Clinics and Research in Hepatology and Gastroenterology (2017) xxx, xxx–xxx

Available online at ScienceDirect www.sciencedirect.com

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ORIGINAL ARTICLE

The association of variations in TLR genes and spontaneous immune control of hepatitis B virus

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KEYWORDS
Toll-like receptors;
Chronic hepatitis B;
Seroconversion;
Polymorphism;
PCR-RFLP

Summary
Background: Toll-like receptors (TLRs) are suspected to play a critical role in liver diseases and the progression of chronic hepatitis B (CHB) infection. In this study, we investigated the possible association between TLRs and hepatitis B virus (HBV) infection chronicity in Turkish population.
Methods: *TLR4* (+896 A→G) (rs4986790), *TLR5* (+1846 T→C) (rs5744174) and *TLR9* (−1237T→C) (rs5743836) polymorphisms were screened in 131 CHB patient and 168 individuals by polymerase chain reaction (PCR) — restriction fragment length polymorphism (RFLP) technique.
Results: Of the screened polymorphisms, TT genotype of the missense variant *TLR5* (rs5744174) (NM_003268.5:c.1846T > C (p.Phe616Leu) is significantly more frequent in the control group than CHB patients ($P < 0.001$), presence of TT genotype of the upstream variant *TLR9* (rs5743836) (NM_017442.3:c.−1237T > C) is more frequent in CHB group ($P = 0.043$). However, no significant association was found for the missense variant *TLR4* (rs4986790) NM_138554.4:c.896A > G (p.Asp299Gly) polymorphism and CHB in Turkish population.
Conclusions: From all three analyzed SNPs association of *TLR5* (rs5744174) with CHB is the most significant. Since *TLR5* is associated with interferon-γ production, a high frequency of TT at rs5744174 in controls subjects suggests that it represents a protective genotype against CHB plausibly associated with an increased interferon-γ production.

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https://doi.org/10.1016/j.clinre.2017.10.002
2210-7401/

Introduction

Chronic hepatitis B (CHB) is a major global health problem and despite national vaccination programs, over 240 million people is infected with hepatitis B virus (HBV) worldwide [1]. Turkey is an intermediate endemicity country with 5% HBsAg positivity [2]. The estimated number of annual deaths due to the consequences of HBV infection is nearly 600.000 [3]. The virus itself is non-cytopathic, and liver damage during chronic infection is due to the host immune reaction against the virus. Several host immune mechanisms have been proposed to be involved in viral persistence, beyond viral factors. An inaccurate, nonselective cytolytic immune reaction directed against infected hepatocytes is believed to cause necroinflammation and further liver fibrosis, rather than eradicating the virus [4].

Increasing evidences suggest that innate immune responses, especially the Toll-like receptor (TLR) signaling pathway, are essential defense mechanisms against various pathogens, including HBV, because they activate downstream inflammatory cascades and proinflammatory cytokines [5]. TLR family involves several subgroups that are either localized in endosomes and recognizes nucleic acids such as viral DNA or RNA or expressed on cell surface to recognize extracellular bacterial and fungal cell wall components besides some viral proteins. Following binding of TLR agonists to their receptors, complex networks of intracellular signal transduction pathways are activated for mediation of the inflammatory response. TLRs undergo various conformational changes and dimerization upon binding with their ligands. These signaling networks involve adaptor proteins and several protein kinases such as extracellular signal—regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3 K), and the transcription factors Interferon regulatory factors 3/5/7 (IRF3/5/7), nuclear factor kappa B (NF-κB), and Activator protein 1 (AP-1). Antiviral response is initiated as a result of the stimulation of type I interferons (IFNs), pro-inflammatory cytokines, or co-stimulatory molecules by activation of these mentioned transcription factors [6,7]. For instance, interferon levels were shown to be affected by nucleotide variation in TLR5 exons [8]. Moreover, studies revealed that variations in cellular immunity against HBV has been associated with differential *TLR2*, *TLR4*, and *TLR9* expression during the course of HBV infection [9—11].

Several studies have also investigated the association between TLR single nucleotide polymorphisms (SNPs) and different HBV outcomes. For instance, *TLR5* rs5744174 (p.Phe616Leu) and *TLR9* rs5743836 promoter area polymorphism were shown to be associated with earlier spontaneous HBeAg seroconversion and *TLR4* rs4986790 (p.Asp299Gly) was shown to be associated with HBsAg seroclearance/seroconversion in chronic HBV patients. Association of TLR5 rs5744174 with higher IFN-γ production in chronic HBV-infected patients was also reported [12]. Another study revealed that the A/A genotype of the TLR4 rs4986790 polymorphism may result in a poorer outcome of chronic HBV infection in HBV-positive Caucasian male patients [13]. However, a recent study has stated a lack of association between the TLR4 rs4986790 and rs4986791 SNPs and susceptibility to infection with HBV and HCV. This study also reported that TLR4 rs4986790 and rs4986791 SNPs are not associated with inflammatory activity, fibrosis, and the presence of cirrhosis upon HBV and HCV infection [14]. In our study, we analyzed the association between TLR4 (+896 A→G) (rs4986790), TLR5 (+1846 T→C) (rs5744174) and TLR9 (−1237T→C) (rs5743836) polymorphisms and susceptibility to HBV infection in Turkish population.

Materials and methods

Study group

This case-control study on chronic hepatitis B in comparison to control group was performed using 299 DNA samples isolated from peripheral blood. All subjects of the study group were recruited from Umraniye Teaching and Research Hospital. The study was reviewed and approved by the local ethics committee and all participants gave written informed consent before recruitment.

The chronic hepatitis B (CHB) group comprised 131 individuals with a mean age of 47.4 ± 13.4 (84 men and 47 women). All of them had a serologically confirmed diagnosis of chronic hepatitis B. The control group was composed of age and gender matched 168 individuals with a mean age of 48.8 ± 12.9 (96 men and 72 women) who are all found to be HBsAg negative, Anti-HBs and Anti-HBcIgG positive without any hepatitis B treatment or vaccination. All the study participants defined themselves as of Turkish descent. Clinical data of CHB group and control group is summarized in Table 1.

Genotyping

DNA was extracted from peripheral blood by using PureLink® Genomic DNA kit according to manufacturer's instructions.

Table 1 Clinical data of CHB and control group.

Clinical data	Mean ± standard deviation (min—max values)
Chronic hepatitis B group (n = 131)	
Age	47.4 ± 13.4
Gender	84 men and 47 women
Log HBV DNA (IU/ml)	5.65 ± 1.97 (0—8.98)
HAI (histology activity index)	7.76 ± 3.01 (0—14)
Ishak F-score (fibrosis score)	3.26 ± 1.47 (0—6)
ALT (alanine transaminase) U/L	118.38 ± 166.37 (12—873)
AST (aspartate aminotransferase) U/L	86.75 ± 147.70 (11—900)
Control group (n = 168)	
Age	48.8 ± 12.9
Gender	96 men and 72 women
HBsAg positivity	0 (0%)
Anti-HBs positivity	168 (100%)
Anti-HBcIgG positivity	168 (100%)

Please cite this article in press as: Katrinli S, et al. The association of variations in TLR genes and spontaneous immune control of hepatitis B virus. Clin Res Hepatol Gastroenterol (2017), https://doi.org/10.1016/j.clinre.2017.10.002