Infection, Genetics and Evolution 12 (2012) 1222–1228

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

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

Relationship between *HLA-DP* gene polymorphisms and clearance of chronic hepatitis B virus infections: Case–control study and meta-analysis

Zehui Yan, Shun Tan, Yunjie Dan, Xiaowen Sun, Guohong Deng *, Yuming Wang *

Institute of Infectious Diseases, Southwest Hospital, Third Military Medical University, 30 Gao Tan Yan Street, Sha Ping Ba District, Chongqing 400038, PR China

ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form 28 March 2012
Accepted 29 March 2012
Available online 19 April 2012

Keywords:
Hepatitis B virus (HBV)
Human leukocyte antigens DP (HLA-DP)
Meta-analysis
Polymorphism

ABSTRACT

Hepatitis B virus (HBV) infection is a serious public health problem worldwide. Two common genetic variants (rs3077 and rs9277535) of human leukocyte antigen DP (HLA-DP) have been reported to be associated with persistent HBV infection in populations of Japan and Thailand. To confirm whether the association can be replicated in Chinese populations, an independent case–control study were conducted, and two polymorphisms (rs3077 and rs9277535) were genotyped using the TaqMan SNP genotyping assay in 282 persistent chronic HBV carriers and 64 spontaneously HBV recovered carriers. To provide a more definitive conclusion, a meta-analysis combining and summarizing five studies was performed by random-effects model using the DerSimonian and Laird's method. By using logistic regression analysis with adjustment for covariates, including age, sex, and alcohol consumption, the results of our independent case–control study showed that the minor allele's homozygote (AA genotype) of rs3077 and rs9277535 was significantly associated with decreasing risk/protection of HBV persistent chronic infection (for rs3077: $P = 0.0017$, OR = 0.29, 95% CI = 0.13–0.62; for rs9277535, $P = 0.0004$, OR = 0.26, 95% CI = 0.12–0.54). The results of meta-analysis pooling all eligible studies also showed that rs3077-A and rs9277535-A alleles were associated with an increased clearance rate to HBV infection (rs3077: OR = 0.57, 95% CI = 0.44–0.75; rs9277535: OR = 0.56, 95% CI = 0.47–0.63). These results further confirmed the strong influence of *HLA-DP* gene variants on risk of spontaneous HBV clearance from persistent HBV infection. Both A alleles of *HLA-DP* SNP rs3077 and rs9277535 showed strong protective effects for spontaneous HBV clearance from persistent HBV infection in the Han Chinese population.

1. Introduction

An estimated two billion people have been infected with the hepatitis B virus (HBV), and more than 350 million have chronic (long-term) liver infections. Although some HBV carriers spontaneously eliminate the virus, 2–10% of the individuals with chronic hepatitis B (CHB) are estimated to develop liver cirrhosis every year, and a subset of these individuals suffer from liver failure or hepatocellular carcinoma (Lai et al., 2003). A very small proportion of patients with persistent HBV infection can spontaneously clear the virus without treatment and are termed spontaneously recovered carriers (SRC). It has been reported that, in western countries, 1–2% of HBV carriers become HBsAg negative each year (Alward et al., 1985), whereas in China, where HBV infection is endemic, the rate of HBsAg clearance is much lower (0.05–0.1% per year) (Yuen et al., 2004). However, the mechanism of spontaneous recovery from chronic persistent HBV infection is not well clarified.

Persistent HBV infection or HBV clearance is influenced by complex factors of viral factors, environmental factors, and genetic makeup, for example, age at infection, gender, viral genotype, ethnicity, host genetic variations and so on (Frodsham, 2005; Segal and Hill, 2003). Candidate gene association studies have implicated HBV clearance or persistence are associated with host gene polymorphisms, including human leukocyte antigen (*HLA*) classes I and II alleles (Singh et al., 2007) and non-*HLA* genes (e.g., *ESR1*) (Deng et al., 2004). However, the results of these candidate gene association studies are not universal, even conflicting for all investigated populations. Recently, Matsuda1 et al. conducted a two-stage genome-wide association study (GWAS) and implicated two single nucleotide polymorphisms (SNPs) (rs3077 and rs9277535) in *HLA-DP* gene associated with risk of persistent HBV infection in Japanese and Thai populations (Kamatani et al., 2009). The same group also performed a second GWAS with an increased sample size for the additional GWAS, and further confirmed that these two *HLA-DP* SNPs contributed to the risk of

Abbreviations: HBV, hepatitis B virus; HLA, human leukocyte antigens; CHB, chronic hepatitis B; SNP, single nucleotide polymorphism.

* Corresponding authors. Tel.: +86 23 68754141; fax: +86 23 65334998 (G. Deng), tel.: +86 23 68754858; fax: +86 23 65334998 (Y. Wang).

E-mail addresses: ghdengsnp@hotmail.com (G. Deng), wym417@163.com (Y. Wang).

1567-1348/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2012.03.026

persistent HBV infection (Mbarek et al., 2011). However, in these two GWAS studies, the data on HBV exposure of controls were unknown, which may introduce information bias in the results. Furthermore, only healthy individuals were used as controls in these two GWAS studies, they did not determine whether the association with persistent infection was attributable to clearance of HBV or resistance to HBV infection. The relationship between *HLA-DP* polymorphisms and HBV clearance needs to be confirmed by other investigators.

A common flaw of genetic-based variant association studies is the failure to confirm findings within further independent populations. China and Japan are highly endemic areas of HBV infection. Similarly in Japan, most patients with CHB in China were infected through vertical transmission and became HBV carriers. Additionally, the frequencies of *HLA-DP* alleles in Chinese populations were similar to those in Japanese populations. Then, it would be necessary to confirm whether there was the association between the *HLA-DP* genetic variation and spontaneous HBV clearance in Chinese populations. In our study, based on the previous GWAS results, we selected the two most strongly associated SNPs of *HLA-DP* (rs3077 and rs9277535) and genotyped these two polymorphisms in a hospital-based case–control study of Chinese Hans from Chongqing (Southwest China). During the period of study and writing the manuscript, six studies which replicated the associations of the two *HLA-DP* SNPs (rs3077 and rs9277535) and chronic HBV infection in Han Chinese have been published (An et al., 2011; Guo et al., 2011; Hu et al., 2011; Lau et al., 2011; Li et al., 2011; Wang et al., 2011). To further test the relationship between these SNPs and spontaneous HBV clearance, we also performed a mini meta-analysis using a dominant genetic model by pooling our current data and the published data.

2. Methods

2.1. Study participants

Individuals with history of persistent HBV infection who were registered and followed up at Southwest Hospital (Chongqing, Southwest China) from January 2006 and June 2011 were included for the primary selection. A uniform questionnaire was used and recorded the self-report of risk factors for HBV transmission, family history of HBV infection, vaccination history, alcohol ingestion etc. The demographic information included gender, birth-date, birthplace, and past and current residency, and was collected by face-to-face interviews. The clinic data were collected from clinical records and short telephone interviews when necessary. All individuals had the obvious history of previous chronic HBV infection. The patients who had serologic evidence for co-infection with hepatitis C virus, hepatitis D virus, and human immunodeficiency virus were excluded from this study. Persons with blood relatives enrolled in the study were excluded.

Finally, a total of 346 unrelated Han Chinese were recruited, and included 282 patients with CHB and 64 individuals (SRC) who spontaneously recovered from previous chronic HBV infection. The diagnostic criteria of CHB was based on the combination of clinical history, physical examination, imaging and laboratory data and/or histology according to related literatures recommended by Chinese Medical Association (Chinese Society of Hepatology, 2007). All patients in the CHB case group of this study should satisfy the following criteria: (1) two positive tests for seropositive HBsAg and anti-HBcAg for at least 6 months, (2) serum HBV DNA $>1 \times 10^5$ copies/ml for HBeAg-positive and $>1 \times 10^4$ copies/ml for HBeAg-negative patients, (3) persistent or intermittent elevation of serum ALT and/or AST levels greater than two times upper limits of normal range before or current; or liver biopsy showing chronic hepatitis with moderate or severe necroinflammation. All subjects in the SRC control group of this study also should satisfy the following criteria: (1) all individuals had the obvious history of previous chronic HBV infection; (2) no history of HBV vaccination; (3) two positive tests for both anti-HBs and anti-HBc antibodies, and two negative tests for HBsAg; (4) normal liver function tests and undetectable serum HBV DNA level in two tests; (5) without any form of antiviral, immune suppressive or immunomodulatory treatment.

All subjects provided informed consent to participate in the study, as approved by the ethical committee of Southwest hospital, Chongqing, China.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from blood leukocytes using standard phenol/chloroform protocols. DNA samples were diluted to 8 ng/μL and distributed into 96-well plates (DNA panels), with 94 samples and 2 controls (DNA-free water) in each plate. The two SNPs on *HLA-DP* (rs3077 and rs9277535) were genotyped using a commercially available TaqMan SNP genotyping assay and GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA), in accordance with the manufacturer's instructions. The sequence detection software was used for allelic discrimination. Genotyping was performed in a blind manner without information of subjects' case/control status. Two DNA-free controls and two duplicate samples were included in each 96-well plate. The rates of successful genotyping calls for the 2 SNPs were all over 99%. The accuracy of genotyping data for SNP obtained from TaqMan SNP genotyping assay was validated by direct sequencing of 30 masked, random samples of patients.

2.3. Statistical analysis of case–control study

Allele frequencies for each SNP were determined by gene counting, and the significance of deviations from Hardy–Weinberg equilibrium was tested using the random-permutation procedure implemented in the Arlequin package (http://lgb.unige.ch/arlequin/). Pair-wise linkage disequilibrium (LD) between SNPs was analyzed by LDA (Ding et al., 2003). Statistical analysis was performed using SPSS software (version 9.0; SPSS Inc, Chicago, IL). A *P*-value less than 0.05 was considered significant. χ^2 tests were performed to examine the differences in the allele frequency and genotype distribution between groups. Multivariable logistic regression analysis was performed to adjust risk factors such as age, sex, and alcohol use. The association between genotyped polymorphisms and the risk of disease was estimated by *P* values, odds ratios (ORs), and 95% confidence intervals (95% CIs).

2.4. Meta-analysis of eligible studies

The eligible studies were searched from electronic medical databases such as Pubmed, Embase and the Cochrane Library. Studies references were also analyzed to find any study not available from the electronic databases. When the same patient population was included in several publications, only the most recent or complete study was used in this meta-analysis. When a study reported results of different subpopulations, we treated each subpopulation as a separate comparison in the meta-analysis.

Data extraction and quality assessment using the Newcastle–Ottawa Scale (NOS) (Stang, 2010), were performed independently by two different investigators (Dr. Yan and Dr. Tan). The articles to be included in this meta-analysis should meet these inclusion criteria: (1) the study was designed as an unrelated case–control study; (2) the study provided the number of patients with CHB cases and individuals spontaneously recovered from previous