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Genetic Variants of PTPN2 Gene in Chinese Children with Type 1 Diabetes Mellitus

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Background: Several studies have reported the association of PTPN2 gene with type 1 diabetes mellitus (T1DM) in many populations but not in the Chinese Han population. Therefore, the goal of our study was to replicate the reported association between 2 single-nucleotide polymorphisms (SNPs; rs478582 and rs2542151) in the PTPN2 gene and T1DM in Chinese Han children.

Material/Methods: This case-control study included 141 Chinese Han children with T1DM and 282 healthy controls. Genetic variants of rs478582 and rs2542151 in PTPN2 gene were performed by PCR amplification followed by restriction fragment length polymorphism method.

Results: No difference was observed in association of rs478582 in The PTPN2 gene and T1DM. The distribution of allele frequency of rs2542151 differed significantly between T1DM patients and healthy controls (OR, 0.6; 95%CI: 0.44 to 0.95; and *P*=0.024). Dominant model of rs254215 also was associated with T1DM (OR, 0.6; 95%CI: 0.40 to 0.96; and *P*=0.032). Younger age at onset in G carriers appeared to increase the risk for T1DM (*P*=0.030).

Conclusions: The findings suggested that rs2542151 SNP in The PTPN2 gene was associated with T1DM in Chinese Han children. Further studies with larger sample sizes involving gene-gene interactions are urgently needed.

MeSH Keywords: **Diabetes Mellitus, Type 1 • Genes, vif • Polymorphism, Single Nucleotide**

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Background

Diabetes mellitus is a class of metabolic diseases characterized by chronic hyperglycemia. It mainly results from defects in insulin secretion, insulin action, or both and mostly falls into 2 types: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). As of 2014, an estimated 387 million people have diabetes worldwide, of which about 10% were diagnosed with T1DM. T1DM is usually caused by cellular-mediated autoimmune destruction of pancreatic β-cells, resulting in lifelong insulin-dependency [1]. Most T1DM cases were in children and adolescents. There has recently been an increasing trend in the incidence rate of T1DM in many populations [2].

As a form of diabetes mellitus, T1DM is also known as insulin-dependent or juvenile diabetes. It is caused by autoimmune destruction of insulin-producing pancreatic islet β-cells, and is a complex, multifactorial disease, which is probably caused by a complex combination of genetic and environmental factors triggering autoimmunity [3]. However, these factors are poorly understood [4,5]. The genes of human leukocyte antigen (HLA) class II were proven to be a major susceptibility locus, which account for 30–50% of the genetic risk for T1DM [6]. Previous studies also have identified several non-HLA *loci* with potential influence on progression of T1DM, such as CTLA4 gene [7], PTPN22 gene [8,9], IL2RA gene [10], insulin gene [11], MAPKAP1 gene [12], and PTPN2 (protein tyrosine phosphatase N2) gene [13–17]. These genes also show potential associations with other autoimmune diseases [18–20].

PTPN2 gene, which is located on chromosome 18, encodes the T-cell-specific protein tyrosine phosphatase, which functions as a negative regulator of inflammation by inhibiting the transcription factor STAT1 in the IFN-γ signaling pathway. The expression of PTPN2 has been identified in several cell types, such as β-cells and intestinal epithelial cells, and was reportedly correlated with inflammatory bowel disease [21], celiac disease [22], rheumatoid arthritis [23], and T1DM [24]. Todd et al. [24] analyzed association of 11 single-nucleotide polymorphisms (SNPs) of the PTPN2 gene in T1DM patients and provided evidence of the association between rs478582 and rs1893217 in the PTPN2 gene and T1DM. Espino-Paisan et al. [15] also found that the rs2542151 in the PTPN2 gene was associated with an earlier onset of T1DM. A strong linkage disequilibrium was detected between rs1893217 and rs2542151 [15,24]. However, a study by Rheinheimer et al. [1] indicated no association of rs1893217 polymorphism with T1DM. The relationship between the PTPN2 gene and T1DM remains controversial.

Previous studies have investigated the association of SNPs of the PTPN2 gene (rs478582 and rs2542151) with T1DM in non-Chinese populations, but there is no evidence of the association of the PTPN2 gene with T1DM in the Chinese Han population, especially in Chinese teenagers. Therefore, the purpose of the present study was to replicate the reported association between the rs478582 and rs2542151 SNP of the PTPN2 gene and T1DM in Chinese Han teenagers.

Material and Methods

Ethics Statement

This case-control study was approved by the institutional review board of Shandong University Affiliated with Shandong Provincial Hospital. Written informed consent was provided by every participant.

Study subjects

A total of 141 unrelated T1DM patients younger than 18 years old were enrolled in this study. A total of 282 healthy individuals were recruited in a 1:2 ratio as matched-pair controls, based on age, sex, and ethnicity. All T1DM patients were first diagnosed using the guidelines of the National Diabetes Data Group (1979). Insulin for glycemic control was used by all T1DM patients. T1DM patients were excluded if they had other autoimmune diseases, including myasthenia gravis, Behcet's disease, psoriasis, or multiple sclerosis.

Data on age, sex, body mass index (BMI), blood pressure, family history of T1DM, blood biochemical markers (e.g., glycated hemoglobin (GHbA1c) and triglyceride), and complications were acquired using a questionnaire survey or collected from medical records. Ten ml of venous blood was collected from each subject for genomic DNA extraction.

Genetic analysis

For both groups, 1.5 ml of whole blood was extracted from each participant and stored at –80°C until further analysis. DNA extraction was carried out using a DNA extraction kit (QIAamp DNA mini Kit, Qiagen, Hilden, Germany) according to manufacturer instructions. Genotyping was determined using TaqMan allelic discrimination assays with an ABI 7900 system (Applied Biosystems, Foster City, CA, USA). The PRIMER sequences in this study were: rs478582, 5-ACTGATAATGTTGCTTCAACGG-3 (forward) and 5-TCACCAGCTTCCTCAACCAC-3 (reverse); and rs2542151, 5-CAGCTGCAGCATTTCACGTGG-3 (forward) and 5-CCACTGCCCTATGCGGTCAATT-3 (reverse). The TaqMan assays were conducted in a final reaction volume of 20 µl containing 0.5 µl primer (25 pmol/µl), 0.5 µl probe, 10 µl PCR mixture reagent, and 100 ng DNA. The PCR reactions initially were conducted at 95°C for 10 min for enzyme activation, followed by 40 cycles at 92°C for 15 s and annealing at 60°C for 1 min. PCR genotyping results were analyzed by SDS allelic discrimination

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Table 1. Demographic characteristics of T1DM patients.

Variables	T1DM (n=141)
Age (y)	8.6±4.2
Gender (M/F)	67/74
BMI	19.4±3.2
Blood pressure	
Normal/hypertension	122/19
LDL cholesterol (mmol/L)	1.8±0.62
HDL cholesterol (mmol/L)	1.3±0.53
Triglyceride (mmol/L)	1.4±0.38
GHbA1c (%)	8.7±3.63
Familiar history	12 (8.5%)
Complications	
None/microalbuminuria	133/8

M – male; F – female; BMI – body mass index; LDL – low-density lipoprotein; HDL – high-density lipoprotein; GHbA1c – glycated hemoglobin A1c.

software (ABI). To ensure 100% concordance, approximately 10% of the sample analysis was duplication.

Statistical analysis

All statistical analyses were performed using the SPSS ver.19.0 software package (SPSS, Chicago, IL, USA). Observed and expected genotype frequencies in patients and controls were compared using Hardy-Weinberg analysis. Distributions of allele type and genotype in the cases and controls were compared by means of 2×3 and 2×2 contingency tables using the chi-square test. Qualitative data, presented as number (%), were examined using chi-square test. Quantitative data, presented as mean ± standard deviation, were analyzed using Kolmogorov-Smirnov test with one-way ANOVA [25,26]. A $P<0.05$ was considered significant for all statistical analyses.

Results

Demographics of T1DM patients are listed in the Table 1. Mean age of T1DM at T1DM diagnosis was 8.6±4.2 years. Distributions of genotypes in controls were in agreement with Hardy-Weinberg equilibrium (rs478582, $P=0.091$; and rs2542151, $P=0.192$, respectively).

We analyzed the rs478582 variation in 141 T1DM patients and 282 healthy controls. There was no association between rs478582 SNP and T1DM (Table 2). However, the PTPN2 rs2542151 demonstrated a significant association with risk of T1DM. Although no significant difference was observed in genotypic model analysis, the distribution of allele frequency differed significantly between T1DM patients and healthy controls (OR, 0.6; 95%CI: 0.44 to 0.95; and $P=0.024$). Moreover, we also found a difference in the dominant model (TT/TC+CC) in rs254215 analysis (OR, 0.6; 95%CI: 0.40 to 0.96; and $P=0.032$) (Table 2).

Subsequently, we investigated the relationship of distribution of rs2542151 genotypes and alleles with the clinicopathological features of T1DM patients (Table 3). There was no statistically significant difference between genotypes and allele frequencies of rs2542151 (T/G) and the variables, including sex, BMI, presence of hypertension and complications, GHbA1c level, family history, and the levels of LDL cholesterol, HDL cholesterol, and triglyceride ($p>0.05$). However, when the T1DM patients were divided into 2 groups according to age at onset (≤10 years; and >10 years), we found that allele G carriers age 10 years and younger had an increased risk of T1DM compared with those who were older than 10 years.

Discussion

The present study indicated an association between rs2542151 polymorphism and T1DM in our sample of Chinese Han teenagers. Individuals with the minor G allele appeared to have greater risk for T1DM. Moreover, the G carriers with younger age seemed to be more susceptible to T1DM.

The PTPN2 gene has been explored in several studies [13–17]. However, the association of the PTPN2 gene with risk of T1DM remains controversial. Some authors [15,27] reported that the PTPN2 gene has a significant association with susceptibility of T1DM, while others [1] have rejected that. Moreover, after searching online for relevant studies, we found no published study that investigated whether the PTPN2 gene has an association with risk of T1DM in Chinese Han, especially in Chinese children. Our aim, therefore, was to examine the association between the PTPN2 gene and the risk of T1DM in the Chinese Han population.

Previous studies have reported that the PTPN2 gene is expressed in β-cells and immune cells [13]. Down-regulated expression of PTPN2 aggravates IL-1β + IFN-γ-induced β-cell apoptosis and turns IFN-γ alone into a proapoptotic signal. Doody et al. [28] indicated that PTPN2-knockout mice had anemia, weight loss, exacerbated thymic involution diarrhea, lymphadenopathy, and systemic inflammatory reaction with increased expression levels of inflammatory markers, including TNF-α, IFN-γ, and IL-12. Therefore, PTPN2 may contribute to the pathogenesis of T1DM due to its influence on physiological state of β-cell and immune system.

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Table 2. Distribution of the PTPN2 genotypes and alleles in Chinese children with T1DM and controls.

	T1DM (n=141)	Controls (n=282)	*P*-value
rs478582			
Genotypic model			0.897
TT	100 (70.9%)	195 (67.8%)	
TC	35 (24.8%)	74 (26.5%)	
CC	6 (4.3%)	13 (5.7%)	
Allele model			0.700
T	235	464	
C	47	100	
Dominant model			0.708
TT/TC+CC	100/41	195/87	
Recessive model			0.868
CC/TC+TT	6/135	13/269	
rs2542151			
Genotypic model			0.088
TT	92 (65.2%)	212 (75.2%)	
GT	42 (29.8%)	62 (22.0%)	
GG	7 (5.0%)	8 (2.8%)	
Allele model			0.024*
T	226	486	
G	56	78	
Dominant model			0.032*
TT/GT+GG	92/49	212/70	
Recessive model			0.256
GG/GT+TT	7/134	8/274	

Table 3. Distribution of PTPN2 gene rs2542151 genotypes and alleles in relation to the clinicopathological features of T1DM patients.

Variables	TT (92)	GT (42)	GG (7)	P-value	T (226)	G (56)	*P*-value
Age at onset (≤10/>10 years)	61/31	35/7	6/1	0.089	157/69	47/9	0.030
Gender (M/F)	45/47	18/24	4/3	0.700	108/118	26/30	0.855
BMI	18.7±4.1	19.1±3.9	19.8±3.7	0.516	18.9±4.3	19.6±3.6	0.271
Hypertension (+/–)	10/82	7/35	2/5	0.232	27/199	11/45	0.131
LDL cholesterol (mmol/L)	1.7±0.68	1.8±0.52	1.8±0.72	0.351	1.8±0.53	1.8±0.71	0.143
HDL cholesterol (mmol/L)	1.3±0.41	1.3±0.52	1.4±0.33	0.438	1.3±0.43	1.3±0.63	0.221
Triglyceride (mmol/L)	1.3±0.46	1.4±0.35	1.4±0.41	0.382	1.3±0.52	1.4±0.39	0.214
GHbA1c (mean ± SD)	8.2±2.11	8.5±2.47	8.8±3.13	0.887	8.8±2.92	8.7±3.32	0.693
Familiar history (+/–)	6/86	4/38	2/5	0.138	16/210	8/48	0.084
Complications (+/–)	3/89	4/38	1/6	0.148	10/216	6/50	0.069

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Todd et al. [24] reported rs1893217 (r^2=1 with rs2542151) and rs478582 independently have associations with T1DM susceptibility. Barrett et al. [4] also provided evidence for the association of the PTPN2 gene with T1DM. In this present study, we also investigated 2 SNPs (rs478582 and rs2542151) of PTPN2 gene. After analysis in a total of 423 subjects, no association was observed in rs478582 polymorphism with T1DM. This result is quite different from that of Todd et al. [24]. However, our findings in rs2542151 polymorphism were in agreement with Todd et al. [24], as well as Espino-Paisan et al. [15]. We found G allele carriers were more susceptible to T1DM and the risk of T1DM might be correlated with age at onset. The findings in our study revealed that younger patients had an increasing risk for T1DM. Similarly, Klinker et al. [29] reported an association of IL2RA polymorphisms with age at T1DM onset.

However, Rheinheimer et al. [1] replicated the association between the rs1893217 SNP and T1DM in white subjects from southern Brazil, finding no association of rs1893217 SNP with risk of T1DM. Moreover, they also showed age at T1DM onset might not contribute to the risk of T1DM. Their findings were inconsistent with our results and that of the studies cited above. Therefore, there is an urgent need for more studies with lager sample sizes involving different ethnicities to replicate and examine the association between the PTPN2 gene and T1DM and to further explore the mechanism by which the PTPN2 gene SNPs affect T1DM risk.

Conclusions

In conclusion, we analyzed the 2 independent SNPs in the PTPN2 gene and found that there was a significant association between the rs2542151*G allele and T1DM. G carriers age 10 and younger were more susceptible to T1DM compared with those older than 10 years. Further studies with larger sample sizes involving gene-gene interactions are needed to confirm these results in the Chinese Han population.

Conflict of interest

The authors declare that they have no conflict of interest.

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