Int J Clin Exp Pathol 2015;8(11):15170-15176
www.ijcep.com /ISSN:1936-2625/IJCEP0017017

Original Article

Interleukin-10 -1082A/G polymorphism is associated with the development of acute pancreatitis in a Chinese population

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Received September 28, 2015; Accepted October 28, 2015; Epub November 1, 2015; Published November 15, 2015

Abstract: We conducted a case-control study to investigate the association between *IL-10* gene polymorphism (-1082A/G, -819T/C, and -592A/C) and risk of acute pancreatitis in a Chinese population. A total of 240 patients with proven acute pancreatitis and 240 control subjects were collected between May 2012 and January 2015. Genotyping of the *IL-10*-1082A/G, -819T/C, and -592A/C gene polymorphisms was conducted by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. By univariate logistic regression analysis, patients with acute pancreatitis were more likely to have higher BMI (OR=2.12, 95% CI=1.45-3.12; P<0.001) and have a habit of alcohol drinking (OR=2.01, 95% CI=1.37-2.95; P<0.001). There were significant differences in the genotype distributions of *IL-10*-1082A/G between patients with acute pancreatitis and control subjects (χ^2=9.97, *P*=0.007). By multiple logistic regression analysis, we found that individuals with the GG genotype of *IL-10*-1082A/G were associated with an increased risk of acute pancreatitis when compared with the AA genotype (OR=2.32, 95% CI=1.20-4.59; *P*=0.007). In dominant and recessive models, the *IL-10*-1082A/G gene polymorphism was significantly correlated with an elevated risk of acute pancreatitis, and the adjusted Ors (95% CI) were 1.50 (1.03-2.20) and 1.99 (1.06-3.79), respectively. However, no significant different was found between *IL-10*-819T/C and -592A/C gene polymorphisms and susceptibility to acute pancreatitis. In conclusion, we suggest that *IL-10*-1082A/G gene polymorphisms contribute to the development of acute pancreatitis in codominant, dominant and recessive models.

Keywords: Interleukin-10, -1082A/G, -819T/C, -592A/C, polymorphism, acute pancreatitis

Introduction

Acute pancreatitis is a sudden inflammation disease, and acute pancreatitis patients have clinical features of a sudden onset of upper abdominal pain, nausea, emesis and an elevated level of pancreatic digestive enzymes in the serum and urine [1-3]. The pathological processes of acute pancreatitis include edema and hemorrhage as well as even necrosis [4]. It is estimated that about 30% of acute pancreatitis patients would develop severe attack with a high mortality rate [5-7]. The mechanisms of developing acute pancreatitis not well understood, and the development of this disease is a complex process, involving in many lifestyle and environmental factors, such as excessive drinking, pancreatic duct stricture, hyperlipemia, infection and etc. [8-12]. However, not all patients who expose to similar risk factors would develop acute pancreatitis, suggesting that genetic factors may contribute to the development of this disease.

Previous studies have indicated that interleukin (*IL*)-1, *IL-6*, and tumor necrosis factor-α (*TNF-α*) levels are associated with the development of acute pancreatitis [13, 14]. IL-10 is an immunoregulatory cytokine, which is produced by Th2 cells, regulatory T cells, and monocytes/macrophages. The encoding gene of *IL-10* is located on chromosome 1 (1q31-1q32). Previous studies have reported the *IL-10* polymorphisms are associated with many inflammation related diseases [15-17]. To date, few studies have reported the association between *IL-10* gene polymorphisms and development of acute pancreatitis [18]. In our study, we conducted a case-control study to investigate the association between *IL-10* gene polymorphisms (-1082A/G, -819T/C,

Table 1. Primers, restriction enzymes and digested fragments for IL-10 gene polymorphisms

Gene polymorphism	Primers	Length of digested fragment	Restriction enzyme	Digested fragment
-1082A/G	5'-TCTGAAGAAGTCCTGATGTCACTG-3'	139 bp	MnlI	G allele: 106 bp and 33 bp;
	5'-ACTTTCATCTTACCTATCCCTACTTCC-3'			A allele: 139 bp
-819T/C	5'-GCTTCTTATATGCTAGTCAGGTA-3'	209 bp	MslI	C allele: 93 bp and 116 bp;
	5'-TGGGGGAAGTGGGTAAGAGT-3'			T allele: 209 bp
-592A/C	5'-GGTGAGCACTACCTGACTAGC-3'	412 bp	RsaI	A allele: 176 bp and 236 bp;
	5'-CCTAGGTCACAGTGACGTGG-3'			C allele: 412 bp

Table 2. Demographic and lifestyle characteristics between patients with acute pancreatitis patients and control subjects

Variables	Patients	%	Controls	%	χ^2 test	OR (95% CI)	*P* value
Age							
≤60	109	45.42	112	46.67		1.0 (Reference)	-
>60	131	54.58	128	53.33	0.08	1.05 (0.72-1.53)	0.79
Gender							
Female	85	35.42	85	35.42		1.0 (Reference)	-
Male	155	64.58	155	64.58	0.00	1.00 (0.68-1.48)	1.00
Family history							
No	220	91.67	229	95.42		1.0 (Reference)	-
Yes	20	8.33	11	4.58	2.79	1.89 (0.84-4.47)	0.09
BMI							
≤25	112	46.67	156	65.00		1.0 (Reference)	-
>25	128	53.33	84	35.00	16.36	2.12 (1.45-3.12)	<0.001
Tobacco smoking							
No	138	57.50	148	61.67		1.0 (Reference)	-
Yes	102	42.50	82	34.17	2.31	1.33 (0.90-1.97)	0.13
Alcohol drinking							
No	112	46.67	153	63.75		1.0 (Reference)	-
Yes	128	53.33	87	36.25	14.16	2.01 (1.37-2.95)	<0.001

and -592A/C) and risk of acute pancreatitis in a Chinese population.

Patients and methods

Study subjects

Patients with proven acute pancreatitis were recruited from our hospital between May 2012 and January 2015. Acute pancreatitis was diagnosed in patients who showed abdominal pain or abdominally localized signs of this disease, had features of acute pancreatitis according to computed tomography (CT) examination, and had serum amylase level more than three times of the upper limit of normal. The exclusion criteria of acute pancreatitis were patients who had serious liver and kidney diseases. A total of 240 patients with acute pancreatitis were collected into our study.

Two hundred and forty control subjects were selected from the hospital clinic during the same period of time and served as controls. All control groups were free of acute pancreatitis and serious liver and kidney diseases. The control subjects were frequently matched with the acute pancreatitis patients by sex and age (±5 years).

The demographic and lifestyle characteristics of patients with acute pancreatitis and control subjects were collected from a self-designed questionnaire. Detailed clinical data included age, gender, body mass index, alcohol consumption and tobacco smoking as well as fam-

Table 3. Genotype distributions of IL-10 -1082A/G, -819T/C, and -592A/C gene polymorphisms in acute pancreatitis patients and controls

IL-10	Patients	%	Controls	%	P for HWE		χ^2 test	*P* value	Minor allele frequency	
	N=240		N=240		In cases	In controls			In database	In controls
-1082A/G										
AA	92	38.33	116	48.33						
AG	113	47.08	105	43.75						
GG	35	14.58	19	7.92	0.97	0.48	7.8	0.02	0.2722	0.2979
-819T/C										
TT	85	35.42	93	38.75						
TC	109	45.42	106	44.17						
CC	46	19.17	41	17.08	0.29	0.26	0.69	0.71	0.4347	0.3917
-592A/C										
AA	87	36.25	95	39.58						
AC	117	48.75	113	47.08						
CC	36	15.00	32	13.33	0.74	0.86	0.66	0.72	0.4349	0.36875

ily history. A blood sample (5 mL) and a signed informed consent forms was obtained from each subject prior to their participation in the study, and our study was approved by our hospital.

DNA isolation

The blood samples were kept in tubes with ethylene diamine tetra-acetic acid (EDTA), and stored at -20°C until use. The genomic DNA was extracted from the peripheral blood samples using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). Genotyping of the *IL-10*-1082A/G, -819T/C, and -592A/C gene polymorphisms was conducted by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primer sequences for genotyping and restriction enzymes for digestion products were shown in **Table 1**. The PCR cycles were using the following program: one cycle of DNA denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 94°C for 40 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 40 seconds; and a final extension at 72°C for 10 minutes. The amplified products were digested with corresponding restriction endonucleases, and confirmed using 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Statistical analysis

The differences in demographic and lifestyle characteristics between patients with acute pancreatitis and control subjects were compared by using chi-square test and univariate logistic regression analysis. The goodness-of-fit χ^2-test was taken to assess whether the genotype distributions in the patients and controls were deviation from the Hardy-Weinberg equilibrium (HWE). Multiple logistic regression analysis was taken to assess the association between *IL-10*-1082A/G, -819T/C, and -592A/C gene polymorphisms and development of acute pancreatitis. The association between *IL-10*-1082A/G, -819T/C, and -592A/C gene polymorphisms and development of acute pancreatitis was estimated by adjusted odds ratio (OR) and their associated 95% confidence intervals (CIs). The wide-type genotype of *IL-10*-1082A/G, -819T/C, and -592A/C was considered as reference for analysis. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). A *P* values <0.05 were taken as a statistically significance.

Results

The demographic and lifestyle characteristics between patients and control subjects were shown in **Table 2**. The mean age of patients with acute pancreatitis and control subjects were 62.12±8.18 and 61.9±8.45 years. There were 85 females and 155 males respectively in patients with acute pancreatitis and control subjects. By univariate logistic regression analysis, patients with acute pancreatitis were more likely to have higher BMI (OR=2.12, 95% CI=1.45-3.12; *P*<0.001) and have a habit of

Table 4. Association between IL-10-1082A/G, -819T/C, and -592A/C gene polymorphisms and development of acute pancreatitis

IL-10	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
-1082A/G						
Codominant						
AA	92	38.33	116	48.33	1.0 (Reference)	-
AG	113	47.08	105	43.75	1.36 (0.91-2.02)	0.12
GG	35	14.58	19	7.92	2.32 (1.20-4.59)	0.007
Dominant						
AA	92	38.33	116	48.33	1.0 (Reference)	-
AG+GG	148	61.67	124	51.67	1.50 (1.03-2.20)	0.03
Recessive						
AA+AG	205	85.42	221	92.08	1.0 (Reference)	-
GG	35	14.58	19	7.92	1.99 (1.06-3.79)	0.02
-819T/C						
Codominant						
TT	85	35.42	93	38.75	1.0 (Reference)	-
TC	109	45.42	106	44.17	1.13 (0.74-1.71)	0.56
CC	46	19.17	41	17.08	1.23 (0.71-2.12)	0.43
Dominant						
TT	85	35.42	93	38.75	1.0 (Reference)	-
TC+CC	155	64.58	147	61.25	1.15 (0.78-1.70)	0.45
Recessive						
TT+TC	194	80.83	199	82.92	1.0 (Reference)	-
CC	46	19.17	41	17.08	1.15 (0.70-1.88)	0.55
-592A/C						
Codominant						
AA	87	36.25	95	39.58	1.0 (Reference)	-
AC	117	48.75	113	47.08	1.13 (0.75-1.70)	0.54
CC	36	15.00	32	13.33	1.23 (0.68-2.23)	0.47
Dominant						
AA	87	36.25	95	39.58	1.0 (Reference)	-
AC+CC	153	63.75	145	60.42	1.15 (0.78-1.70)	0.45
Recessive						
AA+AC	204	85.00	208	86.67	1.0 (Reference)	-
CC	36	15.00	32	13.33	1.15 (0.66-1.99)	0.60

[1]Ajusted for family history and tobacco drinking.

alcohol drinking (OR=2.01, 95% CI=1.37-2.9; *P*<0.001).

The genotype distributions of *IL-10*-1082A/G, -819T/C, and -592A/C in acute pancreatitis patients and controls are summarized in **Table 3**. The genotype distributions of *IL-10*-1082A/G, -819T/C, and -592A/C in patients with acute pancreatitis and controls confirmed with the Hardy-Weinberg equilibrium (All *P* values >0.05). By the chi-square test, there were significant differences in the genotype distributions of *IL-10*-1082A/G between patients with acute pancreatitis and control subjects (χ^2=9.97, *P*=0.007). However, no significant differences were observed in the genotype distributions of *IL-10*-819T/C (χ^2=0.58, *P*=0.75) and -592A/C (χ^2=0.12, *P*=0.94) between acute pancreatitis patients and controls by chi-square test. Moreover, the minor allele frequencies (MAF) of IL-10-1082A/G, -819T/C, and -592A/C in controls were similar with those in dbSNP (http://www.ncbi.nlm.nih.gov/snp/).

By multiple logistic regression analysis, we found that individuals with the GG genotype of *IL-10* -1082A/G were associated with an increased risk of acute pancreatitis when compared with the AA genotype (OR=2.32, 95% CI=1.20-4.59; *P*=0.007) (**Table 4**). In dominant and recessive models, the *IL-10* -1082A/G gene polymorphism was significantly correlated with an elevated risk of acute pancreatitis, and the adjusted Ors (95% CI) were 1.50 (1.03-2.20) and 1.99 (1.06-3.79), respectively. However, no significant differences were found between *IL-10*-819T/C and -592A/C gene polymorphisms and susceptibility to acute pancreatitis in codominant, dominant and recessive models.

Moreover, we conducted stratified analysis between *IL-10*-1082A/G polymorphism and acute pancreatitis risk based on demographic and lifestyle characteristics. However, we did not find significant interaction between *IL-10*-1082A/G polymorphism and gender, age, family history, BMI, tobacco smoking and alcohol

drinking in the susceptibility to acute pancreatitis.

Discussion

Cytokines play an important role in the modification of immune responses, and maintaining a balance between pro-inflammatory and anti-inflammatory stimuli during the development of several kinds of diseases. Single nucleotide polymorphisms (SNPs) is defined by the gene sequence of a single nucleotide bases inserting, missing or replacing to cause the polymorphism of the nucleic acid sequence [19]. SNPs in the cytokine genes could alter the gene expression, structure and quantity of the products, and thus influence the function of the gene [20, 21]. In the present case-control study, we assessed the role of three important SNPs in *IL-10* gene in the susceptibility to acute pancreatitis. We indicated that *IL-10*-1082A/G gene polymorphisms were associated with an increased risk of acute pancreatitis in codominant, dominant and recessive models, which suggested that *IL-10*-1082A/G gene polymorphisms play an important role in the development of this disease.

The *IL-10* gene is a candidate gene in the pathophysiological mechanism of auto-immune/inflammatory disease, since it plays an important role in regulating both the cellular and humoral immunity. Previous studies have reported that the IL-10 gene polymorphism is associated with inflammation-related diseases, such asnephropahty, helicobacter pylori infection, deep venous thrombosis, rheumatic heart disease, and Crohn diseases [15, 22-25].

For the correlation between *IL-10* gene polymorphisms and development of acute pancreatitis, only several studies reported their association, but the results are inconclusive [26-31]. Sargen et al. firstly reported the association between *IL-10* gene loci and the occurrence or severity of acute pancreatitis, and they found that *IL-10* gene polymorphisms did not contribute to the development of acute pancreatitis [26]. Schneider et al. also conducted a study in an American population, and they reported that *IL-10* gene polymorphisms did not play a dominant role in the development of pancreatitis [27, 28]. de-Madaria et al. conducted a study in a Spanish population, and they found that *IL-10* gene polymorphism was not associated with biliary etiology of acute pancreatitis [29]. Bao et al. conducted a case-control study in a Chinese population, and they did not reveal a significant association between *IL-10* gene polymorphism and susceptibility to acute pancreatitis [30]. Li et al. assessed the association between two SNPs in *IL-10* gene and risk of acute pancreatitis, and they found that no association between IL-10 gene polymorphisms and susceptibility to this disease [31]. However, we conducted a study in a Chinese population, and found that *IL-10*-1082A/G polymorphism contributed to the acute pancreatitis risk. The results of our study are different with previous results. The discrepancies of previous studies may be caused by different in populations, sample size, patients and controls selection and also by chance.

Three limitations should be considered in our study. First, selection bias may be caused by only hospital selection of the patients and control subjects. However, the genotype distributions of *IL-10*-1082A/G, -819T/C, and -592A/C are in line with HWE and similar with MAF in dbSNP, suggesting that the samples of our study has good representation of the general population. Second, there are many other cytokine genes should be considered into statistical analysis, which may have interaction with IL-10 gene polymorphisms. Third, we only included 182 patients with acute pancreatitis, and the small sample size may reduce the statistical power to find difference between groups.

In conclusion, we suggest that *IL-10*-1082A/G gene polymorphisms contribute to the development of acute pancreatitis, and *IL-10*-819T/C and -592A/C gene polymorphisms are not correlated with the increased risk of this disease. Further large sample size studies are greatly needed to confirm the finding of our study.

Disclosure of conflict of interest

None.

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