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Host genetic background affects the course of infection and treatment response in patients with chronic hepatitis B

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ABSTRACT

Background: Hepatitis B virus (HBV) utilizes proteins encoded by the host to infect hepatocytes and replicate. Recently, several novel host factors have been identified and described as important to the HBV lifecycle. The influence of host genetic background on chronic hepatitis B (CHB) pathogenesis is still poorly understood.
Objectives: Here, we aimed to investigate the association of *NTCP, FXRα, HNF1α, HNF4α*, and *TDP2* genetic polymorphisms with the natural course of CHB and antiviral treatment response.
Study design: We genotyped 18 single-nucleotide polymorphisms using MALDI-TOF mass spectrometry in 136 patients with CHB and 100 healthy individuals. We investigated associations of the selected polymorphisms with biochemical, serological and hepatic markers of disease progression and treatment response.
Results: No significant differences in genotypic or allelic distribution between CHB and control groups were observed. Within *TDP2*, rs3087943 variations were associated with treatment response, and rs1047782 modified the risk of advanced liver inflammation. Rs7154439 within *NTCP* was associated with HBeAg seroconversion after 48 weeks of nucleos(t)ide analogue treatment. *HNF1α* genotypes were associated with treatment response, liver damage and baseline HBeAg presence. *HNF4α* rs1800961 predicted PEG-IFNα treatment-induced HBsAg clearance in long-term follow up.
Conclusions: This study indicates host genetic background relevance in the course of CHB and confirms the role of recently described genes for HBV infection. The obtained results might serve as a starting point for validation studies on the clinical application of selected genetic variants to predict individual risks of CHB-induced liver failure and treatment response.

1. Background

Chronic hepatitis B virus (HBV) infection is present in more than 350 million people worldwide [1], and might progress to fibrosis, cirrhosis or hepatocellular carcinoma [2]. Evaluation of liver damage by needle biopsy or non-invasive methods, such as elastography, is crucial to estimating chronic liver disease outcomes.

Covalently closed circular (ccc) DNA, a reservoir and episomal form of the HBV genome, cannot be efficiently eradicated with current treatment options, including pegylated interferon alpha (PEG-IFNα) or nucleos(t)ide analogs (NA) [3]. Virus adhesion, entry, cccDNA formation and replication require host factor activities, highlighting their potential as prognostic and predictive markers and as anti-HBV drug targets.

Abbreviations: ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA; CHB, Chronic hepatitis B; FXRα, farnesoid X receptor alpha; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HNF, hepatocyte nuclear factor; LD, linkage disequilibrium; MAF, minor allele frequency; NA, nucleos(t)ide analogs; NTCP, sodium taurocholate cotransporting polypeptide; PEG-IFNα, pegylated interferon alpha; rcDNA, relaxed circular DNA; SNP, single-nucleotide polymorphism; SVR, sustained virological response; TDP2, tyrosyl-DNA phosphodiesterase 2

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Recently, sodium taurocholate cotransporting polypeptide (SLC10A1, known also as NTCP) was identified as an HBV functional receptor on human hepatocytes [4]. This protein is a Na^+-bile acid symporter responsible for bile salt uptake from the enterohepatic circulation [5]. Exogenous expression of NTCP on the surface of HepG2 or Huh7 non-susceptible human hepatoma sensitized these cells to HBV infection [4,6]. Furthermore, a synthetic NTCP inhibitor, Myrcludex-B, was evaluated in a phase 2 clinical trial as an anti-HBV drug and was effective at limiting viral entry [7].

The enhancer II/core promoter region (EnhII/Cp) of the HBV genome contains elements responsive to farnesoid X receptor alpha (FXRα), a liver-enriched nuclear receptor engaged in bile acid metabolism [8]. *FXRα* expression in cells of non-hepatic origin was sufficient to allow HBV transcription and replication upon bile acid or FXRα agonist exposure [9]. Moreover, FXRα regulates the expression of bile acid transporters and export pumps, including NTCP, which underlines the interdependence between bile acid metabolism and HBV replication, and suggests that manipulation of this pathway might be exploited as a potential therapeutic approach [10,11]. Apart from FXRα, the enhancer/core domain of the HBV genome contains binding sites for hepatocyte nuclear factors (HNF), which play important roles in regulating HBV replication. HNF1-alpha (HNF1α) and HNF4-alpha (HNF4α) stimulate viral replication in transgenic HBV mouse models and in cell culture systems [12–14]. Interestingly, CHIP-Seq analyses showed that FXRα cooperated with HNF4α to modulate gene transcription in mouse liver, which suggests that these receptors might collaborate in stimulating HBV replication [15].

After HBV enters a hepatocyte, it utilizes cellular DNA repair machinery to transform its genome from a protein-bound relaxed circular (rc) DNA form to a cccDNA form. This complicated process requires the release of P protein from minus-strand DNA, removal of a short RNA oligomer from plus-strand DNA, removal of a short terminal redundancy from minus-strand DNA, completion of plus-strand DNA, and ligation of both DNA strands [16]. It has been recently reported that the host factor engaged in the first step is tyrosyl-DNA phosphodiesterase 2 (TDP2), an enzyme capable of excising a variety of covalent adducts from DNA through a 5′-phosphodiester bond hydrolysis [17]. RNAi-mediated repression of *TDP2* inhibited the rate of HBV rcDNA to cccDNA conversion in human cells, whereas subsequent ectopic *TDP2* expression restored this conversion [18].

2. Objectives

The aim of this study was to investigate the influence of host genetic background within the *NTCP, FXRα, HNF1α, HNF4α*, and *TDP2* genes on treatment response and liver fibrosis progression in patients with chronic hepatitis B (CHB).

3. Study design

3.1. Patients

The study group included 136 patients with CHB who were admitted to the Department of Infectious Diseases, Medical University of Gdansk, and the Hepatology Outpatients Clinic Pomeranian Centre for Infectious Diseases and Tuberculosis in Gdansk in 2014-2016. Chronicity was defined as persistence of hepatitis B surface antigen (HBsAg) and anti-HBc antibodies (IgG type) for at least 24 months prior to enrolment. Blood tests, including alanine aminotransferase (ALT) activity, HBsAg, HBeAg, anti-HBe, and HBV DNA quantification, were performed on all recruited patients. Liver biopsies were collected from 132 patients and were assessed for inflammation activity and stage of fibrosis according to Scheuer scores. All patients received treatment according to Polish National Health Service (NFZ) recommendations, and 77 (57%) were treated with PEG-IFNα, 39 (29%) with lamivudine, 17 (12%) with entecavir, and 3 (2%) with tenofovir. Treatment response was monitored by the measurement of HBV DNA and HBsAg seroclearance at week 24 after treatment discontinuation for PEG-IFNα-treated individuals and at week 72 of treatment for NA-treated patients. A 48-week follow-up analysis was conducted to further monitor HBsAg seroclearance. Sustained virological response (SVR) was defined as an undetectable HBV DNA level 24 weeks after treatment discontinuation.

The study protocol was approved by the Local Independent Bioethics Committee at the Medical University of Gdansk in compliance with the Declaration of Helsinki. All enrolled participants in the study provided written informed consent.

A local control group included 100 blood donors with confirmed seronegativity to HIV, HBV and HCV from the Gdansk Regional Centre of Blood Donations and Haemotherapy.

3.2. SNP genotyping

Genomic DNA was isolated from whole blood samples using QIAamp DNA Blood Mini Kit (QIAGEN, Germany) according to manufacturer's protocol. A total of 18 single-nucleotide polymorphism (SNPs) were selected for genotyping, 9 from *TDP2*, 3 from *NTCP*, 1 from *FXRα*, 3 from *HNF1α*, and 2 from *HNF4α*. SNP genotyping was performed using a MassARRAY MALDI-TOF MS platform with iPLEX Pro chemistry (Agena Bioscience, USA) following the standard protocol described elsewhere [19–21]. Primers were designed with Assay Design Suite v2 (Table S1). First, DNA regions containing SNPs of interest were amplified and unincorporated dNTPs were digested with shrimp alkaline phosphatase. Next, a single-nucleotide extension reaction was conducted in which allele-specific products of distinct masses were obtained. The reaction products were desalted and dispensed on a SpectroCHIP. Mass spectra were acquired with an Analyzer 4 mass spectrometer, and analyzed with Typer 4.0 software.

3.3. Statistical analysis

Statistical analysis was conducted using STATISTICA 12 (StatSoft, USA), and R version 3.4.2. MIDAS software [22] was used to assess the linkage disequilibrium (LD). Deviations from Hardy-Weinberg equilibrium were assessed with the R HardyWeinberg package. Chi-squared test and Fisher's exact test were applied to analyze the distribution of nominal variables. Quantitative variables were compared with Student's *t*-test and the Mann-Whitney *U*test when applicable. Multiple logistic regression was conducted to determine the associations between analyzed variables adjusted for possible confounders. All statistical tests were two-tailed. P values less than 0.05 were considered significant.

4. Results

4.1. Study group characteristics

The study group constituted 136 patients with CHB, whereas the control group included 100 healthy volunteers. Baseline characteristics of the enrolled individuals are shown in Table 1. Patients treated with NA were significantly older and had higher levels of HBV DNA, ALT, and liver fibrosis stage than the patients treated with PEG-IFNα. HBeAg-positivity was more frequent in NA-treated than in PEG-IFNα-treated patients. Out of 136 treated patients, 12 (9%) became HBsAg-negative after treatment, 5 on NA (8%) and 7 on PEG-IFNα (9%). Older age was the only demographic or clinical variables that was significantly associated with overall and PEG-IFNα-induced HBsAg loss (Table S2). Of the 132 patients with available liver biopsy results, 20 (15%) had advanced (F3/F4 stage) liver fibrosis. Advanced liver fibrosis cases were significantly older, had higher serum ALT values and higher liver inflammation grades than cases with no or mild fibrosis, whereas the levels of HBV DNA and baseline HBeAg seronegativity did not contribute significantly to advanced liver fibrosis (Table S3).

Table 1
Baseline demographic and clinical characteristics of the study and control groups.

	CHB patients			*P value*[a]	Healthy controls (n = 100)	*P value*[b]
	All (n = 136)	PEG-IFNα therapy (n = 77)	NA therapy (n = 59)			
Age, years	40 ± 1	34 ± 1	48 ± 2	< ***0.00001***	24 (18-59)	< ***0.00001***
Sex, % female	46%	45%	46%	*0.82*	22%	***0.0002***
Liver inflammation grade †	1.5 (0-3)	1.5 (0-3)	2 (0-3)	*0.18*	–	–
Liver fibrosis stage †	1 (0-4)	1 (0-4)	1.5 (0-4)	***0.004***	–	–
HBV DNA, kIU/mL	20 609 ± 4 617	6 692 ± 3 767	38 920 ± 8 959	***0.004***	–	–
HBsAg, % positive	100%	100%	100%	*1*	–	–
HBeAg, % positive	23%	8%	42%	< ***0.00001***	–	–
Anti-HBe, % positive	78%	91%	59%	***0.00007***	–	–
ALT, IU/L	77 ± 10	52 ± 7	113 ± 22	***0.0002***	–	–

Data are shown as the mean ± standard error of the mean; [a]PEG-IFNα vs NA therapy, [b]All CHB vs healthy control group, † median value (min-max); ALT – alanine aminotransferase, CHB – chronic hepatitis B, NA- nucleos(t)ide analogs, PEG-IFNα – pegylated interferon alpha. P values less than 0.05 are shown in bold.

Genotypes of 18 SNPs were obtained for all 236 subjects included in the study. Genotypic and allelic frequencies of the analyzed SNPs for the CHB and healthy control groups are shown in Table S4. The distribution of genotypes followed Hardy-Weinberg equilibrium for both the CHB and control groups ($p > 0.05$), except for *HNF4α* rs2144908 for the CHB ($p = 0.006$) and rs1800961 for the control group ($p = 0.01$). The distribution of alleles and genotypes was similar in patients and controls ($p > 0.05$). Rs707887 and rs1047782 were in strong LD ($r^2 = 0.917$). All remaining SNPs were independent ($r^2 < 0.5$).

4.2. TDP2 polymorphisms

Three SNPs within the *TDP2* gene, rs17249952, rs17249973 and rs3212230 were highly conserved in enrolled individuals.

We observed significantly higher major allele frequency in females for rs11559067 (TT vs. TC: OR = 5.29, 95% CI = 1.19–23.38, p = 0.027). The homozygosity of major alleles in rs1047782 predisposed NA-treated patients to higher liver inflammation grades (Table 2). Patients with major alleles in rs3087943 [A/G] had higher chance of HBsAg seroclearance after PEG-IFNα treatment, which is the ultimate goal in HBV infection management (Table 3). Patients with genotype rs3087943 AA were more likely to have HBeAg present prior to treatment than those with other genotypes (Table 4).

No correlation with any clinical or virological parameters was found for rs707887, rs1129644, or rs2294689.

4.3. NTCP and FXRα polymorphisms

Out of the three SNPs selected for NTCP, only rs7154439 [A/G] was variable in the study group. Carriers of the rs7154439 AA genotype had a higher chance of anti-HBe positivity after 48 weeks of NA treatment than non-carriers (GG, GA vs. AA: sex adjusted OR = 13.45, 95% CI = 1.32–107, p = 0.024). No association was found between *FXRα* genotypes and clinical or virological parameters.

Table 2
Gene polymorphisms and risk of advanced liver inflammation.

Genotype	Power*	Odds ratio	95% CI	*P value*
HNF1α †				
rs2464196 [G/A]				
GG vs. AA	0.92	44	1.71-136	*0.018*
rs7310409 [A/G]				
GG vs. AA	0.75	8.62	1.01-73.29	*0.042*
TDP2†				
rs1047782 [G/T]				
TT, GT vs. GG	0.87	4.91	1.16-20.67	*0.025*

Multivariate logistic regression analysis adjusted for sex; advanced liver inflammationwasdefinedas G3 or G4 grading. * Statistical power calculated with G*Power software† NA therapy.

Table 3
Gene polymorphisms and chance of hepatitis B surface antigen (HBsAg) seroclearance in the course of PEG-IFNα treatment.

Genotype	Power*	Odds ratio	95% CI	*P value*
HNF1α				
rs1169288 [G/T]				
GG, GT vs. TT	0.92	10	1.18-99.54	*0.031*
rs7310409 [A/G]				
AA, AG vs. GG	0.94	10	1.18-99.54	*0.031*
TDP2				
rs3087943 [A/G]				
GG, GA vs. AA	0.97	18.93	1.22-293	*0.031*

Multivariate logistic regression analysis adjusted for age; HBsAg seroclearance – HBsAg negativity at week 24 after treatment discontinuation. * Statistical power calculated with G*Power software.

Table 4
Gene polymorphisms and baseline HBeAg presence.

Genotype		Power*	Odds ratio	95% CI	*P value*
		NA therapy			
HNF1α	**rs1169288 [G/T]**				
	TT vs. GT	0.51	3.62	1.44-9.20	*0.007*
	TT vs. GG	0.83	13.12	2.07-82.88	*0.005*
	rs2464196 [G/A]				
	GG vs. AG	0.55	4.77	1.65-13.76	*0.004*
	GG vs. AA	0.92	22.79	2.74-189	*0.003*
	rs7310409 [A/G]				
	GG vs. GA	0.55	2.93	1.25-6.83	*0.014*
	GG vs. AA	0.83	8.57	1.57-46.67	*0.011*
		PEG-IFNα therapy			
TDP2	**rs3087943 [A/G]**				
	GG, GA vs. AA	0.99	0.05	0.003-0.82	*0.032*
HNF1α	**rs1169288 [G/T]**				
	TT, GT vs. GG	0.98	33	1.96-197	*0.003*

Multivariate logistic regression analysis adjusted for sex. * Statistical power calculated with G*Power software.

4.4. HNF1α and HNF4α polymorphisms

PEG-IFNα carriers of major alleles in rs1169288 [G/T] were more likely to have the baseline HBeAg present (Table 4). Additionally, patients with rs1169288 TT genotype had advanced liver fibrosis stage (GG vs. TT: sex adjusted OR = 11.15, 95% CI = 1.36–91, p = 0.022). On the other hand, rs1169288 TT genotype increased the chances of HBsAg seroclearance at week 24 after treatment (Table 3). Moreover, patients with the rs7310409 GG genotype had a higher predisposition to HBsAg seroclearance after PEG-IFNα treatment than patients with AA or GA genotypes (Table 3). Additionally, rs2464196 was associated

with HBV DNA presence at 48 weeks of treatment (GG vs. AA: sex adjusted OR = 0.25, 95% CI = 0.06-0.98, p = 0.044).

All SNPs within the *HNF1A* gene influenced baseline HBeAg presence and anti-HBe negativity in NA-treated patients (Table 4). Rs7310409 and rs2464196 within the *HNF1A* gene impacted the liver inflammation grade (Table 2) in NA-treated patients. The liver fibrosis stage was associated with rs2464196 (GG vs. AA: sex adjusted OR = 0.93, 95% CI = 0.01–0.82, p = 0.023).

HBsAg clearance in long-term follow up (up to 48 weeks after treatment discontinuation) was associated with the minor allele in rs1800961 (CC vs. CT: sex adjusted OR = 11.51, 95% CI = 1.26–104, p = 0.027) in PEG-IFNα-treated patients. No association was found between *HNF4α* rs2144908 and clinical or virological parameters.

5. Discussion

Recently, a few novel genes that seem to be crucial for HBV infection and viral lifecycle have been described: *NTCP* [4], which is responsible for HBV attachment to hepatocytes, *FXRα,* which regulates the expression of *NTCP* [10], and *TDP2*, which is involved in viral cccDNA formation [18]. In this study, we showed that common polymorphic changes in host genes recently associated with HBV infection, *TDP2* and *NTCP*, affect the course of CHB, particularly treatment response and in the case of *TDP2*, also liver injury. Additionally, we demonstrated that SNPs within *HNF1α* and *HNF4α*, which have not been associated with the course of HBV infection before, might influence disease outcome and treatment response.

5.1. NTCP polymorphisms

Human NTCP is polymorphic, with at least some of the variants affecting its activity as a bile acid transporter. One of the best described variants is S267 F (rs2296651), determines lack of susceptibility to HBV infection [23]. Further studies revealed that the NTCP S267 F variant is associated with a lower risk of cirrhosis and HCC in CHB patients [24]. Although the SNP is relatively common in East Asian populations, its prevalence in Caucasians is scarce, with no variant alleles detected in our study and in the Exome Aggregation Consortium database [25]. Rs4646287, an *NTCP* variant previously associated with HCC in Chinese CHB patients [26] and with reduced risk of baseline HBeAg presence [27], was also conserved in the studied population. The third SNP within *NTCP* analyzed in our study, rs7154439, was variable. We observed that the rs7154439 AA genotype was associated with a higher chance of serum hepatitis B e antigen (HBeAg) loss and the development of anti-HBe antibodies (HBeAg seroconversion) after 48 weeks in NA-treated patients. Similarly, *Su* and colleagues demonstrated that rs7154439 AA genotype was slightly more frequent in an HBV clearance group than in persistently infected patients in Chinese Han population [26]. These observations suggest that AA genotype can be a positive predictor of virus clearance. Interestingly, another big group analysis performed by Chen and colleagues in Han Chinese population [28] did not confirm these data and showed no effect of any of examined *NTCP* genetic variants (including rs7154439) on HBV infection and clearance in the studied group after adjusting age, gender, smoking status and alcohol consumption. So far, we do not know what is the reasone of this dicrepancy. Functional studies are therefore needed to reveal the role of this SNP on NTCP expression and function. Considering that rs7154439 is located upstream of the gene, it may act by modifying *NTCP* expression by, e.g. changing binding affinity of transcription factors. To our knowledge this hypothesis has not been validated yet. The only functional studies have been performed for rs111409076 and provided no evidence for its role in regulating NTCP protein expression levels [28]. To the best of our knowledge, this study is the first evidence of a common single-nucleotide variation within *NTCP* that influences the course of HBV infection in Caucasians.

The production of NTCP can be augmented by the liver-enriched nuclear receptor farnesoid X receptor alpha (FXRα) [11]. In our study, however, no associations were found between *FXRα* genotypes and clinical or virological parameters.

5.2. HNF1α and HNF4α polymorphisms

The three *HNF1α* SNPs investigated in this work (rs1169288 – missence mutation, rs7310409 – intron variant, and rs2464196 – missence mutation) were previously associated with phenotypic outcomes concerning lower C-reactive protein levels, diabetes mellitus, cardiovascular risk and lipid metabolism disorders. [29–32], We observed that the frequencies of major alleles of these SNPs were significantly higher in CHB patients with baseline HBeAg presence or with serum HBsAg clearance after treatment. There is no straightforward explanation of the above associations. The murine model of HBV infection showed that the absence of HNF1α protein resulted in elevated serum HBeAg levels [13]. On the other hand, the lack of baseline HBeAg in CHB patients is often caused by two linked viral mutations that introduce a novel HNF1α binding site in the core promoter [33]. In our study, major alleles of SNPs within *HNF1α* increased the risk of baseline HBeAg presence. Lower predisposition to HBsAg seroclearance rates in response to anti-HBV treatment in individuals with minor *HNF1α* alleles might be an effect of altered L-type HBsAg expression [34]. All analyzed SNPs within *HNF1α* significantly modified the risk of liver inflammation and fibrosis, confirming their potential role in the course of CHB. Our finding of a linkage between *HNF4α* rs1800961 missense polymorphism and serum HBeAg clearance after PEG-IFNα treatment may be due to the interference from HBeAg nucleocapsid polypeptides expression, which is regulated by HNF4α.

5.3. TDP2 polymorphisms

To best of our knowledge, there has been no correlation between *TDP2* polymorphism and HBV infection or replication reported so far. Both SNPs that were found significantly correlated with clinical parameters in our study (rs1047782 and rs3087943) are located within the 3′UTR region of *TDP2* gene. As 3′-UTRs often contain regulatory regions, their mutations might affect mRNA stability, localization and translation [35,36]. There is a possibility that the detected 3′UTR variants cause an increased *TDP2* expression, though it has not been verified. Higher expression of *TDP2* may result in an increased cccDNA formation [18]. Our results indicate for the first time that *TDP2* polymorphism associates with patient response to anti-HBV treatment and liver injury, supporting the idea that the rcDNA to cccDNA conversion may be considered a valid target for CHB treatment.

5.4. Study limitations

The major limitation of our study is a relatively small sample size. As one of the main aims was to analyze the impact of a host genetic background on liver outcomes of HBV infection, the availability of liver histopathological examination results was essential. And this is limited among HBV patients in the era of non-invasive elastography. Furthermore, particular HBV infection outcomes, such as spontaneous or treatment-induced HBeAg seroconversion are rare, which might negatively affect validity of statistical analysis results. Still, provided analyses demonstrated sufficient power of conducted statistical tests. Thirdly, we did not perform functional studies to verify the reveled associations. Further biological experiments (starting from the evaluation of the SNPs influence on gene expression) are therefore warranted to clarify mechanisms underlying the associations reported here.

5.5. Conclusions

To conclude, our study supports the hypothesis that host genetic background is crucial for HBV to replicate and might significantly affect

infection course. We provided evidence for the importance of recently identified host factors *NTCP* and *TDP2*, confirming their role in HBV pathogenesis. The genetic variants described in this study, after they are examined in broad-range validation studies, may serve as biomarkers of disease progression, determining an individual's risk of developing late-stage liver injury with cirrhosis and HCC. A deeper understanding of host response to HBV infection might also be useful for defining novel efficient targets for anti-HBV treatment.

Declaration of Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.09.002.

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