-173G/C polymorphism in the promoter of MIF is associated with hepatitis B virus infection in a Chinese Han population


1Department of Hepatobiliary Surgery, The Affiliated Hospital of Qingdao University, Qingdao, China
2Shandong Provincial Key Laboratory of Metabolic Disease, The Affiliated Hospital of Qingdao University, Qingdao, China
3Department of Hepatobiliary Surgery, Jiaozhou People Hospital, Qingdao, China
4Clinical Laboratory, Medical College, Qingdao University Qingdao, China
5Prenatal Diagnosis Center, The Affiliated Hospital of Qingdao University, Qingdao, China

Corresponding author: S.H. Liu
E-mail: qdmc_genetics@163.com

Received January 13, 2015
Accepted April 24, 2015
Published July 28, 2015
DOI http://dx.doi.org/10.4238/2015.July.28.23

ABSTRACT. In addition to the host immune response, genetic and environmental factors play crucial roles in the manifestation of hepatitis B virus (HBV) infection. The macrophage migration inhibitory factor (MIF) -173G/C polymorphism (rs755622), located in the promoter region of MIF, may play integral roles in diverse processes, including the immune response. Thus, the MIF -173G/C polymorphism may influence the immune response to HBV during natural infection. We investigated whether the MIF -173G/C polymorphism was associated with susceptibility to HBV infection in a Chinese Han population. A total of 596 HBV infection cases and 612 age-matched controls were recruited for the study. Genotyping of the MIF -173G/C polymorphism was performed using the allele-specific polymerase chain reaction method. The frequencies of the alleles and genotypes in patients and
controls were compared using the \( \chi^2 \) test. Carriers of the variant C allele in MIF -173 G/C were at significantly higher risk of HBV infection than carriers of the wild-type allele (\( P = 0.032 \), odds ratio = 0.799, 95% confidence interval = 0.651-0.981). However, there was no significant difference in the distribution of MIF -173G/C genotypes between case and control groups in either population (\( P = 0.096 \), degrees of freedom = 2). Our findings indicate that the G to C base change in MIF -173 G/C confers an increased risk of development of HBV infection by altering the expression of MIF in our Chinese Han population.

**Key words:** Hepatitis B virus; MIF-173G/C; Polymorphism

**INTRODUCTION**

Epidemiological studies have provided evidence that more than 350 million people worldwide are chronically infected with the hepatitis B virus (HBV) and that approximately 500,000-700,000 people die annually from this disease. The precise mechanisms of HBV infection have not yet been clearly defined, but host immune and genetic factors are considered to be important factors contributing to HBV infection (Thursz, 2001; Chu and Lok, 2002). Cytokines play a significant role in the HBV immune defense. Several pro-inflammatory cytokines such as T helper (Th) 1 cytokines [including interleukin (IL)-2 and interferon-gamma] have been found to participate in the process of viral clearance and the host immune response to HBV. In contrast, the Th2 cytokine (IL-10) serves as a potent inhibitor of Th1 effector cells in HBV diseases (Gao et al., 2009). Therefore, as genetic predictors of disease susceptibility or clinical outcome, cytokine gene polymorphisms may be important (Ramezani et al., 2012).

Macrophage migration inhibitory factor (MIF), a pro-inflammatory T-cell cytokine mainly produced by immune cells (including T-cells, macrophages, and monocytes), is widely expressed in both immune and nonimmune cells and plays an essential role in the pathophysiology of host immune and inflammatory responses (Calandra and Roger, 2003; Baugh and Bucala, 2002). MIF has the potential not only to activate monocytes and macrophages but also to stimulate T cells. MIF is a significant regulator of the host response to HBV infection (Zhang et al., 2005). The serum MIF level is elevated in different disorders, including various inflammatory and autoimmune diseases (Zhang et al., 2002). As a functional single-nucleotide polymorphism (G/C) of the 5' untranslated region at position 173, MIF -173 G/C (rs755622) has been associated with altered MIF protein expression *in vitro* (Donn et al., 2002). Therefore, we hypothesized that the MIF -173 G/C polymorphism may, by regulating MIF production, lead to activities against pathogens, activate effector cells involved in the cellular interactions that occur during inflammation, is part of the acute and chronic stages of viral hepatitis, and induce target-cell apoptosis. In this study, we examined the genetic association between the MIF -173G/C polymorphism and HBV infection in patients in a Chinese Han population. Our results may help to explain the pathogenic mechanism of chronic hepatitis B and provide insights into the treatment of HBV.

**MATERIAL AND METHODS**

**Study subjects**

HBV-infected patients and controls were recruited from the Affiliated Hospital of the
Qingdao University Medical College. A total of 596 HBV-infected patients and 612 ethnically matched controls were genotyped for the MIF -173G/C polymorphism. The patient age was 36.54 ± 12.76 (means ± SD) years; 384 (64.4%) subjects were male and 212 (35.6%) were female. The age of the controls was 38.48 ± 11.58 (means ± SD) years; 384 (62.7%) controls were male and 228 (37.3%) were female.

Chronic HBV infection was diagnosed based on the Proposal of Prevention and Treatment of Viral Hepatitis, 2005, issued by the Chinese Society of Infectious Diseases and Parasitology and the Society of Hepatology of the Chinese Medical Association (Delaloye et al., 2012). The healthy controls were hepatitis B surface antigen/HBV-DNA/anti-HBc with normal liver function tests. All patients and controls were repeatedly negative for serologic markers of hepatitis C virus and human immunodeficiency virus. All subjects were provided written informed consent, and the study protocol was approved by the Ethics Committee of Affiliated Hospital of the Qingdao University Medical College.

Genotyping

Genomic DNA was extracted from 5 mL peripheral blood leukocytes using standard methods. Genotyping of the MIF -173G/C polymorphism was performed using the allele-specific polymerase chain reaction (PCR) method. The segment was replicated using the following primers: 5’-CGC CAA GTG GAG AAC AGG-3’, 5’-CGC CAA GTG GAG AAC AGC-3’ and 5’-GCA GAG GCA CAG ACG CA-3’. PCR was carried out in a final volume of 20 μL containing 2X PCR MasterMix, 0.4 mM of each primer, and 100 ng genomic DNA. The reaction for MIF -173G/C was carried out as follows: 94°C for 5 min; 30 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 1 min; and 1 cycle at 72°C for 7 min. The PCR products were isolated by 2% agarose gel electrophoresis and visualized with ethidium bromide. The MIF -173G/C polymorphic genotypes were categorized into homozygous wild, heterozygous, and homozygous variant. More than 30 subjects were selected to confirm the genotype using DNA sequencing techniques.

Statistical analysis

In this study, the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The goodness of fit χ² test was used to examine Hardy-Weinberg equilibrium in HBV-infected patients and controls. The Pearson χ² test was used to compare the genotype and allele frequencies between controls and patients (if expected values were below 5, Fisher’s exact test was used). The odds ratios (ORs) and 95% confidence intervals (95%CIs) were used to determine the strength of relationships in the genotype distribution and allele frequencies between the patient cases and controls, and between virus-replication patients and non-virus replication patients. P < 0.05 was considered to be statistically significant.

RESULTS

The genetic contribution of control population and cases were in Hardy-Weinberg equilibrium for MIF -173G/C (P = 0.391 for patients, P = 0.886 for controls). Table 1 lists the genetic distribution of this polymorphism in genotypes and alleles between cases and controls. Evaluation of polymorphisms within the -173G/C region of MIF showed the presence of the C/C genotype in 28 (4.7%) and 18 (2.9%), the G/C genotype in 186 (31.2%) and 171 (28.0%), and
MIF is associated with HBV infection

The G/G genotype in 382 (64.1%) and 423 (69.1%) patients and controls, respectively. Statistical analysis showed that differences in this genotype were not significant ($\chi^2 = 4.681, P = 0.096$, degrees of freedom = 2) and there was no association with genotype frequency. The C allele was present in 242 (20.3%) and 207 (16.9%) patients and controls, respectively. The G allele was observed in 950 (79.7%) patients and 1017 (83.1%) controls. Statistical analysis showed a statistically significant difference in these alleles ($P = 0.032, OR = 0.799, 95%CI = 0.651-0.981$). We also analyzed genotype and allele frequencies in males and females (Tables 2 and 3). There was also no association between genotype frequency and HBV in males and females. In the male population, the MIF -173 C allele frequencies showed an association with HBV infection ($P = 0.026, \chi^2 = 4.928, OR = 0.754, 95%CI = 0.587-0.968$ by alleles), which differed from that in the female population ($P = 0.227, \chi^2 = 1.457, OR = 0.804, 95%CI = 0.565-1.146$ by alleles).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>382 (64.1%)</td>
<td>423 (69.1%)</td>
<td>0.096</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>28 (4.7%)</td>
<td>18 (2.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>186 (31.2%)</td>
<td>171 (28.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>950 (79.7%)</td>
<td>1017 (83.1%)</td>
<td>0.032</td>
<td>0.651-0.981</td>
</tr>
<tr>
<td>C</td>
<td>242 (20.3%)</td>
<td>207 (16.9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Association between the polymorphism MIF -173 G/C according to genotype and allele between cases and controls.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>241 (62.8%)</td>
<td>260 (67.7%)</td>
<td>0.210</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>21 (5.5%)</td>
<td>13 (3.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>122 (31.7%)</td>
<td>111 (28.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>604 (78.6%)</td>
<td>631 (82.2%)</td>
<td>0.026</td>
<td>0.587-0.968</td>
</tr>
<tr>
<td>C</td>
<td>164 (21.4%)</td>
<td>137 (17.8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Association between the MIF -173 G/C polymorphism according to genotype and allele between cases and controls in the male population.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>141 (66.5%)</td>
<td>163 (71.5%)</td>
<td>0.478</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>7 (3.3%)</td>
<td>5 (2.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>64 (30.2%)</td>
<td>60 (26.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>346 (81.6%)</td>
<td>386 (84.6%)</td>
<td>0.227</td>
<td>0.565-1.146</td>
</tr>
<tr>
<td>C</td>
<td>78 (18.4%)</td>
<td>70 (15.4%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Association between the MIF -173 G/C polymorphism according to genotype and allele between cases and controls in female population.

DISCUSSION

In this study, we investigated the association between MIF -173G/C and the susceptibility to persistent HBV infection in a Chinese population. We found that the G to C base change in MIF -173G/C increased the risk of HBV infection.

The HBV infection is known to be largely regulated by immune molecules such as
cytokines and other soluble factors, which are commonly found to be altered in HBV infection (Cheong et al., 2006). Cytokines can balance the immune response and are considered to play a pivotal role in the pathogenesis of HBV infection. T cell derived cytokines are important in the host immune response (Ramezani et al., 2012). MIF can not only activate monocytes/macrophages but also stimulate T cells. MIF has also pro-inflammatory effects both for in vivo and in vitro models of Th1- and Th2-type inflammation. In several model systems, MIF was found to “override” or counter-regulate the broad, immunosuppressive action of glucocorticoids in immune cells (Calandra et al., 1995). The serum MIF level is elevated in different types of disorders, including various inflammatory and autoimmune diseases. Anti-MIF antibodies inhibit T cell proliferation and IL-2 production in vitro and suppress antigen-driven T cell activation and antibody production in vivo (Bacher et al., 1996). Polymorphisms in MIF have been associated with Th2 diseases such as atopic dermatitis, asthma, and several Th1-biased inflammatory conditions (Hizawa et al., 2004; Mizue et al., 2005; Wu et al., 2009). MIF is considered to be the first inflammatory cytokine regulating both innate and acquired immune responses to bacterial and parasitic infections. MIF influences viral diseases such as sepsis and human immunodeficiency virus (Lehmann et al., 2009; Delaloye et al., 2012).

The levels of MIF production determine immune regulation and the balance between inflammatory and anti-inflammatory responses. MIF is an important regulator of the host response to HBV infection (Zhang et al., 2002). There is some evidence that the capacity of MIF production is a major genetic component playing a role in sustaining cell-mediated hepatic injury during the immune-clearance phase of chronic hepatitis B infection (Bacher et al., 1996). Zhang et al. (2002) showed that serum MIF levels in chronic virus hepatitis B patients were significantly higher than those in normal healthy controls. MIF participates in the pathological process of chronic virus hepatitis B and that serum levels of MIF appear to reflect the severity of tissue injury in HBV disease (Zhang et al., 2002). MIF plays a role in sustaining cell-mediated hepatic injury during the immune-clearance phase of chronic hepatitis B infection (Zhang et al., 2005). Several studies have reported the involvement of genetic variation in MIF in enhancing the susceptibility to a large number of autoimmune and inflammatory diseases, such as rheumatoid arthritis (Martínez et al., 2007), inflammatory polyarthritis (Barton et al., 2003), and Crohn’s disease (Donn et al., 2002). Serum levels of MIF were significantly higher in individual subjects carrying the MIF -173C allele (Donn et al., 2002). Furthermore, the MIF -173G/C polymorphism has been shown to be functional variant both in vitro and in vivo (Ziino et al., 2005) and can effect MIF expression (Benigni et al., 2000).

Our results showed that the frequencies of the evaluated (G and C) alleles of MIF -173 differed between HBV-infected patients and healthy controls. Therefore, based on our results, it may be concluded that this polymorphism is associated with HBV infection. To our knowledge, this is the first study to evaluate the MIF -173G/C polymorphism in HBV-infected patients. We suggest that the MIF -173G/C polymorphism affected MIF production. This polymorphism may alter the function of the immune pathways and subsequently enhance the risk of developing HBV infection. In contrast, because of the lower absolute numbers of females among our HBV patients, our study may not be sufficiently powerful to replicate the gender-specific association between the minor MIF -173 C allele and HBV infection observed in females in this study.

The strength of this present study is the high number of the evaluated blood donors. However, there is a lack of a functional link between the polymorphism of MIF -173G/C and its role in regulating MIF expression. In future studies, this can be resolved using luciferase report-
er assays in which reporter expression is studied under the control of the wild-type and disease promoters. It would also be interesting to measure and compare circulating serum levels of MIF in HBV infection patients carrying the MIF -173G/C polymorphism and in healthy controls.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank all probands for their participation. Research supported by the National Natural Science Foundation of China (#81371499 and #30971586).

REFERENCES

Zhang W, Yue B, Wang GQ and Lu SL (2002). Serum and ascites levels of macrophage migration inhibitory factor, TNF-