviations

ALT: alanine aminotransferase; ATGL: adipocyte triglyceride lipase; CHO: cholesterol; CPT1A: carnitine palmitoyltransferase; *CTLA-4*: cytotoxic T lymphocyte-associated antigen-4; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; PBMC: peripheral blood mononuclear cell; SNPs: single nucleotide polymorphisms; T2DM: type 2 diabetes mellitus; TGs: triglycerides.

Competing Interests

The authors have declared that no competing interest exists.

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