



## Association of *PTPN22* gene polymorphism with type 1 diabetes mellitus in Chinese children and adolescents

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**ABSTRACT.** Previous studies have indicated that the protein tyrosine phosphatase nonreceptor type 22 gene (*PTPN22*) is associated with type 1 diabetes (T1DM) in the Caucasian population. In the present study, we investigated the relationship between *PTPN22* genetic polymorphisms and T1DM in Chinese children. A total of 202 children and adolescents with T1DM and 240 healthy control subjects of Chinese Han origin were included in our analysis. Polymerase chain reaction-restriction fragment length polymorphism was used to determine the presence of the C1858T polymorphism in the *PTPN22* gene. We found that the TT +TC genotype and the T allele of C1858T were more frequent in T1DM patients (19.40 and 10.0%, respectively) than in healthy subjects (7.51 and 4.0%, respectively), and the difference was significant (both  $P < 0.001$ ). After adjusting for confounding variables such as gender, age, and family history of T1DM, the difference remained significant ( $P = 0.007$ , odds ratio = 2.88, 95% confidence interval 1.76-4.32). Our results indicate that genetic polymorphisms in the *PTPN22* gene may increase the risk of T1DM in Chinese children and adolescents.

**Key words:** Adolescent; Children; Type 1 diabetes mellitus; *PTPN22*

## INTRODUCTION

Type 1 diabetes mellitus (T1DM) is the second most common chronic childhood disease. The prevalence of T1DM accounts for 5-10% of all diabetes cases (Gale, 2002; Awoniyi et al., 2013; Lipman et al., 2013). Although the exact pathogenesis of T1DM is unknown, T1DM is thought to involve multifactor disorders resulting from genetic polymorphisms as well as various environmental factors (Rich et al., 2006; Ei Wafai et al., 2011; Pehlić et al., 2012; Hadžija et al., 2013). Previous studies have indicated that genetic polymorphisms in genes such as *TRAIL* (Bernardi et al., 2012), *HLA* (Black and Dabelea, 2013), and *CCR5* (Yang et al., 2004) were associated with T1DM risk. However, these results do not fully explain the susceptibility to T1DM.

Protein tyrosine phosphatase non-receptor type 22 (*PTPN22*), a powerful negative regulator of T-cell activation, has been associated with several autoimmune diseases, such as systemic lupus erythematosus (Namjou et al., 2013), T1DM (Giza et al., 2013), juvenile idiopathic arthritis (Dimopoulou et al., 2013), autoimmune thyroid disease (Alkhateeb et al., 2013), and rheumatoid arthritis (Taylor et al., 2013).

*PTPN22*, which is located on chromosome 1, encodes the lymphoid specific tyrosine phosphatase protein Lyp. Lyp is composed of 807 amino acids and plays a major role in regulating the Src family of tyrosine kinases. Src family tyrosine kinases act as a molecular switch to regulate various cellular events, including cell growth, division, differentiation, and programmed death (Reddy et al., 2005).

Recently, the C1858T polymorphism in *PTPN22* was reported to be associated with T1DM in a Caucasian population (Bottini et al., 2004). However, this association was not observed in a Greek population (Giza et al., 2013) or in Japanese subjects (Taniyama et al., 2010). C1858T in *PTPN22* was also associated with T1DM in a recent meta-analysis (Tang et al., 2012); however, the relationship between the C1858T variant and T1DM in Chinese children and adolescents remains unknown.

In this study, we investigated the association between a genetic polymorphism and T1DM in Chinese children and adolescents.

## MATERIAL AND METHODS

### Subjects

We selected 202 children (3-12 years) and adolescents (13-18 years) (121 male, 81 female) with T1DM and 240 age- and gender-matched healthy control subjects (149 male, 91 female) of Chinese Han origin from May 2011 to July 2013. The mean age of participants was  $12.01 \pm 2.11$  years. All patients were diagnosed with T1DM before the age of 15 years and were insulin-dependent. The control group included children and adolescents without T1DM and no family history of T1DM. Informed consent was obtained from parents or guardians of individuals younger than 18 years. The research protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Faculty of Qilu Hospital, Shandong University.

### Methods

Peripheral blood was collected in a test tube containing ethylenediaminetetraacetic

acid (EDTA) as an anticoagulant. DNA was isolated using a DNA Extraction Kit for blood (Bioteke Inc.; Beijing, China) according to the manufacturer protocol. The primers have been described previously (Giza et al., 2013): forward 5'-ACTGATAATGTTGCTTCAACGG-3' and reverse 5'-TCACCAGCTTCCTCAACCAC-3'. The genotyping method was performed according to a previously described protocol (Giza et al., 2013). Briefly, the 40- $\mu$ L polymerase chain reaction (PCR) included 4  $\mu$ L 10X reaction buffer, 3.2  $\mu$ L  $Mg^{2+}$ , 3.8  $\mu$ L dNTPs, 0.5  $\mu$ L upstream primer, 0.5  $\mu$ L downstream primer, 0.3  $\mu$ L *Taq* enzyme, 28.7  $\mu$ L ddH<sub>2</sub>O, and 2.0  $\mu$ L DNA. The PCR conditions were as follows: 94°C initial denaturation for 3 min, 94°C denaturation for 15 s, 57°C annealing for 30 s, and 72°C extension for 30 s. After 35 cycles, we conducted a final extension step at 72°C for 10 min and the reaction was held at 12°C for conservation. A restriction fragment length polymorphism method was used to identify the presence of the target polymorphism, C1858T. The enzymatic system (20  $\mu$ L) included 2  $\mu$ L NEB4 Buffer (New England Biolabs; Ipswich, MA, USA), 0.5  $\mu$ L *RsaI* enzyme (Fermentas; Vilnius, Lithuania), 7.5  $\mu$ L purified PCR products, and 10  $\mu$ L ddH<sub>2</sub>O. PCR products were incubated in a 37°C water bath for 3 h. Next, 10  $\mu$ L enzyme-digested product was analyzed by electrophoresis on a 1.5% agarose gel. The enzyme cuts the C allele into 2 fragments of 172 base pairs (bp) and 46 bp, while the T allele allows the fragment to remain intact (218 bp).

### Statistical analysis

The SPSS 17.0 software (SPSS Institute; Chicago, IL, USA) was used for data analysis. Hardy-Weinberg equilibrium was evaluated using the chi-square test; differences in frequencies of genotypes and alleles between patients and controls were analyzed by chi-square or Fisher's exact tests. Statistical significance was defined as  $P < 0.05$ .

## RESULTS

The distribution of genotypes in patients and controls was found to be in Hardy-Weinberg equilibrium (both  $P > 0.05$ ). The characteristics of the participants are shown in Table 1. There were significant differences between the T1DM group and the control group in family history, body mass index, glucose, triglycerides, total cholesterol, and low-density lipoprotein-cholesterol (all  $P < 0.01$ ). We found no significant difference between groups in gender ratio, age, and high-density lipoprotein-cholesterol (all  $P > 0.05$ ). The frequency of the T allele was higher in patients than in controls (10.0 vs 4.0%;  $P < 0.001$ , odds ratio (OR) = 2.81, 95% confidence interval (CI) = 1.59-4.95). A significant difference was observed between patients and control subjects in the CT genotype distribution ( $P < 0.001$ ). However, we found no significant difference between these 2 groups in the TT genotype frequency. Because of the relatively low frequency of the T allele, the CT and TT genotypes were grouped together for statistical analysis. We observed the TT + CT genotype and T allele more frequently in T1DM patients (19.40 and 10.0%, respectively) than in healthy subjects (7.51 and 4.0%, respectively), and the difference was significant (both  $P < 0.001$ ) (Table 2). After adjusting for other confounders such as gender, age, family history of T1DM, and lipid profiles, the difference remained significant ( $P = 0.007$ , OR = 2.88, 95%CI = 1.76-4.32) (Table 3).

**Table 1.** Characteristics of participants.

Groups	N	Gender ratio (Male/Female)	Age (years)	Family history (N = %)	BMI (Kg/m <sup>2</sup> )	GLU (mM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)
T1DM group	229	136/93	13.3 ± 1.02	47 (20.52)	20.8 ± 2.7	4.52 ± 0.49	0.61 ± 0.27	2.85 ± 0.68	0.89 ± 0.22	1.45 ± 0.49
Control group	213	115/98	13.9 ± 1.01	15 (7.04)	17.2 ± 1.5	4.32 ± 0.43	0.52 ± 0.17	2.64 ± 0.56	0.90 ± 0.21	1.27 ± 0.34
P		0.291	0.102	<0.001	<0.001	<0.001	<0.001	<0.001	0.893	<0.001

T1DM = type 1 diabetes mellitus; BMI = body mass index; GLU = blood glucose; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

**Table 2.** Distributions of *PTPN22* genotype.

Groups	N	Genotypes [N, (%)]			P value	Allele		OR (95%CI)	P value
		TT	TC	CC		T	C		
T1DM group	239	6 (2.51)	38 (15.89)	195 (81.60)	<0.001	50 (0.10)	426 (0.90)	2.81 (1.59-4.95)	<0.001
Control group	213	1 (0.47)	15 (7.04)	197 (92.49)		17 (0.04)	409 (0.96)		

T1DM = type 1 diabetes mellitus.

**Table 3.** Results of logistic regression.

Variables	B	S.E.	Wald	P value	OR (95%CI)
Age	1.121	0.076	4.123	0.011	1.44 (1.01-3.12)
Gender	-0.231	0.233	2.132	0.121	0.87 (0.13-1.66)
Family history	2.322	0.674	4.127	0.010	2.18 (1.45-3.43)
T allele	1.242	0.012	5.329	0.007	2.88 (1.76-4.32)

## DISCUSSION

In the present study, we found that a polymorphism in the *PTPN22* gene was associated with T1DM in Chinese children and adolescents. This is the first study to identify the relationship between the *PTPN22* gene polymorphism and T1DM in Chinese children and adolescents.

The *PTPN22* gene, which encodes Lyp, is located on chromosome 1p13.3-p13.1. Lyp inhibits the activation and proliferation of T lymphocytes, and genetic variants in *PTPN22* may result in the reduction or inactivation of Lyp function. Currently, there are 1073 single-nucleotide polymorphisms of *PTPN22* listed in the NCBI database (<http://www.ncbi.nlm.nih.gov/snp>); rs2476601, which is at position 1858 in the coding region of the *PTPN22* gene, results in substitution of arginine with tryptophan at codon 620 of Lyp (Yu et al., 2007). This mutation may interfere with the Lyp-Csk interaction (Yu et al., 2007), leading to uncontrolled T-cell receptor signaling and inappropriate prolonged activation of T lymphocytes (Yu et al., 2007). This may be the mechanism linking C1858T variants with the risk for T1DM.

Various recent publications have reported a relationship between *PTPN22* and T1DM (Bottini et al., 2004; Kahles et al., 2005; Zheng and She, 2005; Steck et al., 2006; Nielsen et al., 2007; Smyth et al., 2004, 2008; Petrone et al., 2008; Saccucci et al., 2008; Dultz et al., 2009). Bottini et al. (2004) were the first to report an association between the *PTPN22* polymorphism and T1DM. However, in their study, the participants were from North America and Sardinia. Recently, several studies involving different populations have explored the relationship between *PTPN22* and T1DM, including studies from Italy (Petrone et al., 2008; Saccucci et al., 2008), Spain (Santiago et al., 2007), Denmark (Nielsen et al., 2007), North America

(Zheng and She, 2005; Steck et al., 2006), Ukraine (Fedetz et al., 2006), Estonia (Douroudis et al., 2008), Finland (Hermann et al., 2006), the Netherlands (Zhernakova et al., 2005), France (Chelala et al., 2007), Croatia (Korolija et al., 2009), Germany (Kahles et al., 2005; Dultz et al., 2009), Russia (Zhebrun et al., 2011), Colombia (Gomez et al., 2005), Norway (Stene et al., 2010), Poland (Fichna et al., 2010), the United Kingdom (Smyth et al., 2004, 2008), the Czech Republic and Azerbaijan (Cinek et al., 2007), India (Baniyadi and Das, 2008), and Brazil (Chagastelles et al., 2010). However, there was disagreement among these studies regarding the relationship between *PTPN22* and T1DM.

In this study, we investigated the role of *PTPN22* polymorphisms in Chinese children and adolescents with T1DM. We observed the T allele more frequently in T1DM patients than in control subjects after adjusting for other confounders, indicating that the T allele is an independent risk factor for T1DM in Chinese children and adolescents.

In conclusion, our findings agree with the results of similar studies in other populations. However, additional studies including a larger sample are needed to confirm our results.

### Conflict of interests

The authors declare no conflict of interest.

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