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Original Article

Association of IL-10 polymorphisms with acute pancreatitis

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Abstract: Background: Interleukin-10 (IL-10) acts as a vital innate immunity receptors involved in immune response. More and more evidences indicate that polymorphisms of IL-10 gene are associated with acute pancreatitis. The aim of this retrospective study was to investigate the relationship of single nucleotide polymorphisms (SNPs) of IL-10 and the risk of acute pancreatitis (AP). Methods: 3 SNPs of IL-10, including rs1800896, rs1800871 and rs1800872, genotyped in 300 patients with acute pancreatitis and 300 control subjects by polymerase chain reaction-restriction fragment length polymorphism, were investigated in this study. Multiple logistic regression analysis was used to evaluate the association between polymorphisms and AP. Results: We found that rs1800896 genotype GG and allele G were significantly associated with risk of AP, compared to healthy controls (P=0.000, OR=4.683; P=0.000, OR=2.441). Only in dominant model, rs1800896 showed significant correlation with risk of AP (P=0.000, OR=5.765). Conclusions: Genetic polymorphism in IL-10 (rs1800896) might be closely correlated with AP risk.

Keywords: Genetic polymorphism, interleukin-10 (IL-10), acute pancreatitis

Introduction

Acute pancreatitis (AP) is an inflammation disease that can cause serious dysfunction and failure of other organs with a case incidence of 10 to 30 per 100,000 [1-3]. Although most patients are mild, about 20% cases is severe with a mortality rate around 25%-30% [1, 4-6].

It is known that alcohol consumption and gallstones are two risk factors for acute pancreatitis [7, 8]. In males, alcohol abuse is the most common reason for acute pancreatitis, while gallstone in bile duct is a more common reason for acute pancreatitis in females [7]. However, the exact mechanisms of acute pancreatitis are unclear.

Cytokines play a vital role in inflammatory response, which result in organ dysfunction and tissue damage or development of acute pancreatitis. In acute pancreatitis patients, an inflammatory response could release reactive oxygen species, which could result in acinar cells auto-digestion [9, 10]. The inflammatory response could result in pancreatic cells necrosis, and recruit and activate inflammatory cells [9, 10].

IL-10, which locates on chromosome 1, is an immunoregulatory cytokine. It is secreted by monocytes and Th2 cells. Some studies reported genetic polymorphisms of IL-10 were associated with acute pancreatitis. However, the results are inconclusive [11, 12]. Therefore, the current study evaluated the correlations between three common polymorphisms (rs1800896, rs1800871, rs1800872) of IL-10 gene and acute pancreatitis in a Chinese Han population.

Materials and methods

Study populations

In this retrospective study, blood samples were extracted from unrelated Chinese Han participants in Ninbo No. 2 Hospital, Zhejiang, China between March 2012 and January 2016. A total of 400 individuals, including 200 AP patients and 200 healthy controls (HC) were investigated. All patients were newly diagnosed. We defined AP as individuals with AP characterized abdominal pain, AP characterized computerized tomography (CT) manifestation, and serum amylase two times more than the normal level. The healthy control was comparable with

Table 1. Primers for IL-10 gene polymorphisms

Polymorphisms	Forward (5'-3')	Reverse (5'-3')
Rs1800896	TCTGAAGAAGTCCTGATGTCACTG	ACTTTCATCTTACCTATCCCTACTTCC
Rs1800871	GCTTCTTATATGCTAGTCAGGTA	TGGGGGAAGTGGGTAAGAGT
Rs1800872	GGTGAGCACTACCTGACTAGC	CCTAGGTCACAGTGACGTGG

Table 2. Characteristics of the study population

Characteristics	HC n=200	AP n=200	P
Male/female	130/70	130/70	1.00
Age			
≤60	81 (40.5%)	81 (40.5%)	-
>60	119 (59.5%)	119 (59.5%)	1.00
BMI			
≤25	138 (69%)	91 (45.5%)	-
>25	62 (31%)	109 (54.5%)	0.000
Alcohol			
Yes	70 (35%)	102 (51%)	-
No	130 (65%)	98 (49%)	0.001
Tobacco			
Yes	80 (40%)	69 (34.5%)	-
No	120 (60%)	131 (65.5%)	0.260
Family history			
Yes	6 (3%)	14 (7%)	-
No	194 (97%)	186 (93%)	0.066

HC, healthy control. AP, acute pancreatitis. BIM, body mass index.

AP patient in sex and age. A standardized questionnaire was used to collect socio-demographic characteristics of AP patients and HC individuals, including age, sex, and family history of AP, body mass index (BMI), alcohol and tobacco consumption. The exclusion criterion: 1) chronic liver disease; 2) chronic renal failure; 3) active neoplastic diseases; 4) concurrent infectious diseases; 5) chronic inflammatory disease; 6) admission later than 48 hours after the start of the symptoms; 7) under 18 years.

The study protocol was approved by the ethics committee of Ninbo N0. 2 Hospital, Zhejiang province, China. Written informed consents were obtained from all subjects.

DNA extraction and SNP analysis

According to manufacturer's instructions of Wizard® Genomic DNA Purification Kit (Promega, USA), genomic DNA samples were extracted from peripheral blood leukocytes. With the NanoDrop spectrophotometer, we measured the concentration of DNA samples, diluted them to 40 ng/μl, and stored at -80°C for analysis. We genotyped three SNPs (rs1800896, rs1800871, rs1800872) of *IL-10* gene with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers for PCR-RFLP were in **Table 1**. The PCR cycles were as following: one cycle of DNA denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 50 seconds; and a final extension at 72°C for 10 minutes. We used MnlI, MsII and RsaI restriction endonucleases to digest the amplified products, and confirmed with 2.0% agarose gel electrophoresis under ultraviolet light.

Statistical analysis

We used χ^2 test to evaluate the difference in age, sex, and family history of AP, body mass index (BMI), alcohol and tobacco consumption between AP group and HC group. HWE software was used to test Hardy-Weinberg equilibrium in HC group. Associations between polymorphisms and risk of AP were evaluated by multiple logistic regression analysis. *P* value and odds ratio (OR) were adjusted by controlling confounding factors. Haplotype frequencies were identified by Haploview software (v4.2). SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical calculation. $P < 0.05$ indicates statistical significance.

Results

In HC group, all the three genotyped SNPs of *IL-10* gene were in accordance with the Hardy-Weinberg equilibrium.

Characteristics of the study population

Comparison of the basic data of the study population between AP group and HC group were in **Table 2**. There was no statistical difference in gender and age between AP group and HC group ($P>0.05$), and the subjects of two groups were comparable. AP patients were more likely to have a higher body mass index (BMI) and more alcohol consumption than HC group, instead of tobacco consumption and family history.

Table 3. Comparison of the frequency of genotype of rs1800896 between AP group and HC group

Genotype	AP	HC	P	OR
rs1800896				
AA	78 (39%)	103 (51.5%)	-	-
AG	93 (46.5%)	75 (37.5%)	0.075	1.598
GG	29 (14.5%)	22 (11%)	0.000	4.683
A	264 (66%)	281 (70.2)	-	-
G	136 (34%)	119 (29.8%)	0.000	2.441
Dominant model	-	-	0.000	5.765
Recessive model	-	-	0.054	1.566

HC, healthy control. AP, acute pancreatitis.

Table 4. Comparison of the frequency of genotype of rs1800871 between AP group and HC group

Genotype	AP	HC	P	OR
rs1800871				
CC	67 (33.5%)	73 (36.5%)	-	-
CT	90 (45%)	86 (43%)	0.176	1.395
TT	43 (21.5%)	41 (20.5%)	0.420	1.289
C	224 (56%)	232 (58%)	-	-
T	176 (44%)	168 (42%)	0.298	1.179
Dominant model	-	-	0.838	1.059
Recessive model	-	-	0.179	1.365

HC, healthy control. AP, acute pancreatitis.

Table 5. Comparison of the frequency of genotype of rs1800872 between AP group and HC group

Genotype	AP	HC	P	OR
rs1800872				
AA	71 (35.5%)	77 (38.5%)	-	-
AC	98 (49%)	94 (47%)	0.232	1.330
CC	31 (15.5%)	29 (14.5%)	0.378	1.354
A	240 (60%)	248 (62%)	-	-
C	160 (40%)	152 (38%)	0.272	1.192
Dominant model	-	-	0.680	1.137
Recessive model	-	-	0.207	1.335

HC, healthy control. AP, acute pancreatitis.

Correlations of SNPs with AP

After adjusted BMI and alcohol, we conducted multi-variant logistic regressions analysis to evaluate the association between SNPS and AP group as well as HC group, and the strength of association to both groups were compared. We found that rs1800896 genotype GG and allele G were significantly associated with risk of AP compared to HC (P=0.000, OR=4.683; P=0.000, OR=2.441) (**Table 3**). However, rs1800871 and rs1800872 showed no significant correlations with risk of AP (**Tables 4** and **5**).

Genetic model analysis

We conducted dominant model and recessive model to evaluate the correlations between genotypes and AP. Only in dominant model, rs1800896 showed significant correlation with risk of AP (P=0.000, OR=5.765) (**Table 3**). In dominant or recessive models, rs1800871 and rs1800872 showed no significant correlations with risk of AP (**Tables 4** and **5**).

Haplotype analysis

We used Haploview to evaluate haplotypes associated with risk of AP. We found that rs1800871 and rs1800872 were in one block. The frequencies were in **Table 6**. However, none of the three blocks showed significant correlations between AP group and HC group.

Discussion

In the present study, three SNPs of IL-10 were genotyped to investigate the risk of AP in a Chinese Han population. We found that polymorphism of *IL-10* (rs1800896) was correlated with the risk of AP compared to HC.

In many immune-related diseases, cytokines take part in regulating of immune responses and keeping balance between anti-inflammatory and pro-inflammatory stimuli. Single nucleotide polymorphisms (SNPs) is a variant in the gene sequence with a single nucleotide bases replacing, missing or inserting, causing the polymorphisms of the nucleic acid sequence. In 1998, Mukaida *et al.* reported that activation of lymphocytes, macrophages and monocytes could be modified by the interleukin gene polymorphisms [13]. Correlations between polymorphisms of interleukin gene and AP were reported in several studies [14-17]. In 2004, Schneider *et al.* evaluated genetic polymorphisms of IFN-γ, IL-10, TGF-β1 and TNF-α on the risk of alcoholic chronic pancreatitis without finding of genetic model analysis [14]. In 2008,

Table 6. Haplotype analysis of rs1800896, rs1800871 and rs1800872

Group	Block	Rs1800871-rs1800872	Frequency	*P* value	P^a value
AP VS HC	1	A-C	0.570	0.568	0.326
	2	C-T	0.390	0.562	0.927
	3	A-T	0.040	1.000	1.000

HC, healthy control. AP, acute pancreatitis. P^a, after 1000 permutation.

Chen and Nie *et al.* reported that genotype AA of MCP-1-2518A/G could reduce risk of progression of AP in a Chinese Han population [15]. In 2015, Chi *et al.* reported that genotype TT of IL-1β rs1143634 could increase AP risk [17]. Yin *et al.* conducted a meta-analysis including ten studies and found IL-8 -251T/A could increase the risk of AP instead of IL-10 polymorphisms [11].

IL-10 gene plays a vital role in regulating humoral immunity and cellular. It is involved in the mechanisms of inflammatory and auto-immune diseases. There were only two studies reported correlations between IL-10 polymorphisms and AP risk, and the results were inconsistent with each other [11, 18]. A meta-analysis from Yin *et al.* reported that polymorphisms of IL-10 gene (rs1800896, rs1800871, and rs1800872) were not significantly associated with risk of AP [11]. However, rs1800896 in IL-10 was found to be significantly correlated with progression of AP in a Chinese Han population [18].

This was partially consistent with our results that rs1800896 was significantly associated with AP compared to HC group. The possible reasons for this discrepancy might be differences in population, sample size and study design.

rs1800896 locates at the intron of IL-10 gene (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1800896). Intron region, which do not encode protein, could regulate transcription and post-transcription in gene expression. Many intron regions of genes were found to regulation function, such as cis-acting element (enhancer, enhancer and silencer). Some transcription factors could bind to this region to regulate gene expression. SNP in the intron could affect potential transcription factor binding sites or directly regulate the activity of promoter of IL-10 gene [19].

In conclusion, we found rs1800896 in *IL-10* was significantly correlated with AP risk. However, there were several limitations in our study: a relatively small sample size and lack of a validation assay, which could disturb the power to find associations between factors and disease. In the future, we need more prospective longitudinal studies to validate our findings in larger populations.

Disclosure of conflict of interest

None.

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