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Impacts of human leukocyte antigen DQ genetic polymorphisms and their interactions with hepatitis B virus mutations on the risks of viral persistence, liver cirrhosis, and hepatocellular carcinoma

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ABSTRACT

Human leukocyte antigen (*HLA*)-*DQ* genetic polymorphisms have been associated with chronic hepatitis B virus (HBV) outcomes. We aimed to determine impacts of *HLA-DQ* polymorphisms and their interactions with HBV mutations on the risks of liver cirrhosis (LC) and hepatocellular carcinoma (HCC). rs2856718 (A > G) and rs9275319 (A > G) were genotyped in 1342 healthy controls, 327 HBV surface antigen (HBsAg) seroclearance subjects, 611 asymptomatic HBsAg carriers (ASCs), 1144 chronic hepatitis B (CHB) patients, 734 LC patients, and 1531 HCC patients using quantitative PCR. HBV mutations were detected by direct sequencing. Logistic regression analyses were utilized to assess the factors and/or multiplicative interactions significantly associated with liver diseases. rs9275319 variant genotypes were inversely associated with HBV persistence compared to HBV natural clearance subjects. rs2856718 variant genotypes significantly increased LC risk compared to ASCs plus CHB patients (GG *vs.* AA: odds ratio [OR], 1.52, 95% confidence interval [CI], 1.17–1.97 and AG + GG *vs.* AA: OR, 1.27; 95% CI, 1.04–1.54) and decreased HCC risk compared to HCC-free HBV-infected subjects (AG *vs.* AA: OR, 0.76; 95% CI, 0.65–0.89 and AG + GG *vs.* AA: OR, 0.78, 95% CI, 0.68–0.90). rs2856718 variant genotypes were significantly associated with an increased frequency of HBV A1726C mutation, a LC-risk, HCC-protective mutation, in genotype C. A rs9275319 variant genotype (GG) was significantly associated with an increased frequency of preS1 start codon mutation, an HCC-risk mutation, in genotype C. The interaction of rs2856718 AG + GG genotype with T1753V, a HCC-risk mutation, significantly reduced LC risk, with an OR of 0.26 (95% CI, 0.09–0.78); whereas the interaction of rs2856718 AG genotype with C1673T, a LC-risk mutation, significantly increased HCC risk, with an OR of 2.80 (95% CI, 1.02–7.66) in genotype C HBV-infected subjects. Conclusively, the *HLA-DQ* polymorphisms affect the risks of LC and HCC differently in chronic HBV-infected subjects, possibly *via* interacting with the HBV mutations.

1. Introduction

Chronic infection with hepatitis B virus (HBV) affects approximately 350 million people worldwide and up to 40% of the infected subjects will died of the end-stage liver diseases, namely hepatocellular carcinoma (HCC) and decompensated liver cirrhosis (LC) (Lavanchy, 2004). Annually, over 600,000 new HCC cases are diagnosed worldwide and persistent HBV infection remains the major cause of HCC worldwide (Parkin et al., 2005). HBV has been classified into at least 8 genotypes according to nucleotide sequence divergence of >8% in the entire genome. HBV genotypes have distinct geographical distributions. Genotype B and genotype C HBV are endemic in Asia (Cao, 2009). In Taiwan where HBV

Abbreviations: anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen; ASCs, asymptomatic HBsAg carriers; CHB, chronic hepatitis B; CIs, confidence intervals; EnhII/BCP/PC, enhancer II/basal core promoter/precore; GWAS, genome-wide association study; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBV-HCC, HBV-positive hepatocellular carcinoma; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HWE, Hardy–Weinberg equilibrium; LC, liver cirrhosis; ORs, odds ratios; SNPs, single nucleotide polymorphisms.

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genotype B is endemic, the lifetime incidences of HCC for women and men seropositive for hepatitis B surface antigen (HBsAg) only were 7.99% and 27.38% (Huang et al., 2011). Epidemiological studies have demonstrated that male, age, chronic active inflammation, HBV genotype C (*vs.* genotype B), high viral load, and some HBV mutations are significantly associated with increased risks of liver cirrhosis (LC) and/or HCC in chronic HBV-infected subjects (Chou et al., 2008; Lee et al., 2013; Yin et al., 2008). It remains a great challenge to identify the HBV-infected subjects who are more likely to develop the end-stage liver diseases.

HBV experiences evolutionary process since the establishment of chronic infection in teenagers (Li et al., 2014). With the progression of chronic inflammation, HBV accumulates disease-related mutations, predominantly in the enhancer II/basal core promoter/precore (EnhII/BCP/PC) and preS regions of HBV genome (Liu et al., 2009; Yin et al., 2010, 2011). Cytokines storm induced by chronic inflammation can up-regulate the expression of activation-induced cytidine deaminases and analogs that can edit HBV genome and increase HBV mutations. Immunoselection of HBV mutants in the immunocompromised microenvironment facilitates the generation of liver diseases-related HBV mutations. Some of the HBV mutations in combination promote the malignant phenotypes of hepatocytes and HCC cells (Deng et al., 2014). Thus, the generation of HBV mutations represents a process of "mutation-selection-adaptation" during hepatocarcinogenesis.

Human leukocyte antigen (*HLA*) genes, located on 6p21.31, play critical roles in immune recognition and response. The most common HLA class-I molecules could activate a cytotoxic process to eliminate HBV-infected hepatocytes by presenting HBV peptides to $CD8^+$ cytotoxic T lymphocytes. Classical HLA class-II alleles, referring as *HLA-DP*, *HLA-DQ*, and *HLA-DR*, encode MHC II molecules that bind to exogenous antigens and present them to $CD4^+$ T cells. Genetic predisposition of HLA class II antigens may contribute to immune imbalance upon HBV infection, leading to chronic inflammation in liver. Single nucleotide polymorphisms (SNPs) in *HLA-DR*, *DP* and *DQ* regions have been associated with the susceptibility to chronic HBV infection (El-Chennawi et al., 2008; Thursz et al., 1995; Kamatani et al., 2009; Mbarek et al., 2011; Zhang et al., 2013). Recently, two genome-wide association studies (GWAS) carried out in Asian populations also demonstrated that SNPs in *HLA-DQ* region were significantly associated with HBV-HCC risk (Hu et al., 2012; Jiang et al., 2013). We hypothesize that *HLA-DQ* SNPs may predispose chronic inflammation upon chronic stimulation of HBV antigens, which facilitates immune selection of the HBV mutations and promotes the development of LC and HCC.

In the current study, we aimed to clarify the impacts of two previously reported SNPs, rs2856718 and rs9275319, in human *HLA-DQ* region, on the immune selection of disease-related HBV mutations and the risks of HBV persistence, LC and HCC, and evaluate the effect of interactions between the SNPs and HBV mutations on the occurrence of LC and HCC. This study may help better understand the complex interactions of host genetic factors with viral mutations in HBV-caused liver diseases and identify the HBV-infected patients who are more likely to develop LC and HCC.

2. Materials and methods

2.1. Study population

Participants included in this study were divided into five groups: healthy controls and HBV natural clearance subjects (used as controls), asymptomatic HBsAg carriers (ASCs), chronic hepatitis B (CHB) patients, HBV-caused LC patients, and HBV-caused HCC patients. Healthy controls were recruited from September 2009 to November 2012 at Health Examination Center of Changhai Hospital (Shanghai, China). All healthy controls were free of previous or current HBV infection, and had no history of liver diseases. HBsAg seroclearance subjects were defined as seronegative for HBsAg and HBV DNA, seropositive for antibodies to HBsAg and hepatitis B core antigen, and without HBV vaccination history. HBsAg seroclearance subjects and ASCs were recruited from the Health Examination Center of Changhai Hospital and our community-based epidemiology study established in Shanghai Yangpu district in 2010. CHB, LC and HBV-HCC patients were recruited from three affiliated hospitals of this university (Shanghai, China), the 88th Hospital (Shandong, China), Southwest Hospital (Chongqing, China), Zhangjiagang First Hospital (Jiangsu, China), and You-An Hospital (Beijing, China) between September 2009 and October 2013. All participants were seronegative for antibodies against hepatitis C virus (HCV), hepatitis delta virus (HDV), and/or human immunodeficiency virus (HIV). In total, 1342 healthy controls, 327 HBsAg seroclearance subjects, 611 ASCs, 1144 CHB patients, 734 HBV-LC patients, and 1,531 HBV-HCC patients were enrolled in this study. All participants were self-reported Han Chinese and provided written informed consents. The study protocol conformed to the ethical guidelines of the 2000 Declaration of Helsinki and was approved by the ethics committee of Second Military Medical University.

2.2. Serological viral marker testing

Commercial enzyme-linked immunosorbent assay kits (Kehua, Shanghai, China; Wantai Bio-Pharm, Beijing, China) were used to test HBV serological markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc) and HDV antibody, following manufacturer's instructions. Anti-HCV, anti-HIV, and alanine aminotransferase (ALT) tests were examined in the hospitals at the enrollment.

2.3. HBV genotyping and HBV mutation analysis

HBV genomic DNA extraction and quantification, HBV genotyping, amplification of HBV EnhII/BCP/PC region and preS region, and viral mutation analyses were carried out as previously described (Yin et al., 2011; Chen et al., 2007). Circulating HBV DNA level of each participant who had one positive marker indicating HBV infection in the past was routinely measured.

2.4. Selection of HLA-DQ SNPs and genotyping

Two SNPs located at the intergenic region between *HLA-DQA2* and *HLA-DQB1* region (rs2856718 and rs9275319) were selected from GWAS conducted in Japanese and Chinese populations, respectively (Mbarek et al., 2011; Jiang et al., 2013). QIAamp blood kits (QIAGEN, Hilden, Germany) were used to extract genomic DNA from 200 μl peripheral blood samples according to manufacturer's instructions. Genotyping was carried out in a LightCycler™480 (Roche, Basel, Switzerland) using fluorescent-probe real-time quantitative PCR. The sequences of the primers and probes and PCR amplification condition are shown in Supplemental Table 1. For quality control, a blank control (ddH_2O) was included in each 384-well plate, and the samples of 600 participants were randomly selected for duplication, yielding a 100% concordance. Of these participants, 1,342 healthy controls, 327 HBsAg seroclearance subjects, 316 ASCs, 845 CHB patients, 467 HBV-LC patients, and 1,108 HBV-HCC patients have also been genotyped for SNPs in *HLA-DP* region (rs3077, rs9277535, rs3135021, and rs2281388) in our previous study (Zhang et al., 2013).