The association of transporter ABCC2 (MRP2) genetic variation and drug-induced hyperbilirubinemia

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Abstract

Background: Hyperbilirubinemia is a predictor of severe drug-induced liver injury (DILI). Hepatobiliary ATP-binding cassette (ABC) transporters play an important role in the transportation of many drugs and bilirubin; however, little is known about these transporters and the risk of DILI. The aim of this study was to explore associations between genetic variations in important ABC transporters and susceptibility to DILI, with a particular focus on hyperbilirubinemia.

Methods: A total of 200 patients with DILI and 200 healthy controls were enrolled as the training dataset. Another 106 patients with DILI were recruited as the validation dataset. They were genotyped for *ABCB11 (BSEP)* rs2287622, *ABCB1 (MDR1)* rs1128503, rs1045642, *ABCB4 (MDR3)* rs2230028, *ABCC2 (MRP2)* rs1885301, rs717620, rs2273697, rs3740066 and rs8187710 using polymerase chain reaction–based TaqMan genotyping assays.

Results: There were no statistical differences in any of the nine ABC transporter single nucleotide polymorphisms between the DILI and control groups. However, in the DILI group, the patients with hyperbilirubinemia had a higher frequency of the *ABCC2* rs717620 C/T and T/T genotypes than those without hyperbilirubinemia (44.2% vs 20.2%, $p = 0.001$). After adjusting for other confounding factors, the *ABCC2* rs717620 T variant was still associated with an increased risk of hyperbilirubinemia (adjusted odds ratio [OR]: 3.83, 95% confidence interval [CI]: 1.73-8.48, $p = 0.001$). This association was confirmed by the validation dataset (adjusted OR: 3.92, 95% CI: 1.42-10.81, $p = 0.015$). We also found that the mortality group had higher frequencies of the *ABCC2 (MRP2)* rs717620 C/T and T/T genotypes than the survival group (50.0% vs 27.9%, $p = 0.048$).

Conclusion: Carriage of the *ABCC2 (MRP2)* rs717620 T variant may increase the risk of hyperbilirubinemia and mortality in patients with DILI. Screening for this variant may help to prevent and mitigate drug-induced hyperbilirubinemia.

Keywords: ATP-binding cassette (ABC) transporters; ABCC2; Drug-induced liver injury; Hyperbilirubinemia; MRP2

1. INTRODUCTION

Drug-induced liver injury (DILI) is an important and challenging liver disease. Most cases of DILI ultimately resolve completely without residual liver injury; however, some cases may progress to acute liver failure (ALF), chronic liver injury, or cirrhosis, which may result in death or the need for liver transplantation.[1]

Hy's law is a method used to predict the potential of a drug to cause a severe DILI and states that the occurrence of hyperbilirubinemia is an indicator of a poor outcome of DILI.[2] Therefore, understanding the pathogenesis and risk factors of hyperbilirubinemia is crucial in the prevention of a serious DILI. Hyperbilirubinemia occurs when there is a disturbance in the homeostasis of bilirubin and bile acid caused by proteins involved in the synthesis, uptake, detoxification, and transport of bile acid and bilirubin. The superfamily of hepatobiliary ATP-binding cassette (ABC) transporters plays a crucial role in the disposition of bile acid, bilirubin and many drugs.[3–9] Among them, bile salt export pump (BSEP, ABCB11), multidrug resistance 1 (MDR1, ABCB1), MDR3 (ABCB4), and multidrug resistance–associated protein 2 (MRP2, ABCC2) are the most important efflux transporters. Hereditary genetic variations of these ABC transporters may regulate transporter expression and may be related to many hereditary and acquired cholestatic liver diseases.[3–9]

A dysfunctional transporter gene may also determine an individual's susceptibility to the occurrence of DILI.[3–9] Recent studies have explored associations between these ABC genetic variations and DILI; however, the results have been inconsistent and inconclusive.[10–25] The inclusion criteria for DILI, offending drugs, and ethnic populations were different in these studies, which may have affected the results. Therefore, the true relationship between genetic polymorphisms of ABC transporters and DILI remains controversial. The purpose of this study was to evaluate the influence of genetic variations in four major ABC transporters, ABCB11, ABCB1, ABCB4, and ABCC2, on the risk of DILI in Taiwanese patients, with a particular focus on hyperbilirubinemic DILI.

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2. METHODS

2.1. Patients studied

A total of 200 patients with DILI were enrolled in the DILI case group, and 200 healthy adults in the control group. To verify associations between hyperbilirubinemic DILI and genetic variations of ABC transporters, a validation dataset including another 106 cases with DILI was also enrolled.

The inclusion criteria for the DILI cases were as follows: (1) an increase in serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level greater than five times the upper limit of normal value (ULN), or an elevation in serum alkaline phosphatase (ALP) greater than twice the ULN during treatment with the drug, as suggested by the Drug-induced Liver Injury Network (DILIN)[26]; (2) serum ALT or AST level greater than five times the baseline value, or ALP level greater than twice the baseline level, in cases with elevated based line levels; and (3) any elevation of serum ALT, AST, or ALP associated with an increase in the level of serum total bilirubin (>2.5 mg/dL), in the absence of a prior diagnosis of liver disease, Gilbert's syndrome, or evidence of hemolysis. The exclusion criteria for the DILI cases were as follows: (1) patients with possible acute or chronic viral hepatitis, such as positive serum hepatitis B virus surface antigen, IgM antibody to hepatitis B core antigen, IgM antibody to hepatitis A virus, antibody to hepatitis C virus, IgM antibody to Epstein-Barr virus, cytomegalovirus, and herpes simplex virus; (2) autoimmune liver diseases, such as autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis; (3) any other major hepatic or systemic diseases that may induce elevation of liver biochemical tests, such as alcoholic liver disease, nonalcoholic fatty liver disease, hepatobiliary stones or tumors, congestive heart failure, hypoxia, and bacteremia; (4) a RUCAM causality assessment score <5, indicating patients with the least possibility of having DILI;[27] and (5) patients with incomplete clinical or laboratory data.

The inclusion criterion for the controls was adults with normal serum levels of ALT, AST, ALP, and bilirubin on enrollment. The exclusion criteria for the controls were as follows: (1) chronic hepatitis B or C infection; (2) autoimmune liver diseases, such as autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis; and (3) any other major hepatic or systemic diseases that may affect liver biochemical tests, such as alcoholic liver disease, non-alcoholic fatty liver disease, hepato-biliary stones or tumors, congestive heart failure, hypoxia, and bacteremia.

The types of DILI were classified according to the DILIN guidelines.[26] This is characterized based upon the relative elevations of serum ALT and ALP (R ratio of ALT to ALP, both expressed in multiples of the ULN) into hepatocellular ($R \geq 5$), mixed (R = 2-5), or cholestatic ($R \leq 2$).

Hyperbilirubinemia was defined as a serum total bilirubin level >2.5 mg/dL as suggested by the DILIN.[26] ALF was defined as prolongation of prothrombin time, international normalized ratio >1.5 or presence of any degree of hepatic encephalopathy according to the European Association for the Study of the Liver.[28]

The study protocol was approved by the Institutional Review Board of Taipei Veterans General Hospital (No. 2017-01-007B).

2.2. Determination of ABC genotypes

Venous blood was obtained from each subject, and DNA was extracted and deposited in the Biobank of Taipei Veterans General Hospital. Samples and controls were genotyped for the *ABCB11 (BSEP)* c.1331T>C polymorphism (rs2287622), *ABCB1 (MDR1)* c.1236T>C (rs1128503), c.3435T>C (rs1045642), *ABCB4 (MDR3)* c.1954A>G (rs2230028) and *ABCC2 (MRP2)* c.-1549A>G (rs1885301), c.-24C>T (rs717620), c.1249G>A (rs2273697), c.3972C>T (rs3740066), and c.4544G>A (rs8187710). These candidate single nucleotide polymorphisms (SNPs) were selected from previous publications which disclosed associations with DILI and hereditary cholestatic liver diseases.[3–25] Genotyping was performed using TaqMan genotyping assays (Thermo Fisher Scientific Inc., Waltham, MA, USA). TaqMan polymerase chain reactions (PCR) were performed according to the manufacturer's standard protocol. Briefly, 20 ng genomic DNA was mixed with the supplied 2× TaqMan Universal PCR Master Mix No AmpErase UNG and 20× TaqMan Assay Mix to a final volume of 5 μL in a 384-well plate. Each sample underwent 40 amplification cycles on a GeneAmp PCR System 9700 (Thermo Fisher Scientific Inc.). Fluorescent signals of the two probes were analyzed using an ABI PRISM® 7900HT Sequence Detection System (Thermo Fisher Scientific Inc.). The genotypes were determined automatically using Sequence Detection Software (Thermo Fisher Scientific Inc.).

2.3. Statistical analysis

Expected gene frequencies were calculated from respective single allele frequencies according to the Hardy-Weinberg equation using the chi-square goodness-of-fit test. Differences in genotype distributions were compared between the DILI cases and controls, three DILI types, and cases with or without hyperbilirubinemia using the chi-square test with or without Yates' correction. Bonferroni correction was applied for multiple comparisons. Means were compared using the Student's t test or ANOVA. Odds ratios (ORs) and confidence intervals (CIs) were estimated using logistic regression analysis. All analyses were performed using SPSS version 17.0 (SPSS Inc, Chicago, IL, USA), and a $p < 0.05$ was considered to be statistically significant.

3. RESULTS

There were no statistical differences in age and gender among the controls, DILI groups in the training dataset, and DILI groups in the validation dataset (Table 1). There were also no statistical differences in the frequency of three DILI types, ALF, and mortality rates between the DILI training dataset and validation dataset. A total of 86 (43%) patients in the DILI training dataset and 21 (19.8%) patients in the DILI validation dataset had hyperbilirubinemia. Antituberculosis drugs (ATDs) were the most common type of drug that induced liver injury in both the DILI training and validation datasets, which included the standard four combination therapy of isoniazid, rifampicin, pyrazinamide, and ethambutol (Table 2).

All of the ABC transporter genotypes were in Hardy-Weinberg equilibrium. There were no statistical differences in any of the nine SNPs of ABC transporters between the DILI training dataset and control group (Table 3). Furthermore, there were no statistical differences in these SNPs among the hepatocellular, mixed, and cholestatic types of DILI (Table 4). However, the patients with hepatocellular DILI had higher frequencies of hyperbilirubinemia and ALF compared with the patients with the other types.

The patients with hyperbilirubinemia had higher frequencies of *ABCC2 (MRP2)* rs717620 C/T and T/T genotypes than those without hyperbilirubinemia (44.2% vs 20.2%, $p = 0.001$, Table 5), and the difference was still statistically significant after Bonferroni correction (significance was defined as $p < 0.006$). There were no statistical differences in any of the genotypes between the patients with and without ALF (Table 6). However, the mortality group had marginally higher frequencies of *ABCC2 (MRP2)* rs717620 C/T and T/T genotypes than the survival group (50.0% vs 27.9%, $p = 0.048$, Table 7). In the patients with ATD-induced liver injury, there were no statistical differences in the frequency of hyperbilirubinemia in any of the genotypes. Furthermore, if the DILI group was separated

Table 1

Basic characteristics of controls, DILI patients in training dataset and DILI patients in validation dataset

	Controls (n = 200)	DILI training (n = 200)	DILI validation (n = 106)	*p*
Age, y	56.9 ± 12.6	59.2 ± 12.4	58.6 ± 12.7	0.179
Gender (F/M)	95/105	91/109	49/57	0.922
Peak ALT, U/L	30.4 ± 7.7	779.3 ± 967.1	530.8 ± 497.9	<0.001
Peak AST, U/L	33.8 ± 6.5	784.7 ± 945.6	539.3 ± 523.6	<0.001
Peak total bilirubin, mg/dL	1.2 ± 0.3	4.1 ± 5.5	3.0 ± 4.0	<0.001
Peak ALP, U/L	80.4 ± 14.8	157.4 ± 155.0	123.2 ± 91.4	<0.001
Albumin, g/dL	5.0 ± 0.4	4.2 ± 0.8	4.7 ± 0.7	<0.001
Creatinine, mg/dL	1.0 ± 0.2	1.6 ± 0.6	1.2 ± 0.6	<0.001
Platelet (10^3/μL)	289.4 ± 62.8	208.9 ± 88.7	212.5 ± 64.8	<0.001
DILI type (H/M/C)	...	158/25/17	85/13/8	0.990[c]
Hyperbilirubinemia[a]	0	86 (43.0%)	21 (19.8%)	<0.001[c]
ALF[b]	0	37 (18.5%)	14 (13.2%)	0.237[c]
Mortality	0	24 (12%)	11 (10.4%)	0.813[c]

Data were expressed as mean ± SD or numbers. Reference value: ALT: 0-40 U/L, AST: 5-45 U/L, total bilirubin: 0.2-1.6 mg/dL, ALP: 10-100 U/L, albumin: 3.5-5.5 g/dL, platelet: 150-400 × 10^3/μL, international normalized ratio: 0.8-1.2.

ALF = acute liver failure; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; C = cholestatic type; DILI = drug-induced liver injury; H= hepatocellular type; M = mixed type.

[a]Hyperbilirubinemia was defined as serum total bilirubin >2.5mg/dL.

[b]ALF was defined as prolongation of prothrombin time, international normalized ratio > 1.5, or presence of any degree of hepatic encephalopathy.

[c]Compared between DILI training dataset and validation dataset 2 groups.

into subgroups of *ABCC2* rs717620 with or without the T variant, there were no statistical differences in ALT and AST levels between these two subgroups (814.8 ± 988.6 vs 763.7 ± 960.7 U/L, $p = 0.735$ and 742.1 ± 830.3 vs 803.4 ± 994.3 U/L, $p = 0.652$, respectively).

In univariate analysis, *ABCC2* rs717620 T variant, ALT, AST, ALP, and albumin were found to be associated with the risk of hyperbilirubinemia in the DILI training dataset. In multivariable analysis, *ABCC2* T variant was the most significant factor after adjusting for ALT, AST, ALP, albumin, age, and gender (adjusted OR: 3.83, 95% CI: 1.73-8.48, $p = 0.001$, Table 8).

In the validation dataset of another 106 cases with DILI, the patients with the *ABCC2* rs717620 T variant also had a higher risk of hyperbilirubinemia (adjusted OR: 4.91, 95% CI: 1.36-17.73, $p = 0.015$, Table 8). In addition, serum albumin level was associated with hyperbilirubinemia in the univariate and multivariable analyses of both datasets. Although serum ALT, AST, and ALP levels were associated with the risk of hyperbilirubinemia in the univariate analysis, there was no statistical significance in the multivariable analysis.

Table 2

Category of incriminated drugs for drug-induced liver injury in this study

Category of drugs	Training dataset (n = 200)	Validation dataset (n = 106)
Anti-tuberculosis drugs	100 (50.0%)	46 (43.4%)
Nonsteroidal anti-inflammatory drugs	18 (9.0%)	11 (10.4%)
Statins	16 (8.0%)	7 (6.6%)
Antibacterial drugs	15 (7.5%)	9 (8.5%)
Anticonvulsant drugs	11 (10.5%)	8 (7.6%)
Hypouricemic drugs	9 (4.5%)	5 (4.7%)
Antifungal drugs	7 (3.5%)	2 (1.9%)
Antineoplastic drugs	6 (3.0%)	5 (4.7%)
Antiarrhythmic drugs	5 (2.5%)	2 (1.9%)
Hormone-related drugs	5 (2.5%)	4 (3.7%)
Antipsychotic drugs	4 (2.0%)	2 (1.9%)
Others	4 (2.0%)	5 (4.7%)

With regard to mortality, the *ABCC2* rs717620 T variant was associated with mortality in the univariate analysis (Table 9), although this association was not found in the multivariable analysis after adjustments for other variables. However, serum albumin level was associated with mortality in the univariate and multivariable analyses of both datasets.

4. DISCUSSION

Hepatic transporters may influence the disposition of drugs and are associated with the development of DILI. A dysfunctional

Table 3

The distribution of genetic variations of ABC transporters in DILI cases and controls based on training dataset

	DILI (n =200)	Control (n =200)	*p*
ABCB11			
rs2287622			
CC/CT/TT	103/80/17	105/78/17	0.978
ABCB1			
rs1128503			
CC/TC/TT	27/87/86	26/87/87	0.988
rs1045642			
CC/TC/TT	74/92/34	72/90/31	0.851
ABCB4			
rs2230028			
AA/GA/GG	185/15/0	189/11/0	0.417
ABCC2			
rs1885301			
AA/AG/GG	9/55/136	16/70/114	0.058
rs717620			
CC/CT/TT	139/55/6	117/72/11	0.060
rs2273697			
AA/AG/GG	3/38/159	2/36/162	0.863
rs3740066			
CC/CT/TT	133/58/9	113/72/15	0.099
rs8187710			
GG/GA/AA	199/1/0	200/0/0	0.317

No statistical difference between 2 groups in all genetic variations.

ABC = ATP-binding cassette; DILI = drug-induced liver injury.

Table 4

The difference of genetic variations of ABC transporters, hyperbilirubinemia, acute liver failure, and mortality among three types of drug-induced liver injury based on training dataset

	Hepatocellular (n = 158)	Mixed (n = 25)	Cholestatic (n = 17)	*p*
ABCB11				
rs2287622	83/60/15	13/11/1	7/9/1	0.685
CC/CT/TT	(52.5/38.0/9.5)	(52.0/44.0/4.0)	(41.2/52.9/5.9)	
ABCB1				
rs1128503	22/69/67	2/12/11	3/6/8	0.868
CC/TC/TT	(13.9/43.7/42.4)	(8.0/48.0/44.0)	(17.6/35.3/47.1)	
rs1045642	58/73/27	8/14/3	8/5/4	0.559
CC/TC/TT	(36.7/46.2/17.1)	(32.0/56.0/12.0)	(47.1/29.4/23.5)	
ABCB4				
rs2230028	147/11//0	22/3/0	16/1/0	0.651
AA/GA/GG	(93.0/7.0/0.0)	(88.0/12.0/0.0)	(94.1/5.9/0.0)	
ABCC2				
rs1885301	8/43/107	1/6/18	0/6/11	0.832
AA/AG/GG	(5.1/27.2/67.7)	(4.0/24.0/72.0)	(0.0/35.3/64.7)	
rs717620	109/43/6	18/7/0	12/5/0	0.799
CC/CT/TT	(69.0/27.2/3.8)	(72.0/28.0/0.0)	(70.6/29.4/0.0)	
rs2273697	2/31/125	1/4/20	0/3/14	0.819
AA/AG/GG	(1.3/19.6/79.1)	(4.0/16.0/80.0)	(0.0/17.6/82.4)	
rs3740066	105/46/7	16/7/2	12/5/0	0.882
CC/CT/TT	(66.5/29.1/4.4)	(64.0/28.0/8.0)	(70.6/29.4/0.0)	
rs8187710	158/0/0	25/0/0	16/1/0	0.083
GG/GA/AA	(100.0/0.0/0.0)	(100.0/0.0/0.0)	(94.1/5.9/0.0)	
Hyperbilirubinemia	78 (49.4)	8 (32.0)	0 (0.0)	<0.001*
Acute liver failure	35 (22.2)	2 (8.0)	0 (0.0)	0.029*
Mortality	23 (14.6)	1 (4.0)	0 (0.0)	0.090

ABC = ATP-binding cassette.
*$p < 0.05$.

Table 5

The distribution of genetic variations of ABC transporters in drug-induced hyperbilirubinemia based on training dataset

	Hyperbilirubinemia[a] (n = 86)	Non-hyberbilirubinemia (n = 114)	*p*
ABCB11			
rs2287622	47/30/9	56/50/8	0.375
CC/CT/TT	(54.7/34.9/10.4)	(49.1/43.9/7.0)	
ABCB1			
rs1128503	7/38/41	20/49/45	0.136
CC/TC/TT	(8.1/44.2/47.7)	(17.5/43.0/39.5)	
rs1045642	34/39/13	40/53/21	0.744
CC/TC/TT	(39.5/45.3/15.2)	(35.1/46.5/18.4)	
ABCB4			
rs2230028	77/9/0	108/6/0	0.167
AA/GA/GG	(89.5/10.5/0)	(94.7/5.3/0)	
ABCC2			
rs1885301	7/25/54	2/30/82	0.075
AA/AG/GG	(8.1/29.1/62.8)	(1.8/26.3/71.9)	
rs717620	48/34/4	91/21/2	0.001*
CC/CT/TT	(55.8/39.5/4.7)	(79.8/18.4/1.8)	
rs2273697	2/18/66	1/20/93	0.570
AA/AG/GG	(2.3/20.9/76.8)	(0.9/17.5/81.6)	
rs3740066	50/30/6	83/28/3	0.066
CC/CT/TT	(58.1/34.9/7.0)	(72.8/24.6/2.6)	
rs8187710	86/0/0	113/1/0	0.384
GG/GA/AA	(100/0/0)	(99.1/0.9/0)	

Data were expressed as number (%).
ABC = ATP-binding cassette.
*$p < 0.05$.
[a]Hyperbilirubinemia was defined as serum total bilirubin >2.5 mg/dL.

Table 6

The distribution of genetic variations of ABC transporters in drug-induced ALF based on training dataset

	ALF[a] (n = 37)	Non-ALF (n = 163)	*p*
ABCB11			
rs2287622	22/10/5	81/70/12	0.149
CC/CT/TT	(59.5/27.0/13.5)	(49.7/42.9/7.4)	
ABCB1			
rs1128503	3/19/15	24/68/71	0.431
CC/TC/TT	(8.1/41.7/43.6)	(14.7/43.0/39.5)	
rs1045642	9/23/5	65/69/29	0.087
CC/TC/TT	(24.3/62.2/13.5)	(39.9/42.3/17.8)	
ABCB4			
rs2230028	34/3/0	151/12/0	0.550
AA/GA/GG	(91.9/8.1/0.0)	(92.6/7.4/0.0)	
ABCC2			
rs1885301	2/9/26	7/46/110	0.868
AA/AG/GG	(5.4/24.3/70.3)	(4.3/28.2/67.5)	
rs717620	21/14/2	118/41/4	0.157
CC/CT/TT	(56.8/37.8/5.4)	(72.4/25.2/2.5)	
rs2273697	0/7/30	3/31/129	0.706
AA/AG/GG	(0.0/18.9/81.1)	(1.8/19.0/79.1)	
rs3740066	26/9/2	107/49/7	0.770
CC/CT/TT	(70.3/24.3/5.4)	(65.6/30.1/4.3)	
rs8187710	37/0/0	162/1/0	0.815
GG/GA/AA	(100/0.0/0.0)	(99.4/0.6/0.0)	

Data were expressed as number (%). No statistical difference between 2 groups in all genetic variations.
ABC = ATP-binding cassette; ALF = acute liver failure.

Table 7

The distribution of genetic variations of ABC transporters in drug-induced mortality based on training dataset

	Mortality (n = 24)	Survival (n = 176)	*p*
ABCB11			
rs2287622	12/8/4	91/72/13	0.293
CC/CT/TT	(50.0/33.3/16.7)	(51.7/40.9/7.4)	
ABCB1			
rs1128503	2/13/9	25/74/77	0.486
CC/TC/TT	(8.3/54.2/37.5)	(14.2/42.0/43.8)	
rs1045642	7/15/2	67/77/32	0.195
CC/TC/TT	(29.2/62.5/8.3)	(38.1/43.8/18.2)	
ABCB4			
rs2230028	22/2/0	163/13/0	0.697
AA/GA/GG	(91.7/8.3/0.0)	(92.6/7.4/0.0)	
ABCC2			
rs1885301	1/5/18	8/50/118	0.725
AA/AG/GG	(4.2/20.8/75.0)	(4.5/28.4/67.0)	
rs717620	12/10/2	127/45/4	0.048*
CC/CT/TT	(50.0/41.7/8.3)	(72.2/25.6/2.3)	
rs2273697	0/4/20	3/34/139	0.764
AA/AG/GG	(0.0/16.7/83.3)	(1.7/19.3/79.0)	
rs3740066	17/6/1	116/52/8	0.889
CC/CT/TT	(70.8/25.0/4.2)	(66.0/29.5/4.5)	
rs8187710	24/0/0	175/1/0	1.000
GG/GA/AA	(100/0.0/0.0)	(99.4/0.6/0.0)	

Data were expressed as number (%).
*$p < 0.05$.
ABC = ATP-binding cassette

Table 8

Logistic analysis of *ABCC2* rs717620 T variant and the risk of hyperbilirubinemia in drug-induced liver injury

	Odds ratio	95% confidence interval	*p*
Training dataset (n = 200)			
Univariate analysis			
ABCC2 rs717620 T variant (n = 61)	3.13	1.68-5.86	0.001*
Peak ALT (n = 200)	1.01	1.00-1.01	0.001*
Peak AST (n = 200)	1.01	1.00-1.01	0.001*
Peak ALP (n = 200)	0.99	0.99-1.00	0.012*
Albumin (n = 200)	0.18	0.11-0.31	0.001*
Age (n = 200)	1.02	0.99-1.05	0.084
Gender (male = 109)	0.62	0.35-1.08	0.093
Multivariate analysis			
ABCC2 rs717620 T variant (n = 61)	3.83	1.73-8.48	0.001*
Peak ALT (n = 200)	1.00	0.99-1.00	0.904
Peak AST (n = 200)	1.01	1.00-1.01	0.186
Peak ALP (n = 200)	0.99	0.99-1.00	0.006*
Albumin (n = 200)	0.21	0.12-0.37	0.001*
Age (n = 200)	1.03	0.99-1.06	0.090
Gender (male = 109)	0.57	0.27-1.19	0.135
Validation dataset (n=106)			
Univariate analysis			
ABCC2 rs717620 T variant (n = 26)	3.92	1.42-10.81	0.001*
Peak ALT (n = 106)	1.01	1.00-1.01	0.001*
Peak AST (n = 106)	1.01	1.00-1.01	0.008*
Peak ALP (n = 106)	0.99	0.99-1.01	0.813
Albumin (n = 106)	0.22	0.10-0.46	0.001*
Age (n = 106)	1.02	0.98-1.06	0.335
Gender (male = 57)	0.58	0.22-1.52	0.266
Multivariate analysis			
ABCC2 rs717620 T variant (n = 26)	4.91	1.36-17.73	0.015*
Peak ALT (n = 106)	1.00	0.99-1.00	0.302
Peak AST (n = 106)	1.00	1.00-1.01	0.681
Peak ALP (n = 106)	0.99	0.99-1.00	0.440
Albumin (n = 106)	0.27	0.12-0.62	0.002*
Age (n = 106)	1.05	0.99-1.11	0.083
Gender (male = 57)	0.66	0.20-2.23	0.506

*$p < 0.05$.

transporter gene may also determine an individual's susceptibility to the occurrence of severe liver injury. In this study, we showed that ABC transporter genetic variations may play a role in DILI, based on the finding that the *ABCC2 (MRP2)* rs717620 T variant was associated with an increased risk of hyperbilirubinemia and mortality in patients with DILI.

ABC transporters are a superfamily of enzymes that are responsible for the uptake and transport of many xenobiotics, lipids, bile acids, and bilirubin.[3–9] Among them, ABCB11 (BSEP) is responsible for the transport of taurocholate and other cholate conjugates from hepatocytes to the bile canaliculi. In humans, the activity of this transporter is the major determinant of bile formation and bile flow. Mutations of the encoded *ABCB11* gene have been associated with progressive familial intrahepatic cholestasis (PFIC) type 2. The major genetic polymorphism of *ABCB11* is c.1331T>C, V444A (rs2287622), which has been associated with lower BSEP expression levels.[11,14–16,18–22,24,25] In Switzerland, Lang et al[11] first demonstrated an association between the rs2287622 genetic polymorphism of *ABCB11* and drug-induced cholestasis. In addition, Meier et al,[14] also from Switzerland, reported that this variant could increase the susceptibility to contraceptive-induced cholestasis, and Ulzurrun et al[23] from Spain reported that this genetic polymorphism could increase the risk of hepatocellular DILI. However, in a study conducted in France, Roustit et al[20] did not find an association between *ABCB11* genetic variation and bosentan-induced liver toxicity. Similarly, Kagawa et al[24] did not find an association between this variant and drug-induced cholestasis in a study conducted in Japan. In addition, Chen et al[25] did not find an association between this genetic polymorphism and ATD-induced liver injury in a study conducted in China. Consistent with these studies, we did not find an association between this variant and the susceptibility to DILI including hepatocellular and mixed and cholestatic types in the present study.

ABCB1 (MDR1) is an important cell membrane protein that pumps many foreign substances out of cells.[3–9] The major genetic polymorphisms of ABCB1 include c.1236T>C (rs1128503) and c.3435T>C (rs1045642). In the present study, we did not find associations between these genetic variants and the risk of DILI, which is consistent with previous Korean and Spanish studies.[17,23]

ABCB4 (MDR3) is also an important export enzyme associated with PFIC type 3.[3–9] The major genetic polymorphism of ABCB4 is c.1954A>G (rs2230028), and Alfirevic et al[12] reported that this genetic variant was not associated with tacrine-induced liver damage in a study conducted in the UK. Ulzurrun et al[23] also reported that *ABCB4* genetic polymorphisms did not increase the risk of DILI in a study conducted in Spain. Likewise, we found no association between this variant and the susceptibility to DILI in the present study.

ABCC2 (MRP2) is expressed in the canalicular part of hepatocytes and functions in biliary transport.[3–9] Several genetic variations of this gene may affect its activity, including c.-1549A>G (rs1885301), c.-24C>T (rs717620), c.1249G>A (rs2273697), c.3972C>T (rs3740066), and c.4544G>A (rs8187710). Some of these variants have been associated with Dubin-Johnson syndrome.[3–9] In a study conducted in the UK, Daly et al[10] showed that the *ABCC2* rs717620 polymorphism was associated with the susceptibility to diclofenac-induced hepatotoxicity. Choi

Table 9

Logistic analysis of *ABCC2* rs717620 T variant and the association of drug-related mortality in drug-induced liver injury

	Odds ratio	95% confidence interval	*p*
Training dataset (n =200)			
Univariate analysis			
ABCC2 rs717620 T variant (n = 61)	2.59	1.09-6.16	0.031*
Peak ALT (n = 200)	1.01	1.00-1.01	0.003*
Peak AST (n = 200)	1.00	1.00-1.01	0.007*
Peak ALP (n = 200)	0.99	0.99-1.01	0.362
Albumin (n = 200)	0.23	0.12-0.44	0.001*
Age (n = 200)	1.00	0.97-1.04	0.956
Gender (male = 109)	0.46	0.19-1.10	0.080
Multivariate analysis			
ABCC2 rs717620 T variant (n = 61)	1.88	0.68-5.19	0.224
Peak ALT (n = 200)	1.00	0.99-1.01	0.392
Peak AST (n = 200)	1.00	0.99-1.01	0.934
Peak ALP (n = 200)	0.99	0.98-1.01	0.340
Albumin (n = 200)	0.27	0.13-0.54	0.001*
Age (n = 200)	0.99	0.96-1.03	0.722
Gender (male = 109)	0.52	0.20-1.38	0.189
Validation dataset (n=106)			
Univariate analysis			
ABCC2 rs717620 T variant (n = 26)	0.66	0.13-3.26	0.607
Peak ALT (n = 106)	1.01	1.00-1.01	0.001*
Peak AST (n = 106)	1.01	1.00-1.01	0.001*
Peak ALP (n = 106)	0.99	0.99-1.01	0.702
Albumin (n = 106)	0.17	0.07-0.41	0.001*
Age (n = 106)	1.03	0.98-1.09	0.301
Gender (male = 57)	0.45	0.12-1.65	0.230
Multivariate analysis			
ABCC2 rs717620 T variant (n = 26)	0.41	1.36-17.73	0.442
Peak ALT (n = 106)	1.00	0.99-1.01	0.183
Peak AST (n = 106)	1.00	0.99-1.01	0.717
Peak ALP (n = 106)	0.99	0.99-1.01	0.214
Albumin (n = 106)	0.14	0.04-0.49	0.002*
Age (n = 106)	1.05	0.98-1.13	0.188
Gender (male = 57)	0.50	0.08-3.17	0.460

*$p < 0.05$.

et al[13] also reported that the rs717620 polymorphism of this gene was related to the risk of DILI in a study conducted in Korea. However, Kim et al[17] and Ulzurrun et al,[23] from Korea and Spain, respectively, disproved relationships between genetic variations of this gene and DILI. In the present study, we did not find an association with any of the five major SNPs of this gene. However, although *ABCC2* rs1885301 and rs717620 did not significantly differ between the DILI and control groups in the present study, there seemed to be trends of associations ($p = 0.058$ and 0.060, respectively, Table 3). A further large-scale study is needed to elucidate the associations between *ABCC2* genotypes and susceptibility to DILI.

Since patients with DILI and hyperbilirubinemia are associated with a high mortality rate and are a serious concern for the pharmaceutical industry and healthcare providers,[2] we intended to explore the association between ABC transporter genetic polymorphisms and the risk of hyperbilirubinemia in this study. We found that the *ABCC2* rs717620 T variant increased the risk of hyperbilirubinemia in the patients with DILI, and this association was verified by our validation dataset. This finding may be because the rs717620 T variant is associated with lower ABCC2 (MRP2) activity and interferes with the disposition and excretion of drug metabolites and bilirubin. Choi et al[13] demonstrated that the *ABCC2* rs717620 T variant reduced MRP2 promoter activity by approximately 40%.

The association between the *ABCC2* rs717620 T variant and drug-related mortality in this study was tenuous and was demonstrated in chi-square and univariate analyses, but not in the multivariable analysis. This may be due to the limited number of cases of mortality in this study.

Previous studies have reported associations between age and gender with the risk of DILI.[29–31] In the present study, multivariable logistic regression was used to lessen the impact of these confounding factors, and the association between the *ABCC2* rs717620 genetic variation and hyperbilirubinemia in DILI remained after these adjustments.

Although the results of this study should be confirmed by other studies with a larger sample size and more diverse ethnic population, it still proposes a potential pharmacogenetic approach for the surveillance and prevention of severe DILIs. To mitigate serious cases of DILI, avoiding the co-administration of drugs that are substrates of ABCC2, regularly and closely monitoring liver function, and decreasing the dose of drugs may be considered.

The main limitation of this study is that most of our DILI cases had the hepatocellular type, especially in the patients with ATD-induced liver injury, which may have affected the significant association between transporter genetic variants and the cholestatic type of liver injury.

In conclusion, in the present study, we showed that people with the *ABCC2 (MRP2)* rs717620 T variant may have a higher risk of hyperbilirubinemia and mortality if they have DILI. Screening for this important ABC transporter variant may be beneficial in the prevention and mitigation of severe DILI.

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