Association of CD226 polymorphisms with the susceptibility to type 1 diabetes in Chinese children

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ABSTRACT. Polymorphisms in the CD226 gene have been reported to be associated with autoimmune diseases. The aim of our study was to investigate the association between two single nucleotide polymorphisms (SNPs) (rs763361 and rs727088) in the CD226 gene and the risk for developing type 1 diabetes (T1D) in Chinese Han children. This case-control study included a total of 152 Chinese children with T1D and 304 matched-pair, healthy controls based on age and gender. The genetic variants of the rs763361 and rs727088 SNPs in the CD226 gene were determined using the polymerase chain reaction and restriction fragment length polymorphism method. The CD226 rs763361 polymorphism increased the risk of T1D in the genotype [P < 0.001, odds ratio (OR) = 3.9, 95% confidence interval (CI) = 2.24-6.76], dominant (P < 0.001, OR = 2.1, 95%CI = 1.40-3.14), and recessive (P < 0.001, OR = 0.5, 95%CI = 0.30-0.84) models. Additionally, the carriers of the T allele were more susceptible to T1D (P < 0.001, OR =

2.1, 95%CI = 1.58-2.79). Carriers of the T allele who were younger than 10 years of age at disease onset had an increased risk of T1D than those who were older at the disease onset. However, there was no association between the CD226 rs727088 SNP and risk for developing T1D. These findings revealed that CD226 rs763361 polymorphism was significantly associated with susceptibility to T1D and that the presence of the T allele might be a genetic factor for susceptibility to T1D.

Key words: Type 1 diabetes; CD226; Polymorphism; Chinese

INTRODUCTION

Patients with type 1 diabetes (T1D) are reported to be at a high risk for cardiovascular disease and microvascular complications, leading to premature death (Morrish et al., 2001; Makinen et al., 2008). T1D is a complex autoimmune disease that is caused by a T cell-mediated attack on pancreatic β cells (American Diabetes Association, 2013). The incidence of T1D increases by 3-5% each year, resulting in an estimated 65,000 new cases each year in children younger than 15 years of age (Borchers et al., 2010: Bruno et al., 2010; Ehehalt et al., 2010; Jarosz-Chobot et al., 2011).

In addition to environmental factors, it is well recognized that T1D has a genetic component that may contribute to differences in susceptibility. To date, the investigation of genetic variants associated with susceptibility to T1D have identified more than 60 loci across the human genome (Todd et al., 2007; Zheng et al., 2014), including human leukocyte antigen (HLA) on chromosome 6p21, the insulin gene on chromosome 11p15, tyrosine-protein phosphatase non-receptor type 22 (PTPN22) on chromosome 1p13, PTPN2 on chromosome 18p11, interferon induced with helicase C domain 1 (IFIH1) on chromosome 2q24, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on chromosome 2q33, interleukin 2 receptor alpha (IL2RA) on chromosome 10p15, and C-type lectin domain family 16, member A (CLEC16A) to (Hakonarson et al., 2007; Todd et al., 2007). However, single nucleotide polymorphisms (SNPs) in these genes that are associated with T1D risk only explain part of the pathogenesis of the development of T1D, indicating that other loci must be identified.

A recent genome-wide association study (GWAS) in patients with T1D identified SNPs in cluster of differentiation 226 (CD226) that may be associated with T1D risk (Todd et al., 2007). The CD 226 gene is located on chromosome 18q22.3 and encodes CD226, which is a type I transmembrane glycol-protein (67 kDa) and a member of the immunoglobulin superfamily (Shibuya et al., 1996) (Shibuya, Campbell et al. 1996). CD226 plays an important role in the mediation of cell activation and differentiation and is constitutively expressed on the surface of immune cells, including $CD4^+$ and $CD8^+$ T lymphocytes, natural killer cells, macrophages, and platelets (Shibuya et al., 1996; Maier and Hafler, 2009; Xu and Jin, 2010).

Recently, several SNPs in the CD226 gene, such as rs763361 and rs727088, have been reportedly associated with several autoimmune diseases, including rheumatoid arthritis, systemic sclerosis, Graves' disease, systemic lupus erythematosus (SLE), and multiple sclerosis (Hafler et al., 2009; Douroudis et al., 2009; Maiti et al., 2010). The association of the rs763361 or rs727088 SNPs with T1D risk was also investigated in several studies. In their study on a Brazilian population, Mattana et al. (2014) reported that the rs763361 SNP in CD226 is associated with susceptibility to T1D. Moreover, two SNPs (rs763361 and rs727088) in the CD226 gene were associated with SLE and systemic sclerosis-related pulmonary fibrosis (Lofgren et al., 2010). However, in a study

of a Korean population, Kim et al. (2013) suggested that CD226 polymorphisms are not associated with inflammatory demyelinating diseases. Therefore, the association between CD226 SNPs and autoimmune diseases remains unclear and requires additional study.

Although rs763361 and rs727088 polymorphisms in CD226 have been investigated in several previous studies, their roles in T1D risk in the Chinese population remain unknown. Therefore, the purpose of this study was to investigate the contributions of CD226 rs763361 and rs727088 SNPs to T1D susceptibility in Chinese children.

MATERIAL AND METHODS

Study subjects

A total of 152 T1D patients were recruited to participate in the present study. The study design was approved by the Institutional Board Review of Shandong Provincial Hospital, and each participant provided written informed consent. The mean age of the patients ± standard deviation (SD) at the time of disease onset was 8.7 ± 4.4 years. The diagnosis of T1D was based on the guidelines of the American Diabetes Association (ADA), and all of the patients were treated with two or more doses of insulin per day. The 1:2 ratio of the matched-pair control group was comprised of a total of 304 healthy individuals (average age 8.4 ± 4.3 years) with normal fasting plasma glycated hemoglobin (GHbA1c) and glucose levels and without positive history of autoimmune diseases, such as myasthenia gravis, Behcet's disease, psoriasis, and multiple sclerosis.

The demographics of all subjects were obtained using a questionnaire survey or were derived from medical records, including age, gender, body mass index standard deviation score (BMI-SDS), blood pressure, blood biochemical markers (GHbA1c, triglycerides, etc.), family history of T1D, and disease complications. Furthermore, 10 mL venous blood samples for genomic DNA extraction were collected from each participant.

CD226 genotyping

The two SNPs, rs763361 and rs727088, in the CD226 gene were obtained from an Asian (Chinese and Japanese) population database of the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). The primers were designed for the polymerase chain reaction (PCR) assay according to the description of a previous study by Kim et al. (2013). DNA extraction was performed using a DNA extraction kit (QIAamp DNA mini Kit, Qiagen, Hilden, Germany) according to the manufacturer instructions. Genotyping was determined by TaqMan allelic discrimination assays using an ABI 7900 system (Applied Biosystems, Foster City, CA, USA). The primer sequences in this study were as follows: rs763361, 5'-TTGCATAAAGATCCATGCATGAGTAC-3' (forward) and 5'-GATTTCTGTTGCATCTCAGTCAAGAA-3' (reverse); rs727088, 5'-TGTCATTAGGGCTGTCTTTGTCTGAATAG-3' (forward) and 5'-CCAGGTCTAGCCTTAGGAGCAAATGTA-3' (reverse).

The TaqMan assays were determined in a final reaction volume of 20 µL containing 0.5 µL primer (25 µM), 0.5 µL probe, 10 µL PCR mixture reagent and 100 ng DNA. Amplification was initially conducted with a denaturation step at 95°C for 5 min for enzyme activation, followed by 30 cycles of: 95°C for 30 s; 60°C for 23 s for rs763361 or 64°C for 30 s for rs727088; and 72°C for 25 s for rs763361 or 72°C for 23 s for rs727088. The reaction was completed with an annealing step at 72°C for 10 min. The PCR products were verified using SDS allelic discrimination software (ABI, Applied Biosystems). To confirm genotyping quality, approximately 10% of the sample analysis was duplicated.

Statistical analysis

All of the statistical analyses were performed using the SPSS 19.0 software (Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) was separately measured for the patient and control groups. The differences in allele and genotype distributions between the cases and controls were calculated by 2 x 3 and 2 x 2 contingency tables using a χ^2 test. The qualitative data, presented as a number (%), were also analyzed using a χ^2 test. The quantitative data, expressed as the mean ± SD, were determined using a Kolmogorov-Smirnov test with one-way ANOVA (Hu et al., 2014). $P < 0.05$ was considered to be statistically significant.

RESULTS

The characteristics of all of the subjects included in the study are shown in Table 1. The subjects were adequately matched based on age and gender. In the present study, the genotype distributions in the controls were in accordance with HWE (rs763361, P = 0.094; and rs727088, P = 0.939).

Table 1. Demographics of included pediatric T1D cases and age- and gender-matched controls.

Variables	T1D cases (N = 152)	Controls (N = 304)	P value
Age of onset (years)	8.7 ± 4.4	8.4 ± 4.3	0.695
Gender (M/F)	85/67	172/132	0.894
Blood pressure			
Normal/Hypertension	125/27	-	
LDL cholesterol (mM)	1.8 ± 0.64	-	
HDL cholesterol (mM)	1.3 ± 0.55	-	
Triglycerides (mM)	1.4 ± 0.41	-	
GHbA1c (%)	8.8 ± 3.53	-	
Family history	15 (9.8%)	-	
Complications		-	
None/Microalbuminuria	144/8	-	

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

Association between CD226 rs763361 and rs727088 polymorphisms and T1D risk

The genotype frequencies of the CD226 rs763361 polymorphism were 32.9% (CC), 38.8% (CT), and 28.3% (TT) in patients with T1D and 50.6% (CC), 38.2% (CT), and 11.2% (TT) in controls. When the CD226 rs763361 CC homozygous genotype was used as the reference group, the TT genotype ($P < 0.001$, odds ratio (OR) = 3.9, 95% confidence interval (CI) = 2.24-6.76) was associated with a significantly increased risk for T1D. Similarly, we found significant differences in allele ($P < 0.001$, OR = 2.1, 95%CI = 1.58-2.79), dominant ($P < 0.001$, OR = 2.1, 95%CI = 1.40-3.14), and recessive models ($P < 0.001$, OR = 0.5, 95%CI = 0.30-0.84). However, there was no association between the CD226 rs727088 SNP and risk of T1D. Table 2 displays the distributions of genotypes and alleles for CD226 rs763361 and rs727088 polymorphisms. Subsequently, we evaluated the correlation between the distribution of the CD226 rs727088 polymorphism and the clinicopathological features of children with T1D (Table 3). No statistically significant associations were found between the CD226 rs763361 SNP and any of the clinicopathological variables, except age at the time of diagnosis of T1D. When the patients with T1D were divided into two groups based on the children's age at the time of the disease onset (≤ 10 years and > 10 years), the results

revealed that carriers of the T allele who were younger than 10 years of age at disease onset had an increased risk for developing T1D than those who were older than 10 years of age at disease onset.

Table 2. Distributions of the CD226 genotypes and alleles in Chinese children with T1D and controls.

	T1D (N = 152)	Controls (N = 304)	OR (95%CI)	P value
rs763361				
Genotype model				
CC	50 (32.9%)	154 (50.6%)	1.00	
CT	59 (38.8%)	116 (38.2%)	1.6 (1.00-2.45)	0.048
TT	43 (28.3%)	34 (11.2%)	3.9 (2.24-6.76)	<0.001
Allele model				
C	159	424	1.00	Reference
T	145	184	2.1 (1.58-2.79)	<0.001
Dominant model				
CC	50	154	1.00	Reference
CT + TT	102	150	2.1 (1.40-3.14)	<0.001
Recessive model				
TT	43	34	1.00	Reference
CC + CT	109	270	0.5 (0.30-0.84)	<0.001
rs727088				
Genotype model				
GG	38 (25.0%)	66 (21.7%)	1.00	Reference
GA	81 (53.3%)	152 (50.0%)	0.9 (0.57-1.50)	0.753
AA	33 (21.7%)	86 (28.3%)	0.7 (0.38-1.17)	0.159
Allele model				
G	157	284	1.00	Reference
A	147	324	0.8 (0.62-1.08)	0.160
Dominant model				
GG	38	66	1.00	Reference
GA + AA	114	238	0.8 (0.58-1.31)	0.430
Recessive model				
AA	33	86	1.00	Reference
GG + GA	118	218	1.4 (0.89-2.23)	0.141

OR = odds ratio.

Table 3. Distributions of CD226 rs763361 SNP genotypes and alleles in relation to the clinicopathological features of T1D patients.

Variables	CC (50)	CT (59)	TT (43)	P value	C (159)	T (145)	P value
Age of onset (≤10/>10 years)	22/28	38/21	27/16	0.089	82/77	92/53	0.037
Gender (M/F)	28/22	36/23	21/22	0.765	92/67	78/67	0.475
Hypertension (+/-)	8/42	7/52	12/31	0.223	23/136	31/114	0.115
LDL cholesterol (mM)	1.7 ± 0.69	1.8 ± 0.61	1.8 ± 0.71	0.812	1.8 ± 0.54	1.8 ± 0.69	0.734
HDL cholesterol (mM)	1.3 ± 0.56	1.3 ± 0.52	1.4 ± 0.62	0.481	1.3 ± 0.53	1.3 ± 0.61	0.248
Triglycerides (mM)	1.3 ± 0.39	1.4 ± 0.43	1.4 ± 0.51	0.328	1.3 ± 0.40	1.4 ± 0.44	0.334
GHbA1c (mean ± SD)	8.3 ± 3.55	8.6 ± 3.23	9.2 ± 3.56	0.587	8.6 ± 3.59	8.9 ± 3.62	0.493
Family history (+/-)	4/46	4/55	7/36	0.183	12/147	18/127	0.155
Complications (+/-)	1/49	3/56	4/39	0.102	5/154	11/134	0.083

DISCUSSION

The current study demonstrated an association between the CD226 rs763361 polymorphism and T1D in a Chinese population. In our analysis, the minor T allele appeared to be aggressive, with 28.3% of children with T1D carrying the TT genotype compared with 11.2% of the healthy controls. These findings confirm previously published data from Brazilian cohorts published by Mattana et al. (2014), which showed that the presence of the T allele might be one of the genetic risk factors for T1D.

It is well known that interactions between genetic and environmental factors may lead to complications in the development of T1D (Pociot et al., 2010). To date, previous studies have identified several genetic loci that may affect immune function and thereby contribute to the development of T1D. With respect to the HLA region (chromosome 6p21), its roles in the immune response and in T1D risk have been widely demonstrated; however, other genes involved in the immune response might also account for risk associated with T1D (Fichna et al., 2013). Since the exact mechanism of T1D development remains unclear, the detection of gene-gene and gene-environment interactions in the etiology of this disease may contribute to a better understanding of its pathogenesis. Therefore, the use of statistical and computational methods for detecting the effect of genetic variants on disease susceptibility with implementation in a systems biology framework may be conducive to solving the missing heritability problem to (Moore et al., 2010).

CD226 is an important cell-surface receptor molecule involved in the adhesion and activation of T cells (Liu et al., 2012). In previous studies, the rs763361 SNP in the CD226 gene has been reported to be associated with susceptibility to autoimmune diseases (Hafler et al., 2009; Douroudis et al., 2010; Maiti et al., 2010), including T1D in a Brazilian population (Mattana et al., 2014). Moreover, Lofgren et al. (2010) identified the potential interference of the rs727088 SNP on the regulation of CD226 transcription both in T cells and natural killer cells. However, Kim et al. (2013) demonstrated that CD226 polymorphisms may not be associated with disease in a Korean population and that their role may vary between populations. Therefore, the exact effect of polymorphisms in CD226 requires additional investigation.

Mattana et al. (2014) revealed that the gender of the subjects is a factor in T1D risk, as their study found that T1D risk associated with the rs763361 SNP was higher in females than males. This suggests an additive effect of female hormones with this SNP. In our study, we did not find similar results; however, the T1D risk in carriers of the T allele who were younger than 10 years of age at disease onset was evident compared with those who were older than 10 years of age at disease onset. T1D risk is likely to be connected to the levels of T helper 1 cells, which varies with age.

Several limitations in our study should not be ignored. First, because this was a hospital-based case-control study in which the subjects included could not sufficiently represent the general population, selection bias was inevitable. Second, the sample size in this study was small, which might produce insufficient results in the statistical analyses. Finally, the polymorphisms were selected on the basis of their functional considerations in our study so they may not provide a comprehensive perspective regarding the genetic variability of T1D.

To the best of our knowledge, this report is the first to focus on the association between two SNPs, rs763361 and rs727088, in the CD226 gene and T1D risk in Chinese children. We found a significant association between the CD226 rs763361 polymorphism and susceptibility to T1D. The presence of the T allele might be a genetic factor for susceptibility to developing T1D. Allele T carriers who were younger than 10 years of age at the onset of disease appeared to increase the risk for T1D. Additional studies with larger sample size involving different ethnicities are required to confirm our findings.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

American Diabetes Association (2013). Diagnosis and classification of diabetes mellitus. *Diabetes Care* 36 (Suppl 1): S67-S74.

Borchers AT, Uibo R and Gershwin ME (2010). The geoepidemiology of type 1 diabetes. *Autoimmun. Rev.* 9: A355-A365.

Bruno G, Maule M, Merletti F, Novelli G, et al. (2010). Age-period-cohort analysis of 1990-2003 incidence time trends of childhood diabetes in Italy: the RIDI study. *Diabetes* 59: 2281-2287.

Douroudis K, Kingo K, Silm H, Reimann E, et al. (2010). The CD226 Gly307Ser gene polymorphism is associated with severity of psoriasis. *J. Dermatol. Sci.* 58: 160-161.

Ehehalt S, Dietz K, Willasch AM, Neu A, et al. (2010). Epidemiological perspectives on type 1 diabetes in childhood and adolescence in germany: 20 years of the Baden-wurttemberg Diabetes Incidence Registry (DIARY). *Diabetes Care* 33: 338-340.

Fichna M, Zurawek M, Fichna P, Ziolkowska-Suchanek I, et al. (2013). Polymorphic variant at the IL2 region is associated with type 1 diabetes and may affect serum levels of interleukin-2. *Mol. Biol. Rep.* 40: 6957-6963.

Hafler JP, Maier LM, Cooper JD, Plagnol V, et al. (2009). CD226 Gly307Ser association with multiple autoimmune diseases. *Genes Immun.* 10: 5-10.

Hakonarson H, Grant SF, Bradfield JP, Marchand L, et al. (2007). A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. *Nature* 448: 591-594.

Hu YC, Wu L, Yan LF, Wang W, et al. (2014). Predicting subtypes of thymic epithelial tumors using CT: new perspective based on a comprehensive analysis of 216 patients. *Sci. Rep.* 4: 6984.

Jarosz-Chobot P, Polanska J, Szadkowska A, Kretowski A, et al. (2011). Rapid increase in the incidence of type 1 diabetes in Polish children from 1989 to 2004, and predictions for 2010 to 2025. *Diabetologia* 54: 508-515.

Kim JY, Kim HJ, Cheong HS, Bae JS, et al. (2013). Lack of association between CD226 genetic variants and inflammatory demyelinating diseases in Korean population. *Neuro. Endocrinol. Lett.* 34: 402-408.

Liu R, Xu N, Wang X, Shen L, et al. (2012). Influence of MIF, CD40, and CD226 polymorphisms on risk of rheumatoid arthritis. *Mol. Biol. Rep.* 39: 6915-6922.

Lofgren SE, Delgado-Vega AM, Gallant CJ, Sanchez E, et al. (2010). A 3'-untranslated region variant is associated with impaired expression of CD226 in T and natural killer T cells and is associated with susceptibility to systemic lupus erythematosus. *Arthritis Rheum.* 62: 3404-3414.

Maier LM and Hafler DA (2009). Autoimmunity risk alleles in costimulation pathways. *Immunol. Rev.* 229: 322-336.

Maiti AK, Kim-Howard X, Viswanathan P, Guillen L, et al. (2010). Non-synonymous variant (Gly307Ser) in CD226 is associated with susceptibility to multiple autoimmune diseases. *Rheumatology* 49: 1239-1244.

Makinen VP, Forsblom C, Thorn LM, Waden J, et al. (2008). Metabolic phenotypes, vascular complications, and premature deaths in a population of 4,197 patients with type 1 diabetes. *Diabetes* 57: 2480-2487.

Mattana TC, Santos AS, Fukui RT, Mainardi-Novo DT, et al. (2014). CD226 rs763361 is associated with the susceptibility to type 1 diabetes and greater frequency of GAD65 autoantibody in a Brazilian cohort. *Mediators Inflamm.* 2014: 694948.

Moore JH, Asselbergs FW and Williams SM (2010). Bioinformatics challenges for genome-wide association studies. *Bioinformatics* 26: 445-455.

Morrish NJ, Wang SL, Stevens LK, Fuller JH, et al. (2001). Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* 44 (Suppl 2): S14-S21.

Pociot F, Akolkar B, Concannon P, Erlich HA, et al. (2010). Genetics of type 1 diabetes: what's next? *Diabetes* 59: 1561-1571.

Shibuya A, Campbell D, Hannum C, Yssel H, et al. (1996). DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. *Immunity* 4: 573-581.

Todd JA, Walker NM, Cooper JD, Smyth DJ, et al. (2007). Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat. Genet.* 39: 857-864.

Xu Z and Jin B (2010). A novel interface consisting of homologous immunoglobulin superfamily members with multiple functions. *Cell. Mol. Immunol.* 7: 11-19.

Zheng H, Tan Z and Xu W (2014). Immune evasion strategies of pre-erythrocytic malaria parasites. *Mediators Inflamm.* 2014: 362605.