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A Candidate Gene Study for the Association of Host Single Nucleotide Polymorphisms with Liver Cirrhosis Risk in Chinese Hepatitis B Patients

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Background and Aims: Recently, genetic association studies have linked a number of single nucleotide polymorphisms (SNPs) with liver fibrosis risk of hepatitis C. The present study was designed to validate the association of emerging SNPs with development of liver cirrhosis and chronicity in a Chinese population infected with hepatitis B virus (HBV). *Methods:* 714 Chinese subjects with persistent HBV infection (429 with evident liver cirrhosis and 285 without cirrhosis clinically or pathologically) and 280 subjects with spontaneous HBV clearance were studied. Six SNPs in five candidate genes were detected with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method. The distribution of each polymorphism was compared between the age-matched cirrhotic and noncirrhotic subjects, and between subjects with persistent infection and spontaneous HBV clearance. *Results:* The rs2679757 polymorphism of antizyme inhibitor 1 (AZIN1) gene was associated with the risk of cirrhosis (odds ratio [OR] for GG + AG versus AA = 1.47, 95% confidence interval [CI] = 1.08–2.01, $p = 0.01$). So was rs886277 in the transient receptor potential cation channel subfamily M, member 5 (TRPM5) gene (OR for CC versus CT + TT = 1.63, 95% CI = 1.20–2.22, $p = 0.002$). The frequencies of these two SNPs were also associated with the severity of decompensated cirrhosis based on the Child-Pugh classification. Genotype frequencies of other SNPs were not different between the cirrhotic and noncirrhotic groups. No SNPs were associated with the outcome of spontaneous HBV clearance. *Conclusions:* AZIN1 rs2679757 and TRPM5 rs886277 are associated with the risk of HBV-related liver cirrhosis in Chinese. The emerging SNPs warrant further clinical validation in other cohorts or ethnic groups, and could lead to mechanistic studies to reveal their contributions to fibrosis progression.

Introduction

Hepatitis B virus (HBV) infection is a public health threat worldwide, with up to two billion people harboring serological evidence of past or present infection, and 360 million with chronic infection (Shepard *et al.*, 2006). HBV infection is endemic highly in China with an HBV surface antigen sero-positivity rate as high as 5%–12% (Chen *et al.*, 2000). The natural history of HBV infection varies from spontaneous recovery postinfection, chronic asymptomatic carrier, to persistent infection with progression of fibrosis to decompensated cirrhosis and hepatocellular carcinoma, which confer a high mortality rate and heavy economic burden (Lavanchy, 2004; McMahon, 2009). Besides factors, such as alcohol abuse, gender, age, and viral genotypes contributing to this high variability (Powell *et al.*, 2000; Fattovich *et al.*, 2008), host genetic factors could be an important factor determining the outcome in hepatitis B.

A single nucleotide polymorphism (SNP), the stable replacement of a single base in a human gene, is the most common genetic mutation in humans appearing in more than 1% of the population. A SNP may change the amino acid sequence and subsequently the protein function, or alter the gene expression in the level of RNA transcription, splicing, or protein translation based on its location in the genome. Although it is uncommon that a SNP is identified as a disease-causing variant, genetic variation may impact significantly on the disease susceptibility. To date, genome-wide association studies (GWAS) and candidate gene association studies have been performed abundantly to delineate the genetic impact of

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SNPs on human liver diseases (Day, 2005; Karlsen *et al.*, 2010). Notebly, Huang *et al.* (2007) identified 7 SNPs residing in seven genes were associated with liver fibrosis in Caucasian patients infected with hepatitis C virus (HCV). The seven genes, including toll-like receptor 4 (TLR4), degenerative spermatocyte homolog 1, lipid desaturase (DEGS1), syntaxin-binding protein 5-like (STXBP5L), antizyme inhibitor 1 (AZIN1), transient receptor potential cation channel subfamily M, member 5 (TRPM5), adaptor-related protein complex 3, sigma 2 subunit (AP3S2), and aquaporin 2 (AQP2) may be functional in the pathogenesis of liver fibrosis. Cirrhosis risk scores (CRS) built on the seven SNPs were predictive for fibrosis progression in chronic hepatitis C patients in longitudinal studies (Marcolongo *et al.*, 2009; Curto *et al.*, 2011). In the studies on nonalcoholic fatty liver disease and alcoholic liver disease, rs738409 in patatin-like phospholipase domain containing 3 (PNPLA3) has been strongly associated with the disease' susceptibility, severity and clinical outcomes (Romeo *et al.*, 2008; Tian *et al.*, 2009; Valenti *et al.*, 2010). Although several polymorphisms in genes of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), chemokine receptor 5 (CCR5), and HLA-DP were reported to be associated with persistent HBV infection (Cheong *et al.*, 2006; Du *et al.*, 2006; Liu *et al.*, 2006; Suneetha *et al.*, 2006; Kamatani *et al.*, 2009), few studies have investigated the roles of host gene polymorphisms in development of HBV-related liver cirrhosis.

As hepatitis B has similar fibrogenic process as hepatitis C or other etiologies of liver diseases once the liver injuries started, they may share common genetic risk factors for fibrosis progression. Therefore, the present study was designed to evaluate the association of cirrhosis risk SNPs reported in Caucasian hepatitis C patients with the risk of cirrhosis and the likelihood of chronicity in Chinese subjects infected with HBV.

Materials and Methods

Study population

A total of 994 participants were enrolled for this case-control study in Zhong Shan Hospital, Fu Dan University between March 2009 and January 2011. They were all Chinese Han subjects from an epidemic region of HBV in the Eastern coastal region of China. The study was approved by the ethical committee of the hospital and was registered on the Chinese Clinical Trials Register (www.chictr.org) numbered ChiCTR-ONC-00000439. The protocol conformed to the provisions of the Declaration of Helsinki (as revised in Tokyo, 2004). Informed consent was obtained from all subjects.

Information, including hepatitis B history, alcohol consumption, serum HBV markers, liver function test, prothrombin time, platelets count, liver imaging (computed tomography or magnetic resonance imaging), and liver histology if available, was collected for the diagnosis and judging the severity of HBV-related liver diseases. Alcohol doses of >40 g/day in males and >20 g/day in females for at least 5 years were deemed alcoholics and excluded from the study. Serum HBV markers, including hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), and antibody to hepatitis B core antigen (anti-HBc) of IgG type were measured with chemiluminescent assays (Roche Diagnostics). The copy numbers of plasma HBV-DNA were measured with Roche Lightcycler® system. Child-Pugh scores were calculated for patients with advanced liver disease. Patients with evidence of chronic hepatitis C, autoimmune liver diseases, nonalcoholic steatohepatitis, drug-induced liver disease, Wilson's disease, and serious clinical condition of other organs were excluded. The diagnosis of these diseases was referred to current clinical practice guidelines (http://aasld.org/practice guidelines/pages/guidelinelisting.aspx). Patients with previous anti-HBV therapy history were also excluded. Patients with hepatocellular carcinoma, which is one of the major complications of hepatitis B, were enrolled in the case group if they were cirrhotic.

Clinical phenotypes were grouped as persistent infection with evident liver cirrhosis (cirrhotic group) or persistent infection without liver cirrhosis (noncirrhotic group). HBV infection was defined as HBsAg positive for more than 6 months, with anti-HBs negative and anti-HBc positive. Persistent infection denoted an infection status for at least 10 years according to disease's history. Spontaneous HBV clearance was defined as HBsAg negative, HBeAg negative, anti-HBc positive, anti-HBs positive, and HBV-DNA negative in healthy population. Cirrhosis was determined based on the clinical manifestations of decompensated cirrhosis (e.g., ascites, varices hemorrhage, hepatic encephalopathy, spontaneous bacterial peritonitis), typical radiological findings of cirrhosis and portal hypertension (e.g., hepatatrophia, cirrhotic nodules, ascites, varices, portal vein dilation, splenomegaly), cirrhotic pathology in liver biopsy (with the highest fibrosis stage of METAVIR 4), and laboratory features pertaining to portal hypertension and decompensated liver function (e.g., low platelets count, low white blood cell count, hyperbilirubinemia, hypoalbuminemia, prothrombin time prolongation). The cirrhotic group of patients was subgrouped into Child-Pugh A group, and Child-Pugh B plus C group based on the classification. The noncirrhotic group included mainly inactive HBsAg carriers with persistently normal alanine aminotransferase level, positive anti-HBe, and undetectable HBV-DNA in two blood tests within over a-year duration, and without any of the above cirrhosis signs.

Selection of polymorphisms

Six SNPs within five genes as reported by previous studies to be associated with cirrhosis risk were selected. Their analysis was performed using a single Multiplex PCR amplification system. Details of the SNP location (missense, synonymous, promoter, intronic or other changes), known function or potential biological impact of the SNPs are listed in Table 1. (Ternes *et al.*, 2002; Beutler, 2004; Mangold, 2006; Takata, 2006; Huang *et al.*, 2007; Kaske *et al.*, 2007; Kraveka *et al.*, 2007; Seki *et al.*, 2007; Guo *et al.*, 2009).

Preparation of DNA samples

From each subject, 2 mL blood was collected in an EDTA-containing tube. Genomic DNA was extracted with QIAamp DNA Blood Midi Kit (Qiagen). DNA samples were analyzed by nucleic acid spectrophotometer and gel electrophoresis to ensure the purity and for quantification.

SNPs detection

SNPs were genotyped using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-

Table 1. General Information on the Six Candidate SNPs in the Study

Rs number	Gene	SNP location	Reported clinical association	Biological function of gene	Functional linkage with fibrogenesis
rs2878771	AQP2	UTR	HCV-related liver fibrosis	Water reabsorption, vasopressin regulation	Unknown
rs2679757	AZIN1	Intron region	HCV-related liver fibrosis	Polyamine biosynthesis, cell proliferation	Unknown
rs4290029	DEGS1	Intergenic	HCV-related liver fibrosis	Lipid metabolism and transport, cell growth	Unknown
rs960312	TLR4	Intergenic	HCV-related liver fibrosis	LPS-stimulated inflammatory responses, profibrogenic signals	Unknown
rs1927911	TLR4	Intron region	HCV-related liver fibrosis	LPS-stimulated inflammatory responses, profibrogenic signals	Unknown
rs886277	TRPM5	Missense	HCV-related liver fibrosis	Taste responses	Unknown

Clinical association and biological function of genes are based on the references shown in the text.
SNP, single nucleotide polymorphism; AQP2, aquaporin 2; UTR, untranslated region; HCV, hepatitis C virus; AZIN1, antizyme inhibitor 1; DEGS1, degenerative spermatocyte homolog 1, lipid desaturase; TLR4, toll-like receptor 4; LPS, lipopolysaccharides; TRPM5, transient receptor potential cation channel subfamily M, member 5.

TOF MS) (Jurinke *et al.*, 2004; Ragoussis *et al.*, 2006), which determines the allele-specific primer extension products with Sequenom's MassARRAY system and iPLEX technology (Sequenom; www.sequenom.com). Briefly, Multiplex PCR amplifications of amplicons containing SNPs of interest were performed using Sequenom HotStart Taq Polymerase on an Applied Biosystems 9700 Real-Time PCR instrument (Applied Biosystems) with 10 ng genomic DNA of each sample. Enzymatic single-base primer extension reactions were performed to add four mass-modified nucleotides, that is, 2′, 3′-dideoxynucleoside 5′-triphosphate (ddNTP), into the polymorphic site, which allow the production of allele-specific extension products of different mass. The iPLEX reaction products were loaded onto 384-spot SpectroChips (Sequenom) and further analysed by MassARRAY Analyzer Compact System (Sequenom). After laser desorption/ionization, automated spectral acquisition analysis was performed and the spectral data was saved to the MassARRAY database.

Statistical analysis

The parametric Student *t*-test and the nonparametric Wilcoxon rank-sum test were utilized to assess differences in the mean data between groups for the characteristics and liver function profiles of the studied populations. The frequency of genotypes and alleles was determined by direct gene counting method. Pearson χ^2 test or Fisher's exact probability test was used for univariate analysis of the allele distribution in difference groups. The χ^2 test was also used in the evaluation of Hardy–Weinberg equilibrium. Binary logistic regression analysis was performed to determine the adjusted significance, where age and sex were covariables. The genotypic distributions of each SNP between different phenotypic groups were analyzed. Each statistically significant SNP in univariate analysis was further assessed with logistic regression analysis using the dominant, recessive and additive genetic models, and the odds ratio (OR) and 95% confidence interval (CI) were calculated. Furthermore, the association of the significant SNPs with the severity of cirrhosis, which was judged by Child-Pugh classification, was analyzed. Statistics was performed with SPSS version 15.0 software (SPSS). A p-value < 0.05 was considered to be statistically significant.

Results

Population characteristics

The characteristics of the 994 subjects in the study are shown in Table 2. There was no difference between the

Table 2. Characteristics of the Study Population

Parameters	Persistent infection (c)			p-Value	
	Cirrhosis (a)	Noncirrhosis (b)	Spontaneous clearance (d)	a, b	c, d
Total $n = 994$	429	285	280	—	—
Age (year)	49.3 ± 10.5	47.8 ± 13.4	48.1 ± 10.1	0.12	0.73
Male, *n* (%)	334 (77.9)	205 (71.9)	211 (75.4)	0.07	0.97
Platelet (10^9/L)	96.1 ± 59.6	195.3 ± 62.1	210.9 ± 64.9	< 0.01	< 0.01
Total bilirubin (μM)	33.1 ± 64.2	12.9 ± 11.0	11.2 ± 4.5	< 0.01	< 0.01
ALT (IU/L)	77.4 ± 169.0	30.1 ± 15.4	22.4 ± 11.9	< 0.01	< 0.01
Albumin (g/L)	35.1 ± 6.8	41.1 ± 4.2	40.4 ± 3.6	< 0.01	< 0.01
PT (second)	14.0 ± 3.4	11.5 ± 0.90	11.7 ± 0.73	< 0.01	< 0.01
Child-Pugh grades, *n*	A:235, B:145, C:49	A:285, B:0, C:0	A:280, B:0, C:0	< 0.01	< 0.01

Normal numeral ranges for platelet, total bilirubin, ALT, albumin, and PT were 101 to 320 (10^9/L), 3.4 to 20.4 (μM), <75 (IU/L), 35 to 52 (g/L), and 10 to 13 (s), respectively.
Data were expressed as mean ± standard deviation. Letters a, b, c, and d denote data of cirrhosis, noncirrhosis, persistent infection, and spontaneous clearance groups, respectively.
ALT, alanine aminotransferase; PT, prothrombin time.

Table 3. Minor Allele Frequency of Genotyped SNPs

SNP	Allele	Detected MAF in this study	Reported MAF in Caucasians
AQP2 rs2878771	G/C	C: 40.9%	C: 18.6%
AZIN1 rs2679757	A/G	G: 22.9%	G: 40.8%
DEGS1 rs4290029	G/C	C: 39.9%	C: 22.4%
TLR4 rs960312	A/G	G: 27.6%	G: 15.8%
TLR4 rs1927911	C/T	T: 41.5%	T: 26.5%
TRPM5 rs886277	C/T	T: 34.7%	C: 34.8%

MAF in Caucasians are based on the the SNP database of the national center for biotechnology information.
MAF, minor allele frequency.

cirrhotic and noncirrhotic groups in mean age ($p = 0.12$). There is a little higher ratio of male gender in the cirrhotic group when compared with the noncirrhotic group (77.9% vs. 71.9%, $p = 0.07$). There were no significant differences between persistent infection group and spontaneous clearance group in mean age and sex ratio ($p > 0.05$). The blood tests and Child-Pugh grades suggested apparent liver decompensation in the cirrhotic group ($p < 0.01$).

Association of the SNPs with liver cirrhosis and persistent infection

Minor allele frequency of each SNP is shown in Table 3. The allele frequencies of these SNPs corresponded well with those reported in the SNP database of the national center for biotechnology information. The frequencies were different between Chinese and Caucasians.

The genotypic distributions were in Hardy-Weinberg equilibrium except TLR4 rs960312 in this population (Table 4). All SNPs showed no significant association with spontaneous HBV clearance (Table 4). For cirrhosis-related analysis, rs2679757 in AZIN1 gene and rs886277 in TRPM5 gene both showed a significant association with the development of liver cirrhosis ($p = 0.003$ and 0.001, respectively) (Table 5). Regression analysis with adjustment for age and sex revealed that rs2679757 genotypes were associated with a significant risk of liver cirrhosis with the use of either a dominant (OR = 1.47, 95% CI: 1.08–2.01, $p = 0.01$) or an additive genetic model (OR = 1.43, 95% CI: 1.04–1.96, $p = 0.03$) for the G risk allele (Table 6). Similarly, rs886277 genotypes in TRPM5 gene were associated with liver cirrhosis risk in the recessive model for the risk C allele (OR = 1.63, 95% CI: 1.20–2.22, $p = 0.002$) (Table 6). In a further analysis for the association of rs2679757 and rs886277 polymorphisms with the severity of cirrhosis judged by Child-Pugh classification, GG homozygote and AG heterozygote (GG + AG) of AZIN1 rs2679757 as a risk factor was found to have a higher frequency in Child-Pugh B plus C subgroup than in Child-Pugh A subgroup ($\chi^2 = 8.54$, $p = 0.003$). The analysis of CC homozygote of TRPM5 rs886277 as another risk factor also achieved a similar result ($\chi^2 = 8.4$, $p = 0.004$).

Discussion

In the present study, six candidate SNPs in five genes were evaluated for their association with the risk of liver cirrhosis and the outcome of chronicity in a Chinese population infected with HBV. We defined the cases and controls primarily by clinical evidence. As the disease progression thereafter could not be anticipated for healthy HBV carriers at the study time, we enrolled participants with a similar mean age for the case and control groups. We found rs2679757 polymorphism in the AZIN1 gene and rs886277 in the TRPM5 gene were associated with a significant risk of HBV-related liver cirrhosis. None of these cirrhosis-related SNPs were associated with HBV clearance, which may indicate that the chronicity of HBV infection and fibrosis progression are two distinct

Table 4. Genotypic Frequency for Each SNP in Persistent Infection Group and Spontaneous Clearance Group

Gene and SNP	Genotype	Persistent infection, n (%)	Spontaneous clearance, n (%)	p-Value	HWP
AQP2	GG	243 (34.2)	97 (34.9)	0.91	0.54
rs2878771	GC	349 (49.2)	138 (49.6)		
	CC	118 (16.6)	43 (15.5)		
AZIN1	AA	411 (57.8)	170 (61.4)	0.57	0.23
rs2679757	AG	266 (37.4)	96 (34.7)		
	GG	34 (4.8)	11 (4.0)		
DEGS1	CC	107 (15.0)	57 (20.6)	0.10	0.38
rs4290029	CG	340 (47.8)	121 (43.7)		
	GG	265 (37.2)	99 (35.7)		
TLR4	AA	379 (53.2)	158 (56.8)	0.43	0.003
rs960312	AG	267 (37.5)	92 (33.1)		
	GG	66 (9.3)	28 (10.1)		
TLR4	TT	125 (17.6)	51 (18.3)	0.86	0.46
rs1927911	TC	335 (47.1)	134 (48.2)		
	CC	251 (35.3)	93 (33.5)		
TRPM5	CC	310 (43.5)	113 (40.8)	0.65	0.88
rs886277	CT	319 (44.8)	127 (45.8)		
	TT	83 (11.7)	37 (13.4)		

HWP, *p*-value for Hardy-Weinberg equilibrium test.

Table 5. Genotypic Frequency for Each SNP in the Cirrhotic and Noncirrhotic Group

Gene and SNP	Genotype	Cirrhotic group, n (%)	Noncirrhotic group, n (%)	p-Value	HWP
AQP2	GG	143 (33.6)	100 (35.2)	0.86	0.70
rs2878771	GC	210 (49.3)	139 (48.9)		
	CC	73 (17.1)	45 (15.8)		
AZIN1	AA	231 (54.1)	180 (63.4)	0.003	0.28
rs2679757	AG	172 (40.3)	94 (33.1)		
	GG	24 (5.6)	10 (3.5)		
DEGS1	CC	62 (14.5)	45 (15.8)	0.80	0.90
rs4290029	CG	208 (48.7)	132 (46.3)		
	GG	157 (36.8)	108 (37.9)		
TLR4	AA	232 (54.2)	147 (51.8)	0.79	0.06
rs960312	AG	158 (36.9)	109 (38.4)		
	GG	38 (8.9)	28 (9.9)		
TLR4	TT	70 (16.3)	55 (19.5)	0.55	0.47
rs1927911	TC	205 (47.8)	130 (46.1)		
	CC	154 (35.9)	97 (34.4)		
TRPM5	CC	206 (48.1)	104 (36.6)	0.001	0.94
rs886277	CT	176 (41.1)	143 (50.4)		
	TT	46 (10.7)	37 (13.0)		

Table 6. Age- and Sex-Adjusted Odds Ratio for AZIN1 rs2679757 and TRPM5 rs886277 Genotypes in the Cirrhotic Group Versus the Noncirrhotic Group

SNP	Comparison	OR	95% CI	χ^2	p-Value
AZIN1 rs2679757	G vs. A	1.38	1.07–1.79	6.15	0.01
	Dominant model GG+AG vs. AA	1.47	1.08–2.01	6.03	0.01
	Recessive model GG vs. AG+AA	1.67	0.78–3.55	1.65	0.19
	Additive model AG vs. AA	1.43	1.04–1.96	4.79	0.03
TRPM5 rs886277	C vs. T	1.36	1.09–1.69	7.23	0.01
	Dominant model CC+CT vs. TT	1.27	0.80–2.03	0.86	0.31
	Recessive model CC vs. CT+TT	1.63	1.20–2.22	9.20	0.002
	Additive mode CT vs. TT	0.98	0.60–1.60	0.002	0.93

OR, odds ratio; CI, confidence interval.

processes that are attributable to different factors associated with different genetic determinants.

This study provides the first evidence that the rs2679757 and rs886277 were associated with the development and severity of cirrhosis in hepatitis B patients. For rs2679757 in AZIN1 gene, the G allele is the risk factor for cirrhosis. Functionally, AZIN1 stabilizes ornithine decarboxylase (ODC) by blocking the function of antizyme, which suppresses ODC activity and accelerates its proteasomal degradation (Mangold, 2006). ODC is a key enzyme in polyamine biosynthesis induced by various growth and differentiation stimuli. The observed association suggests a possible role of AZIN1-related activity in hepatic pathology and fibrogenesis. It was reported that another AZIN1 polymorphism, rs62522600 was associated with development of cirrhosis in Caucasian hepatitis C patients (Huang *et al.*, 2007; Paris *et al.*, 2011). However, this locus was not shown with diverse genotypes in Chinese. The polymorphic nature of the AZIN1 gene may modulate individual responses to liver damage and serve as a common functional SNP in HBV- and HCV-induced hepatic fibrogenesis. The presence of this SNP in the intronic region of the AZIN1 gene may alter the gene expression and function by creating an alternative splice site and thus affects post-transcriptional regulation of the gene. TRPM5 is one member of the melastatin subfamily of transient receptor potential ion channels that are widely distributed in mammalian tissues (Prawitt *et al.*, 2003). It is proved to be a calcium-activated cation channel that functions in taste signal transduction (Kaske *et al.*, 2007). The specific physiological function of TRPM5 in liver is so far unknown. The variant rs886277 leading to a missense change in amino acid sequence may influence the biological function of the TRPM5 protein. Further research is encouraged to validate these emerging risk SNPs in other hepatitis B population or ethnic groups, and to reveal the mechanism links between these polymorphisms and liver fibrosis.

There was no association of other SNPs with cirrhosis risk. The discrimination between our study and previous reports may be due to the following reasons: (1) This is a cross-sectional study, which may not as accurate as the longitudinal study in the evaluation of the disease occurrence or progression, (2) The candidate genes and SNPs were selected based on the studies on different kinds of viral hepatitis; thus, specific pathogenetic differences among different liver diseases may account for the divergent results, (3) Ethnic differences of allelic frequencies and diseases susceptibility may exist between Caucasians and Chinese.

As the present study was mainly focused on host genetic factors, we didn't test the HBV genotypes and gene polymorphisms of the virus. The participants enrolled in this study are from a centralized area of Chinese mainland where HBV genotypes B and C are most prevalent. The impact of the HBV genotypes B and C on the long-term prognosis of chronic hepatitis B, such as cirrhosis, cirrhosis-related complications, and/or hepatocellular carcinoma is controversial (Yuen *et al.*, 2003; Yin *et al.*, 2010). Further analysis for the association between the risk SNPs and HBV genotypes in larger cohorts is warranted to delineate genotype-specific mechanisms in relation to fibrosis progression.

In this study, we developed a reliable medium-throughput genotyping platform using the Sequenom MassARRAY system for testing gene variants. This methodology allows simultaneous determination of multiple SNPs in one set with hundreds of samples (Jurinke *et al.*, 2004; Ragoussis *et al.*, 2006), and has potential to generate convenient polygenic tests useful in clinical setting.

In conclusion, two polymorphisms, rs2679757 in AZIN1 gene and rs886277 in TRPM5 gene, were verified to be associated with liver cirrhosis risk in Chinese hepatitis B patients. The emerging SNPs related to cirrhosis warrant further basic investigation and clinical validation in hepatitis B. Larger efforts, including GWAS are needed for generating CRS signatures specifically for Chinese hepatitis B patients. These genetic signatures may allow better determination of prognosis, offer opportunities for prevention, and identify new therapeutic targets for the treatment of liver cirrhosis.

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Author Disclosure Statement

No competing financial interests exist.

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