Genetic polymorphisms of interleukin-6 influence the development of hepatitis B virus-related liver cirrhosis

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Research article

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Abstract

Background Interleukin-6 (IL-6) plays an important role in chronic inflammation. Thus, we aimed to investigate the effects of IL-6 polymorphisms in predicting the progression of hepatitis B virus (HBV) - related liver cirrhosis. **Methods** A cross-sectional study was conducted to analysis IL-6 polymorphisms and serum levels of IL-6 in HBV-infected patients of different clinical phases and in healthy controls. IL-6 polymorphisms were detected by Taqman PCR method and plasma IL-6 levels were assessed by ELISA. **Results** Our analysis included 182 chronic hepatitis B (CHB) patients, 190 HBV-infected liver cirrhosis cases, 125 inactive HBsAg carriers, and 246 healthy controls. Seven SNPs in IL-6 including rs10499563, rs17147230, rs1800796, rs2069837, rs1524107, rs2066992, rs2069852 were analyzed. In haplotype analysis between HBV-infected liver cirrhosis cases with CHB patients, inactive HBV-carriers or healthy controls, haplotype CT in block 1 and haplotype GGCGG in block 2 were associated with liver cirrhosis ($P<0.05$). What's more, the genotype or allele frequencies were significantly different in IL-6 rs10499563 and rs2069837 when HBV-infected liver cirrhosis patients compared with CHB patients, inactive HBV-carriers or healthy controls. A further study found that compared with the controls or CHB patients, plasma IL-6 was elevated in HBV-infected liver cirrhosis patients ($P<0.05$). **Conclusion** In conclusion, the polymorphisms of the IL-6 rs10499563 and rs2069837 are associated with the susceptibility of liver cirrhosis may through their effects on IL-6 expressions and these two single nucleotide polymorphisms can be used as potential predicting markers for prognosis of HBV-infected liver cirrhosis.

Background

HBV infection is a global public health issue. What's more, it is the principal reason for hepatitis, liver cirrhosis, and even hepatocellular carcinoma [1]. Natural outcome of this infection varies greatly between individuals, and both viral and host factors contribute to the diversity [2]. Specifically, single nucleotide polymorphisms (SNPs) of a great many genes have been reported to take part in the variety of HBV clinical course [3, 4]. Liver cirrhosis, as a common ending of most HBV infection, is increasingly becoming a global health burden [5]. Thus, early diagnosis of HBV-infected liver cirrhosis becomes necessary.

Cytokines are thought to be the pivotal mediators which regulate many inflammatory immune responses and play an important part in determining the outcome of HBV infection [6]. IL-6, one of primary immunomodulatory cytokines, has been documented to act a pivotal part in regulating the biological processes of many cells including hepatocytes [7]. The gene of IL-6 lies at chromosome 7p21 and it contains 184 amino acids [8]. IL-6 is a multifunctional cytokine produced by diverse cell types and it plays an important role in various biological responses [9]. Many studies have proved that cytokine genetic polymorphisms such as IL-6 are related with HBV infection outcomes [10].

However, the literature is little concerning about IL-6 polymorphisms in HBV-infected liver cirrhosis in China [11]. In this study, we explored the relationships about IL-6 polymorphisms and HBV-infected liver cirrhosis. What's more, compared with other studies, We paid attention to functional SNPs in whole genome of IL-6 gene besides those at the promoter region.

Methods

Study population

We recruited at the First Affiliated Hospital of Zhejiang University (Hangzhou, China) and 246 healthy controls and 497 Han Chinese with HBV infection (125 inactive HBsAg carriers, 182 CHB patients, 190 HBV-infected liver cirrhosis subjects) were enrolled in our study. All participants gave the written informed consents. Individuals with concurrent hepatitis C virus, hepatitis D virus or human immunodeficiency virus infections, alcoholic liver diseases or autoimmune liver diseases were excluded. Chronic hepatitis B patients should fulfill the criteria that elevated alanine aminotransferase (ALT) and positive for HBsAg and HBV DNA. Patients with inactive HBsAg carriers fulfilled the diagnostic criteria: HBsAg-positive, HbeAg-negative and undetectable serum HBV DNA levels with persistently normal ALT. HBV-infected liver cirrhosis patients met the criterions that a histology or clinical evidence of liver cirrhosis and a clear etiology of HBV infections. Meanwhile, controls were negative for HBsAg.

SNP selection

The SNPs were selected according to the following principle: (1) minor allele frequency (MAF) > 0.05; (2) $r^2 > 0.8$; (3) SNPs related to diseases in previous studies. Finally, seven SNPs including rs10499563, rs17147230, rs1800796, rs2069837, rs1524107, rs2066992, rs2069852 (Table 2) in the IL-6 gene were selected from the International HapMap Project (<http://hapmap.ncbi.nlm.nih.gov/>).

DNA isolation and genotyping

We followed the manufacturer's instructions to extract the genomic DNA from the whole blood of all subjects with a DNA extraction kit (QIAamp DNA Blood Mini Kit, USA). After extracting, we stored the DNA samples at a concentration of 100 ng/μl at -80 °C. All seven IL6 SNPs (rs10499563, rs17147230, rs1800796, rs2069837, rs1524107, rs2066992 and rs2069852) were genotyped with TaqMan probes (Applied Biosystems, Foster City, CA, USA). We used 5 % random samples to verify accuracy and repeatability of our experiment.

Detection for IL6

Plasma IL-6 was measured by a commercial ELISA kit (MultiSciences, China). We followed the kit protocol to perform the procedures.

Statistical analysis

All the statistical analyses were performed by SPSS software version 19.0 (SPSS, Inc., Chicago, IL). Chi-square test was used to analyze Hardy–Weinberg (HW) equilibrium. The genotype and allele frequencies were calculated by direct gene counting method and compared by Chi-square test. Student's t-test was used to analyze continuous variables among the groups. Odds ratios (ORs) and 95% confidence intervals (95% CI) were described. Haploview 4.2 software was used for linkage disequilibrium (LD) and haplotype analysis. It was statistically significant when p -value ≤ 0.05 .

Results

Hardy-Weinberg Equilibrium

General characteristics of the subjects are summarized in Table 1. Among the groups, there was no difference in age ($P > 0.05$). The genotype distributions of IL-6 polymorphisms showed no significant difference from Hardy-Weinberg equilibrium in each group ($P > 0.05$) (Table 2) and we could see the relative positions of the seven SNPs in Table 2.

Haplotype Block Structure and Linkage Disequilibrium Analysis

First, we conduct the LD analysis in HBV-infected liver cirrhosis cases with CHB patients (Fig. 1 A), inactive HBV-carriers (Fig. 1 B) and healthy controls (Fig. 1 C). As shown in Fig. 1, LD analysis showed that there were two blocks in the IL-6 gene. Rs10499563 and rs17147230 were in LD block 1 and rs1800796, rs2069837, rs1524107, rs2066992, and rs2069852 were located in LD block 2. In Table 3, we could see that in haplotype analysis between liver cirrhosis cases and CHB patients, haplotype CT ($P = 0.0022$) in block 1 and haplotype GGCGG ($P = 0.0235$) in block 2 were associated with liver cirrhosis. In haplotype analysis between liver cirrhosis cases and HBV-carriers, haplotype CT in block 1 ($P = 9.757E-6$) and haplotype GGCGG in block 2 ($P = 0.0029$) were associated with liver cirrhosis. In haplotype analysis between liver cirrhosis cases and healthy controls, haplotype CT in block 1 ($P = 0.0228$) and haplotype GGCGG in block 2 ($P = 0.0127$) were associated with liver cirrhosis.

The Polymorphisms of IL-6 and The Development of HBV-Related Liver Cirrhosis

Based on the haplotype analysis, we further did the genotype and allele analyses. In Table 4, we could find that the allele frequencies were different in rs10499563 and rs2069837 ($P = 0.0031$, $P = 0.0240$)

between HBV-infected liver cirrhosis cases and CHB patients. And TC genotype of rs10499563 had a significant protective effect on liver cirrhosis patients ($P=0.0246$) (The Bonferroni correction was applied. $P<0.025$). In HBV-infected liver cirrhosis patients and inactive HBV-carriers, TC genotype of rs10499563 had a significant protective effect on liver cirrhosis patients ($P=7.0000 \times 10^{-6}$) (The Bonferroni correction was applied. $P<0.025$). GG genotype of rs2069837 also had protective effects on liver cirrhosis patients ($P=0.0239$) (The Bonferroni correction was applied. $P<0.025$). And the allele frequencies were also different in rs10499563 and rs2069837 ($P=1.4000 \times 10^{-5}$, $P=0.0026$). In addition, the allele frequencies were different between HBV-infected liver cirrhosis patients and control group in rs10499563 and rs2069837 ($P=0.0245$, $P=0.0062$).

Comparison of IL-6 Expressions in Different Groups

For the sake of researching whether there are any differences of IL-6 expressions between controls and HBV-cirrhotic group, we detected the IL-6 expressions in control, CHB and HBV-cirrhotic groups. As shown in Figure 2, there were significantly strong IL-6 expressions in the CHB and HBV-cirrhotic groups compared with healthy control group (0.19 ± 0.15 pg/ml versus 0.14 ± 0.23 pg/ml, $P=0.0299$; 4.85 ± 3.01 pg/ml versus 0.14 ± 0.23 pg/ml, $P=0.0029$ resp.). We next evaluated the association of changes in plasma IL-6 concentrations with the progression of HBV-infected liver cirrhosis. Plasma IL-6 levels were remarkably higher in HBV-cirrhotic groups than that in CHB group (4.85 ± 3.01 pg/ml versus 0.19 ± 0.15 pg/ml, $P=0.0013$) (Figure 2).

Discussion

In the study, we tested our hypothesis that the IL-6 polymorphisms may associate with the progression of HBV-infected liver cirrhosis. Our result showed that the polymorphisms of the IL-6 rs10499563 and rs2069837 were associated with the susceptibility of liver cirrhosis may through their effects on IL-6 expressions and these two SNPs could be used as potential predicting markers for prognosis of hepatitis B development.

As we all know that the interaction between host immunity and viral replication influences the clinical outcome of HBV infection, and nowadays more attentions are paid to the genetic background influence [12, 13]. Gene polymorphisms such as SNP may change the corresponding protein's structure or biological function. An SNP in the promoter region could induce decreased or increased productions of the corresponding protein. Thus, a person may be more resistant or susceptible to a certain disease [14]. Consequently, the impact of IL-6 gene polymorphisms on disease outcomes is attracting more and more attention. It was stated that IL-6 rs10499563 was a functional polymorphism site in the promoter region of IL-6 and could transcriptionally regulate the expression of IL-6 [15]. Recently, several researchers found that IL-6 rs10499563 polymorphism was linked to gastric cancer risk [16, 17]. However, the relationship of IL-6 rs10499563 and the progression of HBV-related liver cirrhosis has not been studied. Based on these, our research explored the relationship between IL-6 rs10499563 and the liver cirrhosis risk in China.

Compared with TT wild-type carriers, we discovered that subjects with TC and CC genotype were related with decreased risk of HBV-related liver cirrhosis. It was reported that in acute inflammatory states, TT wild-type carriers had a higher level of IL-6 while CC genotype was associated with decreased IL-6 concentrations, which was confirmed by in vitro luciferase assay [15]. These results were in line with our IL-6 expression levels in patients. That is, the IL-6 rs10499563 polymorphism with wild-type genotype could produce a higher level of IL-6 and might be related with increased risk of HBV-related liver cirrhosis; the IL-6 rs10499563 polymorphism with mutant-type genotype could produce a lower level of IL-6 and might be related with decreased risk of HBV-related liver cirrhosis. The IL6 rs2069837 SNP was located at the intron region. It has been studied in many diseases, such as nephropathy [18], chronic periodontitis [19], cervical cancer [20] and chronic hepatitis B virus infection [21]. One research predicted that IL6 rs2069837 SNP might participate in IL6 gene regulatory networks [22]. Chen et al. deduced that carriers of rs2069837 A allele had higher plasma levels of IL-6 than in others [23]. However, it needs to be decided by further functional studies whether the IL6 rs2069837 SNP could regulate the expressions of IL-6, which may reveal the exact mechanisms underlying the observed association with the progression of HBV-related liver cirrhosis.

Recently, IL-6 polymorphisms in liver diseases such as hepatitis C and anti-tuberculosis drug-induced hepatitis (ATDH) were focused. Motawi et al. [24] have explored the influences of IL-6, α 1-antitrypsin (A1AT) polymorphisms in HCV-infected liver cirrhosis patients. In their study, 85 chronic HCV-infected patients and 65 cirrhotic patients together with 100 healthy control subjects were genotyped for IL-6 rs1800795, A1AT Z mutation rs28929474 and A1AT S mutation rs17580. However, the results showed that IL-6 rs1800795 was not associated with cirrhotic patients but the A1AT gene polymorphisms were related with the progression of chronic hepatitis C. In addition, Wang et al. investigated the distribution of the IL-6, HSPA1L and STAT3 SNPs in patients with ATDH in Chinese Han population [25]. A total of 356 healthy controls and 89 ATDH patients were enrolled in their study. However, they found that the three selected IL-6 SNPs (rs2066992, rs2069837, rs1524107) did not contribute to ATDH susceptibility and STAT3 polymorphisms were associated with ATDH. The association between IL-6 SNPs and the outcome of HBV infection could be found in earlier studies. Some researchers found that there was no significant relationship between IL-6 rs1800796 polymorphisms and the outcome of chronic hepatitis B disease [26]. However, after adjusting all subjects based on gender, males with the G allele had a higher chance of HCC than those with the CC genotype [10]. Furthermore, Lu et al. reported that IL-6 rs1800796 allele G may be helpful for spontaneous clearance of HBV [27]. They found that compared to CHB patients, controls with spontaneous clearance of HBV had higher GG genotype and allele G frequencies in IL-6 rs1800796 site. Hence, they drawn the conclusion that IL-6 rs1800796 was related with spontaneous clearance of HBV.

In our study, the haplotype analysis showed that the polymorphisms of the IL-6 rs10499563, rs17147230, rs1800796, rs2069837, rs1524107, rs2066992 and rs2069852 were associated with the susceptibility of

liver cirrhosis. This discovery is in accordance with the concept that IL-6 haplotypes are more functionally correlative to some illnesses than single polymorphisms [28]. What's more, based on the genotype and allele analyses, there were definitive evidences pointing to the relationships about IL-6 rs10499563 and rs2069852 in HBV-infected liver cirrhosis cases with CHB patients, inactive HBV-carriers or healthy controls.

Conclusions

In conclusion, it was found that the polymorphisms of the IL-6 rs10499563 and rs2069837 were associated with the susceptibility of liver cirrhosis. Moreover, we found that plasma IL-6 levels were remarkably higher in HBV-cirrhotic groups than that in CHB group and in control group. We assume that the genetic influences of the IL-6 SNPs on susceptibility to liver cirrhosis may through their effects on IL-6 expressions. Our works find a pivotal role of IL-6 SNP in predicting the progression of HBV-related liver cirrhosis in a Chinese population, which is helpful of the existing prognostic and therapeutic strategies in the HBV-related liver cirrhosis disease. Until now, our research is the first one to explore the distribution of functional SNPs in whole genome of IL-6 gene in a Chinese population about the HBV-related liver cirrhosis disease. Nevertheless, larger comprehensive population-based studies with different races are needed to further validate these preliminary observations. What's more, our next study will focus on the functional research whether the IL6 SNP could regulate the expressions of IL-6, which may reveal the exact mechanisms underlying the observed association with the progression of HBV-related liver cirrhosis.

Abbreviations

IL-6	Interleukin-6
HBV	Hepatitis B virus
CHB	Chronic hepatitis B
SNPs	Single nucleotide polymorphisms
ALT	Alanine aminotransferase
MAF	Minor allele frequency
HW	Hardy–Weinberg
ORs	Odds ratios
LD	Linkage Disequilibrium
ATDH	Anti-tuberculosis drug-induced hepatitis

Declarations

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Availability of data and materials

The data used or analysed during the study are available from the corresponding author on reasonable request.

Authors' contributions

MZ, YL and ZC were involved in conceptualization, project administration, supervision of the original draft. CX, WZ and CH took part in methodology, formal analysis and writing the original draft. GL, BY and FC were involved in resources and formal analysis. All authors read and approved the final manuscript.

Notes

Ethics approval and consent to participate

This research was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. Subjects have given their written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest to declare.

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References

- 1 Chang ML, Liaw YF. Hepatitis b flares in chronic hepatitis b: Pathogenesis, natural course, and management. *J Hepatol* 2014, 61: 1407-1417.
- 2 Knolle PA, Thimme R. Hepatic immune regulation and its involvement in viral hepatitis infection. *Gastroenterology* 2014, 146: 1193-1207.
- 3 Thursz M, Yee L, Khakoo S. Understanding the host genetics of chronic hepatitis b and c. *Semin Liver Dis* 2011, 31: 115-127.
- 4 Zeng Z. Human genes involved in hepatitis b virus infection. *World J Gastroenterol* 2014, 20: 7696-7706.

- 5 Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, Abu-Raddad LJ, et al. The global burden of viral hepatitis from 1990 to 2013: Findings from the global burden of disease study 2013. *Lancet* 2016, 388: 1081-1088.
- 6 Tuncbilek S. Relationship between cytokine gene polymorphisms and chronic hepatitis b virus infection. *World J Gastroenterol* 2014, 20: 6226-6235.
- 7 Pan CJ, Wu HL, Kuo SF, Kao JH, Tseng TC, Liu CH, Chen PJ, et al. Serum interleukin 6 level correlates with outcomes of acute exacerbation of chronic hepatitis b. *Hepatol Int* 2012, 6: 591-597.
- 8 Bowcock AM, Kidd JR, Lathrop GM, Daneshvar L, May LT, Ray A, Sehgal PB, et al. The human "interferon-beta 2/hepatocyte stimulating factor/interleukin-6" gene: DNA polymorphism studies and localization to chromosome 7p21. *Genomics* 1988, 3: 8-16.
- 9 Rincon M, Irvin CG. Role of il-6 in asthma and other inflammatory pulmonary diseases. *Int J Biol Sci* 2012, 8: 1281-1290.
- 10 Tang S, Yuan Y, He Y, Pan D, Zhang Y, Liu Y, Liu Q, et al. Genetic polymorphism of interleukin-6 influences susceptibility to hbv-related hepatocellular carcinoma in a male chinese han population. *Hum Immunol* 2014, 75: 297-301.
- 11 Xia C, Liu Y, Chen Z, Zheng M. Involvement of interleukin 6 in hepatitis b viral infection. *Cell Physiol Biochem* 2015, 37: 677-686.
- 12 Wang L, Zou ZQ, Wang K. Clinical relevance of hla gene variants in hbv infection. *J Immunol Res* 2016, 2016: 9069375.
- 13 Zhou C, Jin X, Tang J, Fei J, Gu C, Chen X. Association of cd40 -1c/t polymorphism in the 5'-untranslated region with chronic hbv infection. *Cell Physiol Biochem* 2015, 35: 83-91.
- 14 Matsuura K, Isogawa M, Tanaka Y. Host genetic variants influencing the clinical course of hepatitis b virus infection. *J Med Virol* 2016, 88: 371-379.
- 15 Smith AJ, D'Aiuto F, Palmen J, Cooper JA, Samuel J, Thompson S, Sanders J, et al. Association of serum interleukin-6 concentration with a functional il6 -6331t>c polymorphism. *Clin Chem* 2008, 54: 841-850.
- 16 Yang L, Sun MJ, Liu JW, Xu Q, Yuan Y. Il-6-6331 (t/c, rs10499563) is associated with decreased risk of gastric cancer in northern chinese. *Asian Pac J Cancer Prev* 2013, 14: 7467-7472.
- 17 Zhang JZ, Liu CM, Peng HP, Zhang Y. Association of genetic variations in il-6/il-6r pathway genes with gastric cancer risk in a chinese population. *Gene* 2017, 623: 1-4.

- 18 Chang WT, Huang MC, Chung HF, Chiu YF, Chen PS, Chen FP, Lee CY, et al. Interleukin-6 gene polymorphisms correlate with the progression of nephropathy in chinese patients with type 2 diabetes: A prospective cohort study. *Diabetes Res Clin Pract* 2016, 120: 15-23.
- 19 Farhat SB, de Souza CM, Braosi AP, Kim SH, Tramontina VA, Papalexiou V, Olandoski M, et al. Complete physical mapping of il6 reveals a new marker associated with chronic periodontitis. *J Periodontal Res* 2017, 52: 255-261.
- 20 Shi TY, Zhu ML, He J, Wang MY, Li QX, Zhou XY, Sun MH, et al. Polymorphisms of the interleukin 6 gene contribute to cervical cancer susceptibility in eastern chinese women. *Hum Genet* 2013, 132: 301-312.
- 21 Zhao XM, Gao YF, Zhou Q, Pan FM, Li X. Relationship between interleukin-6 polymorphism and susceptibility to chronic hepatitis b virus infection. *World J Gastroenterol* 2013, 19: 6888-6893.
- 22 MacQuarrie KL, Fong AP, Morse RH, Tapscott SJ. Genome-wide transcription factor binding: Beyond direct target regulation. *Trends Genet* 2011, 27: 141-148.
- 23 Chen M, Li T, Lin S, Bi D, Zhu D, Shang Q, Ma C, et al. Association of interleukin 6 gene polymorphisms with genetic susceptibilities to spastic tetraplegia in males: A case-control study. *Cytokine* 2013, 61: 826-830.
- 24 Motawi T, Shaker OG, Hussein RM, Houssen M. Polymorphisms of alpha1-antitrypsin and interleukin-6 genes and the progression of hepatic cirrhosis in patients with a hepatitis c virus infection. *Balkan J Med Genet* 2016, 19: 35-44.
- 25 Wang J, Chen R, Tang S, Lv X, Wu S, Zhang Y, Xia Y, et al. Analysis of il-6, stat3 and hspa1l gene polymorphisms in anti-tuberculosis drug-induced hepatitis in a nested case-control study. *PLoS One* 2015, 10: e0118862.
- 26 Park BL, Lee HS, Kim YJ, Kim JY, Jung JH, Kim LH, Shin HD. Association between interleukin 6 promoter variants and chronic hepatitis b progression. *Exp Mol Med* 2003, 35: 76-82.
- 27 Lu Y, Peng J, Wang C, Zhu Y, Wang F, Sun Z. Il-6 promoter functional polymorphism -572c/g affects spontaneous clearance of hepatitis b virus infection. *Clin Lab* 2014, 60: 1903-1907.
- 28 Chen J, Liu RY, Yang L, Zhao J, Zhao X, Lu D, Yi N, et al. A two-snp il-6 promoter haplotype is associated with increased lung cancer risk. *J Cancer Res Clin Oncol* 2013, 139: 231-242.

Tables

Table 1 General Characteristics of the Subjects

	healthy control	HBV-carrier	CHB	HBV-cirrhotic
Number of cases	246	125	182	190
Mean Age (y)	43.98±13.03	42.23±7.32	42.32±8.49	43.95±7.78
HBsAg status	All (-)	All (+)	All (+)	All (+)

Table 2 Information of seven selected SNPs of IL-6 gene region

SNP	Chromosome	Allele	MAF ^a	<i>P</i> value ^b
rs10499563	7: 22720869	C: T	0.20	0.79
rs17147230	7: 22722557	A: T	0.19	0.06
rs1800796	7: 22726627	C: G	0.31	0.40
rs2069837	7: 22728408	G: A	0.12	0.99
rs1524107	7: 22728600	T: C	0.31	0.42
rs2066992	7: 22728630	T: G	0.31	0.36
rs2069852	7: 22732641	A: G	0.27	0.57

SNP single nucleotide polymorphism, *MAF* minor allele frequency

^a From both HapMap and dbSNP databases

^b *P* value for Hardy-Weinberg equilibrium test

Table 3 Haplotype analysis between cases and controls

HapMap block	Haplotype	Cases	Controls	Chi Square	P value
Block 1 ^a	TA	200	184	0.328	0.5666
	TT	121	92	3.898	0.0484
	CT	58	88	9.345	0.0022
Block 2 ^a	CATTA	232	233	0.675	0.4114
	GGCGG	58	79	5.133	0.0235
	GACGG	47	29	3.927	0.0475
	CATTG	41	20	6.801	0.0091
Block 1 ^b	TA	200	113	3.386	0.0657
	TT	121	62	3.560	0.0592
	CT	58	75	19.558	9.757E-6
Block 2 ^b	CATTA	232	152	0.004	0.9480
	GGCGG	58	62	8.889	0.0029
	GACGG	47	18	4.353	0.0369
	CATTG	41	17	2.857	0.0910
Block 1 ^c	TA	200	268	0.283	0.5945
	TT	121	119	6.595	0.0102
	CT	58	105	5.186	0.0228
Block 2 ^c	CATTA	232	311	0.425	0.5147
	GGCGG	58	108	6.214	0.0127
	GACGG	47	39	4.758	0.0292
	CATTG	41	26	9.132	0.0025

a: Controls mean CHB patients; b: Controls mean inactive HBV-carriers; c: Controls mean healthy controls

Cases all mean HBV-infected liver cirrhosis patients.

Table 4 Comparison of genotype and allele distributions among HBV-infected liver cirrhosis group and three other groups (CHB patients, inactive HBV-carriers; and healthy controls)

Genotypes/Alleles	Cases (%)	Controls (%)	P value	OR(95%CI)
rs10499563 ^a TT	135 (71.1)	105 (57.7)		1.00 (Ref)
TC	51 (26.8)	66 (36.3)	0.0246 ^d	0.60 (0.38-0.94)
CC	4 (2.1)	11 (6.0)	0.0256 ^d	0.28 (0.09-0.91)
TC+ CC			0.0071	0.56 (0.36-0.85)
T	321 (84.5)	276 (75.8)		1.00 (Ref)
C	59 (15.5)	88 (24.2)	0.0031	0.58 (0.40-0.83)
rs2069837 ^a AA	134 (70.5)	112 (61.5)		1.00 (Ref)
AG	53 (27.9)	60 (33.0)	0.1825 ^d	0.74 (0.47-1.15)
GG	3 (1.6)	10 (5.5)	0.0271 ^d	0.25 (0.07-0.93)
AG+ GG			0.0671	0.67 (0.43-1.03)
A	321 (84.5)	284 (78.0)		1.00 (Ref)
G	59 (15.5)	80 (22.0)	0.0240	0.65 (0.45-0.95)
rs10499563 ^b TT	135 (71.1)	56 (44.8)		1.00 (Ref)
TC	51 (26.8)	63 (50.4)	7E-6 ^d	0.34 (0.21-0.54)
CC	4 (2.1)	6 (4.8)	0.0898 ^d	0.28 (0.08-1.02)
TC+ CC			3E-6	0.33 (0.21-0.53)
T	321 (84.5)	175 (70.0)		1.00 (Ref)
C	59 (15.5)	75 (30.0)	1.4E-5	0.43 (0.29-0.63)
rs2069837 ^b AA	134 (70.5)	70 (56.0)		1.00 (Ref)
AG	53 (27.9)	47 (37.6)	0.0327 ^d	0.59 (0.36-0.96)
GG	3 (1.6)	8 (6.4)	0.0239 ^d	0.20 (0.05-0.76)
AG+ GG			0.0083	0.53 (0.33-0.85)
A	321 (84.5)	187 (74.8)		1.00 (Ref)
G	59 (15.5)	63 (25.2)	0.0026	0.55 (0.37-0.81)
rs10499563 ^c TT	135 (71.1)	152 (61.8)		1.00 (Ref)
TC	51 (26.8)	8 (33.3)	0.0953 ^d	0.70 (0.46-1.07)
CC	4 (2.1)	12 (4.9)	0.0851 ^d	0.38 (0.12-1.19)
TC+ CC			0.0431	0.66 (0.44-0.99)
T	321 (84.5)	386 (78.5)		1.00 (Ref)
C	59 (15.5)	106 (21.5)	0.0245	0.67 (0.47-0.95)
rs2069837 ^c AA	134(70.5)	145 (58.9)		1.00 (Ref)
AG	53 (27.9)	89 (36.2)	0.0366 ^d	0.64 (0.43-0.97)
GG	3 (1.6)	12 (4.9)	0.0340 ^d	0.23 (0.07-0.98)
AG+ GG			0.0125	0.60 (0.40-0.90)
A	321 (84.5)	379 (77.0)		1.00 (Ref)
G	59 (15.5)	113 (23.0)	0.0062	0.62 (0.44-0.87)

a: Controls mean CHB patients; b: Controls mean inactive HBV-carriers; c: Controls mean healthy controls.

Cases all mean HBV-infected liver cirrhosis patients.

d: The Bonferroni correction was applied for multiple comparisons.

Figures

Figure 1

LD (D') plot of IL-6 gene. Two blocks were in the IL-6 gene. D' means the degree of LD between two SNPs. (A) The LD analysis in HBV-infected liver cirrhosis cases with CHB patients. (B) The LD analysis in HBV-infected liver cirrhosis cases with inactive HBV-carriers. (C) The LD analysis in HBV-infected liver cirrhosis cases with healthy controls.

Figure 2

Plasma IL-6 levels in control, CHB and HBV-cirrhotic groups. a: compared with healthy control group $P < 0.05$ b: compared with CHB group $P < 0.01$.