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ORIGINAL ARTICLE

Case Control Study

Interleukin-21 gene polymorphisms and chronic hepatitis B infection in a Chinese population

Jia-Yan Yao, Kang Chao, Min-Rui Li, Yan-Qing Wu, Bi-Hui Zhong

Jia-Yan Yao, Kang Chao, Min-Rui Li, Yan-Qing Wu, Bi-Hui Zhong, Department of Gastroenterology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510080, Guangdong Province, China

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Correspondence to: Bi-Hui Zhong, MD, PhD, Division of Gastroenterology, The First Affiliated Hospital of Sun Yat-Sen University, No. 58 Zhongshan Road II, Guangzhou 510080, Guangdong Province, China. sophiazhong@medmail.com.cn
Telephone: +86-20-87755766
Fax: +86-20-87332916

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Abstract

AIM: To investigate the relationship between interleukin-21 (*IL21*) gene polymorphisms and chronic hepatitis B virus (HBV) infection in a Chinese population.

METHODS: In this case-control study, 366 Chinese HBV-infected patients were recruited and divided into hepatocellular carcinoma (HCC; *n* = 94) and non-HCC (*n* = 272) groups at The First Affiliated Hospital of Sun Yat-Sen University, from April 2009 to December 2012. In the non-HCC group, the patients were classified into three clinical subsets, 76 patients had chronic hepatitis B, 101 were HBV carriers and 95 patients had HBV-related cirrhosis. Two hundred eight unrelated healthy controls were also included. Genomic DNA was extracted from peripheral blood. Single nucleotide polymorphisms (SNPs) rs13143866, rs2221903, and rs907715 were subsequently genotyped using the SNaPshot SNP technique.

RESULTS: There were no significant differences in allele and genotype frequencies of SNPs rs13143866, rs2221903, and rs907715 between chronic HBV-infected patients and control subjects. Furthermore, no significant differences were found in the frequencies of all alleles and genotypes between the HCC group and the non-HCC group. However, in the subgroup analysis, *IL21* rs13143866 genotype AA frequency in the HBV carrier group was higher than in controls (OR = 6.280, 95%CI: 1.238-31.854; *P* = 0.019), and the effect of the recessive model (AA *vs* GG + GA, OR = 6.505, 95%CI: 1.289-32.828) was observed in the HBV carrier group. *IL21* rs2221903 genotype TC frequency in the HBV carrier group was higher than in controls (OR = 1.809, 95%CI: 1.043-3.139; *P* = 0.035). In the haplotype analysis, the ATA haplotype (rs13143866, rs2221903, and rs907715) of *IL21* was more frequent in the HCC group than in the non-HCC group (0.165 *vs* 0.104, *P* = 0.044; OR = 1.700, 95%CI: 1.010-2.863).

CONCLUSION: Genotypes rs13143866 AA and rs2221903 TC are risk factors for carrying HBV; ATA haplotype increases the risk of HBV-related HCC onset

in a Chinese population.

Key words: Chinese population; Chronic hepatitis B virus infection; Interleukin-21 gene; rs13143866; rs2221903; rs907715; Single-nucleotide polymorphism

Core tip: This study is the first to investigate the relationship between single nucleotide polymorphism rs13143866 of the interleukin-21 gene and chronic hepatitis B virus (HBV) infection in a Chinese population. We found that genotypes rs13143866 AA and rs2221903 TC were risk factors for carrying HBV, and the ATA haplotype (rs13143866, rs2221903 and rs907715) increased the risk of HBV-related hepatocellular carcinoma.

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INTRODUCTION

Approximately one-third of the world's population has serologic evidence of past or present infection with hepatitis B virus (HBV), and 350-400 million people are chronic HBV surface antigen (HBsAg) carriers. The spectrum of disease and natural history of chronic HBV infection are diverse and variable, ranging from an inactive carrier state to progressive chronic hepatitis B (CHB), which may evolve to cirrhosis and hepatocellular carcinoma (HCC)[1]. An epidemiologic serosurvey of hepatitis B in China showed that the weighted prevalence of HBsAg in the Chinese population aged 1-59 years was 7.2%[2], which indicated that there were approximately 93 million people infected with HBV in China[3].

Interleukin (IL)-21, mainly produced by a range of differentiated $CD4^+$ T-cell subsets, is a relatively recently discovered multifunctional and pleiotropic cytokine[4,5]. It promotes proliferation and accumulation of Ag-specific $CD8^+$ effector T-cells, and increases their survival and cytolytic potential[6]. It also has a significant influence on the regulation of B-cell functions. It promotes the differentiation of antigen-stimulated B cells into memory and antibody-secreting plasma cells, affects IgE production, and induces Ig switch to IgG1 and IgG3 production[7,8]. In addition, it induces the differentiation of naive T cells into Th17 cells, and is involved in the maturation, activation, and survival of natural killer cells[9-11]. Several *in vivo* studies in animal models have shown that IL-21 is essential for controlling chronic viral infections[12-14]. The adaptive immune response is greatly attenuated in chronic HBV infection. It is likely that the absence of $CD4^+$ T-cells prevents the maturation of a functionally effective $CD8^+$ T-cell response, and is the primary reason for viral persistence[15-18]. Recent investigations have shown that serum levels of IL-21 are increased in CHB patients and associated with severe liver inflammation[19]. The levels of IL-21 expression in the liver tissues are significantly associated with increased degrees of inflammation and fibrosis in CHB patients[20]. High serum IL-21 levels after 12 wk of antiviral therapy predict HBeAg seroconversion in CHB[21]. IL-21 enhances HBcAg-specific interferon-γ^+CD8^+ T-cell proliferation, whereas treatment with anti-IL-21 inhibits expansion *in vitro*[22].

Collectively, these findings suggest that IL-21 may play a critical role in HBV infection. However, the precise mechanisms underlying the effects of IL-21 on hepatitis B pathogenesis have not yet been elucidated. Genetic polymorphisms in *IL21* have been explored in genetic susceptibility to chronic HBV-infected diseases. One report finds that *IL21* rs2221903 TC is less frequent in the HBV patients than in the HBV infection resolvers or in controls[23]. In kidney transplant patients with acute rejection, frequencies of TT homozygote genotype and T allele of IL-21-G1472T (rs2055979) polymorphism and CC homozygote genotype and C allele of IL-21-C5250T (rs4833837) polymorphism are higher in the HBV-infected patients than in the HBV-noninfected patients[24]. Accordingly, this study was conducted to confirm the association of *IL21* rs13143866, rs2221903, and rs907715 gene polymorphisms with susceptibility to chronic HBV infection in a Chinese population.

MATERIALS AND METHODS

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by The First Affiliated Hospital of Sun Yat-Sen University Institutional Review Board. All patients signed an informed consent form for this investigation.

Subjects

According to the guideline for the prevention and treatment of CHB (2010 version) and the diagnostic criteria (modified during the 10th National Conference on Viral Hepatitis and Hepatopathy 2000, China), 366 independent chronic HBV-infected patients (103 female and 263 male; mean age 48.3 ± 11.8 years) were recruited from The First Affiliated Hospital of Sun Yat-Sen University from April 2009 to December 2012 and were divided into an HCC (n = 94) and non-HCC (n = 272) group. All patients with HCC were confirmed by pathology. The non-HCC group was classified into three clinical subsets, CHB (n = 76), HBV carrier (n =

Table 1 Demographic data for all groups

Groups	Sex (M/F)	*P* value[1]	*P* value[2]	Age (yr)	*P* value[1]	*P* value[2]
HBV infection	263/103	0.672		48.3 ± 11.8	0.657	
HCC	64/30	0.713	0.345	48.8 ± 12.2	0.935	0.589
non-HCC	199/73	0.473		48.1 ± 11.7	0.544	
Carrier	71/24	0.416		47.9 ± 11.3	0.570	
CHB	58/18	0.310		46.5 ± 11.0	0.151	
Cirrhosis	70/31	1.000		49.4 ± 12.6	0.621	
Control	146/62			48.7 ± 11.3		

[1]*vs* control group; [2]between HCC and non-HCC groups. HBV: Hepatitis B virus; HCC: Chronic HBV-infected patients with hepatocellular carcinoma; non-HCC: Chronic HBV-infected patients without hepatocellular carcinoma; Carrier: HBV carriers; CHB: Patients with chronic hepatitis B; Cirrhosis: Patients with HBV-related cirrhosis.

101), and HBV-related cirrhosis (n = 95). None of the 366 chronic HBV-infected patients had received any antiviral therapy, including nucleoside analogues or interferon, before diagnosis. This study also included 208 geographically and ethnically matched unrelated healthy controls (62 female and 146 male; mean age 48.7 ± 11.3 years). Exclusion criteria included the presence of autoimmune diseases and other liver diseases, such as alcoholic liver disease, silt hemorrhagic liver disease, autoimmune liver disease, and intra-and extrahepatic bile duct stones.

Selection and genotyping of single-nucleotide polymorphisms

For selection of the single-nucleotide polymorphisms (SNPs), Haploview software (http://www.broad.mit.edu/mpg/haploview) was used to perform linkage disequilibrium and haplotype block analyses using HapMap phase genotype data for the chromosomal region 4:123,750,234..123,764,662 (CHB database, HapMap release 27). The amplicon of interest was a 14.4 kb region within *IL21* and approximately 3 kb upstream and 3 kb downstream of the gene. Three previously reported SNPs (rs13143866, rs2221903, and rs907715) with minor allele frequency (MAF) ⩾ 0.05 were chosen. Genomic DNA from peripheral blood was extracted using a commercially available kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. SNPs rs13143866, rs2221903, and rs907715 were subsequently genotyped by the SNaPshot SNP technique. Briefly, three segments were amplified using three pairs of forward and reverse primers: 5′-AAGTACCCACTGGACCAACTCA-3′ and 5′-TCTAGCTCTGAACCCAAACACT-3′, 5′-GGACCACATATTGCCAGACAC-3′ and 5′-GACACTGACGCCCATATTGAT-3′, and 5′-CACACTGGCATTGAGATGCTA-3′ and 5′-CCTCTTTTCACTTGGAGCATTC-3′ for rs13143866, rs2221903, and rs907715, respectively. All primers were designed using the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Multiplex PCR was carried out using the SNaPshot Multiplex Kit (Applied Biosystems of Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer's instructions. The reaction was performed in a total volume of 20 μL containing 2 μL 10 × PCR buffer (Mg^{2+}-free) (Invitrogen of Thermo Fisher Scientific), 0.8 μL $MgCl_2$ (50 mmol/L; Invitrogen), 0.5 μL dNTP (10 mmol/L; Takara Bio Inc., Shiga, Japan), 0.5 μL Primer Mix, 1 μL DNA, 0.5 μL Platinum Taq (5U; Invitrogen), and 14.2 μL ddH_2O. Cycling conditions were as follows: 95 ℃ for 2 min; 33 cycles of 95 ℃ for 20 s, 56 ℃ for 30 s, and 72 ℃ for 40 s, followed by 72 ℃ for 5 min. PCR products were purified using shrimp alkaline enzyme (Promega Corp., Madison, WI, United States) and exonuclease Ⅰ (EpiCentre, Palmerston North, New Zealand) according to the manufacturer's instructions. Purified PCR products from two panels were mixed and used as a template for extension. Extension was performed in a total volume of 5 μL comprising 2.5 μL of SNaPshot Multiplex reaction mix (Applied Biosystems), 1.5 μL of purified PCR products, 0.7 μL of Probe Mix, and 0.3 μL of GC buffer. Extension was performed under the following conditions: 25 cycles of 96 ℃ for 10 s, 51 ℃ for 5 s, and 60 ℃ for 30 s, and then kept at 4 ℃. Extension products were purified using shrimp alkaline enzyme (Promega) and loaded onto an ABI PRISM 3730 DNA Sequencer (Applied Biosystems) for sequencing.

Statistical analysis

Distributions of the allele and genotype frequencies were calculated by the χ^2 test or Fisher's exact test. ORs and 95%CIs of genotype frequencies were adjusted by logistic regression analysis. All analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, United States). The Hardy-Weinberg equilibrium test and the haplotype analysis were completed by SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). All two-sided $P < 0.05$ were considered statistically significant.

RESULTS

Demographic data on each group are shown in Table 1. The allele and genotype frequencies of the three SNPs in *IL21* in the HBV-infected patients and control subjects are shown in Table 2. The Hardy-Weinberg equilibrium P value in each group was > 0.01.

Association between IL21 polymorphisms and HBV infection

Although there was no statistically difference, our data showed a tendency that SNP rs2221903 was associated with an increased risk of chronic HBV infection. The frequency of allele C was 13.7% in the chronic HBV infection group and 10.3% in the control group. Furthermore, the effect of the dominant model was observed (Table 2).

Table 2 Comparison of interleukin-21 polymorphisms between chronic hepatitis B virus-infected patients and controls *n* (%)

Polymorphism		HBV infection (*n* = 366)	Control (*n* = 208)	*P* value	OR (95%CI)
rs13143866					
Allele frequency	Allele G	621 (85.5)	363 (87.3)		Reference
	Allele A	111 (14.5)	53 (12.7)	0.259	1.224 (0.861-1.741)
Genotype frequency[1]	GG	266 (72.7)	157 (75.5)		Reference
	GA	89 (24.3)	49 (23.5)	0.734	0.933 (0.625-1.392)
	AA	11 (3.0)	2 (1.0)	0.290[2]	0.308 (0.067-1.408)
	HWE	0.294	0.391		
Recessive model	GG + GA	355 (97.0)	206 (99.0)		Reference
	AA	11 (3.0)	2 (1.0)	0.149[2]	3.195 (0.701-14.493)
Dominant model	GG	266 (72.7)	157 (75.5)		Reference
	AA + GA	100 (27.3)	51 (24.5)	0.463	1.157 (0.783-1.711)
rs2221903					
Allele frequency	Allele T	632 (86.3)	373 (89.7)		Reference
	Allele C	100 (13.7)	43 (10.3)	0.101	1.373 (0.939-2.006)
Genotype frequency[1]	TT	272 (74.3)	167 (80.3)		Reference
	TC	88 (24.1)	39 (18.7)	0.130	1.385 (0.907-2.116)
	CC	6 (1.6)	2 (1.0)	0.262[2]	1.842 (0.367-9.232)
	HWE	0.712	0.868		
Recessive model	TT+TC	360 (98.4)	206 (99.0)		Reference
	CC	6 (1.6)	2 (1.0)	0.717[2]	1.715 (0.342-8.547)
Dominant model	TT	272 (74.3)	167 (80.3)		Reference
	CC + TC	94 (25.7)	41 (19.7)	0.105	1.408 (0.930-2.130)
rs907715					
Allele frequency	Allele G	415 (56.7)	234 (56.2)		Reference
	Allele A	317 (43.3)	182 (43.8)	0.884	0.982 (0.770-1.252)
Genotype frequency[1]	GG	110 (30.0)	70 (33.7)		Reference
	GA	195 (53.3)	94 (45.2)	0.160	1.320 (0.896-1.945)
	AA	61 (16.7)	44 (21.1)	0.155	0.882 (0.540-1.440)
	HWE	0.104	0.238		
Recessive model	GG + GA	305 (83.3)	164 (78.8)		Reference
	AA	61 (16.7)	44 (21.2)	0.181	0.746 (0.484-1.148)
Dominant model	GG	110 (30.1)	70 (33.7)		Reference
	AA + GA	256 (69.9)	138 (66.3)	0.372	1.181 (0.820-1.699)

[1]The *P* values of rs13143866, rs2221903, and rs907715 genotype distribution between chronic HBV-infected patients and controls were 0.237, 0.876, 0.358, respectively; [2]Fisher's exact test. HWE: Hardy-Weinberg equilibrium.

Association between IL21 polymorphisms and HCC

No significant differences were found in the frequencies of all alleles and genotypes (rs13143866, rs2221903, and rs907715) between the HCC group and the non-HCC group (Table 3).

Association between IL21 polymorphisms and HBV infection subgroups

The distribution of genotypes and alleles of *IL21* rs907715 polymorphisms showed no significant difference among HBV carriers, patients with CHB, patients with HBV-related cirrhosis, and healthy controls. However, *IL21* rs13143866 and rs2221903 polymorphisms were differently distributed between the HBV carrier group and controls (Table 4). *IL21* rs13143866 genotype AA frequency in the HBV carrier group was higher than in controls (5.9% *vs* 1.0%, *P* = 0.019), and the effect of the recessive model (AA *vs* GG + GA, *P* = 0.017) was observed in the HBV carrier group. *IL21* rs2221903 genotype TC frequency in the HBV carrier group was higher than in controls (29.7% *vs* 18.7%, *P* = 0.035).

Haplotype analysis

The results showed that the ATA haplotype (rs13143866, rs2221903, and rs907715) was significantly associated with HBV-related HCC and appeared to be a risk haplotype (*P* = 0.044) (Table 5).

DISCUSSION

In this study, we analyzed allele and genotype frequencies at three SNPs of the *IL21* gene in 366 chronic HBV-infected patients and 208 healthy controls in a Chinese population. The intronic SNPs may not be the actual risk mutation, but they are likely to be a surrogate marker for a mutation with functional consequences. The intronic SNPs may be in high linkage disequilibrium with a variant that associates with translation of the mRNA[25]. In other words, they may associate with protein expression. For example, synonymous SNPs have a substantial contribution to human disease risk and other complex traits[26]. Literature reports explored *IL21* rs907715 and rs2221903 and *IL21R* T-83C and rs3093301

Table 3 Interleukin-21 polymorphisms among chronic hepatitis B virus-infected patients with hepatocellular carcinoma, patients without hepatocellular carcinoma, and controls *n* (%)

Polymorphism		HCC (*n* = 94)	non-HCC (*n* = 272)	Control (*n* = 208)	HCC *vs* non-HCC		HCC *vs* Control		non-HCC *vs* Control	
					P value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)
rs13143866										
Allele	Allele G	167 (88.8)	454 (83.5)	363 (87.3)		Reference		Reference		Reference
frequency	Allele A	21 (11.2)	90 (16.5)	53 (12.7)	0.078	1.576 (0.949-2.617)	0.586	0.861 (0.503-1.474)	0.102	1.358 (0.941-1.958)
Genotype	GG	74 (78.7)	192 (70.6)	157 (75.5)		Reference		Reference		Reference
frequency[1]	GA	19 (20.2)	70 (25.7)	49 (23.5)	0.231	1.420 (0.800-2.520)	0.522	0.823 (0.453-1.495)	0.470	1.168 (0.766-1.781)
	AA	1 (1.1)	10 (3.7)	2 (1.0)	0.298[2]	3.854 (0.485-30.637)	1.000[2]	1.061 (0.095-11.886)	0.074[1]	4.089 (0.883-18.934)
	HWE	0.857	0.262	0.391						
Recessive	GG + GA	93 (98.9)	262 (96.3)	206 (99.0)		Reference		Reference		Reference
model	AA	1 (1.1)	10 (3.7)	2 (1.0)	0.230[2]	0.282 (0.036-2.231)	1.000[2]	1.108 (0.099-12.367)	0.077[1]	3.931 (0.852-18.139)
Dominant	GG	74 (78.7)	192 (70.6)	157 (75.5)		Reference		Reference		Reference
model	AA + GA	20 (21.3)	80 (29.4)	51 (24.5)	0.129	1.542 (0.882-2.695)	0.539	0.832 (0.463-1.495)	0.234	1.283 (0.852-1.932)
rs2221903										
Allele	Allele T	163 (86.7)	469 (86.2)	373 (89.7)		Reference		Reference		Reference
frequency	Allele C	25 (13.3)	75 (13.8)	43 (10.3)	0.866	1.043 (0.641-1.696)	0.288	1.330 (0.786-2.252)	0.108	1.387 (0.931-2.067)
Genotype	TT	70 (74.5)	202 (74.3)	167 (80.3)		Reference		Reference		Reference
frequency[1]	TC	23 (24.5)	65 (23.9)	39 (18.7)	0.940	0.979 (0.566-1.694)	0.075	1.668 (0.949-2.932)	0.160	1.378 (0.881-2.154)
	CC	1 (1.1)	5 (1.8)	2 (1.0)	0.696[2]	1.733 (0.199-15.087)	1.000[2]	1.193 (0.106-13.369)	0.466[1]	2.067 (0.396-10.790)
	HWE	0.554	0.931	0.868						
Recessive	TT + TC	93 (98.9)	267 (98.2)	206 (99.0)		Reference		Reference		Reference
model	CC	1 (1.1)	5 (1.8)	2 (1.0)	0.696[2]	1.742 (0.201-15.101)	1.000[2]	1.108 (0.099-12.367)	0.479[1]	1.929 (0.700-10.042)
Dominant	TT	70 (74.5)	202 (74.3)	167 (80.3)		Reference		Reference		Reference
model	CC + TC	24 (25.5)	70 (25.7)	41 (19.7)	0.969	0.989 (0.578-1.693)	0.256	1.397 (0.785-2.484)	0.122	1.411 (0.912-2.184)
rs907715										
Allele	Allele G	106 (56.4)	309 (56.8)	234 (56.2)		Reference		Reference		Reference
frequency	Allele A	82 (43.6)	235 (43.2)	182 (43.8)	0.920	0.983 (0.704-1.374)	0.976	0.995 (0.703-1.408)	0.864	0.978 (0.756-1.265)
Genotype	GG	25 (26.6)	85 (31.2)	70 (33.7)		Reference		Reference		Reference
frequency[1]	GA	56 (59.6)	139 (51.1)	94 (45.2)	0.256	0.730 (0.424-1.257)	0.135	0.598 (0.305-1.173)	0.347	1.218 (0.808-1.836)
	AA	13 (13.8)	48 (17.7)	44 (21.1)	0.831	1.086 (0.509-2.317)	0.062	0.827 (0.383-1.785)	0.685	0.898 (0.536-1.507)
	HWE	0.041	0.496	0.238						
Recessive	GG + GA	81 (86.2)	224 (82.4)	164 (78.8)		Reference		Reference		Reference
model	AA	13 (13.8)	48 (17.6)	44 (21.2)	0.393	1.335 (0.688-2.592)	0.135	0.598 (0.305-1.173)	0.334	0.799 (0.506-1.260)
Dominant	GG	25 (26.6)	85 (31.3)	70 (33.7)		Reference		Reference		Reference
model	AA + GA	69 (73.4)	187 (68.7)	138 (66.3)	0.397	0.797 (0.472-1.347)	0.222	1.400 (0.816-2.403)	0.577	1.116 (0.759-1.640)

[1]The *P* values of rs13143866, rs2221903, and rs907715 genotype distribution among hepatocellular carcinoma (HCC) group, non-HCC group, and controls were 0.237, 0.876, and 0.358, respectively; [2]Fisher's exact test. HWE: Hardy-Weinberg equilibrium.

polymorphisms in chronic HBV-infected patients, and *IL17* rs2275913, *IL23R* rs10889677, *IL21* rs4833837, and *IL21* rs2055979 polymorphisms in kidney transplant patients with HBV infection[23,24].

The statistical difference in genotype frequencies in the recessive model (AA *vs* GG + GA) of SNP rs13143866 showed that the A allele, when present in homozygotes, was a risk factor for carrying HBV. Furthermore, *IL21* rs13143866 genotype AA frequency in the HBV carrier group was higher than that in controls. In other studies, rs13143866 was associated with recurrent idiopathic spontaneous miscarriage[27], juvenile idiopathic arthritis[28], and lupus[29]. Thus, rs13143866 is likely a key genetic factor in both autoimmune diseases and chronic HBV-infected diseases.

Previous studies showed a significant association between rs2221903 and systemic lupus erythematosus (SLE) in the Chinese, European-American, and African-American populations[25,29,30]. Although there was no statistical difference, our data showed that this SNP is also associated with an increased risk of chronic HBV infection. The frequency of allele C was 13.7% in the chronic HBV infection group and 10.3% in the control group. In the subgroup analysis, the frequency of the TC genotype in the HBV carrier group was higher than that in controls. However, Li *et al*[24] found the opposite result, where the C frequency in HBV-infected patients was lower than in controls (8.1% *vs* 12.4%, *P* = 0.023, OR = 0.625, 95%CI: 0.415-0.941) and the TC genotype was less frequent in HBV-infected patients than in controls (16.2% *vs* 24.7%, *P* = 0.017, OR = 0.589, 95%CI: 0.381-0.911). Several potential explanations need to be considered. Firstly, the regulatory mechanisms of *IL21* intronic SNPs are still not clear. Secondly, chronic HBV infection is a complex complication related to other genetic and environmental factors[3].

Our data found no significant association between

Table 4 Interleukin-21 polymorphisms among non-hepatocellular carcinoma subgroups and controls *n* (%)

Polymorphism		Carrier	CHB	Cirrhosis	Control	Carrier *vs* control		CHB *vs* control		Cirrhosis *vs* control	
		(*n* = 101)	(*n* = 76)	(*n* = 95)	(*n* = 208)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)
rs13143866											
Allele frequency	Allele G	170 (84.2)	128 (84.2)	156 (82.1)	363 (87.3)		Reference		Reference		Reference
	Allele A	32 (15.8)	24 (15.8)	34 (17.9)	53 (12.7)	0.294	1.289 (0.802-2.073)	0.348	1.284 (0.761-2.166)	0.095	1.493 (0.933-2.388)
Genotype frequency	GG	75 (74.3)	53 (69.7)	64 (67.4)	157 (75.5)		Reference		Reference		Reference
	GA	20 (19.8)	22 (28.9)	28 (29.5)	49 (23.5)	0.600	0.854 (0.474-1.539)	0.345	1.330 (0.736-2.403)	0.227	1.402 (0.811-2.424)
	AA	6 (5.9)	1 (1.3)	3 (3.1)	2 (1.0)	0.019[1]	6.280 (1.238-31.854)	1.0001	1.481 (0.132-16.666)	0.156[1]	3.680 (0.601-22.544)
	HWE	0.01	0.44	0.977	0.391						
Recessive model	GG + GA	95 (94.1)	75 (98.7)	92 (96.8)	206 (99.0)		Reference		Reference		Reference
	AA	6 (5.9)	1 (1.3)	3 (3.2)	2 (1.0)	0.017[1]	6.505 (1.289-32.828)	1.000[1]	1.373 (0.123-15.367)	0.330[1]	3.359 (0.552-20.441)
Dominant model	GG	75 (74.3)	53 (69.7)	64 (67.4)	157 (75.5)		Reference		Reference		Reference
	AA + GA	26 (25.7)	23 (30.3)	31 (32.6)	51 (24.5)	0.816	1.067 (0.618-1.843)	0.330	1.336 (0.746-2.392)	0.142	1.491 (0.875-2.540)
rs2221903											
Allele frequency	Allele T	172 (85.1)	130 (85.5)	167 (87.9)	373 (89.7)		Reference		Reference		Reference
	Allele C	30 (14.9)	22 (14.5)	23 (12.1)	43 (10.3)	0.105	1.513 (0.918-2.495)	0.172	1.468 (0.846-2.547)	0.517	1.195 (0.697-2.046)
Genotype frequency	TT	71 (70.3)	56 (73.7)	75 (78.9)	167 (80.3)		Reference		Reference		Reference
	TC	30 (29.7)	18 (23.7)	17 (17.9)	39 (18.7)	0.035	1.809 (1.043-3.139)	0.324	1.367 (0.729-2.598)	0.926	0.971 (0.516-1.825)
	CC	0 (0.00)	2 (2.6)	3 (3.2)	2 (1.0)	-[2]		0.575[1]	2.982 (0.410-21.668)	0.330[1]	3.340 (0.547-20.405)
	HWE	0.080	0.705	0.121	0.868						
Recessive model	TT + TC	101 (100.0)	74 (97.4)	92 (96.8)	206 (99.0)		Reference		Reference		Reference
	CC	0 (0.0)	2 (2.6)	3 (3.2)	2 (1.0)	-[2]		0.577[1]	2.784 (0.385-20.120)	0.330[1]	3.359 (0.552-20.441)
Dominant model	TT	71 (70.3)	56 (73.7)	75 (78.9)	167 (80.3)		Reference		Reference		Reference
	CC + TC	30 (29.7)	20 (26.3)	20 (21.1)	41 (19.7)	0.052	1.721 (0.996-2.973)	0.232	1.455 (0.787-2.689)	0.787	1.086 (0.596-1.979)
rs907715											
Allele frequency	Allele G	115 (56.9)	89 (58.6)	105 (55.3)	234 (56.2)		Reference		Reference		Reference
	Allele A	87 (43.8)	63 (41.4)	85 (44.7)	182 (43.8)	0.873	0.973 (0.693-1.366)	0.624	0.910 (0.625-1.326)	0.820	1.041 (0.737-1.470)
Genotype frequency	GG	33 (32.7)	24 (3.16)	28 (29.5)	70 (33.7)		Reference		Reference		Reference
	GA	49 (48.5)	41 (53.9)	49 (51.6)	94 (45.2)	0.715	1.106 (0.645-1.896)	0.425	1.272 (0.704-2.298)	0.352	1.303 (0.746-2.277)
	AA	19 (18.8)	11 (14.5)	18 (18.9)	44 (21.1)	0.800	0.916 (0.465-1.806)	0.443	0.729 (0.325-1.634)	0.950	1.023 (0.507-2.064)
	HWE	0.914	0.331	0.674	0.238						
Recessive model	GG + GA	82 (81.2)	65 (85.5)	77 (81.1)	164 (78.8)		Reference		Reference		Reference
	AA	19 (18.8)	11 (14.5)	18 (18.9)	44 (21.2)	0.632	0.864 (0.474-1.573)	0.210	0.631 (0.307-1.296)	0.659	0.871 (0.473-1.606)
Dominant model	GG	33 (32.7)	24 (31.6)	28 (29.5)	70 (33.7)		Reference		Reference		Reference
	AA + GA	68 (67.3)	52 (68.4)	67 (70.5)	138 (66.3)	0.864	1.045 (0.630-1.733)	0.742	1.099 (0.626-1.929)	0.471	1.214 (0.717-2.055)

[1]Fisher's exact test; [2]χ^2 test cannot be conducted due to cross tabulation of zero. CHB: Chronic hepatitis B; HWE: Hardy-Weinberg equilibrium.

rs907715 and chronic HBV infection, which is consistent with Li *et al*[24]. The association between this SNP and immune disease has been widely investigated, though the findings are controversial. In a Chinese population, rs907715 was associated with Graves' disease[31] rather than SLE[25], and the two studies had a similar sample size. This discrepancy may be attributable to different diseases. Two studies investigated the same disease in an African-American population. Hughes *et al*[21] found that G allele increased the risk of SLE, but Sawalha *et al*[29] drew the opposite conclusion. These findings require further replication in other independent cohorts of chronic HBV-infected patients.

The data regarding ATA haplotype frequency (rs13143866, rs2221903, and rs907715) in HCC patients compared to non-HCC patients showed that this haplotype may be a risk for HBV-related HCC. This result suggests that the haplotype, according to the *IL21* polymorphisms, might be one of the most important genetic factors for susceptibility to HBV-related HCC.

Table 5 Haplotype analysis of polymorphisms

Haplotype[1]	Frequency		χ^2	*P* value	OR	95%CI
	HBV infection	Control				
A-T-A	0.150	0.127	1.117	0.291	1.209	0.850-1.720
G-C-G	0.137	0.103	2.725	0.099	1.376	0.941-2.011
G-T-A	0.283	0.31	0.883	0.347	0.882	0.678-1.147
G-T-G	0.429	0.459	0.959	0.327	0.886	0.695-1.129
	HCC	non-HCC				
A-T-A	0.165	0.104	4.057	0.044	1.700	1.010-2.863
G-C-G	0.138	0.133	0.017	0.900	1.033	0.635-1.681
G-T-A	0.267	0.333	3.226	0.072	0.072	0.503-1.031
G-T-G	0.430	0.423	0.009	0.926	1.016	0.726-1.422

[1]Haplotype of rs13143866 (G/A), rs2221903 (T/C), and rs907715 (G/A). HBV: Hepatitis B virus; HCC: Chronic HBV-infected patients with hepatocellular carcinoma; non-HCC: Chronic HBV-infected patients without hepatocellular carcinoma.

In conclusion, our study demonstrates that genotypes rs13143866 AA and rs2221903 TC are risk factors for carrying HBV, and ATA haplotype increase the risk of HBV-related HCC onset in a Chinese population. However, further studies are needed to determine the associations and functional consequences of these polymorphisms in chronic HBV-infection susceptibility.

COMMENTS

Background

The pathogenesis of chronic hepatitis B virus (HBV) infection is complicated, and the adaptive immune response plays a significant role in the pathophysiologic process. Interleukin (IL)-21 is a relatively recently discovered multifunctional and pleiotropic cytokine. Recently, investigations have shown that the serum level of IL-21 was increased in chronic hepatitis B patients and associated with severe liver inflammation. Genetic polymorphisms in *IL21* have been explored concerning genetic susceptibility to autoimmune and chronic HBV-infected diseases.

Research frontiers

The relationship between single-nucleotide polymorphisms (rs13143866, rs2221903, and rs907715) and autoimmune disease varies in different ethnicities. Therefore, studies performed using the same procedures and methods and enrolling more ethnic groups are required. In addition, further studies are needed to determine the associations and functional consequences of these polymorphisms in chronic HBV-infection susceptibility.

Innovations and breakthroughs

This is the first published study to investigate the relationship between the *IL21* rs13143866 polymorphism and chronic HBV-infection susceptibility.

Applications

Physicians should pay more attention to individuals who have the rs13143866 AA or rs2221903 TC genotype, or ATA haplotype (rs13143866, rs2221903 and rs907715), and provide early intervention before chronic HBV infection develops and leads to hepatocellular carcinoma.

Terminology

Single-nucleotide polymorphisms refer to DNA sequence polymorphisms at the genomic level caused by a single nucleotide mutation. HBV is a DNA virus, which belongs to the hepatotropic DNA virus (*Hepadnairidae*) group, and causes hepatitis B disease.

Peer-review

In this study, authors investigated the relationship between three single-nucleotide polymorphisms in *IL21* and chronic HBV infection in a Chinese population. They found that the rs13143866 A allele increases the risk of HBV infection and ATA haplotype (rs13143866, rs2221903, and rs907715) increases the risk of HBV-related hepatocellular carcinoma.

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