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NTCP S267F variant associates with decreased susceptibility to HBV and HDV infection and decelerated progression of related liver diseases

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ABSTRACT

Objectives: To determine potential associations of the rs2296651 variant (c.800C > T, S267F) of NTCP with HBV and HBV plus concomitant HDV infection as well as with the progression of related liver diseases.
Methods: The S267F variant was genotyped by DNA sequencing in 620 HBV-infected patients and 214 healthy controls (HCs). Among the patients, 450 individuals were tested for HDV by a nested PCR assay. Logistic regression was applied to examine the association.
Results: The S267F variant was found more frequently among HCs (16%) compared to HBV-infected (6%) and HBV-HDV co-infected patients (3%) (HBV patients vs HC: OR = 0.32, $P = 0.00002$ and HDV patients vs. HC: OR = 0.17, $P = 0.018$). The frequency of S267F variant was inversely correlated with CHB, LC or HCC patients compared with HCs (OR = 0.31, $P = 0.001$; OR = 0.32, $P = 0.013$; OR = 0.34, $P = 0.002$, respectively). S267F variant was also associated with decreased risk of the development of advanced liver cirrhosis (LC) and hepatocellular carcinoma (HCC) (Child B and C vs. Child A, OR = 0.26, adjusted $P = 0.016$; BCLC B,C,D vs. BCLC A, OR = 0.038, $P = 0.045$, respectively). In addition, patients with the genotype CT had lower levels of AST, ALT, total and direct bilirubin as well as higher platelet counts, indicating an association with a more favorable clinical outcome.
Conclusion: The NTCP S267F variant of the *SLC10A1* gene exhibits protective effects against HBV and HDV infection and is associated with a reduced risk of developing to advanced stages of LC and HCC.

Introduction

Although effective hepatitis B virus (HBV) vaccines are in use worldwide, HBV-related liver diseases are still a major public health concern, causing considerable morbidity and mortality. Approximately 257 million people are currently suffering from chronic hepatitis B and 887,000 deaths have been recorded in 2015 due to HBV infection (WHO, 2017a). HBV causes various clinical conditions, including acute hepatitis B, chronic hepatitis B (CHB), liver cirrhosis (LC) and hepatocellular carcinoma (HCC) (Liaw and Chu, 2009; WHO, 2017a). The risk of HCC development in chronic

Abbreviations: NTCP, sodium taurocholate co-transporting polypeptide; S267F, substitution of serine at position 267 of NTCP with phenylalanine; SNP, single nucleotide polymorphism; HC, healthy control; HBV, hepatitis B virus; HDV, hepatitis delta virus; CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; HBsAg, Hepatitis B surface antigen; HDAg, Hepatitis D antigen; BCLC, Barcelona Clinic Liver Cancer staging; AFP, alpha-fetoprotein; PLT, platelets; AST and ALT, aspartate and alanine amino transferase.

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HBV carriers is approximately 40 times higher than that in non-carriers (Lee et al., 2013).

Hepatitis D virus (HDV), an RNA virus first identified in 1977 (Rizzetto et al., 1977), can cause disease only in the presence of preexisting HBV infection, as it requires HBV envelope proteins for effective infection of hepatocytes (Sureau and Negro, 2016). HDV particles contain a circular single-stranded RNA of 1679 nucleotides and two viral proteins, the small and large hepatitis D antigens, which are surrounded by an outer coat containing HBV-derived envelope proteins and host phospholipids (Hughes et al., 2011). HDV coinfection affects 15–20 million HBV carriers worldwide (Noureddin and Gish, 2014; WHO, 2017b). HDV infection is associated with an increased risk of LC and HCC development (Hughes et al., 2011).

As both HDV and HBV utilize identical proteins, they may enter the hepatocytes through similar mechanisms. Recently, the sodium taurocholate co-transporting polypeptide (NTCP) receptor has been identified as a cellular receptor for both HBV and HDV entry (Ni et al., 2014; Yan et al., 2012). A homozygous non-synonymous Arg252His substitution in the *NTCP* was associated with the impaired uptake of bile salts into hepatocytes, confirming the important role of this hepatic bile acid transporter (Vaz et al., 2015). NTCP is encoded by the *SLC10A1* gene (Solute Carrier family 10, member 1) located on chromosome 14. It is a transmembrane protein and involved in transport of sodium and bile acids across cellular membranes. The N-terminus of the pre-S1 domain of the large HBV envelope protein binds to NTCP, which is predominantly expressed at the basolateral membrane of hepatocytes, supporting HBV and HDV entry into hepatocytes (Ni et al., 2014; Yan et al., 2012).

The missense rs2296651 variant (*c.800C > T*, S267F; substitution of serine by phenylalanine at position 267) of the *SLC10A1* gene may influence HBV infection by modifying the structure of the membrane receptor, resulting in decreased susceptibility of hepatocytes to HBV/HDV infection. Several studies have shown that the S267F variant influences susceptibility to HBV infection, but not in HDV infection and that it is associated with a decreased risk of liver disease progression (Hu et al., 2016; Lee et al., 2017; Peng et al., 2015).

Vietnam is highly endemic for both HBV and HDV infections (Mai et al., 2018; Nguyen et al., 2017; Sy et al., 2013). The functional role of the missense S267F variant in HBV infection has not yet been investigated, and data on whether S267F correlates with susceptibility or resistance to HDV infection is still limited. We conducted a genetic association study on the role of the NTCP S267P variant in HBV and HBV/HDV infection as well as its association with clinical progression of related liver diseases.

Materials and methods

Patients

We randomly recruited 620 HBV-positive patients and 214 healthy controls (HCs) at 108 Military Central Hospital, Hanoi, Vietnam, between 2013 and 2015. Patients and healthy controls represent individuals from the Hanoi metropolitan area and were of Kinh ethnicity. The patients were clinically characterized and HBsAg-positive for at least 6 months. Patients were classified into clinical subgroups, including CHB patients without LC or HCC (n = 176), HBV-related LC patients (n = 144) and HBV-related HCC patients (n = 300). The clinical and diagnostic characteristics applying to each subgroup have been described previously (Hoan et al., 2017). LC and HCC patients were further grouped according to the Child-Pugh scores A, B, and C (Cholongitas et al., 2005). HCC patients were categorized according to the Barcelona Clinic Liver Cancer (BCLC) staging (Diaz-Gonzalez et al., 2016). HCs were civilian individuals and were blood donors tested to be HBsAg seronegative. Both patients and HCs were negative for anti-HCV and anti-HIV antibodies as assessed by routine ELISA assays. None of the study participants had a history of alcohol or drug abuse. Of the 620 HBV-infected patients, 450 individuals were tested for concomitant HDV-infection. Blood sampling of all patients was performed on hospital admission. Whole blood and serum samples were stored at −80 °C until further use.

Ethics statement

Informed written consent was obtained from all study participants after detailed explanation of the study at the time of blood and serum sampling. The study protocol was approved by the Institutional Review Board of the 108 Military Central Hospital, Hanoi, Vietnam. All experiments were performed in accordance with applying guidelines and regulations.

NTCP genotyping

Genomic DNA was isolated from whole blood using a DNA purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Exon 4 of the *SLC10A1* gene was amplified using primers NTCP_4F (5′-CCA TCG CTG CGA AAC TC-3′) and NTCP_4R (5′-GGG CTA CCT GGT TCT TAG TGA-3′). PCR amplification was carried out in 20 μl reaction volumes containing 1 X PCR buffer, 1 X Q solution, 0.2 mM dNTPs, 1 mM $MgCl_2$, 0.15 mM of each primer, 1 unit of Taq Polymerase and 50 ng of genomic DNA. Thermal cycles consisted of initial denaturation (95 °C, 5 min), 36 cycles of denaturation (30 s, 95 °C), annealing (20 s, 55 °C), extension (30 s, 72 °C), followed by a final extension step (10 min, 72 °C). PCR products were visualized on 1.2% agarose gels. Amplicons were purified by Exo-SAP-IT (USB, Affymetrix, CA, USA) and 5 μl of products were used as sequencing templates (BigDye Terminator v.1.1 cycle sequencing kit, ABI 3130XL DNA sequencer; Applied Biosystems, Foster City, CA, USA). Genotypes of SNP rs2296651 were wild-type (*CC*), heterozygous (*CT*) and homozygous (*TT*).

HDV detection

Viral RNA was isolated from serum (QIAamp Viral RNA Mini Kit; Qiagen GmbH, Hilden, Germany) and RNA was subsequently reversely transcribed into cDNA using the High-Capacity cDNA Reversers Transcription Kit (Thermo Fisher Scientific, Foster City, CA, USA) following the manufacturer's instructions. HDV-specific nested PCR was employed for HDV detection as described previously (Mai et al., 2018; Nguyen et al., 2017; Sy et al., 2013).

Statistical analysis

Clinical and demographic data are given in medians with ranges for quantitative variables and categorical data, provided as numbers and percentages. Hardy-Weinberg equilibrium was assessed. Binary logistic regression models adjusted for age and gender were applied to determine NTCP S267F associations with HBV and HBV/HDV-related liver diseases. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated. Chi-square and Fisher's exact tests were used to test for differences in categorical variables. Kruskal–Wallis and Mann–Whitney–Wilcoxon tests were applied to compare quantitative variables. Statistical analyses were performed using SPSS version 22 (SPSS Statistics, IBM, Armonk, NY, USA) and GraphPad Prism 7 (http://www.graphpad.com). Significance was set at a value of $P < 0.05$.

Table 1
Demographic and clinical characteristics of healthy controls and HBV patients.

Characteristics	HC (n = 214)	HBV (n = 620)	CHB (n = 176)	LC (n = 144)	HCC (n = 300)	*P* value
Age (years)	46 [18–69]	55 [18–90]	39 [18–85]	57 [20–86]	60.5 [18–90]	<0.0001‡
Male (%)	66.8	85.8	77.3	83.3	92	<0.0001 β
Child-Pugh	NA					
Child A			NA	50/144	217/300	
Child B			NA	55/144	67/300	
Child C			NA	38/144	16/300	
Clinical parameters						
AST (U/L)	NR	63 [14–6206]	44 [14–6206]	60 [15–1221]	60 [17–983]	0.0096‡
ALT (U/L)	NR	52 [8–3390]	64 [9–3390]	56 [8–1426]	47 [10–934]	<0.0001
Total bilirubin (μmol/L)	NR	19 [4.1–571]	17 [5.5–551]	31.3 [4.1–571]	17 [4.3–392]	<0.0001‡
Direct bilirubin (μmol/L)	NR	6.5 [0.4–349]	6.2 [0.7–349]	12 [0.4–291]	5.4 [0.4–247.3]	<0.0001‡
Albumin (g/L)	NR	39 [12–51]	42 [12–51]	31 [15–47]	38 [18–49]	<0.0001‡
Prothrombin (%)	NR	85 [13–269]	92 [17–267]	58.5 [13–101]	86 [20–269]	<0.0001‡
WBC ($x10^3$/mL)	NR	6.13 [1.7–20.5]	6.2 [4.1–13.44]	5.6 [1.7–20.5]	6.15 [2.7–17.8]	0.0011‡
RBC($x10^6$/mL)	NR	4.51 [1.7–6.8]	4.9 [3.1–6.8]	4.04 [1.9–6.7]	4.5 [1.7–6.3]	<0.0001‡
PLT ($x10^3$/ml)	NR	170 [17–441]	211 [89–360]	90 [17–441]	159 [35–432]	<0.0001‡
HBV DNA (log_{10} copies/ml)	NR	5 [2–10]	6 [2–10]	5 [2–10]	5 [2–9]	0.018‡
AFP (IU/L)	NR	9.8 [0.84–300]	3.5 [1–300]	6.8 [1.18–300]	111.4 [0.84–300]	<0.0001‡

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; HC, healthy control; RBC, red blood cells; WBC, white blood cells; PLT, platelets. AST and ALT, aspartate and alanine amino transferase; AFP, alpha-fetoprotein; NR, normal range, NA, not applicable. Values given are medians and ranges. (‡) Kruskal–Wallis test. (β): chi-square test.

Results

Baseline characteristics of study subjects

The demographic, laboratory and clinical parameters of the 834 participants are summarized in Table 1. In the HC group, the mean age was 46 years (range: 18–69), and the majority of HCs were male (67%). Of the 620 patients, 532 (86%) were male. The mean age of patients was 55 years (18–90), and the median age of patients increased according to the degree of progression of liver diseases ($P < 0.0001$). The albumin and prothrombin levels and platelet counts were higher among CHB patients compared to HCC and LC patients ($P < 0.0001$). We also observed high HBV DNA levels in CHB compared to LC and HCC patients ($P = 0.018$). Higher total bilirubin and direct bilirubin were observed in LC patients compared to the subgroups of CHB and HCC patients ($P < 0.0001$). AFP levels were significantly higher among HCC patients compared to the subgroups of CHB and LC patients ($P < 0.0001$).

Association of the S267F variant with HBV infection and clinical outcome

The genotype and allele frequencies of the S267F variant (rs2296651) in the 620 HBV patients and 214 HCs are shown in Table 2. Genotype frequencies of both HCs and cases were in Hardy Weinberg equilibrium ($P = 0.6$ and 0.09, respectively). The genotype frequency of S267F (*CT/TT*) was significantly lower in HBV patients (6%) compared to HCs (16.4%) (OR = 0.32, 95% CI = 0.19-0.54, adjusted $P = 0.00002$; Table 2); indicating that genotypes *CT* and *TT* are associated with relative resistance to HBV infection. Similarly, the frequency of the *T* allele was also significantly lower among HBV patients (3%) compared to HCs (8.4%), suggesting that the *T* allele exerts a protective role in HBV infection (OR = 0.34, 95% CI = 0.2–0.57, adjusted $P = 0.00004$) (Table 2). In order to confirm the protective role of S267F in HBV infection, we compared the frequency of the *CT* genotype in HBV monoinfection with that found in the HCs. The analysis showed that this genotype contributes to a significantly decreased risk of HBV monoinfection (OR = 0.39, 95% CI = 0.22-0.69, adjusted $P = 0.0012$) (Table 4). Frequencies of genotypes and alleles did not differ between the patient subgroups of CHB, LC and HCC (data not shown).

Next, we analyzed the association of S267F with clinical outcomes of the HBV patients. Individuals carrying the genotype *CT* had significantly higher platelet counts ($P = 0.002$), but lower serum AST as well as total and direct bilirubin levels ($P = 0.012$; 0.031; 0.038, respectively) compared to patients with the genotype *CC* (Figure 1). Although not statistically significant, individuals with genotype *CT* had a similar trend of ALT, prothrombin, total protein and albumin levels compared to those carrying the genotype *CC* (data not shown).

Association of S267F with LC and HCC

We applied a logistic regression model adjusted for age and gender to analyze the association of S267F with LC and HCC. Compared to the HCs, individuals with the *CT* genotype had a 3-fold decreased risk of both LC and HCC (LC vs. HC: OR = 0.32, 95% CI = 0.13–0.79, adjusted $P = 0.013$; HCC vs. HC: OR = 0.34, 95% CI = 0.17–0.68, $P = 0.002$) (Table 2).

Patients with LC were classified into the Child-Pugh subgroups A, B and C. Genotype *CT* was significantly more frequent in patients with more advanced LC compared to patients with less advanced LC (Child-Pugh scores B and C *vs.* Child-Pugh score A: OR = 0.26, 95% CI = 0.09–0.78, $P = 0.016$). Patients with HCC were classified into the BCLC subgroups stage A, B, C and D. The frequency of the genotype *CT* was significantly higher among patients with intermediate and advanced stages of HCC compared to those with an early stage of HCC (BCLC staging B, C and D *vs.* BCLC staging A: OR = 0.38, 95% CI = 0.15-0.97, $P = 0.045$) (Table 3).

S267F in HDV infection

To explore the protective role of genotype *CT* in HDV infection, we compared the frequency of genotype *CT* in HBV-HDV co-infected patients with HCs. The analyses indicated that S267F variant contributes significantly to a decreased risk of HBV-HDV coinfection (OR = 0.17, 95% CI = 0.04–0.74, adjusted $P = 0.018$). Although not statistically significant, chronic HBV patients with genotype CT had a reduced risk of concomitant HDV infection compared to those carrying genotype *CC* (Table 4).

Table 2
Association of NTCP S267F variant with HBV-related liver diseases.

NTCP rs2296651 (S267F)	CHB n = 176 (%)	LC n = 144 (%)	HCC n = 300 (%)	HBV n = 620 (%)	HC n = 214 (%)	HBV patients vs. HCs		CHB vs. HC		LC vs. HC		HCC vs. HC	
						OR (95%CI)	*P* value	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Genotype													
CC	165 (93.8)	137 (95.1)	281 (93.7)	583 (94)	179 (83.6)	Reference		Reference		Reference		Reference	
*CT+TT**	11 (6.2%)	7 (4.9)	19 (6.3)	37 (6)	35 (16.4)	**0.32 (0.19–0.54)**	**0.00002**	**0.31 (0.15–0.64)**	**0.001**	**0.32 (0.13–0.79)**	**0.013**	**0.34 (0.17–0.68)**	**0.002**
Allele													
C	341 (96.9)	281 (97.6)	581 (96.8)	1203 (97)	392 (91.6)	Reference		Reference		Reference			
T	11 (3.1%)	7 (2.4)	19 (3.2)	37 (3)	36 (8.4)	**0.34 (0.2–0.57)**	**0.00004**	**0.32 (0.16–0.63)**	**0.001**	**0.32 (0.13–0.78)**	**0.012**	**0.33 (0.17–0.63)**	**0.001**

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; HC, healthy controls; n, numbers individuals; OR, Odd ratio. *P* values were calculated using binary logistic regression model adjusted for age and gender. (*), one healthy carried TT genotype.
Bold values reflect statistical significance.

Discussion

NTCP is a member of the solute carrier family of transporters. Its major physiological function is the transport of bile acids from portal blood into hepatocytes (Claro da Silva et al., 2013; Hagenbuch and Meier, 1994). NTCP is the hepatocytic receptor for HBV and HDV (Ni et al., 2014; Yan et al., 2012); thus, genetic variation of the gene encoding NTCP might be associated with HBV and HDV susceptibility. Previous studies have indicated the clinical significance of the NTCP variant S267F only in HBV infection (Hu et al., 2016; Lee et al., 2017; Peng et al., 2015; Wang et al., 2017). Our data confirm the protective role of S267F in HBV infection, including the stages of HBV-related liver disease progression and especially also in HBV-HDV coinfection.

Several studies have reported that the genotype and allele frequencies of S267F vary considerably between different study groups and geographical regions (Ezzikouri et al., 2017; Hu et al., 2016; Lee et al., 2017; Li et al., 2014; Pan et al., 2011; Peng et al., 2015; Yang et al., 2016; Zhang et al., 2017). Here, we report that the frequencies of the genotypes CC, CT and TT are 83.6%, 15.9% and 0,5%, respectively, among 214 Vietnamese healthy individuals. The frequency of the S267F genotypes *CT* and *TT* in our study was lower than in other Asian populations, e.g. in the Chinese Han (20.4%) and Taiwanese (18.5%) (Hu et al., 2016; Peng et al., 2015), but more common than in the Korean population (5.7%) (Lee et al., 2017). Although the occurrence of S267F was significantly lower in HBV patients than in HCs in most studies, including our present study, its frequency is regionally different, ranging from 0.9% to 18% (Ezzikouri et al., 2017; Hu et al., 2016; Lee et al., 2017; Li et al., 2014; Pan et al., 2011; Peng et al., 2015; Yang et al., 2016; Zhang et al., 2017).

A protective effect of S267F in HBV infection has been reported in previous studies, indicating that individuals with the S267F variant were 2–5-fold less susceptible to chronic HBV infection (Hu et al., 2016; Lee et al., 2017; Peng et al., 2015). Our study yields corresponding results (OR = 0.3, 95% CI = 0.19–0.54). The protective effect of S267F on HBV infection has also been demonstrated in *in vitro* experiments, showing that in mixed cells of wild-type NTCP and S267F at a 1:1 ratio the efficiency of HBV infection was higher than 70% (Yan et al., 2014), suggesting that the *T* allele of S267F contributes to a certain degree to resist HBV infection. Similarly, we observed that the *T* allele of S267F contributes to reducing the risk of CHB (OR = 0.34).

The earlier studies have also shown that S267F is independently associated with a decreased risk of progression to LC and/or HCC (Hu et al., 2016; Lee et al., 2017; Wang et al., 2017). In our study, when comparing HBV patients with advanced stages of LC and HCC with HCs, those carrying S267F had a significantly decreased risk of developing LC or HCC, indicating a lower probability of unfavorable clinical outcome compared to individuals carrying the wild-type genotype. The substitution of serine, a hydrophilic residue, by phenylalanine, a large hydrophobic residue, alters the structure of NTCP and causes a modification of the HBV/HDV receptor function (Yan et al., 2012), but also results in a reduced function of bile acid transport (Ho et al., 2004). Bile acids are cytotoxic compounds, and as their concentrations increase in the liver, they trigger hepatocyte apoptosis by activating the death receptor interactive signaling pathway, thereby promoting persistent inflammatory injury (Faubion et al., 1999; Ho et al., 2004; Miyoshi et al., 1999). The NTCP S267F variant decreases uptake of bile acids into hepatocytes, thus reducing accumulation of intrahepatic cytotoxic bile salts (Ho et al., 2004). Decreased uptake of bile acids into hepatocytes is associated with mild hypotonia, growth retardation, and delayed motor milestones (Vaz et al., 2015). However, the relationship between reduced bile salt uptake into hepatocytes

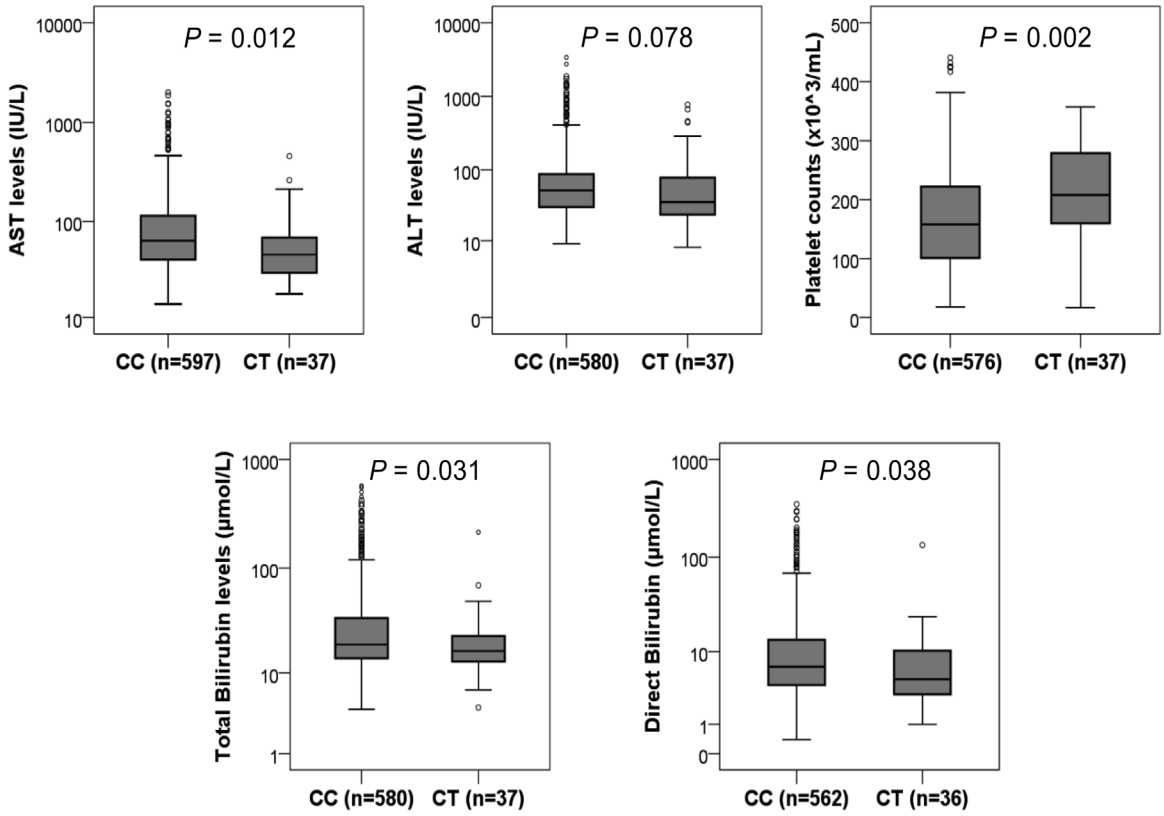

Figure 1. Association of NTCP S267F variant with clinical outcome of HBV infection.
Laboratory parameters were compared between patients with genotype *CC* and those with genotype *CT*. Box plots illustrate medians with interquartile range. *P* values were calculated by using Mann–Whitney–Wilcoxon test.

Table 3
Association of NTCP S267F variant with LC and HCC progression.

Child-Pugh score									
NTCP rs2296651 (S267F)	Child A n = 266 (%)	Child B n = 123 (%)	Child C n = 54 (%)	Child B vs. Child A		Child C vs. Child A		Child B/C vs. Child A	
				OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
CC	245 (92.1)	120 (97.6)	52 (96.3)	Reference		Reference		Reference	
CT	21 (7.9)	3 (2.4)	2 (3.7)	**0.19 (0.04–0.81)**	**0.025**	0.44 (0.1–1.9)	0.28	**0.26 (0.09–0.78)**	**0.016**
BCLC classification									
NTCP rs2296651 (S267F)	Stage A n = 94	Stage B n = 132	Stage C/D n = 55	Stage B vs. Stage A		Stages C/D vs. Stage A		Stages B/C/D vs. Stage A	
				OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
CC	83 (88.3)	127 (96.2)	52 (94.6)	Reference		Reference		Reference	
CT	11 (11.7)	5 (3.8)	3 (5.4)	**0.33 (0.11–0.99)**	**0.049**	0.54 (0.06–4.5)	0.5	**0.38 (0.15–0.97)**	**0.045**

Child A, B, C: Child-Pugh score A, B, C; Stage A, B, C, D: Barcelona Clinic Liver Cancer stage A, B, C, D; *P* values were calculated using binary logistic regression model adjusted for age and gender.
Bold values reflect statistical significance.

Table 4
Association of NTCP S267F variant with HBV and HDV infections.

NTCP rs2296651 (S267F)	HC n = 214 (%)	HBV monoinfection n = 386 (%)	HDV/HBV coinfection n = 64 (%)	HBV monoinfection vs. HC		HDV/HBV coinfection vs. HC		HDV/HBV coinfection vs. HBV monoinfection	
				OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Genotype									
CC	179 (83.6)	361 (93.1)	62 (96.8)	Reference		Reference		Reference	
*CT + TT**	35 (16.4)	25 (6.9)	2 (3.2)	**0.39 (0.22–0.69)**	**0.0012**	**0.17 (0.04–0.74)**	**0.018**	0.44 (0.1–1.9)	0.27
Allele									
C	392 (91.6)	747 (96.8)	126 (98.4)	Reference		Reference		Reference	
T	36 (8.4)	25 (3.2)	2 (1.6)	**0.38 (0.22–0.66)**	**0.0006**	**0.17 (0.04–0.73)**	**0.017**	0.45 (0.11–1.93)	0.28

HC, healthy controls; HBV mono, hepatitis B monoinfection; HDV/HBV, HDV and HBV coinfection, n = numbers of individuals; OR, Odd Ratio. *P* values were calculated using binary logistic regression model adjusted for age and gender. (*), one healthy carried TT genotype.
Bold values reflect statistical significance.

and lower risk of LC and HCC development warrants further investigation.

Regarding the association of the S267F variant with HDV infection, individuals carrying S267F had a lower risk of concomitant HDV infection. However, we did not observe any difference of S267F genotype and allele frequencies when the comparison between HBV monoinfection and HDV/HBV coinfection was considered. This may be explained by the fact that HBV and HDV share NTCP as hepatocytic receptor and the minor allele of S267F contributes to impair entry of both HBV and HDV.

A larger number of HDV-HBV coinfected patients is desirable to confirm any association of S267F with HDV infection as well as to correlate the genetic findings with the clinical outcome of HBV-HDV coinfection. In conclusion, the NTCP S267F variant was frequent in the Vietnamese population. It is associated with decreased susceptibility to HBV and HDV infection, as well as with a decreased occurrence of LC and HCC and advanced stages of HBV-related liver diseases.

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Conflict of interests

All authors have no conflicts of interest to declare.

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Author's contributions

TPV designed, supervised the study, contributed materials and reagents, and wrote the manuscript. PGK contributed materials and reagents. MTB recruited patients and collected samples, performed the experiments, carried out the statistical analyses, interpreted data and wrote the manuscript. NXH recruited patients and collected samples, carried out the statistical analyses and edited the manuscript. HVT contributed to the analysis, interpreted data and edited the manuscript. BTS and NTT contributed to the experimental design. LHS recruited patients. MHB recruited patients. CGM revised the draft and edited the final version of the manuscript. All authors agreed with the results and conclusions.

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