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Short Communication

Association of X-prolyl aminopeptidase 1 rs17095355 polymorphism with biliary atresia in Thai children

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Aim: To investigate *XPNPEP1* rs17095355 polymorphism in biliary atresia (BA) patients and to determine whether there is an association between *XPNPEP1* gene polymorphism and susceptibility to BA in a Thai population.

Methods: A total of 124 cases of BA and 114 controls were genotyped for *XPNPEP1* rs17095355 polymorphism. The *XPNPEP1* rs17095355 C/T genotype was determined by polymerase chain reaction (PCR) and direct sequencing. Allele and genotype frequencies were established by directed counting from the sequences.

Results: Genotype distributions for the *XPNPEP1* rs17095355 polymorphism tested were in Hardy–Weinberg equilibrium for both control and study groups. There were no significant differences in genotype and allele frequencies of the single nucleotide polymorphism between controls and Thai children with BA. Genotype frequencies of rs17095355 of T/T in BA were higher than those of controls (34.68% and 16.67%, $P < 0.002$). Also, the T allele frequencies of BA were higher than those of controls (56.85% and 42.98%, $P < 0.003$).

Conclusion: The association between *XPNPEP1* rs17095355 polymorphism and BA has been demonstrated, particularly with the T allele. We hypothesize that the *XPNPEP1* rs17095355 polymorphism confers increased susceptibility to the disease.

Key words: Biliary atresia, polymorphism, X-prolyl aminopeptidase 1 (XPNPEP1).

INTRODUCTION

BILIARY ATRESIA (BA) is a rare neonatal disease of unknown etiology characterized by progressive fibrosclerosing obstruction of the extrahepatic biliary system that presents in the first months of life, causing severe cholestasis and biliary cirrhosis with a fatal outcome in the first years of life. Initially, hepatoportoenterostomy (Kasai operation) was performed to allow for bile drainage to the intestine in the first 3 months of life. The success of surgery depends on the age at which it is performed.[1] If the operation is unsuccessful, liver transplantation becomes necessary. Potential complications after surgery include portal hypertension and cholangitis. The incidence of disease varies from 5/100 000 to 32/100 000 live births, and is highest in Asia and the Pacific region.[2]

The etiology and pathogenesis of BA are unknown. Several possible mechanisms have been proposed to be involved including genetics, autoimmunity, inflammation and environmental factors such as virus or toxins.[3,4] These factors are likely to contribute to the progressive inflammation and sclerosing processes. Various studies have investigated genetic susceptibility for BA focusing on vascular endothelial growth factor gene (VEGF), intercellular adhesion molecule-1 (ICAM-1), macrophage migration inhibitory factor (MIF), and hepcidin antimicrobial peptide gene, genes involved in the inflammatory processes.[5–7]

Garcia-Barcelo *et al.* from the genome-wide association study (GWAS) have recently conducted research on 200 patients and 481 control individuals aimed at discovering genes involved in BA. Upon investigating approximately 500 000 markers in the Chinese population for single nucleotide polymorphisms (SNPs), this

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group established 10 SNP positions associated with the pathogenesis of biliary atresia. The strongest overall association was found for rs17095355 on 10q24, upstream of *XPNPEP1* ($P = 0.00024$).[8]

XPNPEP1 encodes the cytosolic form of a metalloaminopeptidase or soluble aminopeptidase P (APP1) that specifically catalyzes the cleavage of the N-terminal amino acid adjacent to a penultimate proline residue. It is expressed in epithelial cells of the hepatobiliary system.[9] APP1 is a commonly found enzyme that may play a role in degradation and maturation of tachykinins, neuropeptides, and peptide hormones. It can metabolize bradykinin (BK) and substance P (SP). BK is a potent endothelium-dependent vasodilator, increases vascular permeability and its expression is regulated by the bile acid nuclear receptor, farnesoid X receptor (FXR) known to play a role in the regulation of bile acid metabolism and secretion and inflammatory processes.[10,11] SP belongs to the tachykinin neuropeptide family. It is a neurotransmitter that plays important roles in inflammatory processes and immunological states, and it is also a mediator of tissue injuries and autoimmune diseases.[12]

However, *XPNPEP1* rs17095355 polymorphism in Thai patients with biliary atresia have not been studied. Therefore, this study has been aimed at investigating the *XPNPEP1* gene polymorphism in BA children and to determine whether there is an association between *XPNPEP1* gene polymorphism and susceptibility to BA in a Thai population.

METHODS

Study population

THIS STUDY HAS been approved by the Ethics Committee on Human Research of the Faculty of Medicine, Chulalongkorn University. All parents or legal guardians of the recruited children with BA were informed of the study's purpose. Written informed consent was obtained from the parents prior to the children entering the study.

One hundred and twenty-four patients with established diagnosis of BA (63 males and 61 females), mean age ± standard deviation (SD) = 8.91 ± 5.89 years, were included in the course of a routine follow-up at the Department of Pediatrics, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. All patients with type 3 (uncorrectable) isolated BA had undergone hepatic portojejunostomy with Roux-en-Y reconstruction (original Kasai procedure), and they were generally in good health; no signs of suspected infection or bleeding abnormalities at the time of blood sampling. Based on their jaundice status, BA children were divided into a non-jaundice group (TB < 2 mg/dL) and a persistent jaundice group (TB ≥ 2 mg/dL). The levels of total bilirubin of BA patients were 2.90 ± 7.07 mg/dL. A group of 114 (59 males and 55 females) anonymous, unrelated, healthy Thai children served as a control group (mean age ± SD = 13.02 ± 2.18 years). None had any history of BA, autoimmune, or liver diseases. The case-control study was performed on these 124 children with BA and 114 controls. For definition of Thai, the parents (father and mother) of the biliary atresia and healthy control subjects were born in Thailand.

Polymorphism analysis

Peripheral whole blood from BA children and healthy controls was collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA of healthy controls and BA patients was extracted from buffy coat leukocytes by the standard phenol/chloroform extraction method. The *XPNPEP1* rs17095355 genotype was determined using direct sequencing. Briefly, polymerase chain reaction (PCR) was carried out using a programmable thermal cycler gradient PCR system (Eppendorf, Hamburg, Germany). Samples were subjected to initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s for denaturation, 30 s at 57°C for annealing and 30 s at 72°C for extension, and concluded by a final 7 min extension at 72°C. The primary PCR reaction mixture comprised 100 ng/mL of genomic DNA, 0.5 μL of 10 μM rs17095355F (5′-GGA TTT GAC CCA AGC ACT GT-3′), 0.5 μL of 10 μM rs17095355R (5′-ACT GCT GAC CTG GGA TTC TG-3′), 12.5 μL of 2X Mastermix (Eppendorf, Hamburg, Germany) and distilled water to a final volume of 25 μL. A 140-bp fragment covering the rs17095355 polymorphism upstream of *XPNPEP1* was amplified. The PCR products were subjected to electrophoresis on a 2.0% agarose gel stained with ethidium bromide and visualized under UV light.

Statistical analysis

Allele and genotype frequencies of the *XPNPEP1* rs17095355 were determined by direct counting from the sequences. Odd ratios (ORs) and 95% confidence intervals (CIs) were calculated for the association between *XPNPEP1* rs17095355 genotype and risk of BA by χ^2 from Epi Info version 6.0 (CDC Atlanta, GA, USA). The Hardy–Weinberg equilibrium was evaluated in both control and case groups by χ^2 test analysis. Statistical analysis was performed using SPSS software for

Windows, version 13.0 (SPSS Inc., Chicago, IL, USA). A *P*-value <0.05 was considered to indicate a statistically significant difference.

RESULTS

SAMPLES FROM 124 cases of BA and 114 controls were subjected to genotype analysis for *XPNPEP1* rs17095355 polymorphism. Genotype distributions for the SNP tested were all in Hardy–Weinberg equilibrium for both control and study groups. There were no significant differences in genotype and allele frequencies of the SNP between controls and Thai children with BA.

Allele and genotype frequencies for the *XPNPEP1* rs17095355 polymorphism are shown in Table 1. Genotype frequencies of the rs17095355 among the 124 cases and 114 controls are as follows: C/C 20.97% and 30.70%, C/T 44.35% and 52.63%, T/T 34.68% and 16.67%, respectively. The C and T allele frequencies of BA were 43.15% and 56.85%, as compared to controls with 57.02% and 42.98%, respectively.

In addition, there was no statistically significant difference in the distributions of genotype and allele of *XPNPEP1* ($P = 0.85$ and 0.78, respectively) between BA patients with persistent jaundice and those without jaundice, Therefore, there was no association between the *XPNPEP1* SNP and the occurrence of jaundice.

DISCUSSION

BILIARY ATRESIA IS still one of the most serious liver diseases in children. A number of hypotheses have been proposed to account for the disease. Despite several studies on its pathogenesis, its cause is not clearly understood. One of the hypotheses is that BA is an immune-mediated disease, which possibly occurs following an assault on a genetically susceptible host. However, until recently, the study of gene polymorphisms in BA has received little attention, probably due to its rare incidence. There has been evidence that polymorphisms of the ICAM-1 241R gene are associated with BA but another study could not show any association with K469E ICAM-1 gene polymorphism.[6,13] An interesting study from China using a genome-wide association study (GWAS) from nearly 500 000 SNPs in 200 BA patients and 481 controls, demonstrated that the strongest overall association was found for rs17095355 on 10q24, downstream *XPNPEP1*, a gene involved in the metabolism of inflammatory mediators.[8] In this study, we investigated the frequencies of the polymorphism rs17095355 in the *XPNPEP1* gene in Thai children with BA and in healthy control subjects. We clearly demonstrated that the rs17095355 genotype and T allele were more frequent in BA compared with controls. Since information regarding *XPNPEP1* in BA is still lacking, further interpretation of the association of the rs17095355 polymorphism of *XPNPEP1* with BA (susceptibility to the disease, pathophysiology, or effect on therapeutic outcome and prognosis) is subject to additional research investigations.

XPNPEP1 (X-prolyl aminopeptidase 1), also known as Aminopeptidase P1 (APP1), is a proline-specific metalloaminopeptidase that specifically catalyzes the removal of any unsubstituted N-terminal amino acid adjacent to a penultimate proline residue. Its function contributes to the bradykinin degradation process via hydrolysis.[14] APP1 can metabolize bradykinin and substance P. Bradykinin is a vasoactive protein, which is able to induce vasodilation, increase vascular permeability, cause smooth muscle contraction, and induce pain.

Table 1 Genotype and allele frequencies (%) of *XPNPEP1* rs17095355 polymorphism in biliary atresia (BA) patients and controls

	BA $n = 124$ (%)	Controls $n = 114$ (%)	Odds ratio (95%CI)	*P*-value
Age (years)	8.91 ± 5.89	13.02 ± 2.18		
Male : female	63:61	59:55		
Genotype				
CC	26 (20.97)	35 (30.70)		
CT	55 (44.35)	60 (52.63)		
TT	43 (34.68)	19 (16.67)	2.65 (1.38–5.15)	0.0016
Alleles				
C	107 (43.15)	130 (57.02)		
T	141 (56.85)	98 (42.98)	1.71 (1.20–2.56)	0.0025
HWE *P*-value	0.29	0.43		

CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

These proteins are involved closely in the inflammatory process. Endotoxin levels were reported to be increased in the portal blood of cirrhotic patients.[15] Thus, they could act as a stimulus for local activation of the contact system and liberation of bradykinin. In addition, the role of bradykinin in the pathogenesis of splanchnic hyposensitivity in rats with cirrhosis induced by common bile duct-ligation has recently been documented.[16] Substance P is an excitatory neuropeptide that acts via the neurokinin-1 receptor. Recent study showed that serum substance P level of patients with chronic liver disease with cholestasis was significantly higher than those without cholestasis. There is increased availability of SP in cholestasis.[17] Since BA is a progressive inflammatory cholangiopathy, *XPNPEP1* is likely to be associated with BA. Future studies are warranted to investigate the exact role of bradykinin and substance P in BA patients. This aminopeptidase has been reported to be associated with various conditions including ileitis, breast tumor, and prostatic cancers.[18,19] Nevertheless, to the best of our knowledge, gene polymorphism of *XPNPEP1* has not been reported to be associated with any other diseases.

In conclusion, the association between the *XPNPEP1* rs17095355 polymorphism and BA has been demonstrated, particularly with the T allele. We hypothesize that the *XPNPEP1* rs17095355 polymorphism confers increased susceptibility to the disease.

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