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Original Article

Association of STAT4 genetic polymorphisms with biliary atresia in Chinese patients

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Abstract: Biliary atresia (BA) is a devastating disease of the liver characterized by progressive fibro-inflammatory obliteration in neonates. Without effective treatment, end stage of liver, will ultimately lead to die about 2 years old. *STAT4* is a now known as a susceptibility gene for autoimmune diseases and a *STAT4* minor allele may be associated with several liver diseases. A case-control study was done between 113 patients with BA and 133 healthy controls. The rs7574865 and rs8179673 single nucleotide polymorphisms (SNPs) and rs10168266 SNPs in the *STAT4* gene were selected for genotyping. There was no significant difference in allele distribution between BA and controls (rs7574865, P = 0.6213, odds ratio [OR] = 1.098, 95% confidence interval [CI] = 0.758-1.589; rs8179673, P = 0.5702, OR = 0.898, 95% CI = 0.620-1.301; rs10168266, P = 0.9351, OR = 1.016, 95% CI = 0.699-1.477), and similar results were found for the genotype and haplotype frequencies of these *STAT4* gene polymorphisms. Our results indicated that these *STAT4* gene polymorphisms do not have a major role in the development of BA in Chinese children.

Keywords: Biliary atresia, gene polymorphism, Han Chinese patients, *STAT4*, SNP rs7574865

Introduction

Biliary atresia (BA) is a progressive fibro-inflammatory disease leading to obliteration of the extrahepatic biliary tree during infancy that invariably leads to cirrhosis and liver failure in neonates [1]. The incidence of BA varies widely among populations (from 1/5000 live-births in Asians to 1/18,000 in Caucasians indicating a higher rate in Asia than Western countries [2]. If left untreated, such as a Roux-en-Y hepatic portoenterostomy (Kasai operation), progressive liver cirrhosis will ultimately cause death by the age of 2 years. Failure of the Kasai procedure leaves liver transplantation as the only hope for survival [1, 2].

However, the mechanisms underlying the etiology and pathogenesis of BA remain unknown [3]. Many factors have been proposed to be involved in the etiology of BA including autoimmunity, virus infections, genetic abnormal development, environmental toxins, and abnormal morphogenesis [4-6]. Gene-expression analyses of BA serum or bile ducts and liver tissues indicated that genetic factors may play have a significant role in BA pathogenesis. Multiple genetic variants associated with primary biliary atresia have been reported [7]. Genetic susceptibility studies of BA have reported that *ADD3* with *GWAS* signal or *ADD3* expression gene variants [8, 9]. interleukin *(IL)-18 ITGB2* Gene *3'-UTR+145C/A* [10] and *+276G/T* adiponectin gene polymorphisms are strongly associated with biliary atresia [5]. Histologic examination of bile ducts has revealed an exclusively T-cell-mediated inflammatory response. A number of other studies have shown that the portal tracts of patients with BA are related to macrophages. These findings support the idea that BA is an immune-mediated inflammatory disease [3, 11, 12].

The human *STAT4* gene is located in a chromosomal linkage region, 2q33, where there are another 12 candidate genes. *Stat4* expression is restricted to myeloid cells, thymus, and testis [13, 14]. Signal transducer and activator of transcription 4 (*STAT4*) is a transcription factor belonging to the STAT family and in human T

Table 1. Odds ratios (OR) and 95% confidence intervals (95% CI) for disease association of 3 single-nucleotide polymorphisms (SNP) in the STAT4 gene in Chinese BA population and control population

Gene	SNP rs no.	Allele	Frequency		*p* value	OR	95% CI
			Case	Control			
STAT4	rs7574865	T	83 (0.367)	92 (0.346)	0.6213	1.098	0.758-1.589
		G	143 (0.633)	174 (0.654)			
	rs8179673	T	141 (0.629)	174 (0.654)	0.5702	0.898	0.620-1.301
		C	83 (0.371)	92 (0.346)			
	rs10168266	T	78 (0.348)	91 (0.345)	0.9351	1.016	0.699-1.477
		C	146 (0.652)	173 (0.655)			

cells it is required for the development of Th1 cells from naive CD4+ T cells and the production in response to IL-12. Among *STAT* family members, *STAT4* has common pathogenic pathways, such as IL-12 signaling, and has an essential and non-redundant role in the development of BA and some of its clinical features.

Many single nucleotide polymorphisms (SNPs) in *STAT4* genes are associated with genetic susceptibility to autoimmune diseases [13, 15], including Juvenile Idiopathic Arthritis (JIA) [16], type 1 diabetes [17], inflammatory bowel disease [18], systemic lupus erythematosus [19] (SLE), and rheumatoid arthritis [20] (RA). However, this is the first study to investigate the potential association between BA and *STAT4* SNPs (rs7574865, rs10168266, rs10168266) to the best of our knowledge.

Subjects and methods

Study subjects

The research was approved by the ethics committee of the Children's Hospital of Fudan University (Shanghai, China). Overall, 113 unrelated Chinese children (70 boys, 43 girls) were diagnosed with BA by exploratory laparotomy with operative cholangiography at the Children's Hospital of Fudan University. All patients between August 2014 and July 2015 underwent successful hepatoportoenterostomy (Kasai operation). The mean operation age of these patients was 68.1 ± 20.7 days (mean ± standard deviation) (range 23-163 days). The control group consisted of 133 healthy Han Chinese children (85 boys, 48 girls) who were recruited randomly from the Department of Pediatrics. None of them had a history of BA or any other liver diseases. After obtaining informed consent from their legal guardians, we took blood samples from the recruited children.

Methods and materials

Because this is the first study of single stat4 gene polymorphisms related to BA, SNPs with minor allele frequencies >5% according to the National Center for Biotechnology Information SNP Database (dbSNP) (http://www.ncbi.nlm.nih.bov/SNP/) were selected from previous studies. Genotyping.

Genomic DNA was extracted from whole blood using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). The three SNPs (rs7574865, rs10168266, s10168266) are located on the chromosome 2q33 region of the STAT4 gene. Genotyping was performed by MassARRAY on a matrix-assisted laser desorption ionization time of flight mass spectrometry platform and analyzed using MassARRAY Typer software version 3.4 (Sequenom). Primers for PCR and single-base extension were designed with Assay Designers software, version 3.0 (Sequenom, San Diego, CA, USA), and then synthesized by Benegene Biotech (Shanghai, China; **Table 1**).

Statistical analysis

Each SNP underwent Hardy-Weinberg equilibrium testing for the cases and control groups. Discrepancies in genotype frequencies and alleles between the BA cases and control subjects were evaluated with Fisher's exact test and χ^2 analysis. A *P* value of <0.05 was considered statistically significant. Association between STAT4 SNPs and disease status was expressed as odds ratio (OR) and 95% confidence intervals (CI). Statistical analysis was performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The haplotype frequen-

Table 2. Genotype frequency of the 3 SNPs in case group and control group

Gene	rs	Genotype	Frequency		*p* value
			Case	Control	
STAT4	rs7574865	TT	19 (0.168)	15 (0.113)	
		GG	49 (0.434)	56 (0.421)	
		GT	45 (0.398)	62 (0.466)	0.3632
	rs8179673	CC	19 (0.170)	15 (0.113)	
		TT	48 (0.429)	56 (0.421)	
		CT	45 (0.401)	62 (0.466)	0.3676
	rs10168266	CC	51 (0.455)	58 (0.439)	
		TT	17 (0.152)	17 (0.129)	
		CT	44 (0.393)	57 (0.432)	0.784

cies of STAT4 were estimated with the Haploview 4.2 program (http://www.broad.mitedu/mpg/haploview/).

Results

Overall, 246 subjects (113 BA patients and 133 controls) were successfully genotyped. The genotype distributions of the three SNPs were in Hardy-Weinberg equilibrium for both BA patients and controls (P > 0.05) and the minor allele frequencies of the three SNPs were >5%.

Tables 1 and **2** summarize the allele and genotype frequencies of the three single-nucleotide polymorphisms (SNP) in the *stat4* gene in the Chinese BA population and control population. No significant differences was found between patients with BA and controls (rs7574865, P = 0.6213, OR = 1.098, 95% CI = 0.758-1.589; rs8179673, P = 0.5702, OR = 0.898, 95% CI = 0.620-1.301; rs10168266, P = 0.9351, OR = 1.016, 95% CI = 0.699-1.477). Potential relevant haplotypes were also determined for the three SNPs; no significant differences in the distribution of haplotypes between BA and controls were found.

Discussion

Here, we investigated whether three *STAT4* gene polymorphisms were involved in the development of BA in Han Chinese patients. We concluded there is no association of the SNPs with BA.

The precise cause and pathogenic mechanisms of BA remain unknown. Genetic factors are known to play a significant role in BA; however, because of the low incidence, previous studies investigating possible genes about genome-wide level have been scant. Thus, the main genetic factors associated with BA remain unclear. Most BA-susceptible genes identified to date, including *ICAM-1*, *ITGB2* and *ADD3* [10, 21, 22], not only related with BA, but also have a role in inflammatory and immune responses. Similarly, most studies of human *STAT4* have focused on correlations between variations in the *STAT4* gene and the incidence of autoimmune disease [16-20].

STAT4 is an important signaling molecule for IL-12, IL-17, IL-23 and type I IFNs produced by T cells and NK cells that regulates immune responses, transmits signals from IL-12 (IL-12) and interferons (IFN) to induce IFN-γ production [13, 14]. IL-12, IL-17, and IL-23 are important cytokines that activate *STAT4* and initiate the differentiation of Th1 and Th17 cells [14]. Studies of mouse models of infectious and inflammatory diseases revealed that *STAT4* is an important factor in inflammatory immunity [26]. *STAT4*-deficient mice displayed less severe disease and decreased parameters of inflammation compared with wild type mice.

By collecting references [23-25] from PubMed and Springer, we concluded that several liver diseases were associated with *STAT4*. Findings demonstrate that *STAT4* allele may be associated with the spontaneous clearance of HBV [23], whereas the major allele may be associated with the progress of HBV-related liver disease. The combined data indicate that the *STAT4* rs7574865 polymorphism may be associated with a significantly reduced risk of HBV-induced HCC [24, 25] in Asians. The association of *STAT4* SNPs with BA is less commonly studied, and the molecular mechanisms that underlie the inherited susceptibility of BA remain unclear.

Based on these studies, we hypothesized that a functional *STAT4* SNP may also confer potential susceptibility to BA in Han Chinese patients. *STAT4* is located on chromosome 2q33, which play significant immune-regulatory roles and administrates a potent transcription factor responsible for transmitting signals stimulated with type1 IFN, IL-23, and IL-12 and so on. IL-12,

a cytokine linked to IL-23, enhances the differentiation of autoimmunity regulated by CD4+ T cells, and is involved in the differentiation of Th1 cells via *STAT4* signaling. Therefore, *STAT4*, as reported earlier, may play as a significant role in many stages of human diseases, concluding initiation, formation, and progression.

However, our findings indicated that these negative associations might be because of the limited selection of SNPs investigated, which did not widely cover the gene, and the small sample size. Thus, a secondary investigation with a larger sample size and bigger statistical power should be performed.

In conclusion, our research findings indicate a lack of association between *STAT4* gene polymorphisms (SNPs rs7574865 and rs8179673 and rs10168266) and BA in Chinese Han children. Future studies with a larger dataset and different races are necessary to explore the potential associations between *STAT4* genetic polymorphisms and BA.

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Disclosure of conflict of interest

None.

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