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Original Article

Interleukin gene polymorphisms in Chinese children with biliary atresia

Bin Lai[1*], Kai Huang[2*], Zhengdong Hong[1], Jun Lei[3], Jinshi Huang[3], Qiang Tao[3], Shouhua Zhang[3], Huan Deng[4]

[1]Department of General Surgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China; [2]Department of Gastrointestinal Surgery, Jiangxi Provincial Cancer Hospital, Nanchang, China; [3]Department of General Surgery, Jiangxi Children's Hospital, Nanchang, China; [4]Department of Pathology, The Fourth Affiliated Hospital of NanchangUniversity, Nanchang, China. [*]Equal contributors and co-first authors.

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Abstract: Biliary atresia (BA) is an illness of newborns that develops during the first months of life without the presence of other malformations. It remains one of the major hepatic causes of death in early childhood. The present study aimed to investigate the relationship between polymorphisms of interleukin (IL)-8, IL-10, and IL-18 genes and BA development. Single nucleotide polymorphisms (SNPs) *IL-8* A/T (dbSNP ID: rs4073) and T/C (dbSNP ID: rs2227306), *IL-10* T/C (dbSNP ID: rs1800896) and A/G (dbSNP ID: rs1800871), and *IL-18* T/G (dbSNP ID: rs1946518), T/G (dbSNP ID: rs549908), and G/C (dbSNP ID: rs187238) were genotyped in 62 Chinese BA patients and 80 healthy controls using the matrix-assisted laser desorption ionization/time of flight mass spectrometry method. No differences were observed regarding genotype and carrier frequencies for any of the SNPs between BA patients and controls. These findings suggest that the genetic variation assessed in *IL-8*, *IL-10*, and *IL-18* is unrelated to BA sensitivity in the Chinese population.

Keywords: Genetic variability, biliary atresia, interleukin genes, single nucleotide polymorphisms

Introduction

Biliary atresia (BA) is a birth defect of newborn infants involving idiopathic progressive inflammation and fibrosclerosis of the bile ducts [1] in which one or more bile ducts become abnormally narrow, blocked, or absent. BA remains one of the major hepatic causes of death in early childhood, but its underlying causes are poorly understood.

In a series of studies into BA, the presence of inflammatory infiltrates in the vicinity of damaged bile ducts has been regarded as evidence of immune-mediated injury [2-6]. The process of inflammatory infiltration is accompanied by the up-regulation of interleukins. Further studies indicated that diseased livers have a gene expression signature unique to BA. Among the genes expressed, those encoding interleukin (IL)-8, IL-10, and IL-18 were shown to be relevant to BA [7-10]. Therefore, gaining insight into the roles of IL-8, IL-10, and IL-18 in BA will contribute to our understanding of disease etiology and pathogenesis. To this end, we have evaluated whether specific IL-8, IL-10, and IL-18 SNPs and their haplotypes are associated with BA.

Materials and methods

Patients and controls

Sixty-two BA patients (38 boys and 24 girls, average age, 72.3±22.5 days) with no diversity in genetic background were recruited from Jiangxi Children's Hospital (Jiangxi, China). Eighty unrelated healthy control participants (40 boys and 40 girls, average age, 82±30.6 days) were selected at random from the same hospital. This study was approved by the ethics committee of Jiangxi Children's Hospital.

Blood sample processing and DNA isolation

Blood samples of patients and healthy donors were stored in ethylenediaminetetraacetic acid

Table 1. Oligonucleotide sequences used for genotyping

Gene	SNP rs No.	Primers	Sequences
IL-8	rs4073	Forward	5'-ACGTTGGATGTGTTCTAACACCTGCCACTC-3'
		Reverse	5'-ACGTTGGATGCTGAAGCTCCACAATTTGGT-3'
		Extension	5'-TTTATCTAGAAATAAAAAAGCATACA-3'
	rs2227306	Forward	5'-ACGTTGGATGCCCTTGACCTCAGTTAGTTC-3'
		Reverse	5'-ACGTTGGATGCCATGAAGATGTTGATATTG-3'
		Extension	5'-AGTCATAACTGACAACATTGAAC-3'
IL-10	rs1800896	Forward	5'-ACGTTGGATGATTCCATGGAGGCTGGATAG-3'
		Reverse	5'-ACGTTGGATGGACAACACTACTAAGGCTTC-3'
		Extension	5'-CCTATCCCTACTTCCCC-3'
	rs1800871	Forward	5'-ACGTTGGATGGGTGTACCCTTGTACAGGTG-3'
		Reverse	5'-ACGTTGGATGATGCTAGTCAGGTAGTGCTC-3'
		Extension	5'-CCCTTGTACAGGTGATGTAA-3'
IL-18	rs1946518	Forward	5'-ACGTTGGATGCTCTCCCCAAGCTTACTTTC-3'
		Reverse	5'-ACGTTGGATGTGCTGTATCAGATGCAAGCC-3'
		Extension	5'-ATGTTGCAGAAAGTGTAAAAATTATTA-3'
	rs549908	Forward	5'-ACGTTGGATGCCTTGGTCAATGAAGAGAAC-3'
		Reverse	5'-ACGTTGGATGAATGTTTATTGTAGAAAACC-3'
		Extension	5'-AAGCTTGCCAAAGTAATC-3'
	rs187238	Forward	5'-ACGTTGGATGAATAAAGTGGCAGAGGATAC-3'
		Reverse	5'-ACGTTGGATGACAGAGCCCCAACTTTTACG-3'
		Extension	5'-TGTAATATCACTATTTTCATGAAAT-3'

Table 2. Odds ratios (OR) and 95% confidence intervals (95% CI) for disease association ofsignal-nucleotide polymorphisms (SNP) in IL-8, IL-18, and IL-10 in Chinese

Gene	SNP rs No.	Allele	Frequency		*P* value	OR	95% CI
			Case, n (%)	Control, n (%)			
IL-8	rs4073	A	0.407	0.440			
		T	0.593	0.560	0.464	0.874	0.610-1.252
	rs2227306	T	0.345	0.391			
		C	0.655	0.609	0.294	0.821	0.568-1.187
IL-10	rs1800896	T	0.898	0.917			
		C	0.102	0.083	0.465	0.796	0.431-1.470
	rs1800871	A	0.664	0.711			
		G	0.336	0.289	0.264	0.804	0.548-1.179
IL-18	rs1946518	T	0.509	0.485			
		G	0.491	0.515	0.597	1.1	0.772-1.569
	rs549908	T	0.865	0.848			
		G	0.135	0.152	0.609	1.143	0.686-1.905
	rs187238	C	0.85	0.86			
		G	0.15	0.14	0.747	0.92	0.556-1.523

tubes, at -20°C. Tubes were inverted several times to prevent coagulation, and genomic DNA was extracted using the phenol-chloroform extraction method.

Polymorphism analysis

IL-8 A/T (dbSNP ID: rs4073) and T/C (dbSNP ID: rs2227306), *IL-10* T/C (dbSNP ID: rs1800896) and A/G (dbSNP ID: rs1800871), and *IL-18* T/G (dbSNP ID: rs1946518), T/G (dbSNP ID: rs549908), and G/C (dbSNP ID: rs187238) genotypes were determined using the MassARRAY system (Sequenom) by matrix-assisted laser desorption ionization/time of flight mass spectrometry according to the manufacturer's instructions (Shanghai Benegene Biotechnology Co., Ltd., Shanghai, China). Genotype calling was performed in real time with MassARRAY RT software version 3.0.0.4 and analyzed using MassARRAY Typer software version 3.4 (Sequenom). Primers for PCR are listed in **Table 1**.

Statistical analyses

Statistical analysis was performed using SPSS software version 12.0 (SPSS, Chicago, IL). Allele frequency and genotype distributions of these SNPs were analyzed using Scheffe's test. Two-sided tests were used to evaluate the probability, and $P<0.05$ was considered statistically significant.

Results

Table 2 shows odds ratios (OR) and 95% confidence intervals (95% CI) for the association of *IL-8*, *IL-10*, and *IL-18* SNPs with BA, while **Table 3** summarizes genotype and carrier frequencies of the SNPs in children with BA and normal controls. Genotype frequencies of all seven lociof the three SNPs conformed to Hardy-Weinberg equilibrium. Although the frequency of the A allele was slightly lower than

Table 3. Genotype frequency of the IL-8, IL-18, IL-10 SNPs in case group and control group

Gene	SNP rs No.	Genotype	Frequency		*P* value
			Case, n (%)	Control, n (%)	
IL-8	rs4073	AA	0.159	0.188	
		TT	0.345	0.308	
		AT	0.496	0.504	0.759
	rs2227306	CC	0.425	0.368	
		TT	0.115	0.150	
		CT	0.460	0.481	0.572
IL-10	rs1800896	CC	0.018	0.023	
		TT	0.814	0.857	
		CT	0	0	N/A
	rs1800871	AA	0.425	0.526	
		AG	0.478	0.368	
		GG	0.097	0.105	0.212
IL-18	rs1946518	TT	0.283	0.233	
		GG	0.265	0.263	
		GT	0.451	0.504	0.622
	rs549908	TT	0.739	0.712	
		GG	0.009	0.015	
		GT	0.252	0.273	0.844
	rs187238	CC	0.708	0.735	
		GG	0.009	0.015	
		CG	0.283	0.250	0.775

that of the T allele for *IL-8* rs4073 in BA patients, no significant differences were observed in the distribution of alleles and genotypes between patients and controls.

Discussion

The present study investigated the frequency of *IL-8*, *IL-10*, and *IL-18* SNPs to determine their correlation with BA in the Chinese population. We found no obvious differences in disease association between patients and the control group regarding any of the seven SNPs examined.

Little is known about the etiology of BA, including bile duct injury and obstruction. Previous research revealed the existence of a potent inflammatory reaction in the livers of BA infants, particularly around the portal tracts [4, 11], with significantly increased levels of serum inflammatory mediators such as IL-2, IL-4, IL-10, and IL-18 [12]. Although these can be considered biomarkers of BA, it is not certain whether the expression of inflammatory mediators is associated with, or actually causes, BA in humans.

Previous studies have examined the role of SNPs in BA pathogenesis. A genome-wide association study analyzed 289,118 SNPs in 181 BA patients and 481 controls, revealing a strong correlation between SNP rs17095355 on chromosome 10q24 and BA [13]. Within the region of this SNP, the X-prolyl aminopeptidase P1 and adducin 3 genes both have an association with the inflammatory response of BA. Other studies have also examined the relationship between interleukin gene polymorphisms and BA risk. Donaldson et al. found that *IL-1* and *IL-10* polymorphisms were not risk factors for BA [9], while Lee HC and colleagues investigated the role of *IL-18* SNPs -1297 T/C (rs360719), -607 C/A (rs1946518), -137 G/C (rs187238), and +105 A/C (rs549908), and IL-4 SNPs -590 C/T (rs2243250), -33 C/T (rs2070874), and 8375 A/G (rs2243289) in BA susceptibility in Taiwan [14]. They concluded that the SNPs examined were unrelated to predisposition to BA in Taiwanese children [9, 14].

To confirm the findings of previous studies and to identify novel SNP associations with BA, we genotyped *IL-8* A/T (rs4073) and T/C (rs2227306), *IL-10* T/C (rs1800896) and A/G (rs1800871), and *IL-18* T/G (rs1946518), T/G (rs549908), and C/G (rs187238) SNPs in Chinese children with BA. However, none appeared closely related to BA sensitivity.

Genetic mutations accumulate in populations over time, but some variation occurs within specific groups of people because it is limited by human migration. Therefore, the detection of other ethnicities may draw a positive conclusion. The average SNP frequency is 0.1% in the human genome [15]. While some diseases are closely associated with SNP alleles [16], diseases with complex traits are usually determined by the joint effects of multiple genes [17], which is why we investigated the roles of SNPs from three genes in BA. Our negative results imply that these SNPs do not contribute to BA pathogenesis, and indicate that other variants should be explored. An alternative viewpoint is that because some SNPs control the expression and function of a protein involved in disease progression, SNPs in *IL-8*, *IL-10*, and *IL-18* may play a part in disease progression but not as BA risk factors.

In summary, we found no correlation between the SNPs investigated here and high morbidity in patients with BA. To our knowledge, this is the first time this has been reported for *IL-8* SNPs.

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Disclosure of conflict of interest

None.

Address correspondence to: Shouhua Zhang, Department of General Surgery, Jiangxi Children's Hospital, Nanchang 300006, China. Tel: +86 13979173982; Fax: +86 791 86804014; E-mail: zshouhua416@163.com; Huan Deng, Department of Pathology, The Fourth Affiliated Hospital of Nanchang University, Nanchang 330006, China. Tel: +86 15270966046; Fax: +86 791 87023487; E-mail: huandeng1971@163.com; beandeng@ncu.edu.cn

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