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Original Article

Association of polymorphism variation in interleukin-8 with the risk of developing acute pancreatitis

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Abstract: The etiology of acute pancreatitis has been widely studied, but the mechanisms of this disease is not well clear. In our study, we performed a study in a Chinese population, and firstly evaluate the role of *IL-8* -251A/T, -353A/T and +781C/T polymorphisms in the risk of acute pancreatitis. This study comprises of 315 acute pancreatitis patients and 376 controls from the First Affiliated Hospital of Zhengzhou University between December 2012 and April 2015. The genotyping of *IL-8* -251A/T, -353A/T and +781C/T was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The unconditional logistic regression analysis revealed a relationship between the TT genotype of *IL-8* -251A/T and an increased risk of acute pancreatitis (adjusted OR=2.88, 95% CI=1.84-4.53), compared to the AA genotype. In the dominant and recessive genetic models, the *IL-8* -251A/T polymorphism was significantly correlated with an elevated risk of acute pancreatitis, and the adjusted Ors (95% CI) for dominant and recessive models were 1.57 (1.11-2.24) and 2.62 (1.80-3.82), respectively. However, no significant differences were observed between *IL-8* -353A/T and +781C/T polymorphisms and risk of acute pancreatitis. In conclusion, the present study has shown that the *IL-8* -251A/T polymorphism was significantly associated with an increased risk of acute pancreatitis in a Chinese population.

Keywords: *IL-8*, polymorphism, acute pancreatitis

Introduction

Acute inflammation of the pancreas, known as acute pancreatitis, has an estimated mortality rate between 10% and 30%, based on the severity of this disease and its infectious status [1]. The etiology of acute pancreatitis has been widely studied, but the mechanism of this disease is not well clear. Many previous studies have shown that many environmental factors play a key role in the pathogenic mechanism of acute pancreatitis, such as gallstones, heavy alcohol consumption and overeating [2-4]. In 10-30% of acute pancreatitis, the etiology is unknown [5]. Recently, many studies have reported that genetic polymorphisms are involved in the development of acute pancreatitis, such as *interleukin-10* (*IL-10*), *DEFB1*, *claudin2*, *PRSS1-PRSS2*, *IL-1β*, *IL-6*, *Toll-like receptor*, *TNFAIP3* and *heme oxygenase-1* [6-12].

Interleukin-8 (*IL-8*) is a family member of chemokines, locates on chromosome 4q13-q21 comprising of 4 exons and a proximal promoter region, and it encodes a 99 amino acid polypeptide [13]. It is reported that *IL-8* is expressed in infiltrating cells, proliferating ductular cells and acinar cell, and its expression is associated with the fibrotic process in pancreatitis [14]. Level of *IL-8* is associated with the etiology of acute pancreatitis in a prospective study [15]. Genomic polymorphisms of *IL-8* could influence the structure, expression and quantity of *IL-8*, and ultimately affect the function of genes. Five studies have investigated the relationship between *IL-8* polymorphisms and development of acute pancreatitis, but the results are conflicting [16-20]. Moreover, no study reported the association between *IL-8* -353A/T polymorphism and development of acute pancreatitis risk. Therefore, we performed a study in a Chinese population, and firstly evaluate the role of *IL-8* -251A/T, -353A/T and +781C/T polymorphisms in the risk of acute pancreatitis.

Material and methods

Subjects

In this hospital-based case-control study, we performed a study comprising of 315 acute

pancreatitis patients and 376 controls. These 315 acute pancreatitis patients were collected between December 2012 and April 2015 from the First Affiliated Hospital of Zhengzhou University. The diagnosis of acute pancreatitis was based on the China guidelines for the diagnosis and treatment of acute pancreatitis (2013, Shanghai) Guidelines for the diagnosis of treatment of severe acute pancreatitis made by the Chinese Medical Association.

The control subjects were individuals receiving regular healthy examinations in outpatient clinics. Women with any history of malignant tumors, digestive system disease, endocrine disease, end-stage kidney or liver diseases.

The clinical variables of acute pancreatitis patients and control subjects were collected from medical record. The demographic and lifestyle variables of included subjects were investigated by face-to-face interviews with a structured questionnaire. All the participants obtained their informed written consent. This study was confirmed by the principles outlined in the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

DNA extraction and genotyping

Finally from each subjects, 5 mL blood collected. Genomic DNA was isolated from blood samples by the DNA Purification Kit (Tiangen Biotech, Beijing, China). The genotyping of *IL-8* -251A/T, -353A/T and +781C/T was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The forward and reverse primers for *IL-8* -251A/T were 5'-GTGGTATCACAGAGGATTATGC-3' and 5'-CAGTCATAACTGACAACATTGATC-3', respectively. For the *IL-8* -353A/T, the primers for *IL-8* -353A/T were 5'-ATTGGCTGGCTTATCTTCA-3' (forward) and 5'-CAAATACGGAGTATGACGAAG-3' (reverse), respectively. For the *IL-8* +781C/T, the primers were 5'-GAATTCAGTAACCCAGGCAT-3' and 5'-AAGCTTGTGTGCTCTGCTGTCTCT-3', respectively. The cycling reaction was performed with an initial step of 95°C for 5 minutes, 20 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 40 s, and a final step of 72°C for 6 minutes. For quality control, 10% of the samples were randomly selected to confirm the consistency with previous results.

Following restriction digests, one fragment of 230 bp was observed for the TT genotype of IL-8 -251A/T, while two fragments of 165 and 65 bp were produced from the AA genotype sequence, and three resulted from those of the AT genotype, with lengths of 230, 165, and 65 bp. For *IL-8* -353A/T, one fragment of 360 bp was observed for the TT genotype, while two fragments of 280 bp and 80 bp were observed from the AA genotype sequence, and three fragments were shown for the AT genotype, with lengths of 360 bp, 280 bp and 80 bp. For the *IL-8* +781C/T, one fragment of 162 bp was found for the CC genotype, two fragments of 118 bp and 44 bp were produced from the TT genotype sequence, and three fragments of 162 bp, 118 bp and 44 bp were seem for the CT genotype.

Statistical analysis

The Chi-square test and student t were used to compare the demographic and clinical variables and allele and genotype frequencies between the case and control groups. Hardy-Weinberg equilibrium (HWE) in the case and control groups was calculated. Odds ratio (OR) with 95% confidence interval (95% CI) were calculated for the relationship between *IL-8* -251A/T, -353A/T and +781C/T polymorphism and risk of acute pancreatitis. The co-dominant, dominant and recessive models were used in our analysis. The wide-type genotype of *IL-8* -251A/T, -353A/T and +781C/T was used as a references. All statistical analyses were performed using the SPSS software V17.0 (SPSS Inc., Chicago, Illinois, USA). Two-sided *P* values less than 0.05 were considered statistically significant.

Results

The Pearson chi-square test and student t test were used to compare the demographic and clinical variables between acute pancreatitis patients and controls (**Table 1**). We observed that acute pancreatitis patients were more likely to have a family history of acute pancreatitis (χ^2=10.24, P=0.001), have a higher BMI (χ^2=35.53, P<0.001), have a habit of alcohol drinking (χ^2=37.20, P<0.001), and have higher levels of TC (t=8.27, P<0.001), TG (t=13.37, P<0.001), LDL-c (t=13.37, P<0.001) and blood glucose (t=15.29, P<0.001). However, no sig-

Table 1. Comparison of demographic and clinical variables between acute pancreatitis patients and controls

Variables	Patients N=315	%	Controls N=376	%	χ^2 or t value	*P* value
Age, years		56.35±8.72		55.70±9.15	0.95	0.17
Gender						
Females	112	35.56	141	37.50		
Males	203	64.44	235	62.50	0.28	0.60
Family history of acute pancreatitis						
No	297	94.29	371	98.67		
Yes	18	5.71	5	1.33	10.24	0.001
BMI, kg/m^2						
≤25	165	52.38	279	74.20		
>25	150	47.62	97	25.80	35.53	<0.001
Tobacco smoking						
No	186	59.05	242	64.36		
Yes	129	40.95	134	35.64	2.05	0.15
Alcohol drinking						
No	111	35.24	220	58.51		
Yes	204	64.76	156	41.49	37.20	<0.001
TC		6.57±1.44		5.65±1.47	8.27	<0.001
TG		2.07±0.42		1.63±0.44	13.37	<0.001
HDL-c		1.13±0.26		1.15±0.29	0.95	0.17
LDL-c		4.15±1.09		3.52±1.04	13.37	<0.001
Blood glucose		7.16±1.36		5.70±1.15	15.29	<0.001

BMI: Body mass index; TC: Total cholesterol; TG: Triglyceride; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol.

Table 2. Genotype distributions of *IL-8* -251A/T, -353A/T and +781C/T in patients and controls

IL-8	Patients N=315	%	Controls N=376	%	χ^2 test	*P* value	HWE in patients	*P* value	HWE in controls	*P* value
-251A/T										
AA	55	24.34	71	31.42						
AT	98	43.36	116	51.33						
TT	73	32.30	39	17.26	13.87	0.001	3.66	0.06	0.51	0.47
-353A/T										
AA	228	72.38	251	66.76						
AT	75	23.81	114	30.32						
TT	12	3.81	11	2.93	3.84	0.15	3.23	0.07	0.20	0.65
+781C/T										
CC	135	42.86	176	46.81						
CT	145	46.03	163	43.35						
TT	35	11.11	37	9.84	1.14	0.57	0.18	0.67	0.07	0.93

nificant differences were observed between acute pancreatitis patients and controls with respect to age (t=0.95, P=0.17), gender (t=0.28, P=0.60), tobacco smoking (t=2.05, P=0.15) and HDL-c (t=0.95, P=0.17).

Using the Pearson chi-square test, the genotype frequencies of *IL-8* -251A/T were significantly difference between acute pancreatitis patients and controls (χ^2=13.87, P=0.001), whereas no significant differences were found

Table 3. Association between *IL-8* -251A/T, -353A/T and +781C/T and risk of acute pancreatitis

IL-8	Patients	%	Controls	%	Adjusted OR (95% CI)[1]	*P* value
-251A/T						
Co-dominant						
AA	73	23.17	121	32.18	1.0 (Reference)	-
AT	136	43.17	194	51.60	1.16 (0.80-1.70)	0.42
TT	106	33.65	61	16.22	2.88 (1.84-4.53)	<0.001
Dominant						
AA	73	23.17	121	32.18	1.0 (Reference)	-
AT+TT	242	76.83	255	67.82	1.57 (1.11-2.24)	0.01
Recessive						
AA+AT	209	66.35	315	83.78	1.0 (Reference)	-
TT	106	33.65	61	16.22	2.62 (1.80-3.82)	<0.001
-353A/T						
Co-dominant						
AA	228	72.38	251	66.76	1.0 (Reference)	-
AT	75	23.81	114	30.32	0.72 (0.51-1.03)	0.06
TT	12	3.81	11	2.93	1.20 (0.47-3.07)	0.67
Dominant						
AA	228	72.38	251	66.76	1.0 (Reference)	-
AT+TT	87	27.62	125	33.24	0.77 (0.54-1.08)	0.11
Recessive						
AA+AT	303	96.19	365	97.07	1.0 (Reference)	-
TT	12	3.81	11	2.93	1.31 (0.52-3.34)	0.52
+781C/T						
Co-dominant						
CC	135	42.86	176	46.81	1.0 (Reference)	-
CT	145	46.03	163	43.35	1.16 (0.83-1.61)	0.36
TT	35	11.11	37	9.84	1.23 (0.71-2.13)	0.42
Dominant						
CC	135	42.86	176	46.81	1.0 (Reference)	-
CT+TT	180	57.14	200	53.19	1.17 (0.86-1.60)	0.30
Recessive						
CC+CT	280	88.89	339	90.16	1.0 (Reference)	-
TT	152	48.25	146	38.83	1.26 (0.95-1.68)	0.10

[1]Adjusted for family history of acute pancreatitis, BMI, alcohol drinking, TC, TG, LDL-c and blood glucose.

between the two study groups with respect to *IL-8* -353A/T (χ^2=3.84, P=0.15) and +781C/T (χ^2=1.14, P=0.57) (**Table 2**). Moreover, we found that all the genotype distributions of *IL-8* -251A/T, -353A/T and +781C/T were in agreement with the HWE in both patients and controls.

The unconditional logistic regression analysis revealed a relationship between the TT genotype of *IL-8* -251A/T and an increased risk of acute pancreatitis (adjusted OR=2.88, 95% CI=1.84-4.53), compared to the AA genotype (**Table 3**). In the dominant genetic models, the AT+TT genotype of *IL-8* -251A/T was significantly correlated with an elevated risk of acute pancreatitis (OR=1.57, 95% CI=1.11-2.24), compared with the AA genotype. Moreover, we observed that the TT genotype showed a 2.62 fold higher risk of acute pancreatitis (OR=2.62, 95% CI=1.80-3.82), contrasted with the AA+AT genotype. However, no significant differences were observed between *IL-8* -353A/T and +781C/T polymorphisms and risk of acute pancreatitis in co-dominant, dominant and recessive models.

Table 4. Interaction between *IL-8* -251A/T polymorphism and demographic and clinical variables in the risk of acute pancreatitis

Variables	Spearman correlation coefficient	*P* value
Age	0.034	0.19
Gender	0.045	0.13
Family history of acute pancreatitis	0.061	0.06
BMI, kg/m^2	0.047	0.11
Tobacco smoking	0.032	0.15
Alcohol drinking	0.062	0.06
TC	0.015	0.49
TG	0.027	0.36
HDL-c	0.025	0.38
LDL-c	0.055	0.09
Blood glucose	0.038	0.22

The interaction between *IL-8* -251A/T polymorphism and demographic and clinical variables in the risk of acute pancreatitis was analyzed using Spearman correlation analysis (**Table 4**). However, we observed that *IL-8* -251A/T had no interaction with the age, gender, family history of acute pancreatitis, BMI, tobacco smoking, alcohol drinking, TG, TG, HDL-c, LDL-c and blood glucose.

Discussion

IL-8 could cause an outbreak of respiratory neutrophils, release many proteolytic enzymes and produce large amounts of oxygen free radicals, and participate in local inflammation [21, 22]. Andoh et al. carried out an experimental study, and indicated that *IL-8* gene expression was associated with pathogenesis of acute pancreatitis [23]. Previous studies have revealed an significant association between an upregulated levels of IL-18 in serum and acute pancreatitis in rats [24]. Single nucleotide polymorphisms (SNPs) in the *IL-8* could alter the expression, structure and quantity of this protein, and consequently affect the protein's function [25, 26]. The present study has indicated that the *IL-8* -251A/T polymorphism contributes to the susceptibility to acute pancreatitis.

Only five studies have investigated the relationship between IL-8 polymorphisms and risk of acute pancreatitis in different populations, but the results are conflicting [16-20]. Hofner et al. carried out a study in a Caucasian population with 92 acute pancreatitis patients and 200 healthy controls, and revealed that the TT genotype of *IL-8* was associated with higher risk of acute pancreatitis [16]. Chantsev et al. reported that the genotype A/T of *IL-8* was associated with the destruction of the pancreas in Russian population [18]. Li et al. performed a study in a Chinese population, and revealed a significant relationship of *IL-8* -251A/T polymorphism with the risk of developing acute pancreatitis in a Chinese population [20]. Our study also reported that the TT genotype of *IL-8* -251A/T was significantly related to acute pancreatitis risk in co-dominant, dominant and recessive models.

Some studies reported inconsistent results. Bao et al. reported that the AA genotype of *IL-8* -251A/T carried a 1.55 fold increased risk of acute pancreatitis in a Chinese population [19]. Chen et al. performed a study in a Chinese population, but they found no significant correlation between *IL-8* -251A/T and susceptibility to acute pancreatitis [17]. The discrepancies in these studies may be attributed to the differences in ethnicities, selection of study subjects, design of study, and sample size as well as by chance.

This study was subject to two limitations. Firstly, the study subjects were performed recruited from among the patients of only one hospital in a city of China, and the selection bias could not be excluded from our study. Secondly, some interleukin factors may be involved into the pathogenesis of acute pancreatitis, and gene-gene interaction may have role in the risk of acute pancreatitis.

In summary, the present study has shown that the *IL-8* -251A/T polymorphism was significantly associated with an increased risk of acute pancreatitis in a Chinese population. Further larger sample sizes to confirm the possible use of the IL-8 -251A/T polymorphism as a biomarker and a predictive tool the for detection of acute pancreatitis are greatly needed.

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Disclosure of conflict of interest

None.

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