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Research paper

Protective effects of HLA-DPA1/DPB1 variants against Hepatitis B virus infection in an Indonesian population

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ABSTRACT

Human leukocyte antigen (HLA) DPA1/DPB1 variants have been reported to influence Hepatitis B virus (HBV) infection. HLA-DPA1/DPB1 plays a pivotal role in antigen presentation to $CD4^+$ helper T cells and influences the outcome of HBV infection. To investigate the influence of HLA-DP variants on the outcome of HBV infection in an Indonesian population where it has the third-highest prevalence of HBV infection worldwide, we performed a case–control study of 686 participants, including patients with HBV-related advanced or nonadvanced liver disease, patients with spontaneously resolved HBV, and healthy controls. Single-nucleotide polymorphisms in HLA-DPA1 (rs3077) and HLA-DPB1 (rs3135021, rs9277535, and rs228388) were genotyped using real-time TaqMan® genotyping assays. Because rs2281388 deviated from Hardy–Weinberg equilibrium, it was excluded from subsequent analyses. The results of logistic regression analyses showed that the HLA-DPB1 rs9277535 variants were associated with a reduced risk of persistent HBV infection (odds ratio [OR] 0.70, 95% confidence interval [95% CI] 0.52–0.96, $P = 0.026$, additive genetic model; OR 0.60, 95% CI 0.38–0.96, $P = 0.033$, dominant genetic model). The HLA-DPA1 rs3077 variant was associated with a protective effect increasing the spontaneously resolved HBV infection (OR 0.64, 95% CI 0.41–0.98, $P = 0.039$, dominant genetic model). By contrast, the HLA-DPB1 rs3135021 variant was not associated with the outcome of HBV infection, including susceptibility, spontaneously resolved, or disease progression. Combinations of haplotype markers were also associated with HBV susceptibility (CA for rs3077–rs9277535, OR 0.57, 95% CI 0.36–0.92, $P = 0.021$; GA for rs3135021–rs9277535, OR 0.56, 95% CI 0.36–0.86, $P = 0.0087$). In conclusion, these findings confirm that HLA-DPA1/DPB1 variants were associated with the outcomes of HBV infection in an Indonesian population.

1. Introduction

Hepatitis B infection is a major threat to public health on a global scale. It is estimated that two billion people worldwide are infected with Hepatitis B virus (HBV). The clinical outcomes of HBV infection range from asymptomatic infection to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Furthermore, end-stage liver disease related to HBV infection is a major reason for liver transplantation and is responsible for more than one million deaths each year (Lavanchy, 2008; Lee, 1997; WHO, 2012).

Although HBV was identified as the causative pathogen of liver disease about 50 years ago (Blumberg, 2002), the exact pathogenesis of

Abbreviations: HBV, Hepatitis B virus; SNP ID, single-nucleotide polymorphism identification number; HLA, Human Leucocyte Antigen; MAF, minor allele frequency; HC, healthy controls; SRH, spontaneously resolved HBV infection; ALD, HBV-related advanced liver disease; NALD, HBV-related nonadvanced liver disease; vs., versus; Additive, additive genetic model (minor allele (m) vs. major allele (M)); Dominant, dominant genetic model (heterozygote and minor homozygote (Mm + mm) vs. major homozygote (MM)); Recessive, recessive genetic model (minor homozygote (mm) vs. major homozygote and heterozygote (MM + Mm)); OR, odds ratio; CI, confidence interval.

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HBV infection remains unclear. The development of single-nucleotide polymorphism (SNP) databases and the advances in technology have provided an opportunity to explore the issue of host susceptibility (Nishida et al., 2013). Recent studies have suggested that complex interactions among genetic variations, HBV virulence, and environmental factors determine disease development and progression (Frodsham, 2005; Thio et al., 2000; Yano et al., 2013). One noteworthy finding of genome-wide association studies is that polymorphisms in the Human Leucocyte Antigen (HLA) DPA1/DPB1 genes, which are located on the short arm of chromosome 6, influence HBV infection (Chang et al., 2014; Kamatani et al., 2009; Kim et al., 2013; Mbarek et al., 2011; Nishida et al., 2012).

HLA-DP molecules are heterodimers of specialized glycoproteins that deliver foreign peptides to the surface of the cell, presenting the peptides to T cells, and enhancing the cellular responses to eliminate pathogens such as HBV. Two classes of HLAs, class I and class II, interact with specific $CD8^+$ cytotoxic T cells and specific $CD4^+$ helper T cells, respectively (Blackwell et al., 2009; Murphy, 2011). Consequently, the inability of HLA-DP to adequately present HBV peptide fragments will impair the host's immune response (Schmidt et al., 2013). The first genome-wide association study examined the associations between chronic HBV infection and 11 SNPs in the HLA-DP gene, a major isotype of HLA class II, made up of a 29-kDa α chain and a 34-kDa β-chain, in Japanese subjects (Kamatani et al., 2009). Different risk alleles or genetic models have been identified in different populations, and HLA-DPA1 rs3077 and HLA-DPB1 rs9277535 were important SNPs in terms of the susceptibility to HBV infection in several studies (Al-Qahtani et al., 2014; Guo et al., 2011; Li et al., 2011; Liao et al., 2014; Migita et al., 2012; Posuwan et al., 2014; Vermehren et al., 2012; Wang et al., 2011; Wong et al., 2013; Zhang et al., 2013). Associations between these SNPs and the spontaneously resolved of HBV infection were subsequently reported in Chinese (An et al., 2011; Guo et al., 2011; Li et al., 2011), Japanese, and Koreans populations (Nishida et al., 2012). However, other SNPs have yet to be widely evaluated. HLA-DPB1 rs3135021 and rs2281388 were reported to be associated with hepatocellular carcinoma progression in a Chinese population (Zhang et al., 2013). Unfortunately, the association between HLA-DPA1/DPB1 and the progression of HBV infection was not repeated in other studies (Al-Qahtani et al., 2014; An et al., 2011; Hu et al., 2012; Li et al., 2011; Liao et al., 2014; Migita et al., 2012). These conflicting findings raised our interest.

Indonesia has the third-highest prevalence of HBV infection worldwide, with a moderate to high incidence that affects 242 million people. Therefore, HBV infection places a heavy burden on the healthcare system (WHO, 2013). However, to date, the influence of HLA-DP variants on HBV infection has not been fully investigated in an Indonesian population. Due to the prevalence rate of HBV infection ranges from 2.1% to 10.5% (Utsumi, 2014), this study was performed to investigate the profiles and the genetic influence of HLA-DPA1/DPB1 SNPs on the susceptibility, spontaneously resolved, and progression of HBV-related advanced liver disease in an Indonesian population. We focused on one SNP in HLA-DPA1 (rs3077) and three SNPs in HLA-DPB1 (rs3135021, rs9277535, and rs2281388).

2. Materials and methods

2.1. Subjects

The Indonesian subjects involved in this study were recruited from Dr. Sardjito Hospital, Yogyakarta, Indonesia, between April 2013 and November 2014. A total of 686 participants, including 222 HBV carriers, 228 spontaneously resolved of HBV, and 236 healthy controls, were enrolled from the blood donation unit, inpatient wards, and outpatient clinic of the Subdivision of Gastroenterohepatology, Department of Internal Medicine. To compare the linkage disequilibrium pattern between populations, we also included 86 Japanese subjects who were recruited from Kobe University Hospital.

The HBV status of each subject was determined based on the serological results of Hepatitis B surface antigen (HBsAg) and total antibody against HBV core antigen (anti-HBc) tests. The healthy controls were blood donors from blood donation units who were seronegative for both HBsAg and anti-HBc. HBV infection was considered to have spontaneously resolved in blood donors who were seronegative for HBsAg and seropositive for anti-HBc. Blood donors and patients with seropositive for HBsAg were considered to be HBV carriers. All blood samples were negative for Hepatitis C virus (HCV) and Human immunodeficiency virus (HIV).

The HBV carriers were divided into two groups of patients with nonadvanced liver disease (asymptomatic and chronic Hepatitis B) or advanced liver disease (cirrhosis and hepatocellular carcinoma). Subjects who were seropositive for HBsAg, had normal serum alanine aminotransferase (ALT) levels (<35 U/L), and had no obvious clinical symptoms were considered to be asymptomatic carriers. Chronic HBV infection, cirrhosis, and hepatocellular carcinoma were classified by a gastrohepatologist at the research site using the criteria of the Asian Pacific Association for the Study of the Liver (Liaw et al., 2012; Omata et al., 2010; Shiha et al., 2009). The diagnosis of HBV infection was based on clinical, biochemical, imaging, and/or histological examinations.

Each subject gave his/her written informed consent before enrolment. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committees of Kobe University, Japan, and Gadjah Mada University, Indonesia (code KE/FK/194/EC).

2.2. Serological and biochemical tests

All subjects were screened for HBsAg, anti-HCV antibodies, and anti-HIV antibodies using automated chemiluminescent enzyme immunoassays on an Architect analyzer (Abbott Laboratories, IL, USA). Subjects who were seronegative for HBsAg were also tested for anti-HBc antibodies using a passive hemagglutination assay (Mycell® Anti-rHBc; Institute of Immunology, Tokyo, Japan). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using standard procedures.

2.3. DNA extraction and SNP genotyping

For SNP genotype determination, peripheral blood was drawn and collected into EDTA blood tubes. Genomic DNA was extracted from 200 ml of buffy coats on the day of collection using a DNA extractor kit (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany), in accordance with the manufacturer's instructions.

Genotyping was performed using the Allelic Discrimination Assay on a 7500 Real-Time PCR System with TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA). SNPs were genotyped as previously described with specific primers (Zhang et al., 2013) and FAM- and VIC-labeled probes provided by Sigma-Aldrich (Hokkaido, Japan), in accordance with the recommended protocol (Malkki and Petersdorf, 2012). The four SNPs were successfully genotyped at rates of >97.5%. Samples with known genotypes obtained by direct sequencing were used as the quality controls in each TaqMan® genotyping assay.

2.4. Meta-analysis study

In order to improve the accuracy of the influence of the SNPs on the outcome of HBV infection, we performed a meta-analysis of rs3077 and rs9277535. Relevant studies were screened and identified by a computerized literature search of electronic databases, including Pubmed, Elsevier, and Web of Science, with English only as the language restriction. No manual searches of books, abstracts and conference