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Prognostic Value of NTCP p.Ser267Phe Variant in Patients with Chronic Hepatitis B

Kronik Hepatit B'li Hastalarda NTCP p.Ser267Phe Varyasyonun Prognostik Değeri

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ABSTRACT

Aim: In this study, the aim is to detect polymorphisms in the gene encoding the sodium taurocholate cotransporting polypeptide (NTCP), the functional receptor for hepatitis B virus (HBV) and hepatitis D virus.

Materials and Methods: The study included a total of 293 patients, with 150 diagnosed with chronic HBV (CHB) and 143 undergoing liver parenchyma biopsy procedures due to different clinical indications. Total DNA was isolated from liver biopsy samples. The TaqMan SNP genotyping method was used to determine the rs2296651 polymorphism in the *SLC10A1* gene, which leads to the NTCP S267F variation.

Results: In patients with CHB and the control group, the NTCP-interacting domain was highly conserved, and no variation of the SNP rs2296651 in the *SLC10A1* gene leading to the NTCP S267F variation was detected in any of the patients.

Conclusion: It was thought that in patients with CHB, the impact of the NTCP S267F variation on the progression of HBV-associated diseases and its influence on the therapeutic efficacy of anti-viral agents targeting NTCP blockade may be limited.

Keywords: Hepatitis B virus, chronic hepatitis B, single nucleotide polymorphism, sodium taurocholate co-transporting polypeptide

ÖZ

Amaç: Bu çalışmada hepatit B virüsün (HBV) ve hepatit D virüs fonksiyonel reseptörü sodyum taurokolat kotransporter polipeptitini (NTCP) kodlayan gendeki polimorfizmlerin tespiti amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmaya 150'si kronik HBV (KHB) ve 143'ü farklı klinik endikasyonlar nedeniyle karaciğer parankim biyopsisi işlemi gerçekleştirilen toplam 293 hasta dahil edildi. Karaciğer biyopsi örneklerinden total DNA izole edildi. NTCP S267F varyasyonuna sebep olan *SLC10A1* genindeki rs2296651 polimorfizminin belirlenmesinde TaqMan SNP genotiplendirme yöntemi kullanıldı.

Bulgular: KHB hastaları ve kontrol grubunda NTCP-etkileşim domaini oldukça iyi korunmakta olup, hiçbir hastada NTCP S267F varyasyona sebep olan *SLC10A1* genindeki SNP rs2296651 varyasyonuna rastlanmadı.

Sonuç: KHB'li hastalarda NTCP S267F varyasyonunun HBV ilişkili hastalıkların progresyonu ile NTCP blokajını hedef alan anti-viral terapötiklerin tedavi etkinliğini üzerindeki etkisinin sınırlı olabileceği düşünüldü.

Anahtar Kelimeler: Hepatit B virüs, kronik hepatit B, tek nükleotid polimorfizmi, sodyum taurokolat kotransporter polipeptiti

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INTRODUCTION

Although the development of cirrhosis and liver cancer, which may be related to hepatitis B virus (HBV), can be prevented with current treatments, it remains an important public health problem because there is no treatment that will ensure complete viral clearance and it may cause approximately one million people to lose their lives worldwide every year[1]. Chronic HBV (CHB) infections are a dynamic process shaped by the interaction of viral factors belonging to the virus, host factors belonging to the infected individual and environmental factors. Therefore, viral factors such as genotype and mutations, genetic and immunologic factors of the infected individual and environmental factors are critical determinants of the natural course of chronic viral hepatitis, the development and prognosis of end-stage liver diseases such as cirrhosis and hepatocellular carcinoma (HCC), and treatment responses[2,3].

Sodium taurocholate cotransporter polypeptide (NTCP) is a functional receptor for human HBV and its satellite virus hepatitis D virus (HDV)[4]. NTCP is a cell surface glycoprotein localized on chromosome 14, encoded by the *SLC10A1* gene, the number one member of the solute carrier family 10 (sodium/bile acid cotransporter family, SLC10) and involved in the enterohepatic circulation of bile salts expressed by hepatocytes[5]. In this respect, the entry step involving the interaction of the viral surface protein (surface) PreS1 with the PreS1-specific receptor NTCP on the hepatocyte surface and subsequent transfer into the cell via endocytosis is of critical importance for HBV infections. Therefore, NTCP constitutes one of the new therapeutic targets for preventing the binding and entry of the virus into hepatocytes for anti-HBV treatment[6-8].

On the other hand, it has been reported that the p.Ser267Phe (S267F) polymorphism (single nucleotide polymorphism; SNP) detected on the *NTCP* gene, especially p.Ser267Phe (S267F) polymorphism, may cause changes in the physiological function of NTCP, leading to a decrease in HBV entry into the cell and infection load. Therefore, it is hypothesized that these genetic variants of NTCP may be associated with resistance to HBV infection and the risk of developing HBV infection-related liver cirrhosis and HCC. It is also suggested that sequence differences in the NTCP interaction domain (HBV PreS1) may negatively affect treatment responses to HBV entry inhibitors designed for anti-HBV therapy by causing changes in NTCP binding affinity[9-15].

Therefore, the aim of this study was to identify polymorphisms in the gene encoding the HBV and HDV functional receptor NTCP and to evaluate the effects of variation of the NTCP S267 polymorphism on the clinical outcomes of patients and the prognostic impact of new therapeutics targeting NTCP in predicting treatment efficacy.

MATERIALS AND METHODS

This study was supported by the Scientific and Technical Research Council of Turkey (TÜBİTAK) project number 218S769. Ethical approval of the study was granted by the Ethics Committee of İstanbul University, İstanbul Faculty of Medicine (no: 2018/1251, date: 14.09.2018).

Selection and Identification of the Cases

Patient Study Group

In this study, 150 treatment-naive patients with CHB who were diagnosed with CHB infection due to HBsAg seropositivity for more than 6 months and who were followed up by the Gastroenterohepatology Clinic of İstanbul University, İstanbul Medical Faculty, and in whom liver parenchymal biopsy was planned for histologic and clinical evaluation were included (Table 1).

Control Group

This study included 63 patients diagnosed with chronic hepatitis C infection and 80 patients who underwent liver parenchymal biopsy for different kinetic indications other than viral hepatitis agents (non-viral) by the Gastroenterohepatology Clinic of İstanbul University, İstanbul Medical Faculty (Table 1).

Clinical Material

Liver biopsy samples were obtained from the patients. Liver biopsy procedures were performed in the Gastroenterohepatology Clinic and interventional radiology units of İstanbul Medical Faculty. Total DNA samples obtained from liver biopsy specimens using a commercial kit (QIAamp DNA Mini kit, Qiagen GmbH, Hilden, Germany) were stored at -20 °C for SNP genotyping and sequence analysis to detect viral mutations.

SNP Genotyping (SLC10A1/rs2296651)

Genotype Analysis

Diagnosis and analysis of SNPs from human DNA samples isolated intrahepatically from patients' liver tissue samples were performed using a real-time PCR (RT-PCR) device and method provided by the manufacturer (Applied Biosystems, Foster City, CA, USA). TaqMan SNP genotyping method was used for genotyping the NTCP S267F variant (c.800G>A, SLC10A1/rs2296651). For SNP analyses, StepOne Software programs were used for amplification curves obtained from TaqMan SNP genotyping assay and allelic discrimination.

Demographic and clinical laboratory data: Demographic, other clinical laboratory and pathologic data were obtained from patient files and/or the electronic data recording system of the hospital.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) (16.0 software, SPSS Inc., Chicago, IL). Chi-square test and/or Fisher's tests were used to compare categorical variables between data, and Mann-Whitney U and one-way ANOVA tests were used to compare non-categorical data. Results were expressed as mean and standard deviation. P<0.05 values were accepted as statistically significant differences.

RESULTS

Characteristics of Patients

Demographic, clinical and histological data of the patients included in the study are summarized in Table 1. In summary, 150 CHB patients, 78 males and 72 females, with a mean age of 41.63±13.17 years, were included in this study. The mean necroinflammatory activity (grade) level was 4.96±2.8 and the mean fibrosis stage (stage) level was 2.04±1.30 according to the histologic activity index obtained from the histologic evaluation of liver biopsy specimens. These data indicate that the patients included in the study group generally had limited inflammatory activity and fibrosis consistent with active chronic hepatitis.

Demographic, clinical, clinical laboratory, virologic and histopathologic data of 143 control group patients, including 63 patients with chronic hepatitis C infection and 80 patients with non-viral liver disease, 58 males and 85 females, are summarized in Table 1. The histologic grade of inflammation was 6.00±2.10 and the stage of fibrosis was 2.59±1.13 in patients with chronic hepatitis C. Of the patients who underwent liver biopsy for clinical indications other than viral hepatitis, 32 patients (40%) had fatty liver disease.

SLC10A1/rs2296651 Polymorphism (S267F, NTCP Variant)

In this study, S267F (G/A) genotype and polymorphism in *SLC10A1/rs2296651* gene were investigated in total human DNA obtained from the liver tissue of 150 CHB patients and 143 patients in the control group. No S267F (G/A) polymorphism was found in any of the patients in both CHB and control groups. In the molecular analysis of all samples in the patient and control groups, the allele in the codon encoding the 267th amino acid (AA) of the *SLC10A1/rs2296651* gene was homozygous as G/G. G/A heterozygous or A/A homozygous alleles were not detected in any patient (Table 2).

Table 1. Characteristic features of patients

		Control group		
	CHB, n=150	**CHC, n=63**	**Non-viral liver disease, n=80**	**p**
Age mean±SD (years)	41.63±13.17	56.08±16.38	47.48±13.86	N.S.
Gender (M/F)	78/72	21/42	37/43	N.S.
Cynical laboratory, mean±SD				
AST (U/L)	51.68±54.08	52.08±42.13	96.68±148.84	**<0.05**
ALT (U/L	68.38±89.46	69.03±99.97	114.22±141.29	**<0.05**
ALP (U/L)	74.62±26.85	80.30±27.46	171.40±207.59	**<0.05**
GGT (U/L)	31.26±34.44	56.84±58.14	174.50±246.13	**<0.05**
Viral factors				
Log HBV DNA (IU/mL)	7.29±7.69	NA	NA	
Log HCV RNA (IU/mL)	NA	8.08±8.03	NA	
Histology, mean±SD				
Inflammation (grade)	4.96±2.84	6.00±2.10	NA	**<0.05**
Fibrosis (stage)	2.04±1.30	2.59±1.13	NA	**<0.05**
NASH	NA	NA	21	
NAFLD	NA	NA	11	
Non-specific change*	NA	NA	26	
Cholestatic liver disease**	NA	NA	12	
Cirrhosis	NA	NA	5	
Portal hypertension/venopathy	NA	NA	5	

*Non-structural changes, absence of fibrosis, minimal portal and lobular inflammatory infiltrates.

**Primary biliary cholangitis, primary sclerosing cholangitis.

AST: Aspartate aminotransferase, ALT: Alanin aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, NASH: Non-alcoholic steatohepatitis, NAFLD: Non-alcoholic fatty liver disease, NA: Not applicable, N.S.: Not significant, HBV: Hepatitis B virus, HCV: Hepatitis C virus, SD: Standard deviation, M/F: Male/female

Table 2. SLC10A1/rs2296651 polymorphism (S267F, NTCP variant) results				
	rs2296651 Allel			
Patients (n)	**G/G**	**G/A**	**A/A**	**p**
Chronic hepatitis B (150)	150	0	0	-
Control (143)	143	0	0	-

DISCUSSION

In recent years, detection of genetic variations in the *SLC10A1* gene encoding NTCP and investigation of their effects on the HBV-associated receptor function of NTCP is a current approach. The most common NTCP-associated genetic variant is reported to be the S267F polymorphism in scientific studies conducted predominantly in Asian populations to detect genetic variations in the *SLC10A1* gene. In some of the scientific studies conducted for this purpose, it has been reported that the S267F polymorphism detected on the *SLC10A1* gene may cause a change in the physiological function of NTCP, leading to a decrease in HBV entry into the cell and infection load. Therefore, it is hypothesized that NTCP S267F genetic variant may be associated with resistance to HBV infection and the risk of development of liver cirrhosis and HCC associated with HBV infection[10-13,16,17].

It has also been suggested that the NTCP S267F (c.800G>A, rs2296651) variant, which is located in the 4th exon of the *NTCP* gene and causes missense mutation, causes a decrease in the receptor function of HBV, which may result in loss of HBV binding to the receptor, intracellular entry and decreased replication capacity[9].

In a study conducted to examine the association of the NTCP S267F variant with HBV clearance, HBV-associated cirrhosis, HCC and resistance to HBV infection, the S267F (A allele) variant was detected at a higher rate in the healthy group than in HBV-infected patients. Therefore, it was reported that the S267F variant may be associated with a decreased risk of developing HBV-related cirrhosis and HCC and disease progression, but may not be associated with spontaneous HBV clearance. It has been reported that the S267F variant is generally protective against HBV infection and related diseases; however, this variant does not prevent the progression of cirrhosis towards HCC[10].

In a study involving an Asian (Chinese) population of approximately 2000 patients and 2000 healthy controls investigating the effect of NTCP variations on resistance to CHB infection and clinical outcomes, it was reported that the NTCP Ser267Phe variant was associated with resistance to CHB and a decrease in the incidence of HBV-related liver diseases[12].

Today, Bulevirtide, a synthetic lipoprotein based on the first 47 aa of HBV Pre-S1, has been synthesized and pre-clinical and clinical studies are ongoing to test its therapeutic efficacy[19-21]. Therefore, sequence differences in the NTCP interaction domain (HBV Pres1) and identification of single nucleotide polymorphisms (SNP) in NTCP may be useful in predicting the therapeutic efficacy of HBV cell entry inhibitors planned for clinical use in the near future for the treatment of HBV infections.

In a large-scale study in Taiwan, in which the association of the NTCP Ser267Phe variant with the serostatus of CHB infection and the risk of HBV-associated cirrhosis and HCC development was examined in 3801 people with CHB and 3801 HBsAg-negative people as the control group, the S267F variant was found in 18.5% of the control group, 17.2% in cirrhotic cases and 13.2% in non-cirrhotic HCC patients. It was reported that the risk of developing HCC was 25 times higher in individuals with GG genotype compared to GA and AA genotypes, and AA genotype was statistically significantly associated with HBsAg seronegativity. In conclusion, it was reported that the S267F variant was associated with resistance to CHB infection, HBV-related cirrhosis and decreased risk of development of HCC, and it was also predicted that analyzing the detection of this variant in patients together with HBV DNA levels may be useful in identifying patients with low risk of HBV-related HCC[22].

On the other hand, the data of some scientific studies investigating the effects of these genetic variants on the HBV-related receptor function of NTCP do not confirm the data in this study[23-25]. In a study examining the relationship between HBV infection and S267F (rs2296651) variation, it was reported that rs2296651 variant was not detected in either the infected or control groups and as a result, this SNP may be specific for the Asian population[26]. Therefore, the role of these variants on resistance to HBV infection and development of CHB-related cirrhosis, HCC and anti-HBV therapies is still controversial. Moreover, the prevalence of NTCP SNP rs229665 may vary according to ethnicity, geography and HBV endemicity.

In our study, the SNP rs2296651, which causes the S267F variation in the *SLC10A1* gene encoding the functional receptor NTCP of HBV, was not detected in any of the patients. Therefore, the effects of S267F variation on the clinical and histologic features of the patients could not be evaluated in this study. Therefore, the data obtained in this study suggest that the utility of SLC10A variants as a novel biomarker for identifying patients with CHB with a low risk of CHB-

associated cirrhosis and HCC is limited, and that their role in reducing the efficacy of anti-viral therapeutics targeting NTCP blockade may be quite limited. Therefore, the prognostic value of the SNP rs2296651, which causes the S267F variation in the *SLC10A1* gene, remains unclear.

Study Limitations

The limitations of this study were the small number of patients and samples included in this study and the detection of the S267F variation in the *SLC10A1* gene at the mRNA level.

CONCLUSION

In conclusion, the data obtained from this study suggest that the NTCP interaction domain is highly conserved in patients with CHB, and therefore the potential negative impact of S267F variations in the *SLC10A1* gene encoding NTCP on the progression of HBV-associated liver damage and antiviral therapeutics targeting NTCP may be quite limited.

Ethics

Ethics Committee Approval: Ethical approval of the study was granted by the Ethics Committee of İstanbul University, İstanbul Faculty of Medicine (no: 2018/1251, date: 14.09.2018).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: B.Ç., A.A., B.Ç., M.B., F.A., Concept: B.Ç., F.A., Design: B.Ç., F.A., Data Collection or Processing: B.Ç., A.A., M.P., B.Çav., M.B., M.G., F.A., Analysis or Interpretation: B.Ç., M.P., M.G., F.A., Literature Search: B.Ç., F.A., Writing: B.Ç., F.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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