Infection, Genetics and Evolution 43 (2016) 407–411

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Association of human leukocyte antigen DP/DQ gene polymorphisms with chronic hepatitis B in Chinese Han and Uygur populations

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 31 May 2016
Accepted 7 June 2016
Available online 9 June 2016

Keywords:
Chronic hepatitis B
HLA-DP/DQ
Single nucleotide polymorphism

ABSTRACT

Several genome-wide association studies (GWAS) have shown that human leukocyte antigen (HLA) DP/DQ gene polymorphisms are associated with susceptibility to chronic hepatitis B virus (HBV) infection. We clarified the roles of the HLA-DP/DQ gene in HBV infection in different nationalities. Three single nucleotide polymorphisms (SNPs) in HLA-DP (rs9277471, rs9277535 and rs9277542) and the SNP rs9272346 in HLA-DQ were studied. In total, 779 patients were recruited to this study, including 400 Chinese Han and 399 Uygurs. The rs9277535 variant genotypes were directly associated with HBV persistence compared to healthy controls in an additive model of the Chinese Han population (odds ratio [OR] = 1.88, 95% confidence interval [CI] = 1.03–3.41, $P = 0.040$), and in a recessive model of the Chinese female population (OR = 2.02, 95% CI = 1.26–3.24, $P = 0.003$). In addition, rs9277471 and rs9277542 variant genotypes significantly decreased the risk of HBV infection compared to healthy controls in an additive model of the Chinese Han population (OR = 0.53, 95% CI = 0.29–0.98, $P = 0.042$; OR = 0.53, 95% CI = 0.29–0.97, $P = 0.039$) and in a dominant model of the Chinese female population (OR = 0.50, 95% CI = 0.31–0.80, $P = 0.004$; OR = 0.49, 95% CI = 0.31–0.79, $P = 0.003$). The GG genotype of rs9277346 was associated with HBV infection in the Chinese Han population (additive model: OR = 0.38, 95%CI = 017–0.82, $P = 0.014$; recessive model: OR = 0.41, 95% CI = 0.19–0.86, $P = 0.019$) and in males (additive model: OR = 0.31, 95% CI = 0.14–0.65, $P = 0.002$; dominant model: OR = 0.65, 95% CI = 0.43–0.97, $P = 0.034$; recessive model: OR = 0.36, 95% CI = 0.18–0.73, $P = 0.005$). In addition, allele G of rs9277346 was marginally related to a reduction in risk for HBV infection in the Uygur population. Our study suggests that HLA-DP/DQ polymorphisms can affect susceptibility and resistance to HBV infection in Chinese populations, and are possibly linked to race and sex.

1. Introduction

Hepatitis B virus (HBV), a DNA virus that is mainly found in liver cells, causes liver inflammation, necrosis, and fibrosis (Seeger and Mason, 2000). Chronic hepatitis B (CHB) is an important public health problem, and the World Health Organization estimates that over 400 million people are affected (Lavanchy, 2004), most of whom reside in low-income countries. The main complications of CHB are liver cirrhosis (LC) and hepatocellular carcinoma (HCC), which is defined as the continuous positive occurrence of hepatitis B surface antigen (HBsAg) for 6 months or more. These complications appear in 15–40% of CHB patients (Purcell, 1993), and about 1 million patients die from this disease each year (Dienstag, 2008). Many people infected with HBV have no symptoms and do not realize they are infected, leading to the continuous progression of this disease, which has become a serious threat to global health.

Recently, several genome-wide association studies (GWAS) demonstrated that single nucleotide polymorphisms (SNPs) in human leucocyte antigen (HLA) genes, particularly polymorphisms in HLA-DP/DQ genes, may affect resistance to persistent HBV infection. A two-stage GWAS identified an association between HLA-DP and HLA-DQ variants with CHB in Asians. In the first-stage, CHB was significantly associated with two SNPs (rs3077 and rs9277535) in the HLA-DP locus. In the second-stage, CHB and two SNPs (rs2856718 and rs7453920, both in the HLA-DQ locus) had significant independent effects on CHB susceptibility (Kamatani et al., 2009; Mbarek et al., 2011). Many studies have found that genetic variants rs9277535 and rs7453920, in the HLA-DPB1 and the HLA-DQB2 loci, respectively, are strongly related to the risk of persistent HBV infection (Chang et al., 2014). Other SNPs near the HLA-DP or HLA-DQ region may also be involved in HBV persistence. A Chinese team genotyped rs9275572 in 506 HBV-related HCC patients and 772 CHB patients (Chen et al., 2013), and found a significant association between rs9275572 and HCC.

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http://dx.doi.org/10.1016/j.meegid.2016.06.022
1567-1348/

The prevalence of HBV differs in countries such as Asia, Southeast Asia, sub-Saharan Africa, and the Pacific Islands. The rate of HBsAg in the general population is greater than or equal to 8%, and the HBV infection rate is >60% in these regions. However, in North America, Western Europe, and Australia, only 2% of the general population has HBsAg, and <20% of individuals are infected. The majority of research in China has focused on the Han population (Chang et al., 2012; Hu et al., 2012; Ji et al., 2014; Li et al., 2012; Liao et al., 2015). In addition, a Japanese team also observed a relationship between gene variants and HBV infection in a Japanese population (Mbarek et al., 2011). Previous studies on Japanese, Korean, Hong Kong, and Thai populations found differences in HLA-DP alleles among the different populations (Nishida et al., 2014). Another study confirmed that variation in HLA genes could affect susceptibility to, and clearance of, HBV infection in Saudi Arabian patients (Al-Qahtani et al., 2014).

The above mentioned studies clearly demonstrate that the distribution of CHB has obvious regional and racial differences. The HBsAg carrying rate in the general population is 7% in China, which is a medium-to-high endemic area. Several epidemiological surveys have shown that the prevalence of HBV among the Uygur population is lower than that of the Han population (Mizuki et al., 1996; Zhang et al., 2010). In accordance with previous reports, Liao et al. (2015) found regional differences in gene polymorphisms between Uygurs and Hans.

Therefore, we investigated the association among rs9277471, rs9277535, and rs9277542 in the HLA-DP locus and rs9272346 in the HLA-DQ locus with HBV infection, and identified the role of host genetic factors in Chinese Han and Uygur populations.

2. Materials and methods

2.1. Study population

In total, 799 subjects, including 400 Chinese Hans (200 CHB patients and 200 healthy subjects used as controls) and 399 Uygurs (199 CHB patients and 200 healthy controls) were recruited for this study. Han CHB patients were enrolled from June 2011 to May 2013 in Chinese Medicine Hospital of Xinjiang Autonomous Region. Uygur CHB patients were collected in the First People's Hospital of Kashgar from June 2011 to May 2013. Healthy controls were recruited from September 2009 to November 2012 at the Clinical Test Centre of Chinese Medicine Hospital in Xinjiang Autonomous Region. All of the CHB patients had the presence of serum HBsAg for at least 6 months, with elevated serum levels of alanine transaminase (ALT), and were positive, with no evidence of LC or HCC. The healthy controls were negative for the serum biomarkers of HBV (HBsAg and HBeAg), and had normal liver functions and no history of liver disease. Subjects who were co-infected with hepatitis A, C, D virus and/or human immunodeficiency virus (HIV) were excluded from the study. Informed consent was obtained from each patient. The study procedures conformed to the Helsinki Declaration and were approved by the Ethical Committee of Xinjiang Autonomous Region Chinese Medicine Hospital. The subjects included in this study were unrelated by blood.

2.2. Serological testing

HBV serology markers, including HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb, were analyzed using Elecsys Modular E170 immunoassay (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's instructions.

2.3. Genotyping of HLA SNPs

HLA-DP and DQ SNPs were chosen based on sites related to persistent HBV infection in Chinese populations that had been identified in previous GWAS studies. Five SNPs were chosen (rs9277471, rs9277535, and rs9277542 in HLA-DP; rs2856718 and rs9272346 in HLA-DQ) that mostly correlated with HBV infection. Accurate genomic location information for these SNPs can be found in Supplemental Table 1. This experiment used the Tiangen Biotechnology Co., Ltd. (Beijing, China) blood genome DNA extraction system 0.1–20 ml (centrifugal column method). Genomic DNA from peripheral blood leukocyte cells was extracted using a Blood genomic Extraction Kit (DP319–2) according to the manufacturer's protocol. All of the above SNPs were genotyped using SNaPshot technology (Applied Biosystems, Carlsbad, CA, USA). The primers used in this study are shown in Supplemental Table 2. The PCR product was obtained by multiple PCR reactions (HotStarTaq, Qiagen), and then extension reaction was performed (SNaPshot Multiplex Kit). After obtaining the extension product, the SNP genotyping was performed on an ABI3730xl machine. The experimental process of genotyping is shown in Supplemental Fig. 1.

2.4. Statistical analysis

For clinical data, sex was expressed as an absolute value and a Chi-square test was used for comparison. Age between case and control was analyzed using Student's *t*-test for continuous variables with a normal distribution. A Wilcoxon rank sum test was used to compare continuous variables with skewed distributions. The SNPs in each group were tested for Hardy–Weinberg equilibrium (HWE) using Stata 12.0 software. The absolute value and frequency for the genotype and allele of each SNP were calculated by Pearson's chi-square test in two-by-two cross tables. Associations between SNPs and susceptibility to HBV infection or disease progression in chronic HBV patients were estimated using the unconditional logistic regression model; the results are expressed in terms of odds ratios (ORs) and 95% confidence intervals (CIs). The following models were assessed between cases and controls: additive model (BB vs. AA), dominant model (AB + BB vs. AA), and recessive model (BB vs. AA + AB) (A as the wild-type allele, B as mutant allele), adjusting for age and sex. Linkage disequilibrium (LD) was evaluated using Haploview software. Haplotype analysis was performed using SHEsis online software (Shi and He, 2005; Li et al., 2009) (http://analysis.bio-x.cn/myAnalysis.php) to estimate the haplotype frequencies and capture significant haplotype blocks associated with HBV infection susceptibility. All of the statistical analyses were performed with Stata 12.0, and a P value <0.05 was considered statistically significant.

3. Results

3.1. Characteristics of studied subjects

The detailed clinical characteristics of 799 samples, including Han and Uygur subjects, are shown in Supplemental Table 3. The study included 200 Han patients with CHB, 199 Uygur patients with CHB, 200 healthy Han controls, and 200 healthy Uygur controls. Significant differences between cases and controls with regard to age and sex were controlled for in the multivariate analysis.

3.2. HWE SNP results

We performed genotyping of rs2856178, rs9272346, rs9277471, rs9277535, and rs9277542 in all of the samples. The frequency of the genotype and allele of each SNP are shown in Supplemental Table 4. The genotype frequencies of four SNPs in all of the clinical groups were in HWE ($P > 0.05$). Only rs2856718 was not in HWE in all of the groups, so it was excluded from further analysis (Supplemental Table 5).

3.3. Associations between HLA-DP/DQ polymorphisms and HBV infection in the Chinese Han population

The results of the logistic regression showed that GG-rs9277471 was significant between the two groups under additive model (OR = 0.53,