PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(11):1594-1598
World Journal of Gastroenterology ISSN 1007-9327

Association of polymorphisms of interleukin-18 gene promoter region with chronic hepatitis B in Chinese Han population

Ping-An Zhang, Jian-Min Wu, Yan Li, Xiang-Sheng Yang

Ping-An Zhang, Jian-Min Wu, Department of Laboratory Science, Affiliated Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
Yan Li, Xiang-Sheng Yang, Department of Laboratory Science, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China
Correspondence to: Ping-An Zhang, Department of Laboratory Science, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China. cydyyjyk@public.wh.hb.cn
Telephone: +86-27-88041911-8258
Received: 2004-11-08 Accepted: 2004-11-24

Abstract

AIM: To investigate the polymorphisms of interleukin-18 (IL-18) gene promoters, and to disclose whether such polymorphisms are associated with susceptibility to chronic hepatitis B in Chinese Han population.

METHODS: Using polymerase chain reaction with sequence specific primers (PCR-SSP) method, the single nucleotide polymorphisms (SNPs) of the promoter region of IL-18 gene at position -607 and -137 were detected in 231 patients with chronic hepatitis B and 300 normal controls.

RESULTS: Allele C at position -607 in the promoter of IL-18 gene was detected in 48.7% of normal controls and 51.9% of patients, while allele A at position -607 was detected in 51.3% of normal controls and 48.1% of patients. The frequencies of -607CC, -607 CA and -607AA genotypes in normal controls were 22.0%, 53.3% and 24.7% respectively and in chronic hepatitis B patients were 26.8%, 50.2% and 23.0% respectively. Allele G at position -137 in the promoter of IL-18 gene was detected in 82.3% of normal controls and 88.5% of chronic hepatitis B patients, while allele C at position -137 was detected in 17.7% of normal controls and 11.5% of patients. The frequencies of -137GG, GC and CC genotype were 67.3%, 30.0% and 2.7% in normal controls respectively, while in chronic hepatitis B patients were 78.8%, 19.5% and 1.7% respectively. The frequency of -137GG genotype in chronic hepatitis B groups was significantly higher than that in normal controls (χ^2 = 8.55, P = 0.003 <0.05), whereas the frequencies of -607C/-137C and -607A/-137C haplotypes in chronic hepatitis B groups were significantly lower than that in normal controls. The association between genotypes of IL-18 promoter region polymorphisms and HBV copies showed that the frequency of -607AA genotype in high HBV-DNA copies groups was lower than that in low HBV-DNA copies groups (χ^2 = 6.03, P = 0.014 <0.05).

CONCLUSION: The polymorphisms of the promoter region of IL-18 gene at position -607 and -137 are closely associated with susceptibility to chronic hepatitis B. The people with allele C at position -137 in the promoter of IL-18 gene may be protected against HBV infection; moreover AA genotype at position -607 may be closely linked to inhibit HBV-DNA replication. These findings give some new clues to the study of pathogenesis of chronic hepatitis B.

Key words: Interleukin-18 gene; Polymorphism; Chronic hepatitis B

Zhang PA, Wu JM, Li Y, Yang XS. Association of polymorphisms of interleukin-18 gene promoter region with chronic hepatitis B in Chinese Han population. *World J Gastroenterol* 2005; 11(11): 1594-1598
http://www.wjgnet.com/1007-9327/11/1594.asp

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 5-10% of cases. 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 the persistence of the virus. But it has been thought that genetic associations may also provide clues to the development of HBV infection. Some polymorphisms have been reported to be involved in susceptibility to chronic hepatitis B, in disease severity and progression, or in disease prognosis[3-5].

Several recent advances concerning the polymorphism of cytokines controlling the host response could play an important role in determining HBV infection outcome[6,7]. Being involved in the proinflammatory cytokine network, interleukin-18 (IL-18) is a novel cytokine that is mainly produced by activated macrophages and, like interleukin-12

(IL-12), is able to induce interferon-gamma (IFN-gamma) and tumor necrosis factor-alpha (TNF-alpha) induction, as well as enhancing the cytotoxicity of NK cells and FasL expression[8-10]. Clinical research showed that there was a correlation between the levels of serum IL-18 and disease severity in patients with viral hepatitis B[11]. Meanwhile, *in vitro* IL-18 can improve the peripheral blood monocytes from chronic hepatitis B patients to produce a great deal of IFN-gamma. These findings indicate the evidence of an association between susceptibility to chronic hepatitis B and IL-18 gene. Three single nucleotide polymorphisms (SNPs) in the promoter of IL-18 gene at the position -656G/T, -607C/A and -137G/C have been identified, and the two SNPs at position -607C/A and -137G/C in the promoter were predicted to be nuclear factor binding sites for cAMP-responsive element binding protein and H4TF-1 nuclear factor, respectively; moreover mutation of the two sites can influence the expression of IL-18 and also potentially of IFN-gamma[12]. In order to investigate the possible roles of the SNPs of IL-18 gene promoter region in the development and progression of chronic hepatitis B, we genotyped 231 patients with chronic hepatitis B and 300 control subjects for two IL-18 SNPs, using polymerase chain reaction with sequence specific primers (PCR-SSP) method.

MATERIALS AND METHODS

Subjects

A total of 231 unrelated Chinese subjects with chronic hepatitis B (156 males, 75 females) aged 5-82 years were recruited in Remin Hospital of Wuhan University. The diagnosis of all the patients was confirmed according to the criteria for chronic hepatitis B[13,14], and the patients did not have other viral hepatitis. Three hundred control subjects (183 males, 117 females) aged 18-81 years were randomly selected in Wuhan area, China during the same period, with definitely negative for HBsAg, anti-HBe and anti-HBc and with no history of HBV vaccination. They did not have any abnormalities based on physical examination, chest radiography, electrocardiogram, urinalysis and routine laboratory blood testing. Liver, renal, endocrine and cardiovascular disorders were excluded. All the subjects were Chinese Han people and they were recruited with their informed consent for genetic analysis.

Specimens preparation

Two microliters of peripheral venous blood were drawn from all the subjects after an overnight fasting and collected in an EDTA tube. Genomic DNA was extracted from peripheral blood leukocytes with standard techniques and frozen at -20 ℃.

Determination of the IL-18 genotypes

Polymorphisms were analyzed by using PCR-SSP, at the position -607 and -137 in the promoter of IL-18 gene[12]. For the position -607C/A-specific PCR, a common reverse primer 5'-TAACCTCATTCAGGACTTCC-3' and two sequence-specific forward primers 5'-GTTGCAGAAA GTGTAAAAATTATTAC-3' and 5'-GTTGCAGAAAG TGTAAAAATTATTAA-3' were used. An amplification product of 196 bp was detected. A control forward primer 5'-CTTTGCTATCATTCCAGGAA-3' was used to amplify a 301-bp fragment covering the polymorphic site as an internal positive amplification control. PCR reaction was performed in a final volume of 15 μL consisting of 1.5 μL 10× PCR buffer, 0.2 mmol/L dNTP, 30 ng genomic DNA and 0.5 U Taq polymerase. One sequence specific primer (for allele C or allele A) and the common reverse primer were included in every reaction mixture at a concentration of 0.6 μmol/L. In addition, the internal position control primer was added to the reaction mixture at a concentration of 0.15 μmol/L. Therefore, two PCR reactions were performed for every individual DNA.

Reactions were carried out in a GenAmp PCR system 2 700 thermal cycler. At the first step, denaturation for 2 min at 94 ℃ was performed, followed by seven cycles of 94 ℃ for 20 s, 64 ℃ for 40 s and 72 ℃ for 40 s and 25 cycles 94 ℃ for 20 s, 57 ℃ for 40 s, 72 ℃ for 40 s and 72 ℃ for 5 min. PCR products were visualized by 2% agarose gel electrophoresis stained by ethidium bromide.

For the -137 genotyping, a common reverse primer 5'-AGGAGGGCAAAATG CACTGG-3' and two sequence-specific forward primers 5'-CCCCAACTTTTACGGAA GAAAAG-3' and 5'-CCCCAACTTTTACGGAAGAAAAC-3' were used. An amplification product of 261 bp was detected. A control forward primer 5'-CCAATAGGAC TGATTAT TCCGCA-3' was used to amplify a 446-bp fragment covering the polymorphic site to serve as an internal positive amplification control. PCR reaction was performed in a final volume of 15 μL consisting of 1.5 μL 10× PCR buffer, 0.2 mmol/L dNTP, 30 ng genomic DNA and 0.5 U Taq polymerase. Concentrations were of 0.3 μmol/L of the control primer and 0.5 μmol/L of the reverse primer and sequence-specific primers used. At the first PCR step, denaturation for 2 min at 94 ℃ was performed, followed by five cycles of 94 ℃ for 20 s, 68 ℃ for 1 min and 25 cycles of 94 ℃ for 20 s, 62 ℃ for 40 s, 72 ℃ for 40 s and 72 ℃ for 5 min.

HBV-DNA measurement

Serum HBV-DNA levels in patients with chronic hepatitis B were detected with the real-time fluorescent quantitative PCR method (reagents supplied by Shanghai Fosun Co. Ltd) using a Lightcycler PCR system. Results were considered abnormal when HBV-DNA $>1\times10^3$ copies/mL.

Statistical analysis

The frequencies of genotypes and alleles in the promoter region of IL-18 gene at position -607 and -137 were calculated by counting. Data were analyzed with SPSS11.5 software and the Hardy-Weinberg equilibrium was determined by means of the χ^2 test. Comparison of allelic and genotypes between groups, and association of -607C/A, -137G/C polymorphisms with HBV-DNA replication were examined for statistical significance with χ^2 test. The odds ratio (OR) was calculated by means of logistic regression and the confidence interval (CI) was calculated at the 95% level. Statistical significance was assumed for *P* values less than 0.05.

RESULTS

Polymorphisms in promoter of the IL-18 gene

Polymorphisms at the position -607 and -137 in the promoter of IL-18 gene were analyzed by PCR-SSP. In every polymorphic site, a common reverse primer and two sequence-specific forward primers were used and two PCR reactions were performed for every individual DNA. The specific products of PCR from homozygous individuals were one DNA segment and from heterozygous individuals showed the expected two specific fragments. In total, 531 unrelated Chinese subjects were studied for IL-18 promoter polymorphisms. As shown in Figure 1, there were CC, CA and AA genotypes at position -607, and GG, GC and CC genotypes at position -137.

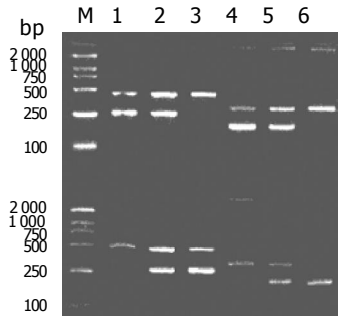

Figure 1 Genotyping for the IL-18 position -137 and -607 polymorphisms. M: DL2000 DNA Marker; lanes 1–3: -137 GG, GC and CC; lanes 4-6: -607 CC, CA and AA.

Frequencies of IL-18 promoter genotypes in both groups

Genotype and allele frequencies for IL-18 polymorphisms are summarized in Table 1. The genotype frequencies were in agreement with the Hardy-Weinberg ($P>0.1$ for all analyses). As the -607 genotypes, of 231 patients with chronic hepatitis B, 62 had the CC type (26.8%), 116 the CA type (50.2%) and 53 the AA (23.0%). Of the 300 control subjects, 66 had the CC type (22.0%), 160 the CA type (53.3%) and 74 the AA (24.7%). No significant difference in the genotype distribution or in the allele frequency between the patients with chronic hepatitis B and the control subjects was observed. As for the -137 genotypes, 182 of the 231 patients with chronic hepatitis B had the GG type (78.8%), 45 the GC type (19.5%) and 4 the AA type (1.7%). Two hundred and two of the 300 control subjects were type GG (67.3%), 90 were GC (30.0%) and 8 were CC (2.7%). There was a significant difference in the genotype distribution and in the allele frequency between the patients with chronic hepatitis B and the control subjects. In genotypes, the GG type at position -137 was present at a significantly higher frequency in the patients with chronic hepatitis B compared to those in the controls. OR of the GG genotype for the comparison with that of the GC and the CC genotype was 1.80 (95%CI 1.21-2.68, $P = 0.01 <0.05$). But in phenotypes, the allele C at -137 was of a significantly lower frequency in the patients with chronic hepatitis B than that in the controls ($\chi^2 = 7.87$, $P = 0.005 <0.05$).

Based on genotypes of IL-18 promoter polymorphisms, haplotype frequencies were estimated by the expectation-maximization method. Four haplotypes of the IL-18 promoter at position -607 and -137 were present in both patients and controls (haplotypes I, II, III and IV in Table 2). The frequencies of haplotype I, II, III and IV in the patients with chronic hepatitis B were 51.7%, 0.2%, 36.8% and 11.3%, respectively. The frequencies of haplotype I, II, III and IV in the controls were 47.4%, 1.3%, 35.0% and 16.3%, respectively. The frequencies of haplotype II and IV, which bear C at -137, in the patients were significantly lower than that in the health control subjects.

Table 1 Comparison of IL-18 gene promoter polymorphism between patients with chronic hepatitis B and controls

Polymorphism	Control $n = 300$ (%)	Patient $n = 231$ (%)	χ^2	P
Position -607				
Genotypes				
CC	66 (22.0)	62 (26.8)	1.67	0.196
CA	160 (53.3)	116 (50.2)	0.51	0.476
AA	74 (24.7)	53 (23.0)	0.21	0.645
Alleles				
C	292 (48.7)	240 (51.9)	1.12	0.842
A	308 (51.3)	222 (48.1)		
Position -137				
Genotypes				
GG	202 (67.3)	182 (78.8)	8.55	0.003
GC	90 (30.0)	45 (19.5)	7.62	0.006
CC	8 (2.7)	4 (1.7)	0.52	0.472
Alleles				
G	494 (82.3)	409 (88.5)	7.87	0.005
C	106 (17.7)	53 (11.5)		

Table 2 Haplotype frequencies of two interleukin-18 bi-allelic polymorphisms in chronic hepatitis B and health controls

Haplotype	-607C/A	-137G/C	Control n (%)	Patient n (%)	χ^2	P
I	C	G	284 (47.4)	239 (51.7)	2.02	0.155
II	C	C	8 (1.3)	1 (0.2)	3.87	0.049
III	A	G	210 (35.0)	170 (36.8)	0.37	0.545
IV	A	C	98 (16.3)	52 (11.3)	5.55	0.018
Total			600	462		

Association of IL-18 gene promoter polymorphism with HBV-DNA replication

For further analysis of the relationship between IL-18 gene promoter polymorphisms and HBV-DNA replication in patients with chronic hepatitis B, the patients were divided into two sub-groups (HBV-DNA $<1\times10^3$ copies/mL as sub-group I, HBV-DNA $\geqslant1\times10^3$ copies/mL as sub-group II). As shown in Table 3, the distribution of AA genotype in the IL-18 gene promoter at position -607 were significantly different between the two sub-groups ($\chi^2 = 6.03$, $P = 0.014 <0.05$).

DISCUSSION

Individuals, with an inadequate primary immune response to HBV, are at increased risk of developing chronic hepatitis

Table 3 The association between genotypes of IL-18 promoter region polymorphisms and HBV copies in the chronic hepatitis B patients

Polymorphism	Group I n = 97 (%)	Group II n = 134 (%)	χ^2	P
Position -607				
Genotypes				
CC	23 (23.7)	39 (29.1)	0.83	0.361
CA	44 (45.4)	72 (53.7)	1.58	0.209
AA	30 (30.9)	23 (17.2)	6.03	0.014
Alleles				
C	90 (46.4)	150 (56.0)	4.14	0.042
A	104 (53.6)	118 (44.0)		
Position -137				
Genotypes				
GG	76 (78.4)	106 (79.1)	0.02[1]	0.890
GC	20 (20.6)	25 (18.7)		
CC	1 (1.0)	3 (2.2)		
Alleles				
G	172 (88.7)	237 (88.4)	0.01	0.940
C	22 (11.3)	31 (11.6)		

Group I: HBV-DNA $<1\times10^3$ copies/mL; Group II: HBV-DNA $\geqslant1\times10^3$ copies/mL. GG compared with both GC and CC.

B. Age is the strongest host feature associated with chronic infection with 90% infants and 5-10% of adults developing chronic hepatitis B after exposure[15,16]. In addition, people belonging to the same age, sex and ethnical groups were exposed to the same HBV strain, which could cause a broad spectrum ranging from no infection to different clinical outcomes. These data suggest that host genetic factors are responsible for the clinical outcomes of HBV infection[17]. Clearance of HBV requires a coordinated innate and adaptive humoral and cell-mediated immune response. Cytokines are soluble polypeptide molecules that mediate cell-to-cell communication and regulate the intensity and duration of the immune response. Previous studies have shown that the maximal capacity of cytokine production varies among individuals and correlates with SNPs in the promoter region of various cytokine genes. Furthermore, cytokine gene polymorphisms were associated with liver disease severity in patients with viral hepatitis B[18-22], which may provide clues to understand the development of end-stage complications such as cirrhosis or hepatocellular carcinoma. In the present study, we compared the distributions of IL-18 gene promoter polymorphisms between patients with chronic B and control subjects.

IL-18 was first described as an IFN-gamma inducing factor, and has multiple functions including induction of the synthesis of IFN-gamma by T cells and NK cells, promotion of Th1-type immune responses, augmentation of proliferative response and cytokine production of activated T cells. Meanwhile, IL-18 leads to activities against pathogens, activate effector cells involved in the cellular interactions that occur during inflammation, and are part of the acute and chronic stages of viral hepatitis, induce target-cells apoptosis[23,24]. Recently, it was reported that injection of a single 10-μg dose of recombinant murine IL-18 rapidly, reversibly and non-cytopathically inhibited HBV replication in the livers of HBV transgenic mice. The anti-viral effect of IL-18 was mediated by its ability to activate resident intrahepatic NK cells and T cells to produce IFN-gamma and by its ability to induce IFN-alpha/beta production in the liver[25]. These results suggest that IL-18 has the potential to contribute to the control of HBV replication during self-limited infection and that it may have therapeutic value for the treatment of patients with chronic hepatitis. The human IL-18 gene is located on chromosome 11q22.2-q22.3, and is composed of six exons and five introns[26]. Giedraitis *et al*[12,27,28] described that there were three SNPs at position -656G/T, -607C/A and -137G/C in the promoter of IL-18 gene first exon. A change from C to A at position -607 disrupts a potential cAMP-responsive element-binding protein binding site and a change at position -137 from G to C changes the H4TF-1 nuclear factor binding site. Cloning and gene expression analysis showed that two SNPs of the promoter of IL-18 gene at position -607 and -137 were suggested to cause the differences in transcription factor binding and have an impact on IL-18 gene activity and potentially also to IFN-gamma. Potentially, the G/C polymorphisms at position -137 could play a main role in the expression of IL-18. Individuals with CC genotype at position -137 had higher levels of IL-18 mRNA compared to other genotypes, that had a clear correlation between IL-18 and IFN-gamma mRNA expression.

In the study, we identified two polymorphisms in the promoter regions of the IL-18 gene and demonstrated the association between these polymorphisms and chronic hepatitis B. The results showed that no significant differences were seen in the distribution of the genotypes or allelic frequencies for polymorphisms of IL-18 gene promoter at position -607 between patients with chronic hepatitis B and control subjects. However, the genotypes distributions and allelic frequencies at position -137 in both groups were statistically different. The genotype frequency of -137GG in chronic hepatitis B groups was significantly higher than that in normal controls ($\chi^2 = 8.55$, $P = 0.003 < 0.05$). The OR was 1.80 for chronic hepatitis B when the genotype at position -137 was GG. Meanwhile, haplotype frequencies' distributions suggested that the frequencies of -607C/-137C and -607A/-137C haplotypes in chronic hepatitis B groups were significantly lower than that in normal controls. These results indicated that the carriage of allele C at position -137 plays a protective role in the development of HBV infection. Further analysis of the relationship between IL-18 gene promoter polymorphism and HBV-DNA replication in patients with chronic hepatitis B showed that AA genotype at position -607 in the IL-18 gene promoter was associated with HBV-DNA replication ($\chi^2 = 6.03$, $P = 0.014 < 0.05$). To our knowledge, there is no published study, concerning the role of the promoter of IL-18 gene in other infection diseases, because of the complication of IL-18 regulation function. Taking into consideration our findings and those of Giedraitis *et al*, there may be a possible link between G→C polymorphism at position -137 of the promoter of IL-18 gene and the increased levels of IL-18, carriage of allele C at position -137 of each of these polymorphisms was related with high production of IL-18, which may augment the production of IFN-gamma, modulate activity of NK and CTL cells, and trigger the complex immunological processes to eliminate HBV and its complex. As for the

frequency of -607AA genotype in low HBV-DNA copies groups was higher than that in high copies groups, whether individualism with -607AA genotype could inhibit HBV-DNA replication, further investigation of the general association of the polymorphisms with cAMP-responsive element-binding protein binding site in an independent data set is needed.

In summary, the findings of this study and others may provide further evidence that genetic factors are important in the pathogenesis of HBV infection. Our results suggest that the carriage of allele C at position -137 in the promoter of IL-18 gene may play a protective role in the development of HBV infection and AA genotype at position -607 may be associated with HBV-DNA replication. However, the real roles of IL-18 gene promoter polymorphisms in the pathogenesis of developing chronic hepatitis B should be further investigated by large population-based studies.

REFERENCES

1 **Perrillo RP**. How will we use the new antiviral agents for hepatitis B? *Curr Gastroenterol Rep* 2002; **4**: 63-71
2 **Wang KX**, Peng JL, Wang XF, Tian Y, Wang J, Li CP. Detection of T lymphocyte subsets and mIL-2R on surface of PBMC in patients with hepatitis B. *World J Gastroenterol* 2003; **9**: 2017-2020
3 **de Andrade DR**, de Andrade DR. The influence of the human genome on chronic viral hepatitis outcome. *Rev Inst Med Trop Sao Paulo* 2004; **46**: 119-126
4 **Thio CL**, Carrington M, Marti D, O'Brien SJ, Vlahov D, Nelson KE, Astemborski J, Thomas DL. Class II HLA alleles and hepatitis B virus persistence in African Americans. *J Infect Dis* 1999; **179**: 1004-1006
5 **Jiang YG**, Wang YM. Association between HLA class II gene and severity of chronic hepatitis B. *Shijie Huaren Xiaohua Zazhi* 2004; **12**: 622-625
6 **Wai CT**, Fontana RJ. Cytokine gene polymorphisms in chronic hepatitis B: a step up the immunology ladder. *Am J Gastroenterol* 2003; **98**: 6-8
7 **Ben-Ari Z**, Mor E, Papo O, Kfir B, Sulkes J, Tambur AR, Tur-Kaspa R, Klein T. Cytokine gene polymorphisms in patients infected with hepatitis B virus. *Am J Gastroenterol* 2003; **98**: 144-150
8 **Hoshino T**, Wiltrout RH, Young HA. IL-18 is a potent coinducer of IL-13 in NK and T cells: A new potential role for IL-18 in modulating the immune response. *J Immunol* 1999; **162**: 5070-5077
9 **Takada T**, Suzuki E, Morohashi K, Gejyo F. Association of single nucleotide polymorphisms in the IL-18 gene with sarcoidosis in a Japanese population. *Tissue Antigens* 2002; **60**: 36-42
10 **Kretowski A**, Mironczuk K, Karpinska A, Bojaryn U, Kinalski M, Puchalski Z, Kinalska I. Interleukin-18 promoter polymorphisms in type 1 diabetes. *Diabetes* 2002; **51**: 3347-3349
11 **Sun Y**, Chen HY, Wang F, Zhang X, Jiang HQ, Shao FJ, Zhu SH. Effect of IL-18 on peripheral blood monocytes from chronic hepatitis B patients. *Zhonghua Ganzangbing Zazhi* 2003; **11**: 470-473
12 **Giedraitis V**, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. *J Neuroimmunol* 2001; **112**: 146-152
13 The branch of infectious diseases, parasitology and hepatology of Chinese Medical Association. The strategy of prevention and cure in viral hepatitis. *Zhonghua Chuanranbing Zazhi* 2001; **19**: 56-62
14 **Lok AS**, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000-summary of a workshop. *Gastroenterology* 2001; **120**: 1828-1853
15 **Cacciola I**, Cerenzia G, Pollicino T, Squadrito G, Castellaneta S, Zanetti AR, Mieli-Vergani G, Raimondo G. Genomic heterogeneity of hepatitis B virus (HBV) and outcome of perinatal HBV infection. *J Hepatol* 2002; **36**: 426-432
16 **Rapicetta M**, Ferrari C, Levrero M. Viral determinants and host immune responses in the pathogenesis of HBV infection. *J Med Virol* 2002; **67**: 454-457
17 **Thio CL**, Thomas DL, Carrington M. Chronic viral hepatitis and the human genome. *Hepatology* 2000; **31**: 819-827
18 **Miyazoe S**, Hamasaki K, Nakata K, Kajiya Y, Kitajima K, Nakao K, Daikoku M, Yatsuhashi H, Koga M, Yano M, Eguchi K. Influence of interleukin-10 gene promoter polymorphisms on disease progression in patients chronically infected with hepatitis B virus. *Am J Gastroenterol* 2002; **97**: 2086-2092
19 **Kim YJ**, Lee HS, Im JP, Min BH, Kim HD, Jeong JB, Yoon JH, Kim CY, Kim MS, Kim JY, Jung JH, Kim LH, Park BL, Shin HD. Association of transforming growth factor-beta1 gene polymorphisms with a hepatocellular carcinoma risk in patients with chronic hepatitis B virus infection. *Exp Mol Med* 2003; **35**: 196-202
20 **Kim YJ**, Lee HS, Yoon JH, Kim CY, Park MH, Kim LH, Park BL, Shin HD. Association of TNF-alpha promoter polymorphisms with the clearance of hepatitis B virus infection. *Hum Mol Genet* 2003; **12**: 2541-2546
21 **Kwon OS**, Song SH, Ju KT, Chung MG, Park DK, Kim SS, Kim YS, Koo YS, Kim YK, Choi DJ, Kim JH, Hwang YJ, Byun KS, Lee CH. Polymorphism in codons 10 and 25 of the transforming growth factor-beta1 gene in Korean population and in patients with liver cirrhosis and hepatocellular carcinoma. *Korean J Gastroenterol* 2003; **42**: 212-219
22 **Wang C**, Tang J, Song W, Lobashevsky E, Wilson CM, Kaslow RA. HLA and cytokine gene polymorphisms are independently associated with responses to hepatitis B vaccination. *Hepatology* 2004; **39**: 978-988
23 **El-Mezzein RE**, Matsumoto T, Nomiyama H, Miike T. Increased secretion of IL-18 *in vitro* by peripheral blood mononuclear cells of patients with bronchial asthma and atopic dermatitis. *Clin Exp Immunol* 2001; **126**: 193-198
24 **Higashi N**, Gesser B, Kawana S, Thestrup-Pedersen K, Expression of IL-18 mRNA and secretion of IL-18 are reduced in monocytes from patients with atopic dermatitis. *J Allergy Clin Immunol* 2001; **108**: 607-614
25 **Kimura K**, Kakimi K, Wieland S, Guidotti LG, Chisari FV. Interleukin-18 inhibits hepatitis B virus replication in the livers of transgenic mice. *J Virol* 2002; **76**: 10702-10707
26 **Kruse S**, Kuehr J, Moseler M, Kopp MV, Kurz T, Deichmann KA, Foster PS, Mattes J. Polymorphisms in the IL 18 gene are associated with specific sensitization to common allergens and allergic rhinitis. *J Allergy Clin Immunol* 2003; **111**: 117-122
27 **Sugiura T**, Kawaguchi Y, Harigai M, Terajima-Ichida H, Kitamura Y, Furuya T, Ichikawa N, Kotake S, Tanaka M, Hara M, Kamatani N. Association between adult-onset Still's disease and interleukin-18 gene polymorphisms. *Genes Immun* 2002; **3**: 394-399
28 **Higa S**, Hirano T, Mayumi M, Hiraoka M, Ohshima Y, Nambu M, Yamaguchi E, Hizawa N, Kondo N, Matsui E, Katada Y, Miyatake A, Kawase I, Tanaka T. Association between interleukin-18 gene polymorphism 105A/C and asthma. *Clin Exp Allergy* 2003; **33**: 1097-1102

Science Editor Li WZ **Language Editor** Elsevier HK