

# Human leukocyte antigen gene polymorphisms are associated with systemic inflammation in hepatitis B virus-related hepatocellular carcinoma

Xiao-long Wu<sup>1,\*</sup>

Zhi-yu Li<sup>1,\*</sup>

Xin-yu Bi<sup>1</sup>

Hong Zhao<sup>1</sup>

Jian-jun Zhao<sup>1</sup>

Jian-guo Zhou<sup>1</sup>

Yue Han<sup>2</sup>

Zhen Huang<sup>1</sup>

Ye-fan Zhang<sup>1</sup>

Jian-qiang Cai<sup>1</sup>

<sup>1</sup>Department of Hepatobiliary Surgery,

<sup>2</sup>Department of Interventional Therapies, National Cancer Center/ Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100021, China

\*These authors contributed equally to this work

**Background:** Systemic inflammation (SI) is associated with tumor progression and overall survival (OS) in patients with hepatocellular carcinoma (HCC). The presence of some single nucleotide polymorphisms (SNPs) in the human leukocyte antigen (HLA) region can influence the prognosis of patients with hepatitis B virus (HBV)-related HCC, although the mechanism remains unknown. This study aimed to analyze the correlations between HLA gene polymorphisms and SI.

**Patients and methods:** This study included 330 patients with HCC. The clinical parameters were reviewed, and five SNPs, namely rs2647073, rs3997872, rs3077, rs7453920, and rs7768538, were genotyped using the MassARRAY system.

**Results:** The rs3997872, rs7453920, and rs7768538 genotypes were found to be significantly associated with OS ( $P < 0.05$ ). The rs7453920 genotype was significantly associated with the neutrophil/lymphocyte ratio (NLR;  $P = 0.001$ ), which was used as an SI index with a threshold determined by receiver operating characteristic analysis. An elevated NLR was also an independent predictor of OS according to univariate and multivariate analyses ( $P < 0.001$ ).

**Conclusion:** Our data show that HLA gene polymorphisms are associated with SI in patients with HBV-related HCC, and the absence of minor allele A (rs7453920) promotes SI and shortens OS.

**Keywords:** human leukocyte antigen, single nucleotide polymorphism, systemic inflammation, hepatitis B virus, hepatocellular carcinoma

## Introduction

Hepatocellular carcinoma (HCC) is among the most prevalent malignancies worldwide and is the fourth most common cancer and the third leading cause of cancer-related deaths in China.<sup>1</sup> Development and prognosis of HCC depend not only on the intrinsic properties of tumor cells but also on several host-related factors. In the Chinese population, most HCCs develop as a complication of chronic liver inflammation and cirrhosis caused by hepatitis B virus (HBV) or hepatitis C virus (HCV) infection.<sup>2-4</sup>

Recent evidence suggests that systemic inflammation (SI) is associated with tumor progression and overall survival (OS) in patients with HCC.<sup>5-8</sup> White blood cell counts and ratios, as well as C-reactive protein levels and the Glasgow prognostic score, are often used as indices of SI. The neutrophil/lymphocyte ratio (NLR) is the most commonly used index to predict prognoses following HCC treatment (which can include surgical resection, liver transplantation, transarterial chemoembolization [TACE], and sorafenib administration).<sup>6,8-12</sup> The neutrophil count in the peripheral blood is associated

Correspondence: Jian-qiang Cai  
Department of Hepatobiliary Surgery,  
National Cancer Center/Cancer Hospital,  
Chinese Academy of Medical Sciences  
and Peking Union Medical College, Beijing  
100021, China  
Tel +86 10 8778 7100  
Email caijianqiang@cicams.ac.cn

with tumor-promoting inflammation, while lymphocyte counts reflect the antitumor immune function. An elevated NLR may indicate an imbalance between SI and the host immune response in favor of the former.<sup>13,14</sup>

Meanwhile, the host immune response plays a key role in liver carcinogenesis and progression. The human leukocyte antigen (HLA) region, which is located on chromosome 6p21.3, is important for the immune response to viral and cancer antigens. Recently, several genome-wide association studies (GWASs) have found that some loci in the HLA region are associated with susceptibility to chronic hepatitis B (CHB) or HBV-related HCC.<sup>15–20</sup> Furthermore, our previous study produced evidence that some of these GWAS-identified susceptibility loci affect the prognosis of patients with HBV-related HCC.<sup>21</sup>

However, the mechanisms by which these susceptibility loci affect the survival of these patients are unknown. Based on the relationship between immunity, inflammation, and cancer, we hypothesized that HLA gene polymorphisms cause an imbalance between SI and immune response that results in poorer outcomes of patients with HBV-related HCC. Finally, this study aimed to analyze the correlation between HLA gene polymorphisms and SI.

## Patients and methods

### Patients

A total of 360 Chinese patients with HBV-related HCC who were admitted to the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China, between January 1999 and December 2012 and had not received any previous treatment were enrolled in this study. A total of 30 patients (8.3%) were excluded because of missing clinicopathological data; thus, 330 patients were ultimately included. Imaging studies including ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and angiography were performed for diagnosis. HCC was diagnosed based on the European Association for the Study of the Liver-European Organization for Research and Treatment of Cancer (EASL-EORTC) clinical practice guidelines; biopsies were acquired if patients did not have typical imaging features.<sup>22</sup> All the enrolled patients were serologically positive for both the hepatitis B surface antigen and core antibody; patients with HCV coinfection were excluded. The patient's baseline clinical characteristics were collected, including white blood cell counts, neutrophil counts, lymphocyte counts, and levels of alanine aminotransferase,  $\gamma$ -glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), albumin, and alpha-fetoprotein (AFP). The type of treatment (surgical resection,

TACE, radiofrequency ablation [RFA], or radiotherapy) was also recorded. The numbers and sizes of hepatic lesions were measured via CT or MRI. Liver function was evaluated using a liver enzyme test and graded according to the Child–Pugh classification. Liver cirrhosis was determined by imaging; the Barcelona Clinic Liver Cancer (BCLC) stage was also determined. This study was performed according to the Declaration of Helsinki and was approved by the institutional review board of the Chinese Academy of Medical Sciences Cancer Institute. All patients provided written informed consent.

### Follow-up

Patients treated with radical resection, RFA, or radiotherapy underwent enhanced CT, ultrasonography, or MRI every 3–4 months to screen for recurrence; appropriate treatment was administered if recurrence was confirmed. For patients treated with TACE, tumor responses were evaluated regularly; if residual lesions were found, another round of TACE was performed after 1 month. TACE was performed until a patient could no longer tolerate the procedure or achieved a complete response. The patients were followed up every 3–4 months with regular laboratory tests and imaging. OS was defined as the interval between the date of initial treatment and that of the last follow-up visit or death. The final follow-up date was January 31, 2017; none of the patients were lost to follow-up.

### Single nucleotide polymorphism (SNP) selection, DNA extraction, and genotyping

According to the previous GWAS findings on the susceptibility to chronic HBV infection or HBV-related HCC in Asian populations, five SNPs located in the HLA region (rs2647073 and rs3997872 at HLA-DRB1, rs3077 at HLA-DPA, and rs7453920 and rs7768538 at HLA-DQB2) were analyzed.<sup>15–20</sup> All the five SNPs were identified with a minor allele frequency of >5% in the Chinese Han population based on the 1000 Genomes browser (<http://www.1000genomes.org/>). Genomic DNA was extracted from whole blood samples obtained from patients using a blood DNA kit (Tiangen, Beijing, China). The extraction procedure was performed following the manufacturer's instructions, which included cell lysis using sodium dodecyl sulfate plus proteinase K, purification using phenol/chloroform, and precipitation using ethanolamine and ice-cold 100% ethanol. SNP genotyping was performed on the Sequenom MassARRAY system (Sequenom, San Diego, CA, USA) according to the manufacturer's protocol. The genotyping primers for the five SNPs are summarized in Table S1, which were designed using the Sequenom Assay Design software v3.1. The resulting data

management and analysis were performed using the Sequenom Typer software v4.0. All assays were performed in 384-well plates, and negative controls were included for quality control. Moreover, a second round of analysis was performed on 20% of the samples, which were selected randomly; all replicate results were found to be completely concordant. The genotyping success rates in the initial 360 patients were 98.9%, 99.2%, 99.4%, 98.6%, and 95.0% for rs2647073, rs3077, rs3997872, rs7453920, and rs7768538, respectively.

## SI index

Several clinical parameters are used to reflect SI. In the current study, NLR was used to evaluate the correlation between SI, HLA gene SNPs, and the survival of patients with HBV-related HCC. The neutrophil and lymphocyte counts were obtained from hematologic blood tests within 1 week before the initial treatment. NLR was calculated by dividing the neutrophil counts by the lymphocyte counts and was considered as a continuous variable.

## Statistical analyses

All statistical analyses were performed using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed using frequencies and percentages, while continuous variables were expressed using mean with SD or median with range. Differences between the subgroups were analyzed using chi-squared test or Fisher's exact test for categorical variables and Student's *t*-test for continuous variables. OS and progression-free survival (PFS) rates between different groups were analyzed using the Kaplan–Meier method, and differences were compared using the log-rank test. The selected SNPs were analyzed using the abovementioned method to confirm the association of unfavorable genotypes with poor outcomes, and the Cox proportional hazards model was used to estimate the hazard ratios. The receiver operating characteristic (ROC) curve was measured to determine the NLR cutoff values to predict the presence or absence of the unfavorable genotype by determining the maximum sensitivity and specificity. Factors analyzed using Cox proportional hazard model for univariate analysis of OS with a *P*-value of <0.05 were subjected to stepwise multivariate analysis. *P*-values of <0.05 were considered statistically significant.

## Results

### Patients' characteristics

As summarized in Table 1, the patients in this study included 282 men (85.5%) and 48 women (14.5%) with a

**Table 1** Baseline clinical variables of patients with HCC

Characteristics	Value (n=330)
Age (years)	54.8±10.5
Sex	
Male	282 (85.5%)
Female	48 (14.5%)
ECOG-PS	
0	104 (31.5%)
1	167 (50.6%)
2	59 (17.9%)
Type of treatment	
Liver resection	90 (27.3%)
TACE	220 (66.7%)
RFA	8 (2.4%)
Radiotherapy	4 (1.2%)
Best supportive care	8 (2.4%)
Liver cirrhosis	
No	128 (38.8%)
Yes	202 (61.2%)
Portal vein involvement	
No	234 (70.9%)
Yes	96 (29.1%)
Number of tumors	
<3	261 (79.1%)
≥3	69 (20.9%)
Size of largest tumor (cm)	7.1 (1.0–21.0)
BCLC stage	
0 or A	153 (46.4%)
B	80 (24.2%)
C	97 (29.4%)
Serum AFP level (ng/mL)	
≤400	182 (55.2%)
>400	148 (44.8%)
HBV DNA, log <sub>10</sub> IU/mL	
≤3	232 (70.3%)
>3	98 (29.7%)
Total bilirubin (μmol/L)	20.24±14.94
Albumin (g/L)	40.0±4.7
PT-INR	1.12±0.12
Child–Pugh	
A	305 (92.4%)
B	25 (7.6%)
ALT (IU/L)	52 (6–328)
GGT (IU/L)	151 (7–1031)
LDH (IU/L)	236 (34–1007)
ALP (IU/L)	125 (37–805)
WBCs (×10 <sup>9</sup> /L)	5.8±2.2
Neutrophils (×10 <sup>9</sup> /L)	3.5±1.8
Lymphocytes (×10 <sup>9</sup> /L)	1.7±0.7
NLR	2.34±1.71
OS time (months)	18.5 (1–162)

**Note:** Continuous variables are expressed as mean ± SD or median (range).

**Abbreviations:** AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; ECOG-PS, Eastern Cooperative Oncology Group performance status; GGT,  $\gamma$ -glutamyl transpeptidase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LDH, lactate dehydrogenase; NLR, neutrophil/lymphocyte ratio; OS, overall survival; PT-INR, prothrombin time-international normalized ratio; RFA, radiofrequency ablation; TACE, transarterial chemoembolization, WBCs, white blood cells.

mean age of  $54.8 \pm 10.5$  years; 262 patients (79.4%) died after a median survival time of 18.5 months. None of the patients had an Eastern Cooperative Oncology Group performance status (ECOG-PS) score of  $>2$ . A total of 90 patients (27.3%) underwent liver resection, while 240 (72.7%) received TACE, RFA, radiotherapy, or best supportive care as initial treatment. Serum AFP levels were elevated ( $>400$  ng/mL) in 148 patients (44.8%), while 305 patients (92.4%) had Child–Pugh class A disease. The mean NLR was  $2.34 \pm 1.71$ .

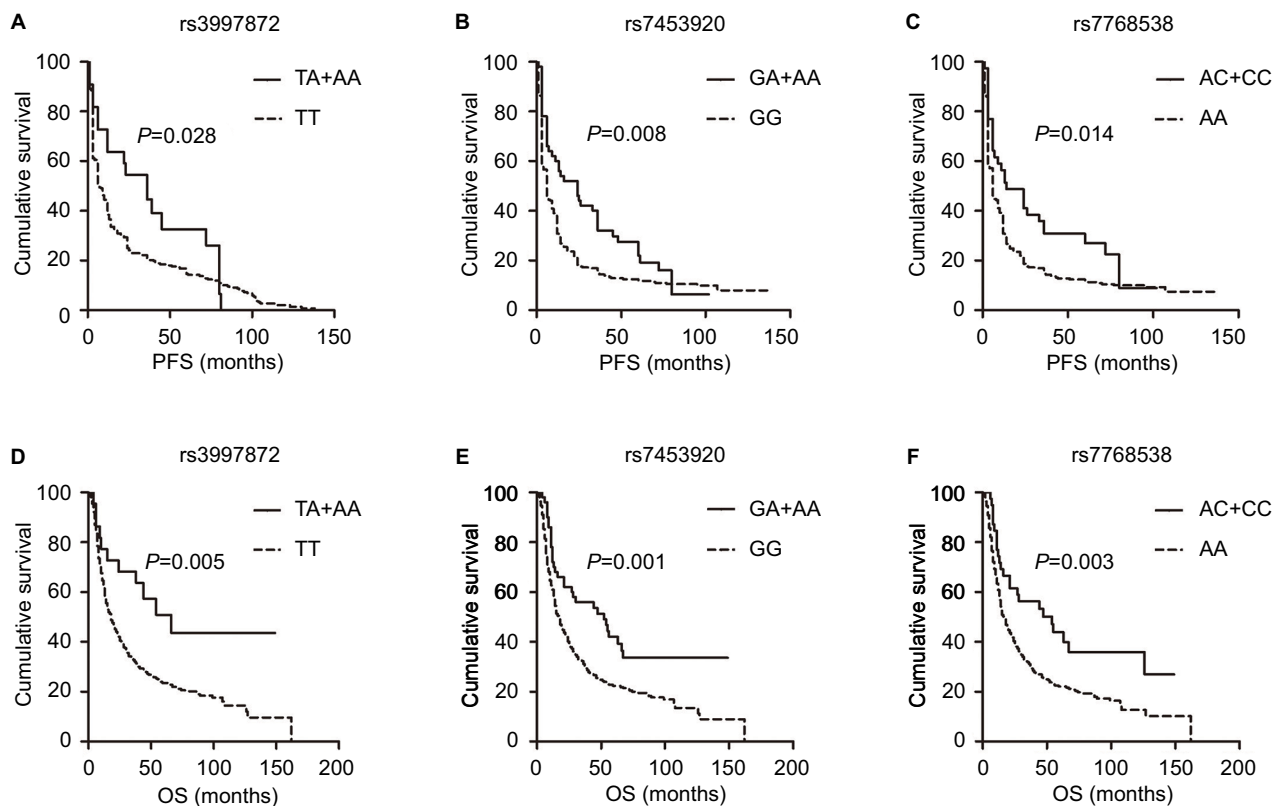
## Association of selected SNPs with the NLR

In univariate analysis using the Cox proportional hazards model, rs3997872, rs7453920, and rs7768538 were significantly associated with OS (all  $P < 0.05$ ; Table 2). Patients with the TT genotype of rs3997872, GG genotype of rs7453920, and AA genotype of rs7768538 had poor OS; hence, these genotypes were considered negative prognostic predictors. Moreover, unfavorable genotypes were associated with poor PFS (Figure 1). However, only the rs7453920 genotype group

**Table 2** Association of the five selected SNPs with OS time

SNP	Nearest gene	Minor allele	Genotype	n	MAF	HR (95% CI)	P
rs3077	HLA-DPA	A	GG/GA/AA	164/131/32	0.38	0.84 (0.69–1.03)	0.087
rs2647073	HLA-DRB1	C	AA/AC/CC	241/64/21	0.08	1.10 (0.90–1.33)	0.361
rs3997872	HLA-DRB1	A	TT/TA/AA	306/22/0	0.06	0.43 (0.23–0.79)	0.006
rs7453920	HLA-DQB2	A	GG/GA/AA	279/47/3	0.12	0.56 (0.39–0.80)	0.001
rs7768538	HLA-DQB2	C	AA/AC/CC	273/36/3	0.19	0.55 (0.37–0.82)	0.003

**Abbreviations:** CI, confidence interval; HLA, human leukocyte antigen; HR, hazard ratio; MAF, minor allele frequency; OS, overall survival; SNP, single nucleotide polymorphism.



**Figure 1** Kaplan–Meier analysis of OS and PFS for patients with HBV-related HCC, grouped by genotypes of the three SNPs.

**Notes:** (A) rs3997872 (TA+AA vs. TT): median PFS, 26 vs. 6 months;  $P=0.028$ . (B) rs7453920 (GA+AA vs. GG): median PFS, 24 vs. 6 months;  $P=0.008$ . (C) rs7768538 (AC+CC vs. AA): median PFS, 14 vs. 6 months;  $P=0.014$ . (D) rs3997872 (TA+AA vs. TT): median OS, 65 vs. 18 months;  $P=0.005$ . (E) rs7453920 (GA+AA vs. GG): median OS, 51 vs. 17 months;  $P=0.001$ . (F) rs7768538 (AC+CC vs. AA): median OS, 54 vs. 17 months;  $P=0.003$ .

**Abbreviations:** HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OS, overall survival; PFS, progression-free survival; SNP, single nucleotide polymorphism.

was significantly associated with the NLR; the GG genotype had a mean NLR of  $2.47 \pm 1.78$ , while the GA+AA genotype had a mean NLR of  $1.61 \pm 0.99$  ( $P=0.001$ ; Table 3).

### Optimal NLR cutoff value

The optimal NLR cutoff value was used to predict the absence or presence of the rs7453920 GG genotype and was determined using ROC curve analysis. The area under the curve (AUC) for the NLR was 0.708 ( $P<0.001$ ), and the optimal cutoff value was 1.43 based on the Youden index (sensitivity, 75.3%; specificity, 60.0%; Figure 2A). ROC curve analysis to predict the median survival time revealed an AUC of 0.753 ( $P<0.001$ ), with an NLR value of 2.19 (sensitivity, 61.2%; specificity, 80.6%) corresponding to the Youden index (Figure 2B). To differentiate between rs7453920 genotypes, patients were divided into two subgroups, such as

NLR $<1.43$  ( $n=100$ ; 30.3%) and NLR $\geq 1.43$  ( $n=230$ ; 69.7%). Kaplan–Meier survival analysis showed that patients in the higher NLR subgroup had significantly poorer OS and PFS ( $P<0.001$ ; Figure 3).

### Association of clinical or genetic variables with the NLR subgroups

As summarized in Table 4, the type of treatment, liver cirrhosis, tumor number, tumor size, and BCLC stage in the two NLR subgroups were significantly different (all  $P<0.05$ ). The levels of total bilirubin, GGT, LDH, and ALP were also significantly different between the NLR subgroups (all  $P<0.05$ ). Interestingly, all three SNPs had significantly different genotype ratios between the two subgroups (all  $P<0.05$ ).

### Association of clinical variables with the rs7453920 genotype

Univariate analysis showed that the BCLC stage, portal vein involvement, Child–Pugh class, and NLR were significantly associated with the rs7453920 genotype. On multivariate analysis for the abovementioned variables, only NLR was independently associated with the rs7453920 genotype (hazard ratio, 4.21; 95% confidence interval [CI], 2.21–8.05;  $P<0.001$ ; Table 5).

### Prognostic factors for OS

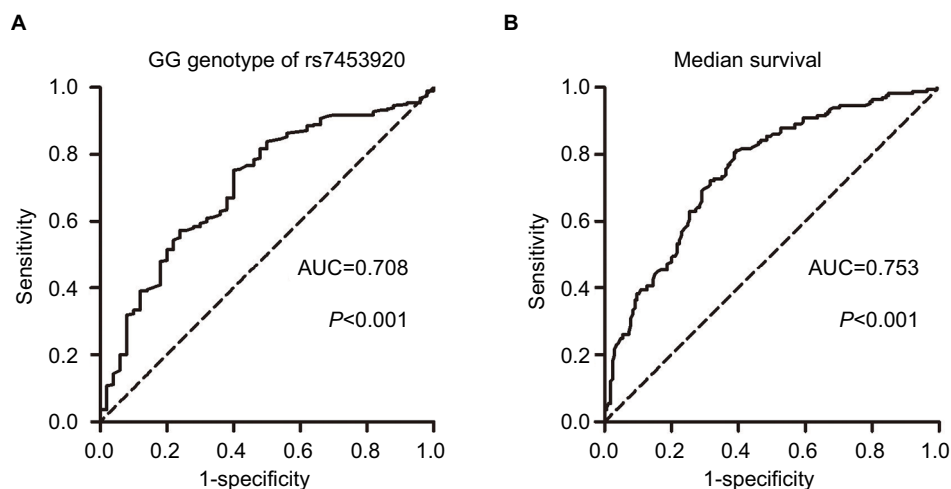
To identify the predictive factors for OS, NLR and other clinical variables were subjected to univariate and multivariate analyses using the Cox proportional hazards model. The results showed that ECOG-PS, BCLC stage, portal vein

**Table 3** Association of genotype of SNPs with NLR

Genotype	n	Median survival time (months)	NLR	P
rs3997872				0.141
TT	306	18	$2.36 \pm 1.74$	
TA+AA	22	65.7	$1.81 \pm 1.12$	
rs7453920				0.001
GG	279	16.7	$2.47 \pm 1.78$	
GA+AA	47	51.5	$1.61 \pm 0.99$	
rs7768538				0.130
AA	273	16.5	$2.39 \pm 1.64$	
AC+CC	39	53.8	$1.95 \pm 2.02$	

**Note:** Continuous variables are expressed as mean  $\pm$  SD.

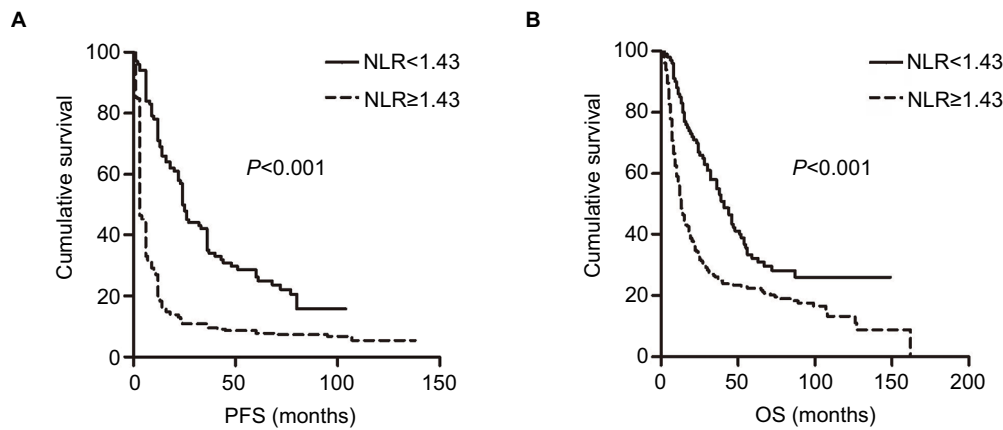
**Abbreviations:** NLR, neutrophil/lymphocyte ratio; SNP, single nucleotide polymorphism.



**Figure 2** (A) ROC curve to determine the cutoff value that predicts the unfavorable genotype (GG) of rs7453920. (B) ROC curve for predicting median survival.

**Abbreviations:** AUC, area under the curve; ROC, receiver operating characteristic.





**Figure 3** Kaplan–Meier curves of OS and PFS for patients with NLR<1.43 vs. NLR≥1.43.

**Notes:** (A) PFS: median 24 vs. 3 months,  $P<0.001$ . (B) OS: median 41 vs. 13 months,  $P<0.001$ .

**Abbreviations:** NLR, neutrophil/lymphocyte ratio; OS, overall survival; PFS, progression-free survival.

involvement, tumor number, tumor size, AFP level, treatment type, and NLR were all significantly associated with OS. Multivariate analysis showed that ECOG-PS, BCLC stage, portal vein involvement, tumor number, tumor size, treatment type, and NLR were independent predictors of OS (all  $P<0.05$ ; Table 6).

## Discussion

In recent years, several GWASs have identified several loci associated with HBV infection and HBV-related HCC. Mbarek et al<sup>15</sup> reported that some SNPs within the HLA-DQ loci were independently associated with susceptibility to CHB and persistent HBV infection in a Japanese population. Clifford et al<sup>20</sup> also reported that 10 SNPs, all located in the gene region involved with immune response, were associated with HCC development. Among the identified SNPs, those located in the HLA region may affect immune response and the clinical outcome of patients with HBV-related HCC. The HLA region plays a key role in HBV clearance, and mutations in HLA genes may result in persistent HBV infection. Such infection, and the cirrhosis that may ensue, can exhaust the host immune system.<sup>23</sup> At the same time, the activation of the immune system increases the production of pro-inflammatory cytokines and the upregulation of cell inflammatory markers.<sup>23–25</sup> Furthermore, the development of liver tumors can affect the balance between SI and immune response, leading to poorer outcomes.<sup>4,26,27</sup> The presence of unfavorable genotypes may indicate that the immune systems of these patients are compromised by HBV infection and HCC. Clinical inflammation indicators are commonly used to evaluate the effects of SI.<sup>5,12,14,28,29</sup> The abovementioned

evidence suggests a close correlation of HLA gene polymorphisms with inflammation indicators.

We found that rs3997872 at HLA-DRB1, as well as rs7453920 and rs7768538 at HLA-DQB2, influenced the clinical outcomes of patients with HBV-related HCC. On a related note, Matoba et al<sup>30</sup> discovered that tumor HLA-DR expression was linked to early intrahepatic recurrence of HCC. Other studies showed that rs7453920 and rs7768538 are associated with persistent HBV infection and HCC development.<sup>31,32</sup> In addition, Li et al<sup>33</sup> discovered that rs7453920 was related to the prognosis of liver transplant recipients. The exact mechanism by which gene polymorphisms in HLA-DRB1 and HLA-DQB2 affect the clinical outcome of patients with HBV-related HCC is unclear. However, both HLA-DR and HLA-DQ molecules are encoded by major histocompatibility complex (MHC) class II genes, and SNPs might alter the MHC class II-mediated immune responses in a manner that is detrimental to the patients' outcomes. MHC class II proteins expressed by antigen-presenting cells such as Kupffer and B cells are crucial for antigen presentation to CD4+ T cells,<sup>34</sup> and Clifford et al<sup>20</sup> reported that rs3997872 might be associated with altered MHC class II protein activity that impairs the T cell response.

Among the three SNPs associated with OS, only the rs7453920 genotype was significantly associated with the NLR, which was elevated in patients with the GG genotype. Mano et al<sup>35</sup> reported that preoperative NLR is a predictor of survival after hepatectomy for patients with HCC; their threshold for high NLR was 2.81. Another study by Pinato et al<sup>8</sup> set the threshold at 5, while Hu et al<sup>36</sup> concluded that an NLR of  $\geq 1.505$  was an independent factor for adverse

**Table 4** Association of clinical or genetic variables with NLR groups

Variables	NLR<1.43 (n=100)	NLR≥1.43 (n=230)	P
Age (years)			0.708
<60	65 (65.0%)	156 (67.8%)	
≥60	35 (35.0%)	74 (32.2%)	
Sex			0.722
Male	87 (87.0%)	195 (84.8%)	
Female	13 (13.0%)	35 (15.2%)	
ECOG-PS			0.906
0 or 1	83 (83.0%)	188 (81.7%)	
2	17 (17.0%)	42 (18.3%)	
Type of treatment			<0.001
Liver resection or RFA	44 (44.0%)	54 (23.5%)	
Others	56 (56.0%)	176 (76.5%)	
Liver cirrhosis			0.022
No	29 (29.0%)	99 (43.0%)	
Yes	71 (71.0%)	131 (57.0%)	
Portal vein involvement			0.001
No	84 (84.0%)	150 (65.2%)	
Yes	16 (16.0%)	80 (34.8%)	
Number of tumors			0.013
<3	88 (88.0%)	173 (75.2%)	
≥3	12 (12.0%)	57 (24.8%)	
Size of largest tumor >5 cm			<0.001
No	57 (57.0%)	56 (24.3%)	
Yes	43 (43.0%)	174 (75.7%)	
BCLC stage			0.002
0 or A	60 (60.0%)	93 (40.4%)	
B or C	40 (40.0%)	137 (59.6%)	
Serum AFP level (ng/mL)			0.295
≤400	60 (60.0%)	122 (53.0%)	
>400	40 (40.0%)	108 (47.0%)	
Total bilirubin (μmol/L)	17.6±10.6	21.4±16.4	0.031
Albumin (g/L)	40.0±4.4	40.0±4.8	0.949
PT-INR	1.11±0.10	1.13±0.12	0.298
Child-Pugh			0.973
A	93 (93.0%)	212 (92.2%)	
B	7 (7.0%)	18 (7.8%)	
ALT (IU/L)	53±39	52±41	0.796
GGT (IU/L)	121±136	165±160	0.017
LDH (IU/L)	189±106	257±230	0.005
ALP (IU/L)	106±48	134±81	0.002
rs3997872			0.022
TT	12 (12.0%)	10 (4.4%)	
TA+AA	88 (88.0%)	218 (95.6%)	
rs7453920			<0.001
GG	30 (30.3%)	20 (8.7%)	
GA+AA	69 (69.7%)	210 (91.3%)	
rs7768538			<0.001
AA	23 (24.7%)	16 (7.3%)	
AC+CC	70 (75.3%)	203 (92.7%)	
OS (months)	41	13	<0.001

**Note:** Continuous variables are expressed as mean ± SD.

**Abbreviations:** AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; ECOG-PS, Eastern Cooperative Oncology Group performance status; GGT,  $\gamma$ -glutamyl transpeptidase; LDH, lactate dehydrogenase; NLR, neutrophil/lymphocyte ratio; OS, overall survival; PT-INR, prothrombin time international normalized ratio; RFA, radiofrequency ablation.

recurrence-free survival (RFS) and an NLR of  $\geq 1.945$  was a predictor of early recurrence. Recent studies have set the threshold in the 2.1–3 range, which was determined mainly from ROC curves for predicting median survival, OS, or RFS; however, the optimal value has yet to be determined.<sup>37–42</sup> In our study, the NLR cutoff of 1.43 was derived from ROC analyses of the effects of rs7453920 and was found to have an AUC, sensitivity, and specificity of 0.708, 75.3%, and 60.0%, respectively. Our value, which ultimately aimed to reflect the effect of HLA gene polymorphisms on SI, was lower than those in previous studies and only had a sensitivity and specificity of 73.3% and 44.1%, respectively, according to ROC analysis of median survival. However, an NLR of  $\geq 1.43$  was still a negative predictor of OS in multivariate analysis. As the optimal NLR cutoff value for predicting median survival was 2.19 in our cohort, we posited that the SI and immune response might be imbalanced in patients with NLR values between 1.43 and 2.19, but not enough to affect their survival. Moreover, it is also possible that other factors such as tumor size, portal vein tumor thrombosis, or the severity of CHB can affect NLR.

The mechanism of how HLA gene polymorphisms affect the immune response and SI remains unknown. However, our study is the first to demonstrate that the rs7453920 genotype at HLA-DQB2 is associated with NLR and that an NLR threshold determined from effects of this genotype is an independent predictor of OS in patients with HBV-related HCC. Although it is unlikely that a single gene locus is a superior predictor of clinical outcomes compared to the BCLC staging system or other identified clinical prognostic factors, our findings highlight a novel aspect of SNP function in the post-GWAS era.

Our study had some limitations. First, only five SNPs in the HLA gene were analyzed, and only one was found to be of clinical significance. A large-scale study of additional SNPs involved in the immune response in other gene regions (such as interleukin-10 or STAT4) is required to validate our results. Second, some BCLC stage C patients were included in our study; most of them were treated with TACE. However, the EASL-EORTC clinical practice guidelines recommend targeted therapies for these patients. Unfortunately, sorafenib was only available for treating HCC in China after 2008, and none of our patients received it as an initial treatment; thus, we did not investigate the association between SI, immune response, and targeted therapies. Third, the study was performed only in a Chinese population, and all participants had HBV infection. Further studies are required to validate our findings in other populations. Moreover, similar studies can

**Table 5** Univariate and multivariate analyses to identify clinical factors associated with the rs7453920 genotype

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Age (years), <60 vs. ≥60	0.94 (0.50–1.78)	0.848		
Sex, male vs. female	1.64 (0.62–4.37)	0.322		
ECOG-PS, 0 or 1 vs. 2	0.85 (0.40–1.82)	0.679		
BCLC stage, 0 or A vs. B or C	1.93 (1.04–3.56)	0.036	1.26 (0.59–2.71)	0.555
Liver cirrhosis, no vs. yes	0.97 (0.52–1.80)	0.924		
Portal vein involvement, no vs. yes	2.42 (1.09–5.37)	0.030	1.42 (0.53–3.80)	0.491
Number of tumors ≥3, no vs. yes	1.76 (0.75–4.10)	0.193		
Size of largest tumor >5 cm, no vs. yes	1.21 (0.65–2.25)	0.555		
AFP >400 ng/mL, no vs. yes	1.14 (0.62–2.09)	0.679		
Child–Pugh class, A vs. B	1.10 (0.96–1.14)	0.020	0.98 (0.52–1.83)	0.944
Treatment type, surgery or RFA vs. others	1.72 (0.92–3.20)	0.089		
NLR ≥1.43, no vs. yes	4.57 (2.44–8.55)	<0.001	4.21 (2.21–8.05)	<0.001

**Abbreviations:** AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; NLR, neutrophil/lymphocyte ratio; RFA, radiofrequency ablation.

**Table 6** Prognostic factors for OS

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Age (years), <60 vs. ≥60	0.86 (0.66–1.11)	0.250		
Sex, male vs. female	1.02 (0.72–1.44)	0.915		
ECOG-PS, 0 or 1 vs. 2	1.88 (1.39–2.55)	<0.001	1.37 (1.00–1.88)	0.048
BCLC stage, 0 or A vs. B or C	3.21 (2.47–4.17)	<0.001	1.44 (1.00–2.06)	0.047
Liver cirrhosis, no vs. yes	0.98 (0.76–1.25)	0.856		
Portal vein involvement, no vs. yes	3.40 (2.61–4.44)	<0.001	1.46 (1.05–2.02)	0.024
Number of tumors ≥3, no vs. yes	2.56 (1.93–3.41)	<0.001	1.39 (1.01–1.90)	0.043
Size of largest tumor >5 cm, no vs. yes	2.68 (2.03–3.53)	<0.001	1.59 (1.17–2.15)	0.003
AFP >400 ng/mL, no vs. yes	1.57 (1.23–2.00)	<0.001	1.25 (0.97–1.61)	0.087
Child–Pugh class, A vs. B	1.43 (0.91–2.23)	0.120		
Treatment type, surgery or RFA vs. others	4.53 (3.27–6.29)	<0.001	2.58 (1.77–3.76)	<0.001
NLR ≥1.43, no vs. yes	1.83 (1.39–2.41)	<0.001	1.55 (1.16–2.07)	0.003

**Abbreviations:** AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; NLR, neutrophil/lymphocyte ratio; OS, overall survival; RFA, radiofrequency ablation.

focus on gene loci associated with the susceptibility to HCC from other etiologies, such as HCV, alcohol, and nonalcoholic fatty liver disease.

## Conclusion

rs7453920 G>A was associated with an elevated NLR, which in turn was an independent factor associated with shorter OS. Our results indicate that the absence of the minor allele A of rs7453920 promotes SI and adversely affects the OS of patients with HBV-related HCC.

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## Disclosure

The authors report no conflicts of interest in this work.

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## Supplementary material

**Table S1** Primers for genotyping of candidate SNPs

SNPs	Forward and reverse primers (5'-3')	Extension primers (5'-3')
rs3077	ACGTTGGATGAGGGTCAGCAATTCAGTCAG ACGTTGGATGTCCAGCTGCCCTACAAACTC	TTATTCTCACTTCATGTGAAAACACTAC(C) TTATTCTCACTTCATGTGAAAACACTAC(T)
rs2647073	ACGTTGGATGAGTAATTTCTTACTGTCCC ACGTTGGATGATATTAGAGAATATCTTCCT	AGTGCATCAGTGGTCACAAATAT(C) AGTGCATCAGTGGTCACAAATAT(A)
rs3997872	ACGTTGGATGCAGTATTGAGTCACAACTGC ACGTTGGATGTGATGATTAGCATCAGGAGG	AGCAGCAACAGAAGG(A) AGCAGCAACAGAAGG(T)
rs7453920	ACGTTGGATGAGAACGCCCTGATCTAAGAG ACGTTGGATGAGCCTGTGAACCAAGTGAAG	AGTGCAGAGGGCAGTCGGACCGATT(C) AGTGCAGAGGGCAGTCGGACCGATT(G)
rs7768538	ACGTTGGATGCTTCAGCCGCTGCCCTGAC ACGTTGGATGGGCCAGCCAAGAACTCAA	GGGAGGCCAAGAAGCTCAATTCGC(G) GGGAGGCCAAGAAGCTCAATTCGC(T)

**Abbreviation:** SNP, small nucleotide polymorphism.

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