Original Article

NTCP polymorphisms were associated with fibrosis development in patients with chronic HBV infection

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Abstract
Introduction: Sodium taurocholate cotransporting polypeptide has been identified as the hepatitis B virus (HBV) entry receptor. However, information regarding the role of sodium taurocholate cotransporting polypeptide variants in the development of HBV-related advanced cirrhosis and hepatocellular carcinoma is limited.
Methodology: Overall, 581 patients with chronic HBV infection were divided into the liver fibrosis or cirrhosis group based on the Fibrosis-4 index. Further, 183 patients with hepatocellular carcinoma were distributed into early/intermediate and advanced/end stage groups based on Barcelona Clinic Liver Cancer Staging approach. Three single nucleotide polymorphisms were genotyped by high resolution melting curve method. Serum biomarkers of liver function were detected, and hepatocellular carcinoma properties were collected as well.
Results: Subjects with GA+AA genotypes at the rs4646287 polymorphism site were associated with a significantly higher rate of fibrosis development (rs4646287 GA+AA genotypes were 13.7% and 20.0% in the non-fibrosis and fibrosis group, respectively; *p* = 0.038). There were no significant differences between sodium taurocholate cotransporting polypeptide polymorphisms and hepatocellular carcinoma progression. The GA+AA genotype carriers of rs7154439 had relatively high albumin levels (*p* = 0.035). The rs2296651 GA genotype carriers tended to have solitary tumor nodule and without metastasis (*p* = 0.004 and 0.015, respectively).
Conclusions: Rs4646287 was associated with HBV-related fibrosis development. Sodium taurocholate cotransporting polypeptide polymorphisms were correlated with serum albumin level as well as hepatocellular carcinoma multifocality and metastasis. Therefore, integrating sodium taurocholate cotransporting polypeptide polymorphisms to a risk stratification algorithm may help clinicians manage the chronic HBV infection patients better.

Key words: Hepatitis B virus; hepatic fibrosis; sodium taurocholate cotransporting polypeptide; hepatocellular carcinoma; single nucleotide polymorphism.

Introduction

Hepatocellular carcinoma (HCC) and hepatic fibrosis culminating into cirrhosis are principal causes of chronic liver disease-related mortality leading to more than 2.1 million deaths annually [1,2]. Both of these disorders process dynamically; thus, screening populations and identifying individuals at a high risk of disease progression could be advantageous for determining the prognosis and translating potential therapeutic targets into clinical practice.

Chronic hepatitis B virus (HBV) infection is highly prevalent in Asia-Pacific and sub-Saharan African regions, which may develop into cirrhosis in approximately 40% of untreated patients and accounts for at least 50% of all HCC cases [3]. China has one of the highest HBV prevalence worldwide [4], and such an epidemic in certain populations is partially influenced by ethnic differences and gene polymorphisms.

Sodium taurocholate cotransporting polypeptide (NTCP) is a liver-specific bile acid transporter, which has been identified as the cellular receptor for HBV [5]. As heritable factors were long considered to have a dominant role in explaining the interindividual variation of human infection susceptibility [6], several genetic association studies have been carried out, which explored the relationship between NTCP polymorphisms and HBV-related clinical outcomes. Most of these studies focused on rs2296651, an Asian-specific non-synonymous variant with a minor allele frequency ranging from 3.1% to 9.2% among different Asian populations [7], which could diminish the virus receptor function of NTCP *in vitro* [8]. Although association studies yielded conflicting results, several suggested that rs2296651 was involved in resisting chronic HBV infection and decreasing the risk for cirrhosis and HCC in Chinese individuals [9–11]. Furthermore, the relationship between rs2296651 and

cirrhosis or HCC progression from early to advanced stage was discussed in a Vietnamese investigation, which verified the variant's protective function [12].

Contrastingly, other variants in NTCP have been less extensively studied. Based on a previous report, two single nucleotide polymorphisms (SNPs) of NTCP (rs4646287 and rs7154439) could influence the natural course of HBV infection. The rs7154439 AA genotype carriers were prone to HBV clearance, while individuals carrying rs4646287 AA genotype tended to HCC [13]. However, both Peng *et al*. and Chen *et al*. indicated that HBV infection was not correlated with rs4646287 nor rs7154439 [14,15]; Jingmin *et al*. even identified that the AA genotype of rs4646287 was inversely related to HCC occurrence [16]. Accordingly, confirming the association between rs4646287 or rs7154439 and end-stage liver disease progression warrants additional studies.

Presently, the knowledge of NTCP variants in developing advanced cirrhosis and HCC is still limited and conflicting. Therefore, this study was conducted to evaluate the association of NTCP polymorphisms with HBV-related fibrosis and cirrhosis development or HCC progression. The relationship between NTCP polymorphisms and several clinical indicators was also examined at the same time.

Methodology
Patients
A total of 581 Han Chinese HBV-persistent carriers were enrolled in the study between 2012 and 2013 from West China Hospital of Sichuan University. All the patients were confirmed positive for hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) over a 6-month interval and were divided into two groups based on the presence (n = 186) or absence (n = 395) of HCC. Patients with other hepatic viral infections or co-infections, anti-human immunodeficiency virus positivity, autoimmune disorders, and other non-HBV liver diseases were excluded.

The study protocol was approved by the Ethical Committee of West China Hospital of Sichuan University and conformed to the guidelines set forth by the Declaration of Helsinki. Written Informed consent was obtained from all patients.

Data collection and laboratory testing
Patients' demographic information and tumor characteristics were obtained from the electronic medical record, including age, sex, ethnicity, family history of liver cancer, tumor number, tumor size (defined as the maximum tumor diameter), presence of portal vein thrombus, and metastasis.

At enrollment, levels of serum HBsAg, antibody to HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), and anti-HBc were determined by electrochemiluminescence immunoassay (E170 immunoassay module, Roche Diagnostics, Basel, Switzerland). HBV DNA was quantified using quantitative real-time polymerase chain reaction (PCR) (Light Cycler480 II, Roche Diagnostics, Basel, Switzerland) with a detection limit of 1.0×10^3 copies/mL. This level was set as the cut-off point for HBV DNA positivity or negativity. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), total protein (TP), albumin, platelet count (PLT) and prothrombin time (PT) were measured at the same time.

Staging of fibrosis and HCC
Considering complications and sampling error with liver biopsy, Fibrosis-4 (FIB-4) index, a noninvasive biomarker for assessing fibrosis, was used in this study to accurately discriminate early fibrosis from advanced fibrosis or cirrhosis. FIB-4 was calculated as follows:

$$FIB\text{-}4 = \frac{age(years) \times AST(IU/L)}{PLT(10^9/L) \times \sqrt{ALT(IU/L)}}$$

Patients were classified into three categories of severity of hepatic fibrosis [17]: non-fibrosis (FIB-4 < 1.45), fibrosis (FIB-4 ≥ 1.45), and cirrhosis (FIB-4 ≥ 3.25).

Patients with HCC were diagnosed based on histological examination (requiring a combination of gross examination, microscopic examination, and immunohistochemistry, with stromal and vascular invasion being common features; the individual tumor cells are often polygonal with nuclear pleomorphism and a high nuclear to cytoplasmic ratio [18]) or high serum alpha fetoprotein (AFP) levels (> 400 ng/mL) with compatible radiological (contrast -enhanced magnetic resonance imaging, computed tomography, or abdominal ultrasound) findings, and were assigned to stage 0 to D described by the Barcelona Clinic Liver Cancer Staging system (BCLC) [19].

Genotyping assay
Whole Blood DNA Extraction Kit (Bioteke Corporation, Beijing, China) was used to isolate the genomic DNA. SNP genotyping was performed with the touchdown PCR and high resolution melting technique, as described previously [13]. The average

call rate for candidate SNPs (rs2296651, rs4646287 and rs7154439) was > 99%, and the failures were verified by sequencing.

All polymorphisms were in Hardy–Weinberg equilibrium in all the patients. Rs2296651 contained only two genotypes (GG and GA), but three genotypes were found in the other two SNPs.

Statistical analysis

All analyses were performed using SPSS, version 25 (IBM, Armonk, NY, USA) and PLINK v1.07 [20]. Two-tailed significance levels were set at 0.05. Approximately normally distributed data were reported as means ± standard deviations (SD), and skewed data were presented as medians with interquartile ranges; comparison between groups were performed using Student's t or Mann–Whitney U test, accordingly. Categorical variables were expressed as numbers with percentages and were compared using the χ^2 test or Fisher's exact test. Binary logistic regression models adjusted for age, sex, HBeAg and HBV DNA were constructed to examine the association of NTCP polymorphisms with HBV-related fibrosis, cirrhosis, or HCC progression.

Results

NTCP polymorphisms were associated with HBV-related fibrosis

Demographic and clinical characteristics of all patients are shown in Table 1. According to the FIB-4 index, patients were divided into non-fibrosis (211, 36.3%) and fibrosis (370, 63.7%) groups; the latter group consisted of non-cirrhosis (215, 58.1%) and cirrhosis patients (155, 41.9%). Majority of the patients were men, and the mean age increased with disease

progression from non-fibrosis to cirrhosis. HBeAg positivity decreased significantly in non-cirrhosis group (24.6%, 8.8%, and 16.1% for non-fibrosis, non-cirrhosis, and cirrhosis group, respectively). In comparison, HBV DNA positivity was higher among cirrhosis patients (29.0%) than those in the other two groups (20.4% for non-fibrosis group and 18.1% for non-cirrhosis group). The multiple serum biochemical indicators of liver function, including ALT, AST, GGT, ALP, TB, albumin, PLT, and PT, deteriorated as the HBV related fibrosis progressed (all $p < 0.05$); only TP appeared to be unaffected.

As shown in Table 2, rs4646287 was significantly associated with susceptibility to HBV-related fibrosis. The frequency of the minor allele A in the fibrosis patients was 10.9%, which was significantly higher than that in the non-fibrosis group (6.9%; $p = 0.013$). Similarly, the GA+AA genotypes were significantly associated with fibrosis development when compare to the GG genotype (GA+AA genotypes were 20.0% and 13.7% in the fibrosis and non-fibrosis group, respectively; $p = 0.038$). However, no significant difference was found in rs4646287 genotype or allele frequency distribution between non-cirrhosis and cirrhosis group ($p = 0.16$ and 0.24, respectively). In addition, there was no association observed between the rs7154439 or rs2296651 variants and HBV-related fibrosis or cirrhosis development (all $p > 0.05$).

Association of NTCP polymorphisms with HCC progression

Rs4646287 was found to be associated with HCC occurrence [13] and was expected to help in evaluating the variant effect on HCC progression continually. The

Table 1. Demographic and clinical characteristics of all the chronic HBV infection patients.

| Characteristics | Non-fibrosis (n = 211) | Fibrosis | | | *p* value | |
		Total (n = 370)	Non-cirrhosis (n = 215)	Cirrhosis (n = 155)	*p1*	*p2*
Age, year	38.50 ± 11.64	52.36 ± 10.44	50.28 ± 10.06	55.26 ± 10.30	< 0.001	< 0.001
Male, n (%)	141 (66.8)	268 (72.4)	155 (72.1)	113 (72.9)	0.15	0.86
HBeAg positivity, n (%)	52 (24.6)	44 (11.9)	19 (8.8)	25 (16.1)	< 0.001	0.033
HBV DNA positivity, n (%)	43 (20.4)	84 (22.7)	39 (18.1)	45 (29.0)	0.51	0.014
ALT, IU/L	25 (18, 38)	34 (21, 54)	29 (19, 46)	43 (24, 75)	< 0.001	< 0.001
AST, IU/L	22 (19, 30)	35 (26, 61)	29 (24, 42)	53 (32, 102)	< 0.001	< 0.001
GGT, IU/L	20 (13, 36)	41 (20, 91)	29 (17, 71)	58 (29, 137)	< 0.001	< 0.001
ALP, IU/L	75 (58, 93)	90 (68, 116)	81 (64, 106)	98 (77, 139)	< 0.001	< 0.001
TB, μmol/L	12.9 (9.5, 16.6)	15.3 (11.7, 20.2)	13.6 (10.7, 17.5)	18.1 (14.6, 25.3)	< 0.001	< 0.001
TP, g/L	71.5 ± 6.6	70.4 ± 6.6	70.5 ± 6.3	70.3 ± 6.9	0.05	0.83
Albumin, g/L	44.5 ± 5.0	42.0 ± 5.3	43.1 ± 4.7	40.4 ± 5.8	< 0.001	< 0.001
PLT, 10^9/L	186 (158, 221)	116 (81, 153)	135 (106, 167)	80 (60, 118)	< 0.001	< 0.001
PT, s	11.2 (10.6, 12.3)	11.9 (11.2, 12.9)	11.7 (11.0, 12.3)	12.3 (11.7, 13.3)	< 0.001	< 0.001

Data were presented as mean ± SD, number (percentage) or median (25th and 75th percentiles); *p1* for comparison between non-fibrosis and fibrosis group; *p2* for comparison between non-cirrhosis and cirrhosis group.

Table 2. Polymorphisms in NTCP associated with HBV-related fibrosis or cirrhosis development.

SNP ID	Model		Non-fibrosis (n = 211)	Fibrosis			Non-fibrosis *vs.* fibrosis		Non-cirrhosis *vs.* cirrhosis	
				Total (n = 370)	Non-cirrhosis (n = 215)	Cirrhosis (n = 155)	OR (95% CI)	*p*	OR (95% CI)	*p*
rs2296651	Genotype	GG	196 (92.9)	339 (91.6)	197 (91.6)	142 (91.6)	1.00	0.53	1.00	0.88
		GA	15 (7.1)	31 (8.4)	18 (8.4)	13 (8.4)	1.29 (0.59 – 2.80)		0.94 (0.43 – 2.06)	
rs4646287	Genotype	GG	182 (86.3)	296 (80.0)	166 (77.2)	130 (83.9)	1.00	0.038	1.00	0.16
		GA+AA	29 (13.7)	74 (20.0)	49 (22.8)	25 (16.1)	1.76 (1.02 – 3.04)		0.67 (0.38 – 1.18)	
	Allele	G	393 (93.1)	659 (89.1)	377 (87.7)	282 (91.0)	1.00	0.013	1.00	0.24
		A	29 (6.9)	81 (10.9)	53 (12.3)	28 (9.0)	1.89 (1.13 – 3.16)		0.75 (0.45 – 1.23)	
rs7154439	Genotype	GG	139 (65.9)	266 (71.9)	151 (70.2)	115 (74.2)	1.00	0.48	1.00	0.31
		GA+AA	72 (34.1)	104 (28.1)	64 (29.8)	40 (25.8)	0.86 (0.55 – 1.32)		0.77 (0.47 – 1.27)	
	Allele	G	346 (82.0)	630 (85.1)	362 (84.2)	268 (86.5)	1.00	0.55	1.00	0.29
		A	76 (18.0)	110 (14.9)	68 (15.8)	42 (13.5)	0.89 (0.59 – 1.32)		0.78 (0.49 – 1.24)	

OR: odds ratio; CI: confidence interval. Data were presented as number (percentage) for each group. OR (95% CI) and *p* value were evaluated after adjustment for age, sex, HBeAg, and HBV DNA status.

Table 3. Demographic and clinicopathological characteristics of the HCC patients.

Characteristics	BCLC stage 0-B (n = 89)	BCLC stage C-D (n = 97)	*p*
Age, year	53.96 ± 11.67	48.95 ± 10.90	0.003
Male	69 (77.5)	85 (87.6)	0.07
HBeAg positive	14 (15.7)	27 (27.8)	0.047
HBV DNA positive	30 (33.7)	44 (45.4)	0.10
AFP, ng/mL			
≤ 400	65 (73.0)	50 (51.5)	0.003
> 400	24 (27.0)	47 (48.5)	
Tumor number			
Single	61 (68.5)	54 (55.7)	0.07
Multiple	28 (31.5)	43 (44.3)	
Tumor size, cm			
≤ 3	40 (44.9)	12 (12.4)	
> 3 and ≤ 10	49 (55.1)	64 (66.0)	< 0.001
> 10	0 (0.0)	21 (21.6)	
Portal vein thrombus	0 (0.0)	41 (42.3)	< 0.001
Metastasis	0 (0.0)	60 (61.9)	< 0.001

Data were presented as mean ± SD or number (percentage).

Table 4. Association of NTCP polymorphisms with HCC staging.

SNP ID	Model		BCLC stage 0-B (n = 89)	BCLC stage C-D (n = 97)	OR (95% CI)	*p*
rs2296651	Genotype	GG	83 (93.3)	86 (88.7)	1.00	0.18
		GA	6 (6.7)	11 (11.3)	2.08 (0.70 – 6.17)	
rs4646287	Genotype	GG	70 (78.7)	80 (82.5)	1.00	0.30
		GA+AA	19 (21.4)	17 (17.5)	0.66 (0.30 – 1.45)	
	Allele	G	156 (87.6)	174 (89.7)	1.00	0.37
		A	22 (12.4)	20 (10.3)	0.74 (0.39 – 1.42)	
rs7154439	Genotype	GG	61 (68.5)	71 (73.2)	1.00	0.30
		GA+AA	28 (31.5)	26 (26.8)	0.70 (0.36 – 1.37)	
	Allele	G	148 (83.1)	167 (86.1)	1.00	0.28
		A	30 (16.9)	27 (13.9)	0.71 (0.39 – 1.31)	

OR: odds ratio; CI: confidence interval. Data were presented as number (percentage) for each group. OR (95% CI) and *p* value were evaluated after adjustment for age, sex, HBeAg, and HBV DNA status.

In the HCC group, 47.8% (n = 89) of patients were in the early or intermediate stage (BCLC stage 0, A or B) and 52.2% (n = 97) of patients were in the advanced or end stage (BCLC stage C or D). A significant difference in tumor size was found across BCLC stages. Furthermore, other features of the tumor, such as serum AFP level, present portal vein thrombus, and metastasis, were significantly different among the two HCC stage groups (Table 3).

Rs4646287 GA+AA genotypes were 21.4% (19 of 89) of the HCC stage 0-B group and 17.5% (17 of 97) of the HCC stage C-D group. This data indicated that rs4646287 was not associated with HCC progression (*p* = 0.30; odds ratio [OR] was 0.66 with 95% confidence interval [CI] from 0.30 to 1.45). No significant differences were found between rs2296651 or rs7154439 polymorphisms and the progression of HCC (Table 4).

Associations between NTCP polymorphisms and laboratory or clinical manifestations

In order to evaluate the influence of NTCP polymorphisms on hepatic function, between-group differences in several laboratory markers were examined in carriers with the distinct genotype of each polymorphic site. The same analyses were also performed for tumor-related indicators in HCC patients.

As shown in Table 5, patients with the variant GA+AA genotypes of rs7154439 had relatively high albumin levels compared to those with the wild-type GG genotype (*p* = 0.035). Furthermore, the rs2296651 GA genotype was associated with decreasing the propensity for HCC multifocality and metastasis (*p* = 0.004 and 0.015, respectively). No other associations

between polymorphism distribution and clinical (tumor size, portal vein thrombus, and cirrhosis) or laboratory (HBV DNA, AFP, ALT, AST, GGT, ALP, TB, TP, PLT, and PT) features of HBV-related diseases were found.

Discussion

Although chronic HBV infection has long been considered to significantly elevate the risk for developing liver cirrhosis and HCC [21,22], the underlying molecular mechanism remains complex and unknown. Since the HBV life cycle begins with viral attachment to hepatocytes, which is mediated by the preS1 peptides of the large HBV surface protein and NTCP [5], this study focused on the mutation of NTCP to give new clues about the influence of host genetic factor on the HBV-related cirrhosis or HCC progression.

Recently, rs4646287 AA genotype was reported to play a positive role in HCC occurrence [13]. However, in the current study, after dividing HCC patients into early/intermediate and advanced/end stage by BCLC approach, no association was found between rs4646287 and HBV-related HCC progression, nor among the other studied SNPs (rs2296651 and rs7154439). In contrast, rs4646287 genotype GA+AA and allele A were slightly more frequent in the fibrosis group. Nevertheless, their distribution was not significantly different between non-cirrhosis and cirrhosis patients.

It is widely accepted that immunologically mediated events play an important role in the pathogenesis and outcome of HBV infection [23]. In addition, observations in immune suppression patients with chronic hepatitis B and immune-deficient

Table 5. Associations between clinical characteristics and NTCP polymorphisms.

Characteristics	rs2296651			rs4646287			rs7154439		
	GG	**GA**	**p**	**GG**	**GA+AA**	**p**	**GG**	**GA+AA**	**p**
Laboratory tests									
TP, g/L	70.8 ±6.6	70.6 ±6.8	0.82	70.9 ±6.6	70.5 ±6.7	0.56	70.6 ±6.6	71.4 ±6.5	0.16
Albumin, g/L	42.9 ±5.3	42.6 ±5.5	0.70	43.0 ±5.3	42.3 ±5.4	0.24	42.6 ±5.5	43.6 ±4.9	0.035
HBV DNA positive	120 (22.4)	7 (15.2)	0.26	100 (20.9)	27 (26.2)	0.24	81 (20.0)	46 (26.1)	0.10
Tumor features									
Number									
Single	99 (58.6)	16 (94.1)	0.004	89 (59.3)	26 (72.2)	0.15	82 (62.1)	33 (61.1)	0.90
Multiple	70 (41.4)	1 (5.9)		61 (40.7)	10 (27.8)		50 (37.9)	21 (38.9)	
Size, cm									
≤ 3	46 (27.2)	6 (35.3)		42 (28.0)	10 (27.8)		37 (28.0)	15 (27.8)	
>3 / ≤ 10	103 (60.9)	10 (58.8)	0.75	91 (60.7)	22 (61.1)	1.00	81 (61.4)	32 (59.3)	0.90
>10	20 (11.8)	1 (5.9)		17 (11.3)	4 (11.1)		14 (10.6)	7 (13.0)	
Metastasis	59 (34.9)	1 (5.9)	0.015	51 (34.0)	9 (25.0)	0.30	43 (32.6)	17 (31.5)	0.88
Cirrhosis*									
Presence	78 (46.2)	9 (52.9)	0.59	73 (48.7)	14 (38.9)	0.29	64 (48.5)	23 (42.6)	0.46
Absence	91 (53.8)	8 (47.1)		77 (51.3)	22 (61.1)		68 (51.5)	31 (57.4)	

Data were presented as mean ± SD or number (percentage). *Cirrhosis was assessed using Fibrosis-4.

transgenic mouse models of HBV infection suggest that hepatocellular injury may also be induced by intracellular surface protein accumulation in the absence of adaptive immune responses [24,25]. NTCP was responsible for HBV entering cells [5], so it was supposed that rs4646287 non-GG genotype may be associated with high expression of NTCP, assisting HBV infection and re-infection, which resulted in viral particle overproduction. These infected hepatocytes would easily become degenerated and necrotic, aided by immune-mediated destruction of cells. Then, hepatic fibrosis would develop as a reversible wound-healing response [26]. In contrast, HBV patients, who do not carry rs4646287 A allele were unlikely to develop fibrosis.

As for HCC progression, after NTCP has been identified as the functional receptor of HBV, several studies found that HBV susceptibility was consistent with the expression of NTCP in different cells [27]. NTCP was significantly expressed in HBV-susceptible cells, including primary human hepatocytes and differentiated HepaRG cells, but was weakly expressed or absent in HCC cell lines HepG2 and Huh-7, which show little to no infection [8]. Recent evidence suggested that NTCP mRNA levels in HCC tumor tissues were markedly lower than those in the peritumoral tissues [16,28]. Rs4646287 minor allele carriers even have lower NTCP expression than the wild-type ones [16]. Accordingly, it was assumed that once HCC formation occurred, the NTCP expression on hepatocytes would decrease dramatically or may even be absent, which means HBV could no longer enter the cells through NTCP. In this case, NTCP polymorphisms could no longer infect HCC progress and clinical stages. Because of this, although it was found that rs4646287 was associated with HCC development [13], no association between NTCP and HCC progression from early to end stage can be further found in this genetic study. Individuals with rs2296651 GA genotype having a decreasing risk of advanced HCC have been described by Binh *et al.* [12] in the Vietnamese population. The discrepancies of the studies may have been due to race and genotyping method variation.

Cirrhosis was a precancerous condition of HCC, and liver parenchyma was substituted by scar tissue [29]. It was inferred that the NTCP expression would decrease similarly as HCC tumor cells. Accordingly, no association was found between receptor polymorphisms and cirrhosis development.

As for laboratory indicators, the report concluded that rs7154439 mutant genotypes were linked to the expression of albumin, but not significantly correlated with the serum total protein concentration. As known, patients with fibrosis have impaired hepatocellular function and reduced albumin synthesis, reaching a 60–80% reduction in advanced cirrhosis [30]. Furthermore, in the bloodstream, transport of bile acids was facilitated by their binding to serum proteins [31]; where the major bile acid carrier was albumin [32]. Based on the previous findings, NTCP was important for bile acids enterohepatic circulation and HBV infection [5]; therefore, it is hypothesized that rs7154439 might affect the bile acid transport function of NTCP, which caused increased bile acid in the peripheral circulation. During the compensatory state of the liver, albumin increased through positive feedback cycle. Meanwhile, rs7154439 may disrupt HBV entering hepatocytes, thereby reducing subsequent liver inflammation and damage. Accordingly, subjects with the mutant genotype have a better function for albumin synthesis than the wild-type.

HCC was a highly invasive tumor, where distant metastasis was frequently observed; meanwhile, intrahepatic metastasis characterized by multifocal nodules was also readily detected [33]. The present study found that rs2296651 GA genotype was linked to reducing tumor aggressiveness. Specifically, there were fewer multinodular tumors that happened in certain genotype carriers (GG *vs*. GA: 41.1% *vs*. 5.9%), and the metastasis rate was lower (GG *vs*. GA: 34.9% *vs*. 5.9%). Such clinical manifestation may support the previous conclusion that rs2296651 was associated with resistance to chronic HBV infection [9]. Rs2296651 may reduce the chance of HBV attaching and entering into hepatocytes, and decrease lesion growth and tumor formation, consequently reducing the possibility of distant metastasis.

Several aspects must be considered for the correct interpretation of the results. Firstly, the findings were preliminary results from a limited number of HBV patients, especially the sample size of the two different HCC stage groups were relatively small. Secondly, only the association of NTCP variants with cross-sectional disease status in chronic HBV infection was analyzed. Lastly, exploring the functions of these gene polymorphisms were needed in the future.

Conclusions

This is a novel study exploring rs7154439 and rs4646287 with HBV-related fibrosis or cirrhosis development. The relationship between NTCP variants and HBV-related HCC clinical progression in the Chinese population was first evaluated. It was found

that NTCP was significantly associated with fibrosis development in chronic HBV infection. NTCP polymorphisms were also correlated with serum albumin level as well as HCC multifocality and metastasis. Therefore, integrating NTCP polymorphisms to a risk stratification algorithm may help clinicians manage the chronic HBV infection patients better. Maximizing the sample size and performing function study are needed to confirm the present results in future research.

Acknowledgements

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