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Interaction effects among IFN-γ+874, IL-2-330, IL-10-1082, IL-10-592 and IL-4-589 polymorphisms on the clinical progression of subjects infected with hepatitis B virus and/or hepatitis C virus: a retrospective nested case–control study

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ABSTRACT

Background The natural outcomes of hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infections vary considerably among individuals The infection may heal naturally, or patients may succumb to chronic liver diseases, including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The mechanism is not fully understood.

Objectives To evaluate the interaction among four single nucleotide polymorphisms (SNPs) and their influence on different clinical outcomes.

Methods 277 individuals infected with HBV and/or HCV, including 81 patients with chronic hepatitis B and C, 122 asymptomatic HBV and/or HCV carriers and 74 controls who cleared HBV and HCV spontaneously, were involved in this study. The SNPs of four genes (*rs2069762/–330 G/T of IL-2*, *rs2430561/+874A>T* of *IFN-γ*, *rs1800896/–1082G>A* and *rs1800872/–592C>A of IL-10* and *rs2243250/–589C>T* of *IL-4*) were analysed using restriction fragment length polymorphism-polymerase chain reaction or sequence-specific primer PCR. The gene–gene interactions were assessed using the multifactor-dimensionality reduction method.

Results Interleukin (IL)-10-592 AC and IL-4-589 CC/CT showed a synergistic effect on liver inflammatory injury ($p<0.01$), whereas interferon (IFN)-γ+874 AA and IL-2-330 TT had a synergistic impact ($p<0.05$). IFN-γ+874 AA and IL-10-1082 AA had an antagonistic effect ($p<0.01$) on the clinical progression, including asymptomatic HBV and HCV carriers and chronic hepatitis. IL-2-330 TT and IL-10-1082 AA synergistically influenced the clinical outcome ($p<0.05$). IFN-γ+874AA, IL-2-330 TT and IL-10-1082 AA interactively affected the clinical outcome including asymptomatic HBV and HCV carriers and chronic hepatitis ($p<0.05$).

Conclusions Interactions among polymorphisms of IFN-γ+874AA, IL-2-330 TT, IL-10-1082 AA, IL10-–592 AC and IL-4-589 CC/CT significantly influenced the clinical progression of the subjects with HBV and/or HCV infection.

Strengths and limitations of this study

- As far as we know, this is the first study of interactions between single nucleotide polymorphisms among the four cytokines on the clinical outcomes for individuals with HBV and/or HCV infections.
- The natural history of the community-based blood donation infected with HBV, HCV and both HBV and HCV made the results more reliable.
- This study used a retrospective nested case–control method and the long-term data collection and analysis strengthened the rigour of the study.
- This study lacked stratification into subgroups of patients with HBV and/or HCV infections and biases might have affected the results.
- A liver biopsy in asymptomatic HBV and/or HCV carriers was not done to evaluate the inflammation.

INTRODUCTION

The outcome of HBV and/or HCV infections varies considerably from person to person.[1 2] Some individuals overcome the infection and are immune for the rest of their lives, whereas most people become disease carriers. Some carriers may have chronic liver diseases, such as chronic hepatitis (CH), liver cirrhosis and hepatocellular carcinoma.[3 4] The mechanisms explaining these differences have not yet been determined. Evidence has shown that the different immune responses to the HBV/HCV infection in individuals result in different clinical outcomes.[5 6]

Helper T cells (Th) are known to play an important role in immunomodulation. The major cytokines, including interleukin 2 (IL-2) and interferon-gamma (IFN-γ), are secreted by Th1 cells. Th1 cytokines are

associated with cell-mediated immunity to control intracellular pathogens, such as viruses, and participate in cytotoxic T lymphocyte proliferation and activation and activation of natural killer cells. The major lymphokines secreted by Th2 cells are interleukin 4 (IL-4) and interleukin 10 (IL-10). Th2 cytokines are essential for antibody-mediated immunity through proliferation and activation of the B lymphocytes.[7] The cytokines from Th1 cells inhibit Th2 cells and vice versa. The imbalance of Th1 and Th2 disturbs the balance between the cell-mediated and antibody-mediated immunity, which might be the key factor in the clinical outcome of chronic HBV and HCV infections.[8] Additionally, studies have shown that the different outcomes of patients with HBV and HCV infections are due to different levels of the Th1 and Th2 cytokines present.[9 10] Many researchers have shown a correlation between the immunity level of the host and the relevant gene polymorphisms, especially single nucleotide polymorphisms (SNPs) in the promoter region that regulate the gene expression.[11 12]

In our previous research, we reported that *rs2069762/–330 G/T of IL-2*, *rs2430561/+874 A>T* of *IFN-γ*, *rs1800896/–1082 G>A* and *rs1800872/–592 C>A of IL-10* and *rs2243250/–589 C>T* of *IL-4* were strongly associated with the clinical outcome of subjects infected with HBV and/or HCV.[13] However, the interleukins and SNPs of cytokines may not have independent roles, but may interact with each other in establishing the clinical consequences. In this study, we analysed the effect of gene–gene interactions of *rs2069762/–330 G/T of IL-2*, *rs2430561/+874 A>T* of *IFN-γ*, *rs1800896/–1082 G>A* and *rs1800872/–592 C>A of IL-10* and *rs2243250/–589 C>T* of *IL-4* on the clinical outcomes of subjects with HBV and/or HCV infections using generalised multifactor-dimensionality reduction.[14]

METHODS

Study subjects

Two hundred and seventy-seven people with HBV and/or HCV infection (137 male, 140 female), with a mean (SD) age of 50.2 (10.4) years (range 30–70 years), were included in the study. Of these, 81 patients were diagnosed with chronic hepatitis B (CHB) and/or chronic hepatitis C (CHC) (38 male, 43 female, mean (SD) age 49.8 (10.9) years), 122 patients were asymptomatic HBV and/or HCV carriers (ASC) (65 male, 57 female, mean (SD) age 51.3 (10.6) years) and 74 participants were controls who had been infected with HBV or HCV in the late 1980s because of plasma donation, but tested negative to HCV or HBV in 2007 (34 male, 40 female, mean (SD) age 48.6 (9.4) years). Subjects were classified into the following groups: (1) a control group; (2) asymptomatic hepatitis B surface antigen carriers and hepatitis C virus carriers, who fulfilled the following criteria: (i) seropositive for HBsAg and anti-HCV for at least 15 years, (ii) no evidence of liver cirrhosis based on the clinical criteria and ultrasound examination and (iii) normal alanine aminotransferase (ALT) (upper limit 80 U/L[15]); (3) patients diagnosed with chronic hepatitis B and/or hepatitis C, based on the following factors: (i) seropositive for HBsAg and anti-HCV for at least 15 years, (ii) serum HBV DNA positive and serum HCV RNA positive, (iii) persistent or intermittent elevation in ALT level and (iv) liver biopsy showing chronic hepatitis with moderate or severe necroinflammation. Patients who had other possible causes of hepatitis, including fatty liver and other concurrent illness, were excluded from this study. This study was approved by the ethics committees of the Centre for Disease Prevention and Control of Shijiazhuang and written consent was obtained from all voluntary subjects in accordance with the Declaration of Helsinki.

Genotyping of SNPs

Genomic DNA was extracted from the peripheral blood using a commercially available kit (Tiangen Biotech, Beijing, China). The genotype polymorphisms of *rs2069762/–330 G/T of IL-2*, *rs2243250/–589 C>T* of *IL-4* and *rs1800872/–592 C>A of IL-10* were examined using restriction fragment length polymorphism-polymerase chain reaction PCR. The primer sequences, PCR reaction volume and conditions for restriction enzyme digestion were the same as reported in our previous study.[13] The polymorphisms of *rs1800896/–1082 G>A* of *IL-10* and *rs2430561/+874 A>T* of *IFN-γ* were detected using the sequence-specific primer-PCR technique. The allele-specific primers, the PCR reaction volume and the internal control primers were used based on our previous publication.[13]

Statistical analysis

Gene–gene interactions were analysed by multifactor-dimensionality reduction.[14] The interaction between *rs1800872/-592 C*>A of *IL-10* and *rs2243250/-589 C>T* of *IL-4* and its relationship with abnormal ALT levels was evaluated. Similarly, the interactions among *rs2430561/+874 A>T* of *IFN-γ*, *rs2069762/-330 G/T* of *IL-2* and *rs1800896/-1082 G>A* of *IL-10*, and their association with the clinical outcome were assessed. Appropriate models were used to test accuracy, cross-validation (CV) consistency and significance. The interaction indexes, including synergy index (S), attributable proportion of interaction (API) and relative excess risk of interaction (RERI) were evaluated based on the Rothman measures[16]: (1) $S = (RR_{11}-1)/[(RR_{10}-1)+(RR_{01}-1)]$, (2) $API = [RR_{11}-(RR_{10}+RR_{01})+1]/RR_{11}$, (3) $RERI = RR_{11} - (RR_{10} + RR_{01})+1$. The 95% CI of RERI was calculated by $RERI^{(1\pm Z/\sqrt{\chi 2})}$. Frequencies of the genotypes among different groups were compared using the χ^2 test. The odds ratio (OR) and its 95% CI were also estimated. A p value of <0.05 was considered as statistically significant. All statistical tests were two sided.

RESULTS

Percentage homogeneity of patients infected with HCV, HBV combined HBV/HCV within each group

There were 37 cases (30.3%) of HCV infection, 43 (35.2%) of HBV infection and 42 (34.4%) of combined HBV and

Table 1 The IL-10-592 and IL-4-589 genotypes and allele distribution of subjects with different alanine aminotransferase (ALT) levels

Genotype and allele	ALT level <80 U/L (n=244)	ALT level ≥80 U/L (n=33)	χ^2	p Value
IL-10-592 AA	110 (45.1)	8 (24.2)	6.32	p<0.05
CC	27 (11.1)	3 (9.1)		
AC	107 (43.9)	22 (66.7)		
A	327 (67.0)	38 (57.6)	2.30	p>0.05
C	161 (33.0)	28 (42.4)		
IL-4-589 CC	6 (2.5)	3 (9.1)	12.46	p<0.05
CT	106 (43.4)	22 (66.7)		
TT	132 (54.1)	8 (24.2)		
C	118 (24.2)	28 (42.4)	9.97	p<0.01
T	370 (75.8)	38 (57.6)		

HCV infection in the ASC group, while in the group with CH, there were 18 cases (22.2%) of HCV infection, 36 cases (44.4%) of HBV infection and 27 (33.3%) patients with combined HBV/HCV infection. The population under study was therefore, shown to be homogeneous (χ^2=2.256, p=0.324).

Genotype and allele distributions in the subjects with HBV and/or HCV infections

rs1800872/–592 C>A of IL-10 and *rs2243250/–589 C>T* of *IL-4* correlated with abnormal ALT levels. While IL-10-592 AC showed an increased risk, IL-10-592 AA presented a reduced risk of unusual levels of ALT (OR (95% CI)=2.83 (1.21 to 6.63)). However, the IL-10–592 A/C alleles were not associated with such abnormal ALT levels (p>0.05). Although IL-4-589 CT/CC corresponded with an increased susceptibility, IL-4–589 TT showed a reduced risk of abnormal amounts of ALT [OR (95% CI)=3.43 (1.47 to 8.00) and 8.25 (1.74 to 39.22), respectively]. IL-4-589 C projected a higher risk; conversely, IL-4-589 T indicated a reduced possibility of a deviant ALT concentration (OR (95% CI)=2.31 (1.36 to 3.93)) (table 1).

rs2069762/–330 G/T of IL-2, *rs2430561/+874 A>T* of *IFN-γ* and *rs1800896/–1082 G>A* of *IL-10* were observed to correlate with the clinical outcome. IL-2-330 TT/T, OR (95% CI)=3.33 (1.42 to 7.79)/1.86 (1.22 to 2.82); IFN-γ+874 AA, OR (95% CI)=2.97 (1.47 to 5.99); and IL-10-1082 AA, OR (95% CI)=2.04 (1.05 to 3.97) were associated with an increased risk of ASC, CHB and CHC. In contrast, IL-2-330 GG/G, OR (95% CI)=3.31 (1.11 to 9.83)/1.71 (1.03 to 2.82); IFN-γ+874 TA, OR (95% CI)=3.71 (1.67 to 8.29); and IL-10-1082GA, OR (95% CI)=2.67 (1.24 to 5.75), were linked with a reduced risk of ASC, CHB and/or CHC (table 2).

Effect of gene–gene interaction and interaction indexes of IL-10-592 AC and IL-4-589 CC/CT on liver inflammatory injury

rs1800872/–592 C>A of IL-10 and *rs2243250/–589 C>T* of *IL-4* revealed significant interactions with liver inflammatory injury in the subjects infected with HBV and/or HCV (p<0.01, testing accuracy=0.7112 and CV consistency=10/10) (table 3). IL-10-592 AC and IL-4-589 CC/

Table 2 The IL-2–330, IFN-γ+874 and IL-10–1082 genotypes and allele distribution of the subjects with different clinical outcomes

Genotype and allele	Clinical outcome: Controls (n=74)	ASC (n=122)	CHB/C (n=81)	χ^2	*P*
IL-2–330 TT	22 (29.7)	54 (44.3)	23 (40.4)	13.46	p<0.05
GG	19 (25.7)	14 (11.4)	6 (10.5)		
TG	33 (44.6)	54 (44.3)	28 (49.1)		
T	77 (52.0)	163 (66.8)	74 (64.9)	11.69	p<0.01
G	71 (48.0)	81 (33.2)	40 (35.1)		
IFN-γ+874TT	8 (10.8)	14 (11.5)	5 (8.8)	14.8	p<0.05
AA	14 (18.9)	48 (39.3)	26 (45.6)		
TA	52 (70.3)	60 (49.2)	26 (45.6)		
T	68 (45.9)	90 (36.9)	36 (31.6)	5.68	p>0.05
A	80 (54.1)	154 (63.1)	78 (68.4)		
IL-10-1082GG	1 (1.4)	1 (0.8)	1 (1.8)	7.03	p<0.05
AA	16 (21.6)	44 (36.1)	24 (42.1)		
GA	57 (77.0)	77 (63.1)	32 (56.1)		
G	59 (39.9)	79 (32.4)	34 (29.8)	5.85	p>0.05
A	89 (60.1)	165 (67.6)	80 (70.2)		

ASC, asymptomatic HBV and/or HCV carriers; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 3 Gene–gene interactions of IFN-γ+874, IL-2-330, IL-10-1082, IL-10-592 and IL-4-589 in the clinical outcome of the subjects infected with HBV and HCV

Clinical types	Interaction among genes	Testing accuracy	CV consistency	P
Abnormal ALT	IL-10-A592C*IL-4-C589T	0.7112	10/10	<0.01
Clinical outcome	IFN-γ+T874A*IL-2-T330G	0.6313	10/10	<0.01
	IFN-γ+T874A*IL-10-G1082A	0.6496	10/10	<0.01
	IL-2-T330G*IL-10-G1082A	0.6182	10/10	<0.01
	IFN-γ+T874A*IL-10-G1082A*IL-2-T330G	0.5902	9/10	≤0.01

ALT, alanine aminotransferase; CV, cross-validation.

CT had a significant synergistic effect on the liver inflammatory injury, with a p value <0.01, OR (95% CI)=5.47 (1.93 to 15.49) and S=7.10. Among patients infected with HBV and/or HCV, the 67.6% of those who were in risk (as shown by the API score) of developing liver inflammatory injury was due to the interaction between IL-10-592 AC and IL-4-589 CC/CT genotypes. The extra risk of progression related to the interaction between these two genotypes was 3.70 times higher than that of other factors; RERI (95% CI)=3.70 (2.01 to6.80) (table 4).

Impact of gene–gene interaction and interaction indexes of IFN-γ+874 AA, IL-2-330 TT and IL-10-1082 AA on the clinical outcomes of the subjects

rs2430561/+874 A>T of *IFN-γ*, *rs2069762/−330 G/T of IL-2* and *rs1800896/−1082 G>A* of *IL-10* showed a significant impact on the clinical outcomes of subjects infected with HBV and/or HCV (p<0.01). Testing accuracy and CV consistency of *rs2430561/+874 A>T*rs2069762/−330*G/T were 0.6313 and 10/10, respectively; and that of *rs2430561/+874 A>T*rs1800896/−1082 G>A* were 0.6496 and 10/10, respectively. While *rs2069762/−330* G/T**rs1800896-1082 G>A* presented a testing accuracy and CV consistency of 0.6182 and 10/10, respectively, the values for *rs2430561/+874 A>T*rs1800896/−1082 G>A*rs2069762/−330*G/T were 0.5902 and 9/10, respectively (table 3).

IFN-γ+874AA and IL-2–330 TT significantly progressed and had a synergistic effect on the clinical outcome of ASC or CH, with p<0.01; OR (95% CI)=10.13 (2.25 to 45.68) and 11.40 (2.40 to 54.22), respectively; and S=13.04and 5.59, respectively (table 5). The clinical manifestation of ASC and CH with 83.2% and 74.9% risks (in terms of the API), respectively, were due to the interaction between IFN-γ+874AA and IL-2-330 TT genotypes. The additional risk of the clinical outcome resulting from the interaction between the two genotypes was higher than due to other factors, as reflected by the RERI (95% CI)=8.43 (2.95 to 24.06) for ASC, 8.54 (2.97 to 24.53) for CH.

IFN-γ+874AA and IL-10-1082 AA interactively impeded the progression of HBV and HCV infections to ASC and CH [OR (95% CI)=3.27 (1.10 to 9.72) and 5.97 (1.92 to 18.56), respectively; p<0.05] (table 6); and showed a significant antagonistic effect on these clinical outcomes in the subjects infected with HBV and/or HCV (S=0.47, 0.67, respectively). Furthermore, the protective actions could be attributed to the interaction between the genotypes, IFN-γ+874AA and IL-10-1082 AA (API=−79.21%, −41.21%, respectively). Meanwhile, the excess protection resulting from the interaction was −2.59 (-1.80 to −3.73) and −2.46 (−1.74 to −3.47) times lower than due to other factors, as indicated by these RERI (95% CI) scores.

The IL-2–330 TT and IL-10–1082 AA had a significant and synergistic impact on the clinical developments to ASC and CH; p<0.05, OR (95% CI)=3.34 (1.24 to 9.01), 4.73 (1.66 to13.47), respectively, S=1.65, 1.40 (table 7). In the clinical outcome of the subjects as ASC and CH, 27.54% and 22.41% of the risks (as signified by the API scores) were due to the interaction between IL-2–330 TT and IL-10–1082 AA genotypes. Furthermore, the RERI scores indicated that the excess risk of this interaction results in the progression of the infection was 0.92 (0.88 to 0.96) and 1.06 (1.03 to 1.09), respectively.

IFN-γ+874AA, IL-2-330 TT and IL-10-1082 AA presented certain associations with the clinical outcome, such as ASC or CH in patients infected with HBV and HCV; OR (95% CI)=9.60 (1.15 to 80.39), 17.23 (1.92 to 154.30), respectively, p<0.05 (table 8). However, no interactions developed into ASC, they progressed only to CH (S=1.06,

Table 4 Effect of the interaction of IL-10-592 AC and IL-4-589 CC/CT on serum alanine aminotransferase (ALT) levels of the subjects infected with HBV and/or HCV

IL-10-592 AC	IL-4-589 CC/CT	ALT<80 U/L	ALT≥80 U/L	p Value	OR (95% CI)
−	−	82	5	−	1.00
−	+	55	6	>0.05	1.79 (0.52 to 6.15)
+	−	50	3	>0.05	0.98 (0.23 to 4.30)
+	+	57	19	<0.01	5.47 (1.93 to 15.49)

Table 5 Effect of the interaction of IFN-γ+874 AA and IL-2-330TT on the clinical outcome of the subjects infected with HBV/HCV (n)

IFN-γ+874 AA	IL-2–330 TT	Clinical outcome						
		Control[1]	ASC[2]	p Value	OR (95% CI)[2vs1]	CHB/C[3]	p Value	OR (95% CI)[3vs1]
–	–	38	45	–	1.00	25	–	1.00
+	–	13	23	>0.05	1.49 (0.67 to3.34)	20	=0.05	2.34 (1.00 to 5.54)
–	+	21	30	>0.05	1.21 (0.60 to 2.44)	21	>0.05	1.52 (0.69 to 3.34)
+	+	2	24	<0.01	10.13 (2.25 to 45.68)	15	<0.01	11.40 (2.40 to 54.22)

1Control; [2]ASC; [3]CHB/C.ASC, asymptomatic HBV and/or HCV carriers; HBV, hepatitis B virus; HCV, hepatitis C virus.

1.27; API=4.79%, 20.08%; RERI (95% CI)=0.46 (0.34 to 0.62), 3.46 (2.45 to 5.58), for ASC and CH respectively).

DISCUSSION

The associations of the SNPs: *rs2069762/–330 G/T of IL-2*, *rs2430561/+874 A>T* of *IFN-γ*, *rs1800896/-1082 G>A* and *rs1800872/-592 C>A of IL-10* and *rs2243250/-589 C>T* of *IL-4* with the clinical outcome of HBV and/or HCV infections were reported in our previous study.[16] Ramos *et al*[17 18] have reported similar findings. In our study, the gene–gene interactions of these SNPs, mediating the clinical outcome of the subjects infected with HBV and/or HCV were evaluated.

IL-10 is an important anti-inflammatory cytokine, while IL-4 performs the function of modulating inflammatory responses by downregulating the production of proinflammatory mediators and preventing the liver from inflammatory injury.[19 20] In a recent study, Saxena *et al*[21] reported the significance of IL-4 in the progression of the HBV disease to development of cirrhosis. Additionally, they identified the IL-4(-590) CT genotype as a vital protective factor for the development of hepatitis, among carriers. In this study, we observed that IL-10-592 AC and IL-4589 CC/CT interactively increased the risk of, and had a significant synergistic effect on, the liver inflammatory injury of the patients infected with HBV and/or HCV; OR (95% CI)=5.47 (1.93 to 15.49), S=7.1. The interaction of IL-10-592 AC and IL-4-589 CC/CT presented a risk as 67.64% for the manifestation of liver inflammatory injury. However, the excess risk from this interaction, as assessed by the RERI score, was found to be 3.70 times higher than that of other factors. This result suggested that it might be worth exploring IL-10 and IL-4 to cure the liver inflammatory injury in patients with hepatitis with different genotypes of IL-10-592 AC and IL-4-589 CC/CT.

IL-2 has a powerful immunoregulatory effect on the stimulation of proliferation and activation of T lymphocytes, natural killer cells and B lymphocytes.[22] IFN-γ plays an important role in modulating almost all the immune responses, including T cell differentiation, anti-proliferation, anti-tumour and antiviral activities.[23 24] It shows that IFN-γ+874 AA genotype and IL-2-330 TT genotype interactively led to the progression of the HBV/HCV infections to ASC [OR (95% CI)=10.13 (2.25 to 45.68)] and CH [OR (95% CI)=11.40 (2.40 to 54.22)]. The risks of the subjects with these genotypes for developing ASC and CH were greater than the IFN-γ+874 AA genotype (6.80 and 4.87 times, respectively) and the IL-2-330 TT genotype (8.37 and 7.50 times, respectively). IFN-γ+874 AA genotype and IL-2-330 TT genotype showed significant synergy towards the clinical manifestation of ASC and CH (S=13.04, 5.59). Furthermore, Bei *et al*[25] reported that the interaction between IL-2-330 and IFN-γ-1615 was associated with an increased risk of hepatocellular carcinoma (OR=1.078, 95% CI=1.022 to 1.136). In our study, the excess risk presented by the interaction between IFN-γ+874 AA and IL-2-330 TT for the clinical outcome suggested that it was worth investigating IFN-γ and IL-2 to provide a cure for patients with hepatitis with different genotypes of IFN-γ+874 AA and IL-2-330 TT. In a study by Wang *et al*,[26] the concentrations of IL-2 and IFN-γ were found to be significantly elevated in the patients with CHB. IFN-γ levels increased significantly in the patients with CHC.

Table 6 Effect of the interaction of IFN-γ+874 AA and IL-10-1082 AA on the clinical outcome of the subjects infected with HBV/HCV (n)

IFN-γ+874 AA	IL-10-1082 AA	Clinical outcome						
		Control[1]	ASC[2]	p Value	OR (95% CI)[2vs1]	CHB/C[3]	p Value	OR (95% CI)[3vs1]
–	–	49	45	–	1.00	23	–	1.00
+	–	9	33	<0.01	3.99 (1.72 to 9.26)	21	<0.01	4.97 (1.97 to 12.53)
–	+	11	29	<0.01	2.87 (1.29 to 6.41)	23	<0.01	4.46 (1.86 to 10.66)
+	+	5	15	<0.05	3.27 (1.10 to 9.72)	14	<0.01	5.97 (1.92 to 18.56)

1Control; [2]ASC; [3]CHB/C.ASC, asymptomatic HBV and/or HCV carriers; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 7 Effect of the interaction of IL-2-330 TT and IL-10-1082 AA on the clinical outcome of the subjects with HBV/HCV (n)

IL-2-330 TT	IL-10-1082AA	Clinical outcome						
		Control[1]	ASC[2]	p Value	OR (95% CI)[2vs1]	CHB/C3	p Value	OR (95% CI)[3vs1]
–	–	41	47	–	1.00	26	–	1.00
+	–	17	31	>0.05	1.59 (0.77 to 3.28)	18	>0.05	1.67 (0.73 to 3.81)
–	+	10	21	>0.05	1.83 (0.77 to 4.34)	19	<0.05	3.00 (1.21 to 7.44)
+	+	6	23	<0.05	3.34 (1.24 to 9.01)	18	<0.01	4.73 (1.66 to 13.47)

1Control; [2]ASC; [3]CHB/C.ASC, asymptomatic HBV and/or HCV carriers; HBV, hepatitis B virus; HCV, hepatitis C virus.

In a study on the correlation between IL-10 and outcome of the HBV or HCV infections, Sofian *et al*[27] reported a greater prevalence of IL-10-1082 GG in the group with persistent HBV. Similarly, da Silva *et al*[28] detected higher frequencies of IL-10-1082 GG/G in patients infected with HCV and Akcam *et al*[29] revealed that the frequency of IL-10 was significantly higher in the hepatitis B and C groups than in controls. In our study, IFN-γ+874AA genotype and IL-10-1082 AA genotype interactively led to the progression of patients with HBV and/or HCV infections to asymptomatic HBV and/or HCV carriers and CH [OR (95% CI)=3.27 (1.10 to 9.72), 5.97 (1.92 to 18.56)]. Nevertheless, the SNPs of IFN-γ+874AA and IL-10-1082 AA had a significant antagonistic effect on the clinical outcome of subjects (S=0.47, 0.67). The interaction of the IFN-γ+874AA genotype and IL-10-1082 AA genotype offered excess protection, as indicated by the RERI scores (−2.59 and −2.46, respectively). Our data provided the evidence that IL-10 and IFN-γ are associated with the clinical outcome of the subjects infected with HBV and/or HCV,[30 31] and offered a molecular basis for the antagonism between the Th1 and Th2 cytokines. Simultaneously, these findings indicated that IL-10 and IFN-γ could be combined and studied for the treatment of patients with different genotypes of IL-10-1082 AA and IFN-γ+874AA.

The interaction of IL-2-330 TT genotype with the IL-10-1082 AA genotype had a significant impact on the clinical outcome (p=0.045). The SNPs of the IL-2-330 TT genotype and IL-10-1082 AA genotype acted synergistically to increase the risk of the subjects infected with HBV and HCV [S=1.65 (ASC) and 1.40 (CH), RERI=0.92 (0.88~0.96) for ASC and 1.06 (1.03~1.09) for CH, API=27.54% (ASC) and 22.41% (CH)]. The interaction between IL-2-330 TT and IL-10-1082 AA was, however, weaker than that of IFN-γ+874AA and IL-10-1082 AA genotypes.

The genotypes, IFN-γ+874AA, IL-2-330 TT and IL-10-1082 AA showed significant associations with the clinical outcome of HBV and HCV infections, such as asymptomatic HBV and HCV carriers (OR (95% CI)=9.60 (1.15 to 80.39)), CH (OR (95% CI)=17.23 (1.92 to 154.30); p=0.027). Although no interactions led to the development of asymptomatic HBV and HCV carriers, interactions towards the manifestation of chronic hepatitis were observed; S=1.06, 1.27; API=4.79%, 20.08%; RERI (95% CI)=0.46 (0.34 to 0.62), 3.46 (2.45 to 5.58), respectively. However, these findings need further confirmation using a larger sample. Nevertheless, this result reaffirmed that Th1 cytokines and Th2 cytokines regulate each other.[32] At the same time, it indicated that IL-10, IFN-γ and IL-2 could be combined to find a remedy for patients with the genotypes IFN-γ+874AA, IL-2-330 TT and IL-10-1082 AA.

In summary, the natural outcome of HBV and/or HCV infections was not completely dependent on one factor

Table 8 Effect of the interaction of IFN-γ+874AA, IL-2-330 TT and IL-10-1082 AA genotypes on the clinical outcome of the subjects with HBV/HCV (n)

IFN-γ +874 AA	IL-2-330 TT	IL-10-1082 AA	Clinical outcome						
			Control[1]	ASC[2]	p Value	OR (95% CI)[2vs1]	CHB/C[3]	p Value	OR (95% CI)[3vs1]
–	–	–	32	30	–	1	13	–	1
–	–	+	6	15	>0.05	2.67 (0.92 to 7.77)	12	<0.01	4.92 (1.52 to 15.91)
+	–	–	9	17	>0.05	2.02 (0.78 to 5.21)	13	<0.05	3.56 (1.22 to 10.33)
–	+	–	15	17	>0.05	1.21 (0.51 to 2.84)	10	>0.05	1.64 (0.59 to 4.59)
+	+	–	2	14	<0.01	7.47 (1.56 to 35.64)	8	<0.01	9.85 (1.84 to 52.74)
–	+	+	5	14	=0.05	2.99 (0.96 to 9.30)	11	<0.01	5.42 (1.57 to 18.68)
+	–	+	4	6	>0.05	1.60 (0.41 to 6.23)	7	<0.05	4.31 (1.08 to 17.25)
+	+	+	1	9	<0.05	9.60 (1.15 to 80.39)	7	<0.01	17.23 (1.92 to 154.30)

1Control; [2]ASC; [3]CHB/C.ASC, asymptomatic HBV and/or HCV carriers; HBV, hepatitis B virus; HCV, hepatitis C virus.

alone but depended on interactions among multiple factors. In this study, we found that IL-10-592 AC and IL-4-589 CC/CT had a synergistic effect on the liver inflammatory injury in the subjects. IFN-γ+874 AA and IL-2-330 TT synergistically affected the risk of clinical progression of HBV and/or HCV infections. In contrast, IFN-γ+874 AA and IL-10-1082 AA had noticeable antagonistic effects on the clinical outcome. IL-2-330 TT and IL-10-1082 AA displayed a weaker impact on the clinical outcome. The interactions among IFN-γ+874 AA, IL-2-330 TT and IL-10-1082 AA showed no influence on asymptomatic HBV and HCV carriers, but they had a weak synergistic effect on chronic hepatitis. These results suggest that different countermeasures for treatment should be investigated in patients with hepatitis with the genotypes IFN-γ+874 AA, IL-2-330 TT, IL-10-1082 AA, IL-10-592 AC and IL-4-589 CC/CT.

In this study, the size of the subgroups of individuals with HBV and/or HCV infections varied, which might have led to biases. More ideally, the samples should have been assessed by stratifying individuals into subgroups with individuals infected only by HCV and only by HBV. In addition, the correlation between interactions among the SNPs and the level of cytokines needs further investigation.

Contributors Q-JG designed the research, performed most of the experiments and drafted the manuscript; J-xX analysed the data and edited the paper; L-MW took part in the investigation and edited the paper; QZ performed the experiments; S-YZ designed the research and analysed the data.

Competing interests None.

Patient consent Obtained.

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