Genetics and Molecular Research

Investigation of the association between Interleukin-10 polymorphisms and risk of acute pancreatitis in a Chinese population

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Genet. Mol. Res. 14 (4): 15876-15881 (2015)
Received July 25, 2015
Accepted October 18, 2015
Published December 1, 2015
DOI http://dx.doi.org/10.4238/2015.December.1.39

ABSTRACT. We conducted a case-control study to investigate the possible association between three common single nucleotide polymorphisms in interleukin-10 (IL-10) and the development of acute pancreatitis in a Chinese population. Between January 2013 and December 2014, 255 patients with acute pancreatitis and 255 control subjects were recruited for the study. Genotyping of IL-10 rs1800896, rs1800871, and rs1800872 was performed using polymerase chain reaction coupled with restriction fragment length polymorphism. Using logistic regression analysis, we found that the AA genotype of IL-10 rs1800896 was correlated with an increased risk of acute pancreatitis in a codominant model (OR = 2.44, 95%CI = 1.28-4.77). In a dominant model, we found that the GA+AA genotype of IL-10 rs1800896 was associated with an elevated risk of acute pancreatitis (OR = 1.51, 95%CI = 1.05-2.18). In a recessive model, the AA genotype of IL-10 rs1800896 was correlated with an increased risk of acute pancreatitis (OR = 1.98, 95%CI = 1.06-3.77). In conclusion, IL-10 rs1800896 was correlated with an increased risk of acute pancreatitis in codominant, dominant, and

recessive models.

Key words: Interleukin-10; Polymorphism; Acute pancreatitis

INTRODUCTION

Acute pancreatitis is an acute inflammatory condition of the pancreas that can cause serious extrapancreatic organ dysfunction and failure. It is estimated that about 30% of patients with acute pancreatitis suffer a severe attack and have a high mortality rate (Hritz et al., 2015). The real etiology of acute pancreatitis is not well understood. The development of acute pancreatitis is a complex, multistep, and multifactorial process. Many environmental and genetic factors are involved in the development of acute pancreatitis, such as gallstones, heavy alcohol consumption, and overeating. However, not all subjects with similar risk factors develop acute pancreatitis, which suggests that genetic variations could influence the development of the condition. Several studies have reported that genetic polymorphisms play an important role in the development of acute pancreatitis such as interleukin-8 (IL-8), IL-1β, IL-6, toll-like receptor, and tumor necrosis factor-alpha (TNF-α) genes (Yin et al., 2012; Yin et al., 2013; Bao et al., 2015; Chi et al., 2015; Matas-Cobos et al., 2015).

Previous studies have reported that chemokines play a critical role in systemic inflammatory response syndrome, remote organ complications, and multiple organ dysfunction syndrome (Sugita et al., 1997; Hotz and Reber, 1999; Sun and Bhatia, 2007). IL-10 is an immunoregulatory cytokine located on chromosome 1 (1q31-1q32), and this protein is produced by Th2 cells and monocytes. A few studies reported an association between IL-10 gene polymorphisms and acute pancreatitis, but the results are inconclusive (Yin et al., 2013; Bao et al., 2015). Therefore, we conducted a case-control study to investigate the possible association between three common single nucleotide polymorphisms in IL-10 and the development of acute pancreatitis in a Chinese population.

MATERIAL AND METHODS

Study subjects

Patients with acute pancreatitis were recruited from the Second Affiliated Hospital of Henan College of Traditional Chinese Medicine during the period of January 2013 to December 2014. All cases were newly diagnosed. The diagnostic criteria of acute pancreatitis were as follows: individuals who presented with abdominal pain or abdominally localized signs of acute pancreatitis, had features of acute pancreatitis based on CT scan diagnosis, and had serum amylase levels at least three times above the upper normal limit. In total, 286 patients with acute pancreatitis were enrolled from the Second Affiliated Hospital of Henan College of Traditional Chinese Medicine. A final group of 255 patients agreed to participate in this study, and the participation rate was 89.16%.

A group of 255 control subjects was randomly selected from the health examination center of the Second Affiliated Hospital of Henan College of Traditional Chinese Medicine, and all the control subjects were free of acute pancreatitis. Each control subject was matched by sex and

age with a patient diagnosed with acute pancreatitis. Informed consent was obtained from all participants in this study. The ethics committee of the Second Affiliated Hospital of Henan College of Traditional Chinese Medicine approved the protocol used in this study.

Socio-demographic characteristics of patients with acute pancreatitis and control subjects were collected from a standardized questionnaire. This included sex, age, body mass index (BMI), use of tobacco, alcohol consumption, and any family history of acute pancreatitis.

DNA extraction and genotyping analysis

All the patients with acute pancreatitis and control subjects were required to provide a blood sample that was kept at -20°C until use. Genomic DNA was isolated from the peripheral blood sample by using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). Genotyping of IL-10 rs1800896, rs1800871, and rs1800872 was performed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primers for IL-10 rs1800896, rs1800871, and rs1800872 were designed using the Sequenom Assay Design 3.1 software. The PCR reaction was conducted at 94°C for 15 min, followed by 45 cycles at 94°C for 20 s, 56°C for 30 s, 72°C for 1 min, and a final incubation at 72°C for 3 min. PCR products were confirmed using 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Statistical analysis

The demographic and clinical characteristics of patients with acute pancreatitis and control subjects were compared by the chi-square test and *t*-test. Departures from the Hardy-Weinberg equilibrium for IL-10 rs1800896, rs1800871, and rs1800872 genotype distributions in controls were assessed by Fisher's exact test. The association of IL-10 rs1800896, rs1800871, and rs1800872 gene polymorphisms with the risk of acute pancreatitis was assessed by conditional logistic regression analysis, and the results were assessed using odds ratios (OR) and 95% confidence intervals (CI). The results were adjusted for potential confounding factors, and the most common control homozygote was taken as a reference group. Statistical analysis was conducted using the SPSS 17.0 package (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a significant difference.

RESULTS

The demographic and clinical characteristics of patients with acute pancreatitis and control subjects are shown in Table 1. Because of matching by gender and age, no significant difference was found between patients with acute pancreatitis and control subjects in terms of gender and age ($P > 0.05$). By the chi-square test, patients with acute pancreatitis were more likely to have a higher BMI and consume alcohol ($P < 0.05$).

The genotype distributions of IL-10 rs1800896, rs1800871, and rs1800872 between patients with acute pancreatitis and control subjects are shown in Table 2. The genotype distributions of IL-10 rs1800896, rs1800871, and rs1800872 were in line with the Hardy-Weinberg equilibrium in the control group, and the P values were 0.56, 0.19, and 0.62, respectively. By the chi-square

test, the genotype distributions of IL-10 rs1800896 was significantly different between patients with acute pancreatitis and control subjects (χ^2 = 9.01, P = 0.01). However, no significant differences were found in the genotype distributions of rs1800871 and rs1800872 between patients with acute pancreatitis and control subjects (P > 0.05).

Using logistic regression analysis, we found that individuals with the AA genotype of IL-10 rs1800896 were correlated with an increased risk of acute pancreatitis in a codominant model (OR = 2.44, 95%CI = 1.28-4.77) (Table 3). In a dominant model, we found that the GA+AA genotype of IL-10 rs1800896 was associated with an elevated risk of acute pancreatitis (OR = 1.51, 95%CI = 1.05-2.18). In a recessive model, the AA genotype of IL-10 rs1800896 was correlated with an increased risk of acute pancreatitis (OR = 1.98, 95%CI = 1.06-3.77). However, no significant differences were found between IL-10 rs1800871 and rs1800872 gene polymorphisms and the risk of acute pancreatitis in codominant, dominant, and recessive models.

Table 1. Demographic characteristics of patients with acute pancreatitis and control subjects.

Variables	Patients	%	Controls	%	Chi-square or *t*-test	P value
Gender						
Females	111	43.53	111	43.53		
Males	144	56.47	144	56.47	0.00	1.00
Age (years)						
<60	81	31.76	83	32.55		
≥60	174	68.24	172	67.45	0.04	0.85
Body mass index (kg/m^2)						
<24	123	48.24	72	28.24		
≥24	132	51.76	183	71.76	21.60	<0.001
Tobacco smoking						
Non-smoker	137	53.73	156	61.18		
Smoker	118	46.27	99	38.82	2.90	0.09
Alcohol consumption						
Non-drinker	131	51.37	161	63.14		
Drinker	124	48.63	94	36.86	7.21	0.007
Family history of acute pancreatitis						
No	243	95.29	248	97.25		
Yes	12	4.71	7	2.75	1.37	0.24

Table 2. Genotype distributions of IL-10 rs1800896, rs1800871, and rs1800872 between patients with acute pancreatitis and control subjects.

SNP	Patients	%	Controls	%	P for HWE	Chi-square value	P value
rs1800896							
GG	106	41.57	128	50.20			
GA	114	44.71	108	42.35			
AA	35	13.73	19	7.45	0.56	9.01	0.01
rs1800871							
TT	108	42.35	116	45.49			
TC	112	43.92	105	41.18			
CC	35	13.73	34	13.33	0.19	0.53	0.77
rs1800872							
AA	95	37.25	106	41.57			
AC	119	46.67	114	44.71			
CC	41	16.08	35	13.73	0.62	1.18	0.55

Table 3. Association between IL-10 rs1800896, rs1800871, and rs1800872 gene polymorphisms and risk of acute pancreatitis.

Model	SNP	Patients	%	Controls	%	OR (95%CI)[1]P value	
	rs1800896						
Codominant	GG	106	41.57	128	50.20	1.0 (Ref.)	-
	GA	114	44.71	108	42.35	1.34 (0.92-1.98)	0.11
	AA	35	13.73	19	7.45	2.44 (1.28-4.77)	0.004
Dominant	GG	106	41.57	128	50.2	1.0 (Ref.)	-
	GA+AA	149	58.43	127	49.8	1.51 (1.05-2.18)	0.02
Recessive	GG+GA	220	86.28	236	92.55	1.0 (Ref.)	-
	AA	35	13.73	19	7.45	1.98 (1.06-3.77)	0.02
	rs1800871						
Codominant	TT	108	42.35	116	45.49	1.0 (Ref.)	-
	TC	112	43.92	105	41.18	1.15 (0.77-1.69)	0.48
	CC	35	13.73	34	13.33	1.11 (0.62-1.97)	0.72
Dominant	TT	108	42.35	116	45.49	1.0 (Ref.)	-
	TC+CC	147	57.65	139	54.51	1.14 (0.79-1.64)	0.48
Recessive	TT+TC	220	86.27	221	86.67	1.0 (Ref.)	-
	CC	35	13.73	34	13.33	1.46 (0.82-2.66)	0.17
	rs1800872						
Codominant	AA	95	37.25	106	41.57	1.0 (Ref.)	-
	AC	119	46.67	114	44.71	1.16 (0.78-1.73)	0.43
	CC	41	16.08	35	13.73	1.31 (0.74-2.30)	0.32
Dominant	AA	95	37.25	106	41.57	1.0 (Ref.)	-
	AC+CC	160	62.75	149	58.43	1.20 (0.83-1.74)	0.32
Recessive	AA+AC	214	83.92	220	86.28	1.0 (Ref.)	-
	CC	41	16.08	35	13.73	1.20 (0.72-2.03)	0.46

DISCUSSION

In the present study, our study found that IL-10 rs1800896 gene polymorphisms were correlated with an increased risk of acute pancreatitis in a Chinese population.

It is reported that interleukin gene polymorphisms could modify the activation of monocytes, macrophages, and lymphocytes (Mukaida et al., 1998). Several previous studies have reported the association between interleukin gene polymorphisms and acute pancreatitis (Schneider et al., 2004; Chen and Nie, 2008; Bao et al., 2015; Chi et al., 2015). Schneider et al. (2004) investigated the role of TNF-α, TGF-β1, IL-10, and IFN-γ gene polymorphisms in the development of alcoholic chronic pancreatitis, but they did not find that genetic variants played a dominant role in alcoholic chronic pancreatitis. Chen and Nie (2008) studied the association between MCP-1-2518A/G and IL-8-251A/T polymorphisms and acute pancreatitis in a Chinese population, and they found that the AA genotype of MCP-1-2518A/G may play a protective role in the development of acute pancreatitis. Chi et al. (2015) conducted a case-control study in a Chinese population and reported that the IL-1β rs1143634 TT genotype was associated with an increased risk of acute pancreatitis. A recent meta-analysis with ten studies reported that the IL-8 -251 T/A polymorphism was associated with an increased risk of acute pancreatitis (Yin et al., 2013), but no significant associations were identified between IL-1β, IL-6, and IL-10 gene polymorphisms and acute pancreatitis risk.

Only two previous studies have reported the association between IL-10 gene polymorphisms and the development of acute pancreatitis (Yin et al., 2013; Bao et al., 2015), but the results are inconclusive. Yin et al. (2013) conducted a meta-analysis and reported that no significant associations existed between IL-10 rs1800896, rs1800871, and rs1800872 gene polymorphisms and acute pancreatitis risk. Additionally, Bao et al. (2015) did not find any significant association between IL-10 rs1800896 and rs1800871 gene polymorphisms and susceptibility to acute pancreatitis in a Chinese population. The discrepancies of the above-mentioned results

could be explained by differences in ethnicities, study design, and sample size.

Two limitations in our study should be taken into consideration. First, patients with acute pancreatitis and control subjects were selected from only one hospital, which would cause selection bias in our study. However, the genotype distributions of IL-10 rs1800896, rs1800871, and rs1800872 confirm with the Hardy-Weinberg equilibrium in controls, which suggests that our population may represent the general population. Second, the sample size of our study is relatively small, which could reduce the statistical power to find differences between groups. Therefore, further studies with participants from multiple locations and a larger sample size are needed to confirm our results.

In conclusion, we suggest that IL-10 rs1800896 gene polymorphism is correlated with an increased risk of acute pancreatitis in codominant, dominant, and recessive models. However, IL-10 rs1800871 and rs1800872 gene polymorphisms have no association with the risk of acute pancreatitis.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank the help of nurses for collecting blood samples in the Second Affiliated Hospital of Henan College of Traditional Chinese Medicine.

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