The Influence of Polymorphisms in the MxA Promoter and the elf-2α Regulatory Region 2 on the Natural outcome of HBV Infection

Xin-su Wei1, Ping-an Zhang1*, Fang-ii Ye1, Yan Li1, Bing Deng2
1Department of Laboratory Science, Renmin Hospital of Wuhan University, Wuhan, China
2Department of infection disease, Renmin Hospital of Wuhan University, Wuhan, China

Abstract

Interferon (IFN) stimulates the expression of a number of genes encoding enzymes with antiviral activities, including myxovirus resistance A (MxA) and double-stranded RNA-dependent protein kinase (PKR). PKR is also activated by dsRNA, this leads to the phosphorylation of eukaryotic initiation factor 2α (eIF-2α), which halts viral replication. We investigated whether polymorphisms in MxA promoter and elf-2α regulatory region 2 (elf-2α reg2) influenced the natural outcome of hepatitis B virus (HBV) infection. A total of 243 patients with chronic HBV infection and 160 patients with self-limited HBV infection were used to genotype and identify this single-nucleotide polymorphism (SNP) by polymerase chain reaction-restriction fragment length polymorphism and sequencing, respectively. The distribution of the genotypes (GG, GT, and TT) at position -88 in the MxA promoter was 52.7%, 44.4%, and 2.9% in patients with chronic HBV infection and 41.3%, 43.1%, and 15.6% in patients with self-limited HBV infection, respectively. The frequencies of the TT genotype at position -88 in the MxA promoter were significantly higher among patients with self-limited HBV infection compared with patients with chronic HBV infection (odds ratio = 6.24, 95% CI: 2.63-14.81; P = 0.001). However, the polymorphisms both at position -123 in the MxA promoter and in the elf-2α reg2 were not significantly different between the two groups (P > 0.05). In conclusion, Polymorphism at position -88G/T in the MxA promoter influences the natural outcomes of HBV infection to some extent. This SNP of MxA promoter may be used as a clinical prognostic marker of HBV infection.

Keywords: MxA promoter and the elf-2α regulatory region 2

Introduction

Hepatitis B virus (HBV) infection is one of the most important chronic viral diseases in the world. An estimated 400 million people worldwide are carriers of HBV, and approximately 250,000 deaths occur each year as a consequence of fulminant hepatic failure, cirrhosis, and hepatocellular carcinoma [1-2]. When HBV is acquired in adulthood, the majority of infections are cleared, with chronic infection occurring in 5% to 10% of cases [3]. However, the dynamic interaction of the host inflammatory response with HBV, and the subsequent impact of this interaction on the clinical outcome of HBV infection, are not yet fully understood, nor are the underlying mechanisms for persistence of the virus [4-5]. But it has been thought that genetic associations may also provide clues to the development of HBV infection [6-7]. Some polymorphisms have been reported to be involved in susceptibility to chronic hepatitis B, in disease severity and progression, or in disease prognosis [8-11].

Interferon (IFN) is an important cytokine for resistance to HBV infection as well as for the clinical treatment of Hepatitis B [12-13]. The antiviral mechanisms of IFN are predominately mediated through the induction of antiviral proteins [14-16]. Therefore, this study investigated the IFN-induced antiviral protein myxovirus resistance protein A (MxA) and the eukaryotic initiation factor 2α regulatory region 2 (elf-2α reg2). MxA is considered to be the strongest IFN-specific index that can directly suppress HBV replication, the SNP at the -88 and -123 positions of the MxA promoter can affect MxA mRNA expression. Protein kinase-activated elf-2α reg2 is also an important factor in IFN signal transduction, but elf-2α reg2 gene single nucleotide polymorphisms (SNPs) correlated with IFN treatment efficacy. To explore the relationship between genotype and HBV infection outcome, polymerase chain reaction-restriction fragment length polymorphism analysis was utilized to detect SNPs in the MxA promoter at positions -88 and -123 and in elf-2α reg2 in samples from patients in Hubei area of China with self-limited HBV infection and chronic HBV infection.

Table 1: Characteristics of patients with chronic HBV infection.

<table>
<thead>
<tr>
<th>Category</th>
<th>Cases(n)</th>
<th>Gender(male/female)</th>
<th>Age(mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-limited infection group</td>
<td>160</td>
<td>106/54</td>
<td>56.4±14.0</td>
</tr>
<tr>
<td>Chronic infection group</td>
<td>243</td>
<td>178/65</td>
<td>54.7±14.8</td>
</tr>
<tr>
<td>P Value</td>
<td>0.807</td>
<td>0.569</td>
<td></td>
</tr>
</tbody>
</table>

Materials and Methods

Subjects

According to epidemiological expert advice, a total of 243 patients with chronic HBV infection (the chronic infection group) and 160 cases of self-limited HBV infection (the self-limited infection group) from Renmin Hospital of Wuhan University in Hubei, China were studied (Table 1). All patients were diagnosed according to the diagnostic criteria for viral hepatitis issued by the 2000 Chinese Medical Association Infectious and Parasitic Diseases Committee that was jointly revised by the Hepatology Committee [17-18]; additionally, patients were screened for other hepatitis virus infections. Patients in the self-limited infection group were not vaccinated against HBV, and the lab results for hepatitis B surface antigen (HBsAg) negative, anti-HBs antibody (anti-HBs) positive, blood count and biochemical parameters were within the reference range, which excluded the presence of liver.

*Corresponding author: Ping-an Zhang, Department of Laboratory Science, Renmin Hospital of Wuhan University, Wuhan, China, E-mail: zhangpingan@yahoo.com.cn

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kidney, endocrine, or cardiovascular disease. As also shown in Table 1, differences in gender and age between chronic infection group and self-limited infection group were of no significance. Informed consent was obtained from each patient before inclusion into the study. Likewise, the study conformed to the ethical guidelines of the Helsinki declaration was approved by the Renmin Hospital of Wuhan University ethics committees.

**Specimens preparation**

Blood samples were collected from the patients in the two groups. Using Genomic DNA Extraction Kits (SBS Biological Engineering Co., Ltd, Shanghai, China), we extracted whole blood genomic DNA, which was stored at -20°C until use.

**Primer design and main reagents**

Gene sequences and the corresponding SNP sites for the MxA promoter and the elf-2a reg2 gene were obtained from the U.S. National Center for Biotechnology Information’s Gene Bank (GenBank). Primer3.0 and a reference paper [19] were used to design the necessary primers. The primers were synthesized by SBS Biological Engineering Co., Ltd. The primer sequences for the MxA promoter -88 and -123 positions amplified a 350 bp fragment of DNA. The sequences of the primers utilized to amplify a 563 bp fragment of the elf-2a reg2 gene were as shown in Table 2. Taq DNA polymerase, dNTPs, and DNA molecular weight markers were purchased from Takara Biotechnology Co., Ltd (Dalian, China). HhaI, PstI, and Spl restriction enzymes were purchased from Shanghai Biological Engineering Technology Services Co., Ltd. (Shanghai, China) which distributes Amersham Life Sciences products. Molecular weight markers were purchased from Takara Biotechnology Co., Ltd. The primer sequences for the elf-2a reg2 locus were as follows: promoter -123 C/A sites and the elF-2a-reg2 were calculated manually in 160 patients with self-limited HBV infection and 243 patients with chronic HBV infection. The differences in distribution of the genotypes and alleles between groups and the Hardy-Weinberg equilibrium were tested using chi-square tests and Fisher’s exact tests. The genotype distribution comparisons between two groups were done after adjusting genotype distribution for potential confounding factor such as age and gender using analysis of covariance (ANCOVA). The odds ratio (OR) with 95% CI was also calculated. For statistical analysis, we used SPSS 11.0 software to perform. A P value of < 0.05 was considered statistically significant.

**Results**

**Analysis of MxA promoter gene polymorphism**

Consistent with expectations, the MxA promoter PCR products were 350 bp in size. Products of individual digestions with HhaI (digestion of the MxA promoter -88 G/T position) and PstI (digestion of the MxA promoter -123 C/A position) are in Figures 1 and 2. DNA products size and genotypes of the three digestion sites in the MxA promoter and elf-2a were as shown in Table 2. Taq DNA polymerase, dNTPs, and DNA molecular weight markers were purchased from Takara Biotechnology Co., Ltd (Dalian, China). HhaI, PstI, and Spl restriction enzymes were purchased from Shanghai Biological Engineering Technology Services Co., Ltd. (Shanghai, China) which distributes Amersham Life Sciences (Cleveland, OH, USA) products.

**SNP analysis**

The PCR amplifications were performed in a final volume of 25-μL using the following reagents: 2.5μL of 10×PCR reaction buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 9.0, 1 g/L Triton X-100), 1.0 U of Taq DNA polymerase, 200μmol/L of each dNTP, 2.0 mmol/L of MgCl2, 0.4μmol/L of each primer, and 50-100 ng of template. For the amplification of the MxA promoter -88 and -123 positions, the conditions were as follows: denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 1 min with a final extension at 72°C for 5 min. The amplification conditions for the elf-2a reg2 locus were as follows: denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min with a final extension at 72°C for 7 min. PCR amplification products were assessed using agarose gel electrophoresis, ethidium bromide (EB) staining, and observation under ultraviolet light. Mixtures of 8-μL of the reaction products and 2-μL of loading buffer (300 mL/L glycerol, 0.75 g/L bromophenol blue) were run on gels with DNA molecular weight standards to enable product identification. For digestions, PCR products (10-μL), 10× buffer (2-μL), restriction enzyme (1-μL of a 2 U/μL mix), and 10 × BSA (2-μL) were mixed with deionized water for a total volume of 20-μL per reaction. Digestions were performed at 37°C for 4 h. Digestion products (12-μL) were run in 20 g/L agarose gels and visualized under UV light with reference to DNA standards to identify fragments. The three digestion sites are listed in Table2. Additionally, selected PCR products were analyzed by DNA sequencing to confirm the PCR-RFLP results.

**Statistical methods**

Alleles and genotype frequencies in MxA promoter -88 G/T and -123 C/A sites and the elf-2a-reg2 were calculated manually in 160 patients with self-limited HBV infection and 243 patients with chronic HBV infection. The differences in distribution of the genotypes and alleles between groups and the Hardy-Weinberg equilibrium were tested using chi-square tests and Fisher’s exact tests. The genotype distribution comparisons between two groups were done after adjusting genotype distribution for potential confounding factor such as age and gender using analysis of covariance (ANCOVA). The odds ratio (OR) with 95% CI was also calculated. For statistical analysis, we used SPSS 11.0 software to perform. A P value of < 0.05 was considered statistically significant.

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**Table 2**: DNA products size and genotypes of the three digestion sites in the MxA promoter and elf-2a.

<table>
<thead>
<tr>
<th>Digestion sites</th>
<th>PCR Primer sequences (direction 5’ to 3’)</th>
<th>Restriction enzymes</th>
<th>Digestion products (bp)</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MxA promoter -88 G/T</td>
<td>F: TGAAGACCCCCAATTACCAA, R: CTCCTGTTCGCTGCTTTCAC</td>
<td>Hha I</td>
<td>259</td>
<td>GG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>310</td>
<td>TT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>310, 259</td>
<td>GT</td>
</tr>
<tr>
<td>MxA promoter -123 C/A</td>
<td>F: TGAAGACCCCCAATTACCAA, R: CTCCTGTTCGCTGCTTTCAC</td>
<td>Pst I</td>
<td>225, 125</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>350</td>
<td>AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>350, 225, 125</td>
<td>CA</td>
</tr>
<tr>
<td>elf-2a reg2 A/G</td>
<td>F: TGCTGCTAGTTTGTCCAC, R: GCCATGTACGTCAC</td>
<td>Ssp I</td>
<td>476</td>
<td>AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>563</td>
<td>GG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>563, 476</td>
<td>AG</td>
</tr>
</tbody>
</table>

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*Figure 1*: Results of the MxA promoter -88 G/T position gene typing. Lane M: DNA molecular weight maker; Lane 1: TT genotype; Lane 2: GT genotype; Lane 3: GG genotype.
Table 3: Comparisons of three polymorphisms between patients in self-limited and chronic HBV infection.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gender (male/female)</th>
<th>Age (mean±SD)</th>
<th>Gender (male/female)</th>
<th>Age (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>42/24</td>
<td>57.6±13.4</td>
<td>98/30</td>
<td>55.9±13.1</td>
</tr>
<tr>
<td>GT</td>
<td>46/23</td>
<td>55.2±14.7</td>
<td>75/33</td>
<td>53.3±16.6</td>
</tr>
<tr>
<td>TT</td>
<td>18/7</td>
<td>56.5±13.9</td>
<td>5/2</td>
<td>53.9±13.8</td>
</tr>
</tbody>
</table>

Table 4: Gender and age in different genotype of MxA promoter -88 G/T between patients in self-limited and chronic HBV infection.

Table 5: Haplotype analysis of SNPs located at MxA promoter -88 G/T and -123 C/A.

Figure 2: Results of the MxA promoter -123 C/A position gene typing. Lane 1: AA genotype; Lane 2: CC genotype; Lane 3: CA genotype.

Figure 3: Results of the elF-2a reg2 position gene typing. Lane M: DNA molecular weight maker; Lane 1: AG genotype; Lane 2: GG genotype; Lane 3: AA genotype.
addition to the molecular characteristics of the virus and the biology of infection may not play a major role in viral clearance. Therefore, the relationship between MxA promoter and eIF-2α reg2 gene polymorphisms and the natural outcome of HBV infection only shows one aspect of the diverse nature of the host genetic background and its complexity in relationship to viral infection. The evaluation of the relationship between the IFN signaling pathway gene SNP and HBV infection requires the large-scale detection of SNPs. The accumulation of more data that take various factors into consideration is needed to reach a definitive conclusion. In short, because host genetic factors produce changes in the natural outcome of HBV infection, it is worth intensive future study. Compared to eIF-2α reg2 gene SNPs, the G/T polymorphism in the MxA promoter at position -88 is expected to become a predictor of HBV infection outcome and drug treatment responsiveness.

Acknowledgments

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References

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