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

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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 χ^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 an important 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 χ^2 test was used to examine Hardy-Weinberg equilibrium in HBV-infected patients and controls. The Pearson χ^2 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

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

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

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

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

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

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

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.

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REFERENCES

Bacher M, Metz CN, Calandra T, Mayer K, et al. (1996). An essential regulatory role for macrophage migration inhibitory factor in T- cell activation. *Proc. Natl. Acad. Sci. U. S. A*. 93: 7849-7854.

Barton A, Lamb R, Symmons D, Silman A, et al. (2003). Macrophage migration inhibitory factor (MIF) gene polymorphism is associated with susceptibility to but not severity of inflammatory polyarthritis. *Genes Immun*. 4: 487-491.

Baugh JA and Bucala R (2002). Macrophage migration inhibitory factor. *Crit. Care Med*. 30: S27-S35.

Benigni F, Atsumi T, Calandra T, Metz C, et al. (2000). The proinflammatory mediator macrophage migration inhibitory factor induces glucose catabolism in muscle. *J. Clin. Invest.* 106: 1291-1300.

Calandra T and Roger T (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat. Rev. Immunol*. 3: 791-800.

Calandra T, Bernhagen J, Metz CN, Spiegel LA, et al. (1995). MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* 377: 68-71.

Cheong JY, Cho SW, Hwang IL, Yoon SK, et al. (2006). Association between chronic hepatitis B virus infection and interleukin-10, tumor necrosis factor-alpha gene promoter polymorphisms. *J. Gastroenterol. Hepatol.* 21: 1163-1169.

Chu CJ and Lok AS (2002). Clinical significance of hepatitis B virus genotypes. *Hepatology* 35: 1274-1276.

Delaloye J, De Bruin IJ, Darling KE, Reymond MK, et al. (2012). Increased macrophage migration inhibitory factor (MIF) plasma levels in acute HIV-1 infection. *Cytokine* 60: 338-340.

Donn R, Alourfi Z, De Benedetti F, Meazza C, et al. (2002). Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. *Arthritis Rheum*. 46: 2402-2409.

Gao QJ, Liu DW, Zhang SY, Jia M, et al. (2009). Polymorphisms of some cytokines and chronic hepatitis B and C virus infection. *World J. Gastroenterol*. 15: 5610-5619.

Hizawa N, Yamaguchi E, Takahashi D, Nishihira J, et al. (2004). Functional polymorphisms in the promoter region of macrophage migration inhibitory factor and atopy. *Am. J. Respir. Crit. Care Med*. 169: 1014-1018.

Lehmann LE, Book M, Hartmann W, Weber SU, et al. (2009). A MIF haplotype is associated with the outcome of patients with severe sepsis: a case control study. *J. Transl. Med*. 7: 100.

Martínez A, Orozco G, Varadé J, López MS, et al. (2007). Macrophage migration inhibitory factor gene: influence on rheumatoid arthritis susceptibility. *Hum. Immunol*. 68: 744-747.

Mizue Y, Ghani S, Leng L, McDonald C, et al. (2005). Role for macrophage migration inhibitory factor in asthma. *Proc. Natl. Acad. Sci. U. S. A*. 102: 14410-14415.

Ramezani A, Banifazl M, Mamishi S, Sofian M, et al. (2012). The influence of human leukocyte antigen and IL-10 gene polymorphisms on hepatitis B virus outcome. *Hepat. Mon*. 12: 320-325.

Thursz M (2001). Genetic susceptibility in chronic viral hepatitis. *Antiviral Res*. 52: 113-116.

Wu J, Fu S, Ren X, Jin Y, et al. (2009). Association of MIF promoter polymorphisms with childhood asthma in a northeastern Chinese population. *Tissue Antigens* 73: 302-306.

Zhang HY, Nanji AA, Luk JM, Huang XR, et al. (2005). Macrophage migration inhibitory factor expression correlates with inflammatory changes in human chronic hepatitis B infection. *Liver Int*. 25: 571-579.

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

alpha and IL-6 in patients with chronic virus hepatitis B and hepatitis cirrhosis. *Hepatobiliary Pancreat. Dis. Int.* 1: 577-580.

Ziino O, D'Urbano LE, De Benedetti F, Conter V, et al. (2005). The MIF -173G/C polymorphism does not contribute to prednisone poor response *in vivo* in childhood acute lymphoblastic leukemia. *Leukemia* 19: 2346-2347.