



# Association of IL-1 $\alpha$ gene polymorphism with susceptibility to type 1 diabetes in Chinese children

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**ABSTRACT.** The interleukin-1 $\alpha$  (IL-1 $\alpha$ ) gene appears to play a role in the pathogenesis of type 1 diabetes (T1D). Therefore, the aim of this study was to investigate the contribution of the IL-1 rs1800587 gene polymorphism to susceptibility to T1D in Chinese children. This case-control study included 332 Chinese children with T1D and 332 healthy controls. Identification of genetic variants of rs1800587 in the IL-1 $\alpha$  gene was performed by polymerase chain reaction amplification. The IL-1 $\alpha$  rs1800587 polymorphism demonstrated a significant association with T1D risk. The allelic frequency significantly differed between the T1D and control groups [odds ratio (OR) = 0.7; 95% confidence interval (CI) = 0.52-0.86; P = 0.002]. Furthermore, significant differences were observed in the dominant model (CC/CT + TT; OR = 0.6; 95%CI =

0.46-0.85;  $P = 0.003$ ). In T1D patients, the prevalence of hypertension in T allele carriers was 4.2-fold higher than that in C allele carriers, (95%CI = 2.67-6.58;  $P < 0.001$ ). In conclusion, the present study found evidence of a significant association between the rs1800587 polymorphism in the IL-1 $\alpha$  gene and T1D.

**Key words:** Interleukin-1 $\alpha$ ; Rs1800587; Type 1 diabetes; Polymorphism

## INTRODUCTION

Type 1 diabetes (T1D) is a complex, multigenetic autoimmune disease characterized by destruction of pancreatic  $\beta$  cells, resulting in insulin-dependence of patients (Tang et al., 2015). It was estimated that as of 2014, 38.7 million people have T1D worldwide, accounting for about 10% of all patients diagnosed with diabetes (Buchmann et al., 2015). Diabetes can, at the very least, double a person's risk of death (Kalra et al., 2015). The incidence of T1D varies among different populations (Bluestone et al., 2010) owing to the interplay between multiple genetic and environmental risk factors, which are still poorly recognized (Noble et al., 1996; Gale, 2002; Hober and Sauter, 2010).

Human leukocyte antigen (HLA) class II genes at chromosome 6p21 were proven to be a major susceptibility locus, accounting for 30-50% of the genetic risk for T1D (Noble et al., 1996). Genome-wide association studies have identified several non-HLA loci with smaller effects on T1D risk, including the insulin gene, the protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene, and the protein tyrosine phosphatase N2 (PTPN2) gene (Todd et al., 2007; Steck et al., 2012).

Interleukin-1 $\alpha$  (IL-1 $\alpha$ ) is a pro-inflammatory cytokine that plays a role in the regulation of immune responses, inflammatory processes, hematopoiesis, and induction of apoptosis in response to cell injury. IL-1 $\alpha$ , which mainly comes from activated macrophages, neutrophils, epithelial cells, and endothelial cells, is considered to have an effect in the pathogenesis of disc degeneration by increasing the production of extracellular matrix (ECM) degradation enzymes and by inhibiting ECM synthesis (Ye et al., 2007; Phillips et al., 2013). As a pro-inflammatory molecule, IL-1 $\alpha$  is mainly produced by macrophages and natural killer cells as a result of T cell-derived cytokines (e.g., interferon- $\gamma$ ) or bacterial stimulation. IL-1 $\alpha$  binds to the IL-1 receptor, eliciting signal transduction and the corresponding biological effects (Luheshi et al., 2009; Risbud and Shapiro, 2014).

Different single nucleotide polymorphisms (SNPs) may lead to structurally different proteins, altered biological roles, or abnormal transcription rates. The SNP rs1800587 in IL-1 $\alpha$  has been shown to be associated with many autoimmune diseases, including systemic sclerosis (Abtahi et al., 2015). Luotola et al. (2011) have identified the positive association of the rs1800587 polymorphism in IL-1 $\alpha$  with risk of type 2 diabetes (T2D). However, no study has investigated the association of this SNP with T1D risk. Therefore, the purpose of the present study was to investigate the contribution of the IL-1 $\alpha$  gene polymorphism rs1800587 to susceptibility to T1D in a Chinese population.

## MATERIAL AND METHODS

This case-control study was approved by the Institutional Board Review of Shandong Provincial Hospital Affiliated to Shandong University.

## Study subjects

A total of 332 T1D patients, who were diagnosed according to the criteria established by the American Diabetes Association (2015), were recruited from Shandong Provincial Hospital Affiliated to Shandong University. There were 332 health volunteers without family history of diabetes and any autoimmune diseases in the control group (1:1 ratio of T1D patients to controls). All T1D patients were managed with two or more doses of insulin per day. T1D patients who had other autoimmune diseases, including myasthenia gravis, Behcet's disease, psoriasis, and multiple sclerosis, were excluded. Each participant gave written informed consent. All characteristics of patients were collected from medical records or using questionnaires, including age, gender, body mass index (BMI), family history, blood pressure, serum biomarkers [glycated hemoglobin (GHbA1c), triglyceride levels, cholesterol levels, etc.], and medical complications.

## Genetic analysis

A total of 10 mL venous blood was obtained from each participant for genomic DNA extraction and stored at  $-80^{\circ}\text{C}$  until further analysis. DNA was extracted using a DNA extraction kit (QIAamp DNA mini Kit, Qiagen, Hilden, Germany) according to manufacturer instructions. Samples were genotyped for the ADD1 rs1800587 polymorphism with TaqMan allelic discrimination assays with an ABI 7900 system (Applied Biosystems, Foster City, CA, USA). The primer sequences in this study were as follows: 5'-GAGAAGACAAGATGGCTGAACTCT-3' (forward) and 5'-GTCTTCGACTTGGGACTGCTT-3' (reverse). The TaqMan assays utilized 100 ng genomic DNA in reaction volumes of 20  $\mu\text{L}$ . The polymerase chain reactions (PCR) were performed as follows: enzyme activation step of 10 min at  $95^{\circ}\text{C}$ , followed by 40 cycles of  $92^{\circ}\text{C}$  for 15 s and annealing and extension at  $60^{\circ}\text{C}$  for 1 min. PCR genotyping results were tested using the SDS allelic discrimination software (Applied Biosystems). Approximately 10% of the samples were duplicated for analysis to ensure 100% concordance.

## Statistical analysis

All statistical analyses were performed using the SPSS ver. 19.0 software package (SPSS, Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) was examined using the  $\chi^2$  test. Comparisons of allele and genotype distributions in the cases and controls were determined by means of 2 x 3- and 2 x 2-contingency tables using the  $\chi^2$  test. The  $\chi^2$  test and Kolmogorov-Smirnov test with one-way ANOVA were performed to compare baseline characteristics of subjects between genotypes. A  $P < 0.05$  was considered significant for all statistical analyses.

## RESULTS

The main clinical and laboratory characteristics of T1D patients are listed in Table 1. Mean age of T1D patients at diagnosis was  $9.6 \pm 5.3$  years and the mean BMI was  $19.2 \pm 4.1$   $\text{kg}/\text{m}^2$ . Males comprised 41.0% of the T1D patients. Genotype frequencies in controls were in agreement with HWE ( $P = 0.192$ ).

**Table 1.** Demographic characteristics of T1D patients.

Variables	T1D (N = 332)
Age (years)	9.6 ± 5.3
Gender (M/F)	136/196
BMI	19.2 ± 4.1
Blood pressure	
Normal/hypertension	285/47
LDL cholesterol (mM)	1.83 ± 0.71
HDL cholesterol (mM)	1.37 ± 0.61
Triglycerides (mM)	1.43 ± 0.42
GHbA1c (%)	8.8 ± 4.18
Familial history	32 (9.63%)
Complications	
None/microalbuminuria	313/19

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

Genotype and allele frequencies of the IL-1 $\alpha$  rs1800587 polymorphism in T1D patients and healthy controls are depicted in Table 2. We compared the rs1800587 variation between 332 T1D patients and 332 healthy controls from Chinese teenagers. After comparison of genotype and allele frequencies, the IL-1 $\alpha$  rs1800587 polymorphism demonstrated a significant association with T1D risk. Although we did not observe significant differences in genotype model analysis, the allelic frequency differed significantly between the groups [odds ratio (OR) = 0.7; 95% confidence interval (CI) = 0.52-0.86; P = 0.002]. Furthermore, significant difference was also observed in the dominant model (CC/CT + TT; OR = 0.6; 95%CI = 0.46-0.85; P = 0.003; Table 2).

**Table 2.** Distribution of rs1800587 genotypes and alleles in Chinese children with T1D and controls.

	T1D (N = 332)	Controls (N = 332)	P value
Genotype model			0.082
CC	171 (70.9%)	209 (67.8%)	
CT	140 (24.8%)	112 (26.5%)	
TT	21 (4.3%)	11 (5.7%)	
Allele model			0.002
C	482	530	
T	182	134	
Dominant model			0.003
CC/CT + TT	171/161	209/123	
Recessive model			0.070
TT/CT + CC	6/135	13/269	

Next, we analyzed the correlation of the distributions of IL-1 $\alpha$  polymorphism genotypes and alleles with the clinicopathological features in T1D patients (Table 3). No statistically significant differences were present between frequencies of IL-1 $\alpha$  alleles (C/T) and each variable, including gender, BMI, presence of complications, GHbA1c level, family history, and the levels of LDL cholesterol, HDL cholesterol, and triglycerides (P > 0.05). However, the prevalence of hypertension in T allele carriers was 4.2-fold higher than in C allele carriers in T1D patients (95%CI = 2.67-6.58; P < 0.001; Table 3).

**Table 3.** Distribution of rs1800587 genotypes and alleles in relation to the clinicopathological features of T1D patients.

Variables	CC (171)	CT (140)	TT (21)	P value	C (482)	T (182)	P value
Age at onset ( $\leq 10 / > 10$ years)	96/75	90/50	14/7	0.089	282/200	118/64	0.137
Gender (M/F)	72/99	54/86	10/11	0.700	198/284	74/108	0.922
BMI	18.7 $\pm$ 4.1	19.1 $\pm$ 3.9	19.8 $\pm$ 3.7	0.516	18.9 $\pm$ 4.3	19.6 $\pm$ 3.6	0.271
Hypertension (+/-)	10/161	22/118	15/6	0.001*	42/440	52/130	<0.001*
LDL cholesterol (mM)	1.73 $\pm$ 0.68	1.84 $\pm$ 0.52	1.81 $\pm$ 0.72	0.351	1.82 $\pm$ 0.53	1.84 $\pm$ 0.71	0.143
HDL cholesterol (mM)	1.34 $\pm$ 0.41	1.36 $\pm$ 0.52	1.42 $\pm$ 0.33	0.438	1.34 $\pm$ 0.43	1.35 $\pm$ 0.63	0.221
Triglycerides (mM)	1.37 $\pm$ 0.46	1.46 $\pm$ 0.35	1.42 $\pm$ 0.41	0.382	1.33 $\pm$ 0.52	1.41 $\pm$ 0.39	0.214
GHbA1c (mean $\pm$ SD)	8.2 $\pm$ 2.11	8.5 $\pm$ 2.47	8.8 $\pm$ 3.13	0.887	8.8 $\pm$ 2.92	8.7 $\pm$ 3.32	0.693
Familiar history (+/-)	15/156	12/128	5/16	0.138	42/440	22/160	0.189
Complications (+/-)	10/161	6/134	3/18	0.448	26/456	12/170	0.553

\*Significant difference. M = male; F = female; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein; GHbA1c = glycated hemoglobin A1c.

## DISCUSSION

In this population-based report, we demonstrated that the IL-1 $\alpha$  rs1800587 polymorphism is associated with T1D risk in Chinese children. Our results showed that the presence of the T allele could increase risk of T1D. Moreover, for the T1D patients, T allele carriers had a higher prevalence of hypertension than the C allele carriers. While there have been many reports concerning the association of this IL-1 $\alpha$  gene polymorphism with T2D, no studies have investigated its role in susceptibility to T1D. To our knowledge, this is the first study to investigate the role of the IL-1 $\alpha$  rs1800587 polymorphism in T1D risk.

T1D is a complex, chronic autoimmune disease featuring T cell-mediated destruction of pancreatic  $\beta$  cells and subsequent dependence on exogenous insulin (Nokoff et al., 2012). Significant familial aggregation and convincing demonstrations of multiple genetic linkages suggest a genetic component as a risk factor in the pathogenesis of T1D (Noble and Erlich, 2012). Although numerous previous studies have investigated the association between genetic variants and the susceptibility to T1D, the exact mechanisms by which these genes confer T1D susceptibility remain unclear. Therefore, it is necessary to identify more potential genes playing roles in T1D susceptibility.

Diabetes has also been recognized as a disease mediated by inflammatory and immune responses, which lead to impaired signaling of insulin and selective destruction of  $\beta$  cells. Therefore, cytokines play an important role in this mechanism. IL-1 has a central role in regulation of inflammatory and immune responses, and IL-1 $\alpha$  and IL-1 $\beta$  are pro-inflammatory cytokines (Banerjee and Saxena, 2012). Polymorphisms in the IL-1 gene have been reported to be associated with obesity, which is a risk factor for the onset of diabetes (Carter et al., 2008), and play a role in glucose homeostasis and diabetes prevalence (Luotola et al., 2009). The IL-1 gene cluster on chromosome 2q, particularly IL-1 $\alpha$ , has been implicated in susceptibility to a large number of neoplastic, autoimmune, and chronic inflammatory disorders (Timms et al., 2004; Han et al., 2010; Liu et al., 2010). Luotola et al. (2009) reported that the rs1800587 polymorphism in IL-1 $\alpha$  was associated with higher blood glucose in a cross-sectional study (Luotola et al., 2009). Therefore, we conducted this study to investigate whether the rs1800587 polymorphism in the IL-1 $\alpha$  gene plays a role in T1D risk.

Luotola et al. (2011) demonstrated that the genetic variations of rs1800587 in the IL-1 $\alpha$  gene might have an association with the risk of T2D (Luotola et al., 2011). Batool et

al. (2014) also reported that rs1800587 was associated with T2D at the haplotype level in a Pakistani population (Batoool et al., 2014). In our study, the results indicated an association between the rs1800587 polymorphism in the IL-1 $\alpha$  gene with T1D risk. The presence of the T allele significantly increased the risk of T1D. Moreover, the diabetic patients who were carriers of the T allele had a higher risk of hypertension compared with those who were C allele carriers.

In the present study, several limitations should be noted when interpreting the results. First, the relatively small sample size may affect the power in statistical analysis. Second, we should replicate the results in additional individuals, which may decrease potential false positives. Third, other potentially relevant SNPs that might also play important roles in T1D should be examined. Last, we have not performed a functional study to further reveal the mechanism of how the genetic polymorphisms in IL-1 $\alpha$  affect T1D risk.

In conclusion, the present study identified a significant association between the rs1800587 SNP in the IL-1 $\alpha$  gene and T1D. Further multicenter studies, including larger sample sizes, and analysis of gene-gene interactions are necessary to confirm these results in the Chinese population.

### Conflicts of interest

The authors declare no conflict of interest.

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