GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association between *IL-1β*, *IL-8*, and *IL-10* polymorphisms and risk of acute pancreatitis

D. Li[1], J. Li[1], L. Wang[1] and Q. Zhang[2]

[1]Department of Emergency, Beijing Tongren Hospital, Capital Medical University, Beijing, China
[2]Department of Hepatobiliary Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing, China

Corresponding author: Q. Zhang
E-mail: lidapeng667@126.com

Genet. Mol. Res. 14 (2): 6635-6641 (2015)
Received May 30, 2014
Accepted October 28, 2014
Published June 18, 2015
DOI http://dx.doi.org/10.4238/2015.June.18.6

ABSTRACT. We assessed the possible correlation between genetic polymorphisms in interleukin (*IL*)-*1β*, *IL-8*, and *IL-10* and risk of acute pancreatitis. Polymorphisms of *IL-1β* +3954C/T (rs1143634), *IL-1β* -511C/T (rs16944), *IL-8* -251T/A (rs4073), *IL-10* -1082A/G (rs1800896), and *IL-10* -819C/T (rs1800871) were assessed by polymerase chain reaction-restriction fragment length polymorphism. Patients with acute pancreatitis were more likely to have a family history of acute pancreatitis and a habit of tobacco smoking and alcohol drinking. Conditional logistic regression analyses showed that subjects carrying *IL-10* -1082A/G and *IL-8* -251 AA genotype with the A allele were significantly associated with an increased risk of acute pancreatitis, with adjusted odds ratio (95% confidence interval) of 1.82 (1.01-3.31) and 1.39 (1.02-1.90), respectively. However, we did not observe that *IL-1β* +3954C/T, *IL-1β* -511C/T, *IL-10* -1082A/G, and *IL-10* -819C/T polymorphisms were associated with the risk of acute pancreatitis. We found that the *IL-8* -251T/A polymorphism is associated with an increased risk of acute pancreatitis, and no significant

association between *IL-1β* and *IL-10* gene polymorphisms and risk of acute pancreatitis was detected.

Key words: Acute pancreatitis; Inflammatory cytokines; Polymorphism

INTRODUCTION

Acute pancreatitis is an inflammatory condition that can cause severe extrapancreatic organ dysfunction and failure. Although advanced treatment has been used to treat acute pancreatitis, this disease is also serious and potentially lethal, with a 30-day survival rate of 10% (Andersen et al., 2008). A specific treatment regimen for acute pancreatitis is not well established.

Two factors are known risk factors of acute pancreatitis, including alcohol consumption and gallstones (Dufour and Adamson, 2003; Venneman et al., 2005). Alcohol abuse is a common cause of acute pancreatitis in males, whereas gallstone migration into the bile duct is the leading cause of acute pancreatitis in females (Dufour and Adamson, 2003). However, the etiology of acute pancreatitis is not completely understood.

Cytokines play an important role in the development of acute pancreatitis by causing an inflammatory response that leads to tissue damage and organ dysfunction or failure in patients with severe acute pancreatitis. It has been reported that an inflammatory response of unknown origin in acute pancreatitis can cause the release of reactive oxygen species, leading to autodigestion of acinar cells (Sah et al., 2012; Sit et al., 2014). The inflammatory response can cause pancreatic necrosis, as well as trigger both recruitment and activation of inflammatory cells (Sah et al., 2012; Sit et al., 2014).

Local recruitment and activation of inflammatory cells in acute pancreatitis can induce the production of proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-8, and IL-10 as well as tumor necrosis factor-alpha (Berney et al., 1999; Devière et al., 2001; Stimac et al., 2006; De Waele and Blot, 2007; Yuan et al., 2007). However, few studies have investigated the pathological development or severity of acute pancreatitis in the Chinese population. Therefore, we assessed the correlation between gene polymorphisms in *IL-1β*, *IL-8*, and *IL-10* and risk of acute pancreatitis.

MATERIAL AND METHODS

Patients

A total of 176 consecutive patients were included in our study from Beijing Tongren Hospital of Capital Medical University. The diagnosis of acute pancreatitis was established based on the following 3 criteria: 1) abdominal pain or abdominal localizing signs of acute pancreatitis; 2) serum amylase and/or lipase that was 3 times the upper limit of normal, and 3) characteristic findings of acute pancreatitis based on computed tomography scan.

A total of 176 subjects were collected from individuals who received a routine health check-up in the health examination center and served as a control group. Control subjects were matched with cases by gender and age. Informed consent was obtained from all cases and control subjects or their relatives before enrollment into the study. The protocol of this study was approved by the Ethics Committee of the Beijing Tongren Hospital of Capital Medical University.

Each case and control was asked to provide 5 mL blood for DNA sequencing after agreeing to participate in the study.

DNA isolation

To examine the polymorphisms in *IL-1β*, *IL-8*, and *IL-10*, genomic DNA was purified from peripheral blood. All study participants provided 5 mL venous blood, which was stored at -20°C until use in 0.5 mg/mL ethylenediaminetetraacetic acid as an anticoagulant. Genomic DNA was isolated from peripheral blood leukocytes using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to manufacturer instructions, and the genomic DNA was stored at -20°C until use.

Identification of *IL-1β, IL-8*, and *IL-10* polymorphisms

Polymorphisms in *IL-1β* +3954C/T (rs1143634), *IL-1β* -511C/T (rs16944), *IL-8* -251T/A (rs4073), *IL-10* -1082A/G (rs1800896), and *IL-10* -819C/T (rs1800871) were assessed by polymerase chain reaction (PCR)-restriction fragment length of polymorphism. Primers for *IL-1β* +3954C/T (rs1143634), *IL-1β* -511C/T (rs16944), *IL-8* -251T/A (rs4073), *IL-10* -1082A/G (rs1800896), and *IL-10* -819C/T (rs1800871) were designed using the Sequenom Assay Design 3.1 software (San Diego, CA, USA) and are shown in Table 1. The PCR was performed in a 50-μL reaction solution containing 25 mM $MgCl_2$, 2 mM 4X dNTP, 20 μM primer, and 5 U/μL *Taq* DNA polymerase. The PCR was performed using the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 45 s, annealing at 62°C for 60 s, and extension at 72°C for 60 s, with final extension at 72°C for 10 min. The PCR products were visualized by 1.0% agarose gel electrophoresis and stained using ethidium bromide staining under UV light. For quality control, a randomly chosen group of 10% of the cases and control subjects were selected, and the results of repeated analysis showed 100% concordance.

Table 1. Primers for used for *IL-1β*, *IL-8*, and *IL-10* polymorphisms.

Polymorphisms	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
IL-1β +3954C/T	GCCTGCCCTTCTGATTTTATACC	CATCGTGCACATAAGCCTCGTTA
IL-1β -511C/T	TTGAGGGTGTGGGTCTCTACCT	AGGAGCCTGAACCCTGCATAC
IL-8 -251T/A	TAAAATACTGAAGCTCCACAATTTGG	ATCTTGTTCTAACACCTGCCACTCT
IL-10 -1082A/G	GATAGGAGGTCCCTTACTTTCCTCTTA	CACACACAAATCCAAGACAACACTAC
IL-10 -819C/T	ATGGTGTACAGTAGGGTGAG	TTTCCACCTCTTCAGCTGTC

Statistical analysis

Continuous variables are reported as means ± standard deviation (SD), and categorical variables are expressed as N(%) of study participants. The Student *t*-test was used to compare continuous variables between patients and control subjects, and the χ^2-test was used to compare categorical variables between patients and control subjects. Hardy-Weinberg equilibrium among controls was compared using the χ^2-test. Unconditional logistic regression was conducted to assess the effects of the *IL-1β*, *IL-8*, and *IL-10* polymorphisms on the risk of acute pancreatitis, with results expressed as odds ratios (ORs) and corresponding 95% confidence intervals (CIs) on acute pancreatitis risk. Homozygotes of the most frequent genotype were regarded as the reference group. All P values were 2-sided, and $P < 0.05$ was considered to be statistically significant. All statistical analyses were conducted using SPSS statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows.

RESULTS

The demographic and clinical characteristics of study subjects are shown in Table 2. Of the 176 patients with acute pancreatitis, 115 subjects (65.34%) were male and 61 (34.66%) were female, with a mean age of 51.6 ± 11.3 years. For the control group, the mean age was 52.3 ± 10.9 years. Patients with acute pancreatitis had a significantly higher mean body mass index than the control subjects (P = 0.003). Patients with acute pancreatitis were more likely to have a family history of acute pancreatitis, as well as a habit of tobacco smoking and alcohol consumption (P < 0.05).

The allele and genotype distributions of the *IL-1β* +3954C/T (rs1143634), *IL-1β* -511C/T (rs16944), and *IL-10* -819C/T (rs1800871) polymorphisms were in Hardy-Weinberg equilibrium in the control group (Table 3). However, the genotype distributions of *IL-8* -251T/A (rs4073) and *IL-10* -1082A/G (rs1800896) were not. Moreover, we found that minor allele frequencies of the 5 gene polymorphisms in the control group were similar to those in the dbSNP database.

Table 2. Demographic and characteristics of acute pancreatitis and controls.

Indexes	Cases (N = 176)	%	Control (N = 176)	%	χ^2 or *t* test	P value
Mean age (years)	51.6 ± 11.3		52.3 ± 10.9		0.59	0.27
Age						
<50	81	46.02	82	46.59		-
≥50	95	53.98	94	53.41	0.01	0.92
Gender						
Male	115	65.34	115	65.34		-
Female	61	34.66	61	34.66	0	1.0
Body mass index						
<25	73	41.48	101	57.39		
≥25	103	58.52	75	42.61	8.91	0.003
Family history						
No	162	92.05	173	98.30		-
Yes	14	7.95	3	1.70	7.48	0.006
Smoking habit						
Never	113	64.20	133	75.57		-
Yes	63	35.80	43	24.43	5.40	0.02
Drinking habit						
Never	86	48.86	110	62.50		-
Yes	90	51.14	66	37.50	6.63	0.01

Table 3. Genotype characteristics of the *IL-1β*, *IL-8*, and *IL-10* polymorphisms.

Gene	SNP	Alleles	MAF[1]		HWE[2] (P value) in controls
			Control group	From dbSNP	
IL-1β +3954C/T	rs1143634	C/T	0.1465	0.1455	0.07
IL-1β -511C/T	rs16944	G/A	0.463	0.4651	0.10
IL-8 -251T/A	rs4073	A/T	0.4895	0.4972	0.002
IL-10 -1082A/G	rs1800896	A/G	0.2985	0.3026	0.004
IL-10 -819C/T	rs1800871	C/T	0.4085	0.4086	0.06

[1]MAF = minor allele frequencies; [2]HWE = Hardy-Weinberg equilibrium.

Conditional logistic regression analyses showed that subjects carrying the *IL-10* -1082A/G and *IL-8* -251 AA genotypes and A allele were significantly associated with an increased risk of acute pancreatitis, with adjusted ORs (95%CI) of 1.82 (1.01-3.31) and 1.39 (1.02-1.90), respectively (Table 4). However, the *IL-1β*+ 3954C/T, *IL-1β* -511C/T, *IL-10* -1082A/G, and *IL-10* -819C/T polymorphisms were not associated with the risk of acute pancreatitis.

Table 4. Association between polymorphisms of *IL-1β*, *IL-8*, and *IL-10* polymorphisms and acute pancreatitis risk.

SNPs		Cases (N = 176)	%	Controls (N = 176)	%	Adjusted OR (95%CI)[1]	P value
IL-1β +3954C/T	CC	121	68.75	133	75.57	1.0 (Ref.)	-
	CT	41	23.3	34	19.32	1.33 (0.78-2.30)	0.28
	TT	14	7.95	9	5.11	1.71 (0.66-4.64)	0.22
Allele	C	283	80.4	300	85.23	1.0 (Ref.)	-
	T	69	19.6	52	14.77	1.41 (0.93-2.13)	0.09
IL-1β -511C/T	GG	50	28.41	56	31.82	1.0 (Ref.)	-
	GA	81	46.02	78	44.32	1.16 (0.69-1.96)	0.38
	AA	45	25.57	42	23.86	1.17 (0.64-2.15)	0.03
Allele	G	181	51.42	190	53.98	1.0 (Ref.)	-
	A	171	48.58	162	46.02	1.11 (0.82-1.51)	0.5
IL-8 -251T/A	TT	37	21.02	53	30.11	1.0 (Ref.)	-
	TA	77	43.75	74	42.05	1.49 (0.85-2.62)	0.13
	AA	62	35.23	49	27.84	1.82 (1.01-3.31)	0.04
Allele	T	151	42.9	180	51.14	1.0 (Ref.)	-
	A	201	57.1	172	48.86	1.39 (1.02-1.90)	0.03
IL-10 -1082A/G	AA	84	47.73	91	51.7	1.0 (Ref.)	-
	AG	67	38.07	65	36.93	1.12 (0.69-1.80)	0.48
	GG	25	14.2	20	11.36	1.35 (0.67-2.77)	0.28
Allele	A	235	66.76	247	70.17	1.0 (Ref.)	-
	G	117	33.24	105	29.83	1.17 (0.84-1.63)	0.33
IL-10 -819C/T	CC	59	33.52	66	37.5	1.0 (Ref.)	-
	CT	80	45.45	76	43.18	1.18 (0.72-1.94)	0.5
	TT	37	21.02	34	19.32	1.22 (0.65-2.27)	0.61
Allele	C	198	56.25	208	59.09	1.0 (Ref.)	-
	T	154	43.75	144	40.91	1.12 (0.82-1.53)	0.45

[1]Adjusted for gender, age, body mass index, family history, smoking, and drinking habits.

DISCUSSION

Acute pancreatitis is a severe disease that significantly affects human health. The incidence of acute pancreatitis is estimated to be 30 in 1,000,000 individuals. Although gallstones, alcohol consumption, and over-eating are considered to be risk factors of acute pancreatitis, the detailed etiology is not well understood. In our study, we assessed the association between *IL-1β*, *IL-8*, and *IL-10* polymorphisms and risk of acute pancreatitis. Our study found that the *IL-8* -251 AA genotype and A allele were associated with the risk of acute pancreatitis, indicating that the *IL-8* -251T/A polymorphism can be used to predict the individual risk of acute pancreatitis and influence the pathogenesis of developing acute pancreatitis.

In recent years, several studies have reported the association between gene polymorphisms and risk of acute pancreatitis (Ozhan et al., 2010; Yin et al., 2012, 2013). In this study, we found that *IL-8* -251T/A polymorphisms were associated with risk of acute pancreatitis. Five previous studies investigated the association between *IL-8* -251T/A polymorphisms and acute pancreatitis (Hofner et al., 2006; Li et al., 2007; Chen and Nie, 2008; Cao and Xiao, 2010; Tang et al., 2010), but the results are inconsistent. Hofner et al. (2006), who conducted a study in Hungary, found that the frequency of *IL-8* -251T/A heterozygote mutant variants was significantly higher in patients with severe pancreatitis than among healthy blood donors, and the *IL-8* polymorphism has a role in the severe form of this disease. Tang et al. (2010) reported an association between the *IL-8* -251T/A polymorphism and the development of acute pancreatitis, and found that the *IL-8* -251T/A polymorphism can influence the risk of acute pancreatitis. However, Chen and Nie (2008) conducted a case-control study in China and investigated the association between the *IL-8* -251T/A polymorphism and risk of acute pancre-

atitis, and reported that the *IL-8* -251T/A polymorphism cannot predict the risk of severe acute pancreatitis. Discrepancies in ethnicities, sample size, control selection, and study design may have caused the difference in the results of different studies. Further studies are needed to clarify the association between *IL-8* polymorphisms and the risk of acute pancreatitis.

In our study, we found no significant association between *IL-1β* and *IL-10* gene polymorphisms and risk of acute pancreatitis. Three previous studies reported an association between *IL-1β* polymorphism and risk of acute pancreatitis, but the association was not significant (Smithies et al., 2000; Powell et al., 2001; Zhang et al., 2005). Regarding the association between *IL-10* gene polymorphisms and risk of acute pancreatitis, 2 previous studies reported a non-significant association (Sargen et al., 2000; Zhang et al., 2005). These study results agree with the results of this study, and further large sample studies are needed to confirm their association.

There were 2 limitations to our study. First, our relatively small sample size limited the statistical power to identify the difference between groups. Second, the genotype distributions of *IL-8* -251T/A and *IL-10* -1082A/G were not in line with Hardy-Weinberg equilibrium in the control group, and thus the controls did not represent the distribution of the general population. Therefore, selection bias may have existed in our study. Third, other genetic polymorphisms may influence the risk of acute pancreatitis, except for the inflammatory cytokines. Therefore, further large sample and multicenter studies are needed to investigate the association between inflammatory cytokines and the risk of acute pancreatitis.

In summary, we found that the *IL-8* -251T/A polymorphism is associated with an increased risk of acute pancreatitis. We found no significant association between *IL-1β* and *IL-10* gene polymorphisms and risk of acute pancreatitis.

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