

Lack of association between human leukocyte antigen polymorphisms rs9277535 and rs7453920 and chronic hepatitis B in a Brazilian population

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ABSTRACT. Hepatitis B virus (HBV) infection is a serious public health problem worldwide. The progression of the disease depends on several host and viral factors and may result in fulminant hepatitis (very rare), acute hepatitis with spontaneous clearance, and chronic hepatitis B infection. Previous studies demonstrated that variations in the human leukocyte antigen (HLA) class II (*HLA-DPB1* and *HLA-DQB2* genes) are related to the chronic HBV infection. This study aimed to investigate the association of two single nucleotide polymorphism (SNPs), one in the *HLA-DPB1* (rs9277535) and one in the *HLA-DQB2* (rs7453920), with chronic hepatitis B infection in a southern Brazilian sample. This case-control study included 260 HBV patients attended in a Specialized

Center for Health in Caxias do Sul (Brazil) between 2014 and 2016. The same number of controls (matching for age, gender, and ethnicity) was obtained in a University Hospital in the same city and period. Blood samples were collected and genomic DNA was extracted. Genotyping were performed by real-time Taqman PCR method. Odds ratios with 95% confidence intervals and significance level of 5% ($P < 0.05$) were calculated. Allele frequencies in the SNP rs9277535 were 72.6% for A and 27.4% for G nucleotides in cases and 75.0% for A and 25.0% for G in controls. Allele frequencies in the SNP rs7453920 were of 25.7% for A and 74.3% for G in cases and 28.8% for A and 71.2% for G in controls. No statistically significant association was found between both SNPs and chronic hepatitis B ($P > 0.05$).

Key words: Hepatitis B virus; Single nucleotide polymorphism; Chronic hepatitis B; HLA antigens

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious public health problem worldwide (Tang et al., 2014). Approximately 240 million of the world population has chronic HBV infection, which is the leading cause of chronic hepatitis (WHO - World Health Organization, 2014). Moreover, HBV chronic disease can progress to hepatic dysfunction, cirrhosis, and hepatocellular carcinoma (Zeng et al., 2014; Poh et al., 2015). More than 600,000 people die every year due to these clinical complications of HBV infection (Nelson et al., 2014; Niederau, 2014). Most of the HBV-infected people live in countries with high endemicity in East Asia and sub-Saharan Africa (WHO - World Health Organization, 2016). In South America, endemicity ranges from intermediate (e.g., southern Brazil and Ecuador) to high (e.g., North Brazil and Peru) (Roman et al., 2014). In Brazil, 120,343 cases of hepatitis B were reported in 2012. South Brazil region represents around 31.6% of the total case notifications in the country (Brasil, 2012).

HBV transmission frequently occurs by contact with infected blood and body fluids (Zeng et al., 2014). The course of the disease depends on several host and viral factors and may result in fulminant hepatitis (<1%), acute hepatitis with spontaneous clearance, and chronic hepatitis B infection. The progression of hepatitis B infection to a chronic disease is not usual (less than 10%) when infection occurs in an adult, but it is common (around 90%) with the HBV transmission in the birth or childhood (WHO - World Health Organization, 2016). This high progression rate in infants is associated with an immune system not yet fully consolidated (Tang et al., 2014).

Human leukocyte antigen (HLA) complex helps the immune system to detect antigens of bacterium and virus particles. HLA complex acts on the antigen processing and presentation in the humoral or cellular responses (Mohamadkhani et al., 2015). HLA biomolecule classes I and II are expressed on antigen-presenting cells (APC), acting in antigen presentation to T lymphocytes. This immune response mediated by T lymphocytes is essential in resolving HBV infections (Mohamadkhani et al., 2015; Wang et al., 2016). HLA complex comprises genes located basically on chromosome 6. The peptides that comprise the HLA class I complex are encoded by the genes *HLA-A*, *-B*, *-C*, and *-D*. Moreover, HLA class II complex consist of α

and β chains that are organized into heterodimers. *HLA-DPBI* and *HLA-DQB2* genes encode β subunits 1 and 2, respectively (McCanlies et al., 2003; Shiina et al., 2004).

Genetic polymorphisms in the HLA classes I and II were well investigated and have been strongly associated with HBV persistence and progression to chronic hepatitis B infection (Singh et al., 2007; Mbarek et al., 2011; Jiang et al., 2014; Zeng et al., 2014). Recent genome-wide association studies (GWAS) have demonstrated two single nucleotide polymorphisms (SNPs) located in the genes *HLA-DPBI* (rs9277535, A/G) and *HLA-DQB2* (rs7453920, A/G). Both are associated with the persistence of HBV infection, mainly in Asian populations (Kamatani et al., 2009; Mbarek et al., 2011; Wong et al., 2013; Al-Qahtani et al., 2014). These SNPs result in an A to G transition in two different gene regions: rs9277535 in the 3'-untranslated region (3'-UTR) of the *HLA-DPBI* gene and rs7453920 in the intron 1 of the *HLA-DQB2* gene. These SNPs may affect the regulation of gene expression and stability of mRNA (Mohamadkhani et al., 2015) with the G allele conferring a higher risk to HBV chronic infection (Mbarek et al., 2011; O'Brien et al., 2011; Thomas et al., 2012; Hu et al., 2013; Mohamadkhani et al., 2015). The aim of this study was to investigate possible associations of these two SNPs with chronic hepatitis B in a population sample in southern Brazil.

MATERIAL AND METHODS

Patients and controls

Adult HBV patients and controls were from the city of Caxias do Sul, Rio Grande do Sul State, South Brazil. The sample size was estimated as previously described (Lwanga and Lemeshow, 1991), with statistical power of 80%, significance at the 5% level (two-tailed test), average exposure frequency of SNPs in controls of 50.7% (Ensembl Genome Browser, 2016), and an odds ratio (OR) of 1.8 at a 95% confidence interval (CI). Therefore, the total sample was initially calculated to include 191 cases and the same number of controls. More 138 samples (26.5%) were added for potential confounders, totaling 260 cases and 260 controls. The case group was composed of individuals routinely attended in the Specialized Center for Health in the city of Caxias do Sul (Centro Especializado em Saúde - CES) from September 2014 to March 2016. They were visiting the service for periodic exams and/or treatment for chronic hepatitis B (all were HBsAg-positive). Controls were obtained by convenience sampling at a University Hospital (Hospital Geral) in the same city and period. All control individuals were negative for HBsAg and they did not have primary sclerosing cholangitis, Wilson's disease, hepatitis C virus (HCV), cirrhosis, acquired immunodeficiency syndrome, hypertension, cancer, hepatitis, and autoimmune diseases (criteria for exclusion). The samples from both groups were matched (for age, gender, and ethnicity) as previously described (Pereira et al., 2017).

SNP genotyping

Blood samples were collected in tubes with EDTA anticoagulant and stored at -70°C for further molecular analysis. Genomic DNA was extracted by silica adsorption method as described by Boom et al. (1990). SNPs rs9277535 and rs7453920 were detected by commercial real-time TaqMan polymerase chain reaction (PCR) assays with reference codes C__29715274_20 and C__27015605_10 (Applied Biosystems, Foster City, CA, USA). The reactions were carried out in a StepOne Plus™ platform (Applied Biosystems) with cycling and

temperature conditions according to the manufacturer. Allelic discrimination was performed using the StepOne™ software (Version 2.3, Applied Biosystems). Controls were used in all experiments aimed at the reliability of technical procedures.

Statistical analysis

The data were analyzed using the SPSS (Statistical Package for Social Sciences, version 17.0, Chicago, IL, USA) software. Genotype and allele frequencies were determined by direct counting and Hardy-Weinberg equilibrium was evaluated by the chi-square test. Statistical Power calculation was performed using the software Epi Info™ version 7.1.5.2. In bivariate analysis, the OR at 95%CI was estimated in order to detect the possible association of the SNPs with chronic hepatitis B. All estimates were bilateral with a significance level of 5% ($P < 0.05$).

RESULTS

Socio-demographic information and clinical history of HBV infection were obtained from all participants of the survey ($N = 520$). The overall average age of the 260 HBV patients was 47.62 ± 12.22 years and there were 143 (55%) males and 229 (88%) self-defined whites.

All the 520 samples were analyzed for the SNPs rs9277535 and rs7453920, but only 420 samples (210 cases and respective controls) could be effectively genotyped. Table 1 shows the genotypes and alleles observed for SNPs rs9277535 and rs7453920 in cases and controls. In a general analysis for the SNP rs9277535, the overall genotype frequencies were 55.2% for A/A, 37.1% for A/G, and 7.6% for G/G. The allele frequencies for this SNP were 73.8% for A and 26.2% for G nucleotides. The SNP rs7453920 presented genotype frequencies of 8.3% for A/A, 37.9% for A/G, and 53.8% for G/G and allele frequencies were 27.3% for A and 72.7% for G nucleotides (Table 1).

Table 1. Bivariate analysis of the distribution of genotypes and alleles in *HLA-DPB1* (rs9277535) and *HLA-DQB2* (rs7453920) SNPs in the case and control groups.

Polymorphisms	Cases (N = 210)		Controls (N = 210)		Total (N = 420)		OR (95%CI)	P
	N	%	N	%	N	%		
<i>HLA-DPB1</i> (rs9277535)								
Genotypes								
A/A	111	52.9	121	57.6	232	55.2	1.00	-
A/G	83	39.5	73	34.8	156	37.1	1.24 (0.83-1.86)	0.300
G/G	16	7.6	16	7.6	32	7.6	1.09 (0.52-2.28)	0.819
Alleles								
A	305	72.6	315	75.0	620	73.8	1.00	-
G	115	27.4	105	25.0	220	26.2	1.13 (0.83-1.54)	0.432
<i>HLA-DQB2</i> (rs7453920)								
Genotypes								
A/A	18	8.6	17	8.1	35	8.3	1.00	-
A/G	72	34.3	87	41.4	159	37.9	0.78 (0.38-1.63)	0.509
G/G	120	57.1	106	50.5	226	53.8	1.07 (0.53-2.18)	0.854
Alleles								
A	108	25.7	121	28.8	229	27.3	1.00	-
G	312	74.3	299	71.2	611	72.7	1.17 (0.86-1.58)	0.314

The genotype frequencies in the case group for the SNP rs9277535 were 52.9% (A/A), 39.5% (A/G), and 7.6% (G/G), while allele frequencies were 72.6% (A) and 27.4%

(G). In the control group, genotype frequencies were 57.6% (A/A), 34.8% (A/G), and 7.6% (G/G), while allele frequencies were 75.0% (A) and 25.0% (G). The SNP rs7453920 presented genotype frequencies in the case group of 8.6% (A/A), 34.3% (A/G), and 57.1% (G/G) and allele frequencies of 25.7% (A) and 74.3% (G). In the control group, genotype frequencies of 8.1% (A/A), 41.4% (A/G), and 50.5% (G/G) and allele frequencies of 28.8% (A) and 71.2% (G) were observed. The frequencies of the genotypes and alleles were in agreement with Hardy-Weinberg equilibrium ($P > 0.05$) for both SNPs evaluated in cases and controls. No associations were observed between the G alleles for SNP rs9277535 (OR = 1.13; 95%CI = 0.83-1.54; $P = 0.432$) and rs7453920 (OR = 1.17; 95%CI = 0.86-1.58; $P = 0.314$) in relation to chronic hepatitis B. In addition, none of the genotypes evaluated demonstrated any statistically significant association with this disease ($P > 0.05$) (Table 1).

DISCUSSION

This study investigated the associations between HBV chronic infection and two SNPs, one in *HLA-DPBI* (rs9277535) and one in *HLA-DQB2* (rs7453920), in a sample of South Brazilian patients. There is enough evidence that these SNPs play important roles in the persistence of the HBV infection (Mohamadkhani et al., 2015). However, the complete mechanism of HBV infection and progression to hepatitis B are not fully understood (Kim et al., 2013). In general, host genetic background influences the immune processing of APC cells, necessary for an effective immune response. SNPs rs9277535 and rs7453920 affect the gene expression of the *HLA-DPBI* and *HLA-DQB2* molecules and they were demonstrated to be important in the recognition and processing of viral particles (Mohamadkhani et al., 2015).

In this scenario, the possible associations between SNPs located in the *HLA-DPBI* (rs9277535) and *HLA-DQB2* (rs7453920) genes with the HBV chronic infection were analyzed in a population from southern Brazil. However, no statistically significant association was found ($P > 0.05$). World studies demonstrated that the *HLA-DPBI* (rs9277535) polymorphism is often found in Asians (≈ 0.61) and less frequent in other populations, such as Europeans (≈ 0.27), South Americans (≈ 0.29), and Africans (≈ 0.19). The *HLA-DQB2* (rs7453920) polymorphism is often found in Asia (≈ 0.87), Europe (≈ 0.62), South America (≈ 0.73), and Africa (≈ 0.70). In this sense, the frequency of variant alleles (26.2% for rs9277535 and 72.7% for rs7453920) observed in the present study were consistent with previous observations in South America (Ensembl Genome Browser, 2016).

Several studies, especially analyzing Asian samples, found association between the SNP rs9277535 and chronic HBV infection (Kamatani et al., 2009; Lau et al., 2011; Mbarek et al., 2011; Png et al., 2011; Nishida et al., 2012; Yan et al., 2012; Kim et al., 2013; Seto et al., 2013; Al-Qahtani et al., 2014; Chang et al., 2014; Liao et al., 2014; Pan et al., 2014; He et al., 2015; Yu et al., 2015). Additionally, the study conducted by Thomas et al. (2012) found an association between the SNP rs9277535 and the elimination rate of HBV in patients Euro-American and African-Americans ($P = 0.0001$). However, Vermehren et al. (2012) did not find an association of this SNP with chronic hepatitis B in Caucasians. In the study conducted by O'Brien et al. (2011), the SNP rs9277535 was associated with a reduction in *HLA-DPBI* gene expression in European patients. In Asian studies, the SNP rs7453920 was associated with the immune response to HBV (Mbarek et al., 2011; Al-Qahtani et al., 2014; Liao et al., 2014; He et al., 2015). However, no studies have associated this SNP in samples from other regions of the world.

This study has some limitations. First, the double or triple pairing was not performed and, second, it was not possible to analyze SNPs in all samples (N = 520) due to technical laboratory restrictions. Anyway, the findings seem to demonstrate clearly the lack of association of these SNPs with chronic hepatitis B. This is very important to the understanding of this issue in a population from South Brazil with higher rates than the average (Menegol and Spilki, 2014). Further research with larger samples can generate more detailed knowledge of the frequencies of the SNPs evaluated in this study, as well as the contribution of these for chronic hepatitis B.

CONCLUSION

In this study, no associations were observed between the SNPs (rs9277535/rs7453920) and progression to chronic hepatitis B. Our data suggest that SNPs in *HLA-DPB1* (rs9277535) and *HLA-DQB2* (rs7453920) genes do not influence the chronicity of hepatitis B in the analyzed sample.

Conflicts of interests

The authors declare no conflict of interest.

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