Genetic polymorphisms of interleukin-6 influence the development of hepatitis B virus-related liver cirrhosis in the Han Chinese population

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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 on predicting the progression of hepatitis B virus (HBV)-r elated liver cirrhosis.Methods A cross-sectional study was conducted to analyse IL-6 polymorphisms and serum levels of IL-6 in HBV-infected patients at different clinical phases and in healthy controls. IL-6 polymorphisms were detected by the 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, and rs2069852 were analysed. In a haplotype analysis between HBV-infected liver cirrhosis cases and CHB patients, inactive HBV carriers or healthy controls, haplotype CT in block 1 and haplotype GGCGG in block 2 were associated with liver cirrhosis ($P<0.05$). Moreover, the genotype or allele frequencies were significantly different in IL-6 rs10499563 and rs2069837 when HBV-infected liver cirrhosis patients were compared with CHB patients, inactive HBV carriers or healthy controls. A further study found that compared with that in the healthy controls, inactive HBV carriers or CHB patients, plasma IL-6 was elevated in HBV-infected liver cirrhosis patients ($P<0.05$).Conclusion In conclusion, the IL-6 rs10499563 and rs2069837 polymorphisms are associated with susceptibility to liver cirrhosis may through their effects on IL-6 expression and these two single nucleotide polymorphisms can be used as potential prognostic markers of HBV-related liver cirrhosis.

Background

Hepatitis B virus (HBV) infection is a global public health issue. Moreover, it is the principal reason for hepatitis, liver cirrhosis, and even hepatocellular carcinoma [1-3]. The natural outcome of this infection varies greatly between individuals, and both viral and host factors contribute to the diversity [4]. Specifically, single nucleotide polymorphisms (SNPs) of great many genes have been reported to take part in the variability of the HBV clinical course [5, 6]. Liver cirrhosis, as a common endpoint of most HBV infections, is increasingly becoming a global health burden [7]. Thus, early diagnosis of HBV-infected liver cirrhosis patients becomes necessary.

Cytokines are thought to be pivotal mediators that regulate many inflammatory immune responses and play an important role in determining the outcome of HBV infection [8, 9]. IL-6, a primary immunomodulatory cytokine, has been documented to play a pivotal role in regulating the biological processes of many cells including hepatocytes [10, 11]. The IL-6 gene is located on chromosome 7p21 and encodes 184 amino acids [12]. IL-6 is a multifunctional cytokine produced by diverse cell types and plays an important role in various biological responses [13-15]. For example, Jing et al. found that IL-6 levels were closely related to myocardial infarction-induced myocardial remodelling [16]. Keshari et al. revealed that IL-6 mediated calcium phosphate-induced pruritus [17]. Moreover, many studies have demonstrated that cytokine genetic polymorphisms such as those in IL-6 are related to HBV infection outcomes [18-21].

However, little is known about IL-6 polymorphisms in HBV-related liver cirrhosis in China. In this study, we explored the relationships between IL-6 polymorphisms and HBV-related liver cirrhosis. Moreover, compared with other studies, we focused on functional SNPs in the whole genomic sequence of the IL-6 gene in addition to those in the promoter region.

Methods

Study population

A total of 246 healthy controls and 497 Han Chinese with HBV infection (125 inactive HBsAg carriers, 182 CHB patients, and 190 HBV-infected liver cirrhosis subjects) were recruited and enrolled in our study at the First Affiliated Hospital of Zhejiang University (Hangzhou, China). All participants gave written informed consent. 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 had to fulfil the criteria of elevated alanine aminotransferase (ALT) and HBsAg positive status for more than six months. Patients that were inactive HBsAg carriers fulfilled the following diagnostic criteria: HBsAg-positive, HBeAg-negative and undetectable serum HBV DNA levels with persistently normal ALT. HBV-infected liver cirrhosis patients met the criteria of histology or clinical evidence of liver cirrhosis and a clear aetiology of HBV infection. Moreover, healthy 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 shown to be related to diseases in previous studies. Finally, seven SNPs, including rs10499563, rs17147230, rs1800796, rs2069837, rs1524107, rs2066992, and rs2069852 (Table 2), in the IL-6 gene were selected from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/), and in the Allele column of Table 2, the first one is the mutant allele and the next is the wild-type allele.

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 (Qiagen, USA). After extraction, we stored the DNA samples at a concentration of 100 ng/μl at -80 °C. All seven IL-6 SNPs (rs10499563, rs17147230, rs1800796, rs2069837, rs1524107, rs2066992 and rs2069852) were genotyped with TaqMan probes (Applied Biosystems, Foster City, CA, USA). We randomly selected 5% of the samples to verify the accuracy and repeatability of our experiment.

Detection of IL-6

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

Statistical analysis

All statistical analyses were performed using SPSS software version 19.0 (SPSS, Inc., Chicago, IL). A chi-square test was used to analyse the Hardy–Weinberg equilibrium (HWE). The genotype and allele frequencies were calculated by the direct gene counting method and compared by the Chi-square test. The student's t test was used to analyse age among the groups. A non-parametric test (Mann-Whitney U) was used to analyse IL-6 levels among the groups. Data are presented as the means (± standard deviation, SD/standard error, SE) and counts (percentage). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were determined. Haploview 4.2 software was used for linkage disequilibrium (LD) and haplotype analysis. The Bonferroni correction was applied for multiple comparisons. Differences were statistically significant when *p*-value ≤ 0.05, except for Bonferroni correction.

Results

Clinical information of study population

The general characteristics of the subjects are summarized in Table 1. Among the groups, there were no differences in age (P>0.05), and the genotype distributions of IL-6 polymorphisms showed no significant departure from HWE in each group (*P*>0.05) (Table 2). The relative positions of the seven SNPs are shown in Table 2.

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

SNP	Chromosome	Allele	MAF[a]	*P* 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 HWE test in the whole group.

Haplotype block structure and LD analysis

First, we conducted the LD analysis of HBV-infected liver cirrhosis cases and 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. Table 3 shows that in the 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 the 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 the 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.

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

Cases all mean the HBV-infected liver cirrhosis patients.

The polymorphisms of IL-6 and the development of HBV-related liver cirrhosis

Based on the haplotype analysis, we further performed genotype and allele analyses. As shown in Table 4, we found that the allele frequencies were different in rs10499563 and rs2069837 (*P*=0.0031, *P*=0.0240, respectively) between HBV-infected liver cirrhosis cases and CHB patients. The 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, the TC genotype of rs10499563 had a significant protective effect on liver cirrhosis patients (*P*=7.0000E-6) (the Bonferroni correction was applied; *P*<0.025). The GG genotype of rs2069837 also had protective effects on liver cirrhosis patients (*P*=0.0239) (the Bonferroni correction was applied; *P*<0.025). The allele frequencies were also different in rs10499563 and rs2069837 (*P*=1.4000E-5, *P*=0.0026, respectively). In addition, the allele frequencies were different between HBV-infected liver cirrhosis patients and the

healthy control group in rs10499563 and rs2069837 (P=0.0245, P=0.0062, respectively). However, there were no significant differences in rs17147230, rs1800796, rs1524107, rs2066992 and rs2069852 between the HBV-infected liver cirrhosis group and the other three groups (see Supplementary Table 2).

Table 4 Comparisons of genotype and allele distributions between the HBV-infected liver cirrhosis group and the other three 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)	82(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 the CHB patients; b: Controls mean the inactive HBV carriers; c: Controls mean the healthy controls.

Cases all mean the HBV-infected liver cirrhosis patients.

d: The Bonferroni correction was applied for multiple comparisons.

Comparisons of IL-6 expression in different groups

To determine whether there were any differences in IL-6 expression between the controls and the HBV-cirrhotic group, we detected the IL-6 expression levels in the healthy controls, the inactive HBV-carrier group, the CHB group and the HBV-cirrhotic group. As shown in Table 5, we could not detect IL-6 levels in the plasma of the healthy control group or the inactive HBV-carrier group. There were significantly high IL-6 expression levels in the CHB group and HBV-cirrhotic group compared with the healthy control group and the inactive HBV-carrier group. We next evaluated the association of changes in the plasma concentrations of IL-6 with the progression of HBV-related liver cirrhosis. Plasma IL-6 levels were remarkably higher in the HBV-cirrhotic group than in the CHB group. Furthermore, we also analysed the plasma concentrations of IL-6 associated with different genotypes and alleles of rs10499563 and rs2069837 in each group (Figure 2). For rs10499563, plasma IL-6 levels in the TT genotype and T allele were higher than those in the CT and CC genotypes and C allele in the CHB group and HBV-cirrhotic group. For rs2069837, plasma concentrations of IL-6 in the AA genotype and A allele were lower than those in the AG genotype and G allele in the CHB group and HBV-cirrhotic group. However, there were no differences between each group.

Table 5 Plasma IL-6 levels in each group

Groups	IL-6 (pg/ml)
Healthy control group	n.d.
HBV-carrier group	n.d.
CHB group	1.87±2.81
HBV-cirrhotic group	10.54±13.16[a]

n.d., not detected; a: compared with the CHB group, $P<0.05$.

Data are presented as mean ± SD.

Discussion

In this study, we tested our hypothesis that IL-6 polymorphisms may be associated with the progression of HBV-associated liver cirrhosis. Our results showed that the IL-6 polymorphisms rs10499563 and rs2069837 were associated with susceptibility to liver cirrhosis may through their effects on IL-6 expression, and these two SNPs could be used as potential prognostic markers of hepatitis B development.

It is well known that the interaction between host immunity and viral replication influences the clinical outcome of HBV infection, and more attention is currently paid to the genetic background influence [22, 23]. Gene polymorphisms such as SNPs may change the structure or biological function of the corresponding protein. An SNP in the promoter region could induce decreased or increased production of the corresponding protein. Thus, a person may be more resistant or susceptible to a certain disease [24]. Consequently, the impact of IL-6 gene polymorphisms on disease outcomes is attracting increasing attention [25]. It has been shown that IL-6 rs10499563 is a functional polymorphism site in the promoter region of IL-6 and could transcriptionally regulate the expression of IL-6 [26]. Recently, several researchers have found that the IL-6 rs10499563 polymorphism is linked to gastric cancer risk [27, 28]. However, the relationship between IL-6 rs10499563 and the progression of HBV-related liver cirrhosis has not been studied. Based on these findings, our research explored the relationship between IL-6 rs10499563 and liver cirrhosis risk in China. Compared with TT wild-type carriers, we discovered that subjects with the TC and CC genotypes had a decreased risk of HBV-related liver cirrhosis. It has been reported that in acute inflammatory states, TT wild-type carriers have a higher level of IL-6, while the CC genotype has been associated with decreased IL-6 concentrations, which has been confirmed by an in vitro luciferase assay [26]. These results are in line with our IL-6 expression levels in patients. That is, the IL-6 rs10499563 polymorphism with the wild-type genotype could produce a higher level of IL-6 and might be related to an increased risk of HBV-related liver cirrhosis; the IL-6 rs10499563 polymorphism with the mutant-type genotype could produce a lower level of IL-6 and might be related to a decreased risk of HBV-related liver cirrhosis (Figure 2). IL-6 rs2069837 is located in the intron region. It is known that introns can increase gene expression through intron-mediated enhancement [29]. For example, introns can not only influence the rate of transcription and nuclear export but also enhance the effectiveness of mRNA translation [30]. As an SNP within the intron region, IL-6 rs2069837 has been studied in many diseases, such as nephropathy [31], chronic periodontitis [32], cervical cancer [33] and chronic hepatitis B virus infection [34]. One study predicted that the IL-6 rs2069837 SNP might participate in IL-6 gene regulatory networks [35]. Chen et al. deduced that carriers of the rs2069837 A allele had higher plasma IL-6 levels than others [36]. However, in our study, carriers of the rs2069837 G allele had higher plasma levels of IL-6 than the A allele (Figure 2 D). Therefore, further functional studies are needed to determine whether the IL-6 rs2069837 SNP could regulate the expression 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 virus infection and anti-tuberculosis drug-induced hepatitis (ATDH) have become the focus of research studies. Motawi et al. [37] explored the influences of IL-6 and 1-antitrypsin (A1AT) polymorphisms in HCV-infected liver cirrhosis patients in Egyptians. 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 to the progression of chronic hepatitis C. Nevertheless, a study in a population from Rio de Janeiro, Brazil found that the IL-6 rs1800795 C allele was associated with protection from chronic hepatitis C and could decrease inflammation scores [38]. In addition, Wang et al. investigated the distribution of IL-6, HSPA1L and STAT3 SNPs in patients with ATDH in a Chinese Han population [39]. 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, and rs1524107) did not contribute to ATDH susceptibility and that STAT3 polymorphisms were associated with ATDH. The association between IL-6 SNPs and the outcome of HBV infection can 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 [40]. However, after adjusting for gender, males with the G allele were at a higher risk of HCC than those with the CC genotype [41]. Furthermore, Lu et al. reported that the IL-6 rs1800796 G allele may be helpful for spontaneous clearance of HBV [42]. The authors found that compared to CHB patients, controls with spontaneous clearance of HBV had higher GG genotype and allele G frequencies in the IL-6 rs1800796 site. Hence, they concluded that IL-6 rs1800796 was associated with spontaneous clearance of HBV. What's more, in a recent study in Egyptians, researchers concluded that IL-6 (rs1800795, rs1800796 and rs1800797) gene polymorphisms were associated with susceptibility to HBV infection [43].

In our study, the haplotype analysis showed that the polymorphisms of the IL-6 rs10499563, rs17147230, rs1800796, rs2069837, rs1524107, rs2066992 and rs2069852 SNPs were associated with susceptibility to liver cirrhosis. This discovery is in accordance with the concept that IL-6 haplotypes are more functionally correlative to some illnesses than single polymorphism [44]. Moreover, based on the genotype and allele analyses, there was definitive evidence indicating the relationships of IL-6 rs10499563 and rs2069852 in HBV-infected liver cirrhosis cases and CHB patients, inactive HBV carriers or healthy controls.

Conclusions

In conclusion, it was found that the IL-6 rs10499563 and rs2069837 polymorphisms were associated with susceptibility to liver cirrhosis. Moreover, we found that plasma IL-6 levels were remarkably higher in the HBV-cirrhotic group than in the CHB group, in the inactive HBV-carrier group and in the healthy control group. We assume that the genetic influences of the IL-6 SNPs on susceptibility to liver cirrhosis may occur through their effects on IL-6 expression. Our works found a pivotal role of IL-6 SNPs in predicting the progression of HBV-related liver cirrhosis in a Han Chinese population, which is helpful for the existing prognostic and therapeutic strategies for the HBV-related liver cirrhosis. To date, our research is the first to

explore the distribution of functional SNPs in the whole genomic sequence of the IL-6 gene in a Han Chinese population and their association with HBV-related liver cirrhosis. Nevertheless, larger comprehensive population-based studies with different races are needed to further validate these preliminary observations. Furthermore, our next study will focus on functional research on whether IL-6 SNPs could regulate the expression 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

SNP Single nucleotide polymorphism

ALT Alanine aminotransferase

MAF Minor allele frequency

HWE Hardy–Weinberg equilibrium

SD Standard deviation

SE Standard error

OR Odds ratio

CI Confidence interval

LD Linkage Disequilibrium

n.d. Not detected

ATDH Anti-tuberculosis drug-induced hepatitis

A1AT 1-antitrypsin

Declarations

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Availability of data and materials

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

Authors' contributions

MZ, YL and ZC were involved in the conceptualization, project administration, and supervision of the original draft. CX, WZ and CH participated in the methodology, formal analysis and writing of the original draft. GL, BY and FC were involved in providing 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. All subjects provided 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 Faure-Dupuy S, Delphin M, Aillot L, Dimier L, Lebosse F, Fresquet J, Parent R, et al. Hepatitis b virus-induced modulation of liver macrophage function promotes hepatocyte infection. J Hepatol. 2019; doi: 10.1016/j.jhep.2019.06.032.

2 Yen CJ, Yang ST, Chen RY, Huang W, Chayama K, Lee MH, Yang SJ, et al. Hepatitis b virus x protein (hbx) enhances centrosomal p4.1-associated protein (cpap) expression to promote hepatocarcinogenesis. J Biomed Sci. 2019; 26: 44.

3 Chang ML, Liaw YF. Hepatitis b flares in chronic hepatitis b: Pathogenesis, natural course, and management. J Hepatol. 2014; 61: 1407-1417.

4 Knolle PA, Thimme R. Hepatic immune regulation and its involvement in viral hepatitis infection. Gastroenterology. 2014; 146: 1193-1207.

5 Li M, Li F, Li N, Sang J, Fan X, Deng H, Zhang X, et al. Association of polymorphism rs1053005 in stat3 with chronic hepatitis b virus infection in han chinese population. BMC Med Genet. 2018; 19: 52.

6 Liu K, Chen L, Zhao G, Cao Z, Li F, Lin L, Zhu C, et al. Ips-1 polymorphisms in regulating interferon response in hbv infection. Biosci Trends. 2019; 13: 130-135.

7 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.

8 Jang Y, Kim JH, Lee H, Lee K, Ahn SH. A quantile regression approach to explain the relationship of fatigue and cortisol, cytokine among koreans with hepatitis b. Sci Rep. 2018; 8: 16434.

9 Yoshio S, Mano Y, Doi H, Shoji H, Shimagaki T, Sakamoto Y, Kawai H, et al. Cytokine and chemokine signatures associated with hepatitis b surface antigen loss in hepatitis b patients. JCI insight. 2018; 3: e122268.

10 Fazel Modares N, Polz R, Haghighi F, Lamertz L, Behnke K, Zhuang Y, Kordes C, et al. Il-6 trans-signaling controls liver regeneration after partial hepatectomy. Hepatology. 2019; doi: 10.1002/hep.30774.

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 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.

13 Kalkman HO. Novel treatment targets based on insights in the etiology of depression: Role of il-6 trans-signaling and stress-induced elevation of glutamate and atp. Pharmaceuticals (Basel). 2019; 12: 113-135.

14 Taheri M, Noroozi R, Rakhshan A, Ghanbari M, Omrani MD, Ghafouri-Fard S. Il-6 genomic variants and risk of prostate cancer. Urology journal. 2018; doi: 10.22037/uj.v0i0.4543.

15 Araki M. Blockade of il-6 signaling in neuromyelitis optica. Neurochem Int. 2018; doi: 10.1016/j.neuint.2018.10.012.

16 Jing R, Long TY, Pan W, Li F, Xie QY. Il-6 knockout ameliorates myocardial remodeling after myocardial infarction by regulating activation of m2 macrophages and fibroblast cells. Eur Rev Med Pharmacol Sci. 2019; 23: 6283-6291.

17 Keshari S, Sipayung AD, Hsieh CC, Su LJ, Chiang YR, Chang HC, Yang WC, et al. The il-6/p-btk/p-erk signaling mediates the calcium phosphate-induced pruritus. FASEB J. 2019; doi: 10.1096/fj.201900016RR.

18 Wei Y, Tian Q, Li L, Zhang D. Association between ifn-gamma genetic polymorphisms and susceptibility to hepatitis b virus infection: A meta-analysis. Ann Hum Biol. 2016; 43: 527-536.

19 Al-Anazi MR, Matou-Nasri S, Al-Qahtani AA, Alghamdi J, Abdo AA, Sanai FM, Al-Hamoudi WK, et al. Association between il-37 gene polymorphisms and risk of hbv-related liver disease in a saudi arabian population. Sci Rep. 2019; 9: 7123.

20 Talaat RM, Abdelkhalek MS, El-Maadawy EA, Abdel-Mageed WS, El-Shenawy SZ, Osman MA. Association of tnf-alpha gene polymorphisms and susceptibility to hepatitis b virus infection in egyptians. Hum Immunol. 2017; 78: 739-746.

21 Chang L, Lan T, Wu L, Li C, Yuan Y, Liu Z. The association between three il-6 polymorphisms and hbv-related liver diseases: A meta-analysis. Int J Clin Exp Med. 2015; 8: 17036-17045.

22 Motawi TK, Mady AE, Shaheen S, Elshenawy SZ, Talaat RM, Rizk SM. Genetic variation in microrna-100 (mir-100) rs1834306 t/c associated with hepatitis b virus (hbv) infection: Correlation with expression level. Infect Genet Evol. 2019; 73: 444-449.

23 Laaribi AB, Hannachi N, Ben Yahia H, Marzouk M, Mehri A, Belhadj M, Yacoub S, et al. Human leukocyte antigen (hla-f) polymorphism is associated with chronic hbv infection. 3 Biotech. 2018; 8: 49.

24 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.

25 Fu J, He HY, Ojha SC, Shi H, Sun CF, Deng CL, Sheng YJ. Association of il-6, il-10 and tgf-beta1 gene polymorphisms with brucellosis: A systematic review with meta-analysis. Microb Pathog. 2019; 135: 103640.

26 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.

27 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.

28 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.

29 Shaul O. How introns enhance gene expression. Int J Biochem Cell Biol. 2017; 91: 145-155.

30 Hoshida H, Kondo M, Kobayashi T, Yarimizu T, Akada R. 5 -utr introns enhance protein expression in the yeast saccharomyces cerevisiae. Appl Microbiol Biotechnol. 2017; 101: 241-251.

31 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.

32 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.

33 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.

34 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.

35 MacQuarrie KL, Fong AP, Morse RH, Tapscott SJ. Genome-wide transcription factor binding: Beyond direct target regulation. Trends Genet. 2011; 27: 141-148.

36 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.

37 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.

38 Fabricio-Silva GM, Poschetzky BS, de Mello Perez R, Dos Santos RC, Cavalini LT, Porto LC. Association of cytokine gene polymorphisms with hepatitis c virus infection in a population from rio de janeiro, brazil. Hepat Med. 2015; 7: 71-79.

39 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.

40 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.

41 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.

42 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.

43 El-Maadawy EA, Talaat RM, Ahmed MM, El-Shenawy SZ. Interleukin-6 promotor gene polymorphisms and susceptibility to chronic hepatitis b virus in egyptians. Hum Immunol. 2019; 80: 208-214.

44 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.

Figures

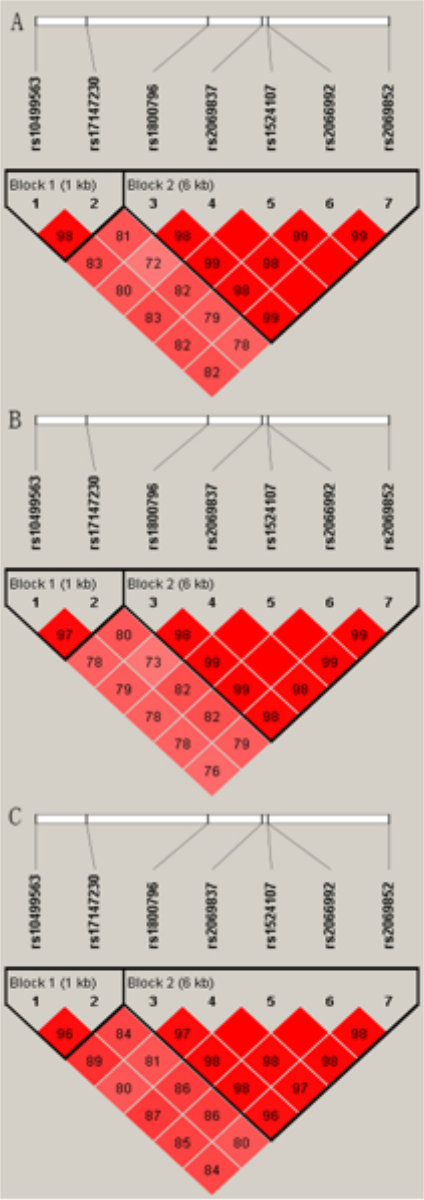

Figure 1

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

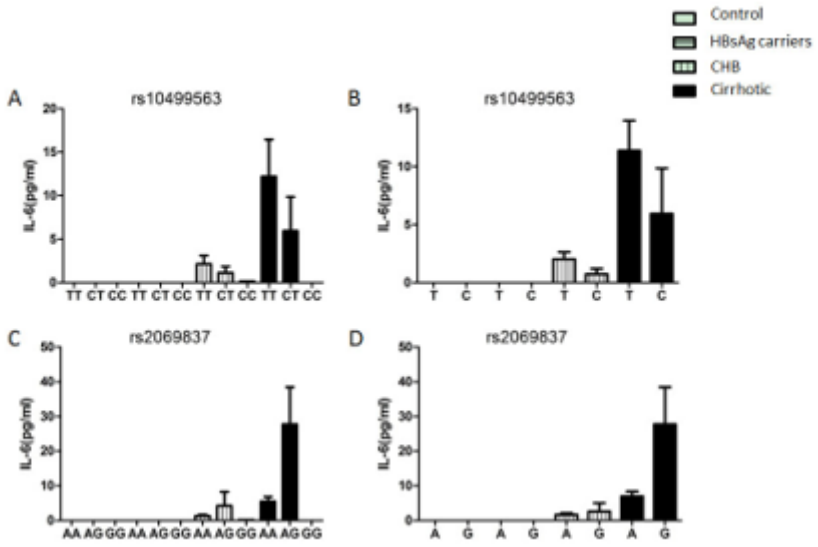

Figure 2

Plasma IL-6 levels in different genotypes and alleles of rs10499563 (A, B) and rs2069837 (C, D). Data are presented as the mean ± SE. IL-6 was not detected in the plasma of the healthy control group or the inactive HBV-carrier group.

Supplementary Files

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- SupplementaryMaterials.pdf