Association Analysis of KIR/HLA Genotype with Liver Cirrhosis, Hepatocellular Carcinoma, and NUC Freedom in Chronic Hepatitis B Patients

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Abstract

Natural killer cells are modulated through the binding of killer cell immunoglobulin-like receptors (KIRs) with human leukocyte antigen (HLA) class I ligands. This study investigated the association of KIR/HLA pairs with progression to liver cirrhosis, hepatocellular carcinoma (HCC) development, and nucleot(s)ide (NUC) treatment freedom in hepatitis B virus (HBV) infection. KIR, HLA-Bw, and HLA-C were genotyped in 280 Japanese HBV patients for clinical comparisons. The proportion of the KIR2DS4/HLA-C2 pair was significantly higher in patients with liver cirrhosis (n = 40) than in those without (n = 240) (12.5% vs. 4.2%, odds ratio [OR] 3.29, P = 0.029). The KIR2DS3 positive rate was significantly higher in patients with HCC (n = 39) than in those without (n = 241) (30.8% vs. 14.9%, OR 2.53, P = 0.015). The KIR3DL1/HLA-Bw4 pair rate was significantly lower in the NUC freedom group (n = 20) than in the NUC continue group (n = 114) (25.0% vs. 52.6%, OR 0.30, P = 0.042). In conclusions, this study revealed significant KIR/HLA associations with progression to liver cirrhosis (KIR2DS4/HLA-C2), HCC development (KIR2DS3), and freedom from NUC therapy (KIR3DL1/HLA-Bw4) in HBV patients. KIR/HLA pairs may therefore play a role in HBV patient status.

Introduction

Hepatitis B virus (HBV) infection is a global health concern, with possible life-threatening liver infection in both acute and chronic disease forms. There are over 250 million estimated HBV carriers in the world, of whom approximately 600,000 die annually from HBV-related liver disease. Chronic HBV infection often leads to liver cirrhosis and eventual hepatocellular carcinoma (HCC) [1–4]. Accordingly, it is the second-leading cause of liver cirrhosis and cancer in Japan after hepatitis C virus (HCV) [5]. In order to prevent disease progression, therapeutic agents including nucleot(s)ide analogues (NUCs) and pegylated interferon are currently available [6–9]. However, it remains difficult to eliminate the covalently closed circular DNA (cccDNA) of HBV in the liver with current standard therapies, supporting a lifelong commitment to HBV treatment with NUCs and careful HCC surveillance.

Natural killer (NK) cells provide rapid responses to viral infection and play a key role in tumor immunosurveillance by directly inducing the death of cancer cells [10]. Accordingly, the functional impairment of NK cells has been associated with chronic disease onset and cancer development. NK cell function is centrally controlled by activating and inhibitory killer immunoglobulin-like receptors (KIRs), which bind to human leukocyte antigen (HLA) class I molecules [11–13]. We earlier demonstrated that KIR/HLA receptor-ligand combinations were associated with chronic liver disease progression and HCC development in HCV-infected patients [14, 15]. However, no reports have addressed such associations in Japanese patients with HBV to date even though a few studies suggested such associations in other ethnicities [16, 17].

The present study investigated the association of KIR/HLA pairs with disease progression to liver cirrhosis (Study 1), HCC development (Study 2), and freedom from NUCs (Study 3) in patients with chronic HBV infection in the Japanese.

Results

Patient characteristics

The cohort’s characteristics are summarized in Table 1. Median age was 62 years, and 52.5% of subjects were male. Regarding HBV markers, HBsAg-positive and HBeAg-positive patients totaled 78.9% and 14.3%, respectively. Clinically, 240 patients were at the chronic hepatitis stage and 40 patients were at the liver cirrhosis stage. Thirty-nine patients had experienced HCC by the end of 2018. Of the 134 patients (47.9%) with prior or ongoing NUC treatment, 20 patients had achieved freedom from NUCs by the end of 2018.

Table 1
Demographic and clinical characteristics of patients

	Total (n = 280)	Patients with cirrhosis (n = 40)	Patients without cirrhosis (n = 240)	P-value	Patients with HCC (n = 39)	Patients without HCC (n = 241)	P-value
Age, y	62 (49–71)	72 (64–76)	59 (47–70)	< 0.001	69 (61–77)	59 (48–71)	< 0.001
Male, n (%)	147 (52.5)	30 (75.0)	117 (48.8)	0.002	29 (74.4)	118 (49.0)	0.003
AST, U/L	22 (18–27)	25 (23–30)	21 (17–26)	< 0.001	24 (21–29)	21 (18–26)	0.009
ALT, U/L	18 (14–25)	18 (14–25)	18 (14–25)	0.177	16 (13–23)	18 (14–25)	0.340
PLT, $\times 10^9$/L	19.3 (15.3–23.7)	13.5 (9.8–17.3)	20.2 (16.5–24.5)	< 0.001	15.0 (11.5–19.0)	20.1 (16.3–24.0)	< 0.001
AFP, ng/mL	2.4 (1.8-4.0)	2.2 (1.7–7.2)	2.5 (1.8–3.7)	0.705	2.1 (1.7–12.3)	2.5 (1.8–3.7)	0.812
DCP, mAU/mL	20.0 (16.0–24.0)	21.0 (15.8–30.5)	19.5 (16.0–23.0)	0.144	21.0 (16.5–32.5)	19.0 (16.0–23.0)	0.148
FIB-4	1.6 (1.0-2.4)	3.2 (2.2–5.3)	1.4 (1.0-2.1)	< 0.001	2.7 (1.8–4.5)	1.5 (1.0-2.2)	< 0.001
APRI	0.29 (0.20–0.40)	0.51 (0.35–0.77)	0.27 (0.20–0.36)	< 0.001	0.40 (0.30–0.68)	0.27 (0.20–0.37)	< 0.001
M2BPGi, -/1+/2+	193/71/16	13/15/12	180/56/4	< 0.001	19/13/7	174/58/9	< 0.001
HBsAg positive, n (%)	221 (78.9)	28 (70.0)	193 (80.4)	0.002	33 (84.6)	188 (78.0)	0.348
HBeAg positive, n (%)	40 (14.3)	5 (12.5)	35 (14.6)	0.662	5 (12.8)	35 (14.5)	0.778

Abbreviations: HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; FIB-4, fibrosis index-4; APRI, aspartate aminotransferase to platelet ratio index; M2BPGi, MAC-2 binding protein glycosylation isomer; HBV, hepatitis B virus; NUC, nucleot(s)ide analogue; CH, chronic hepatitis; LC, liver cirrhosis

	Total (n = 280)	Patients with cirrhosis (n = 40)	Patients without cirrhosis (n = 240)	P-value	Patients with HCC (n = 39)	Patients without HCC (n = 241)	P-value
HBV DNA, LIU/mL	0.0 (0.0-2.4)	0 (0.0–0.0)	1.3 (0.0-2.5)	< 0.001	0.0 (0.0–1.0)	1.3 (0.0-2.5)	0.001
NUC treatment, n (%)	134 (47.9)	28 (70.0)	106 (44.2)	0.002	31 (79.5)	103 (42.7)	< 0.001
NUC free, n (%)	20 (7.1)	0 (0.0)	20 (8.3)	0.014	0 (0.0)	20 (8.3)	0.014
CH/LC, n	240/40	-	-	-	17/22	223/18	< 0.001
HCC (+), n (%)	39 (13.9)	22 (55.0)	17 (7.1)	< 0.001	-	-	-

Abbreviations: HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; FIB-4, fibrosis index-4; APRI, aspartate aminotransferase to platelet ratio index; M2BPGi, MAC-2 binding protein glycosylation isomer; HBV, hepatitis B virus; NUC, nucleot(s)ide analogue; CH, chronic hepatitis; LC, liver cirrhosis

Clinical characteristics and KIR/HLA genotyping of patients with HBV-induced cirrhosis (Study 1)

Age, male frequency, FIB-4, APRI, and M2BPGi 1 + or 2 + were significantly higher in patients at the liver cirrhosis stage, while platelet count and HBV DNA were significantly lower (Table 1). To clarify the impact of KIR/HLA pairs on disease progression to liver cirrhosis, KIR and HLA genes were genotyped and their frequencies were compared between patients with and without cirrhosis. Although no significant differences were detected for any KIR or HLA frequency between the groups, the KIR2DS4/HLA-C2 pair rate was significantly higher in the cirrhosis group than in the non-cirrhosis group (odds ratio [OR] 3.29, P = 0.047) (Table 2).

Table 2
Frequency of HLA alleles, KIR genes, and KIR/HLA pairs in patients with and without cirrhosis

Genetic factor	Patients with cirrhosis (n = 40)		Patients without cirrhosis (n = 240)		OR	P-value
	n	%	n	%		
HLA						
HLA-Bw4	26	65.0	137	57.1	1.40	0.347
HLA-Bw6	33	82.5	214	89.2	0.57	0.226
HLA-C1	40	100.0	237	98.8	1.19	0.477
HLA-C2	5	12.5	42	17.5	0.67	0.433
KIR						
KIR2DL1	40	100.0	240	100.0	0.17	-
KIR2DL2	7	17.5	30	12.5	1.48	0.387
KIR2DL3	40	100.0	240	100.0	0.17	-
KIR2DL4	40	100.0	240	100.0	0.17	-
KIR2DL5	19	47.5	100	41.7	1.27	0.490
KIR2DS1	18	45.0	87	36.3	1.44	0.290
KIR2DS2	8	20.0	33	13.8	1.57	0.301
KIR2DS3	6	15.0	42	17.5	0.83	0.698
KIR2DS4	35	87.5	206	85.8	1.16	0.778
KIR2DS5	18	45.0	77	32.1	1.73	0.110
KIR3DL1	39	97.5	219	91.3	3.74	0.174
KIR3DL2	40	100.0	239	99.6	0.51	0.683
KIR3DL3	40	100.0	240	100.0	0.17	-
KIR3DS1	19	47.5	95	39.6	1.38	0.345
KIR/HLA pairs						
KIR2DL1/HLA-C2	5	12.5	42	17.5	0.67	0.433

Abbreviations: HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; OR, odds ratio

Genetic factor	Patients with cirrhosis (n = 40)		Patients without cirrhosis (n = 240)		OR	P-value
KIR2DS1/HLA-C2	2	5.0	12	5.0	1.00	1.000
KIR2DL2/HLA-C1	7	17.5	30	12.5	1.48	0.387
KIR2DS2/HLA-C1	8	20.0	33	13.8	1.57	0.301
KIR2DL3/HLA-C1	40	100.0	237	98.8	1.19	0.477
KIR2DS4/HLA-C1	35	87.5	203	84.6	1.28	0.632
KIR2DS4/HLA-C2	5	12.5	10	4.2	3.29	0.047
KIR3DL1/HLA-Bw4	25	62.5	122	50.8	1.61	0.171
KIR3DS1/HLA-Bw4	11	27.5	51	21.3	1.41	0.378
Abbreviations: HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; OR, odds ratio						

Clinical characteristics and KIR/HLA genotyping of patients with HBV-induced HCC (Study 2)

Age, male frequency, FIB-4, APRI, and M2BPGi 1 + or 2 + were significantly higher in patients with HCC, whereas platelet count and HBV DNA were significantly lower (Table 1). There were no significant differences in the gene frequencies of HLA-Bw4, HLA-Bw6, HLA-C1, or HLA-C2 in patients with and without HCC. However, the KIR2DS1 and KIR2DS3 positive rates were significantly higher in the HCC group (53.8% vs. 34.9%; OR 2.18, P = 0.023, and 30.8% vs. 14.9%; OR 2.53, P = 0.015, respectively). No significant differences in KIR/HLA proportions were observed between the groups (Table 3).

Table 3
Frequency of HLA alleles, KIR genes, and KIR/HLA pairs in patients with and without HCC

Genetic factor	Patients with HCC (n = 39)		Patients without HCC (n = 241)		OR	P-value
	n	%	n	%		
HLA						
HLA-Bw4	25	64.1	138	57.3	1.33	0.422
HLA-Bw6	33	84.6	214	88.8	0.69	0.452
HLA-C1	39	100.0	238	98.8	1.16	0.484
HLA-C2	6	15.4	41	17.0	0.89	0.801
KIR						
KIR2DL1	39	100.0	241	100.0	0.16	
KIR2DL2	8	20.5	29	12.0	1.89	0.147
KIR2DL3	39	100.0	241	100.0	0.16	
KIR2DL4	39	100.0	241	100.0	0.16	
KIR2DL5	21	53.8	98	40.7	1.70	0.122
KIR2DS1	21	53.8	84	34.9	2.18	0.023
KIR2DS2	8	20.5	33	13.7	1.63	0.264
KIR2DS3	12	30.8	36	14.9	2.53	0.015
KIR2DS4	32	82.1	209	86.7	0.70	0.434
KIR2DS5	18	46.2	77	32.0	1.83	0.082
KIR3DL1	35	89.7	223	92.5	0.71	0.548
KIR3DL2	39	100.0	241	100.0	0.16	
KIR3DL3	39	100.0	241	100.0	0.16	
KIR3DS1	21	53.8	93	38.6	1.85	0.072
KIR/HLA pairs						
KIR2DL1/HLA-C2	6	15.4	41	17.0	0.89	0.801
KIR2DS1/HLA-C2	2	5.1	11	4.6	1.13	0.877

Abbreviations: HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; HCC, hepatocellular carcinoma; OR, odds ratio

Genetic factor	Patients with HCC (n = 39)		Patients without HCC (n = 241)		OR	P-value
KIR2DL2/HLA-C1	8	20.5	29	12.0	1.89	0.147
KIR2DS2/HLA-C1	8	20.5	33	13.7	1.63	0.264
KIR2DL3/HLA-C1	39	100.0	238	98.8	0.46	0.484
KIR2DS4/HLA-C1	32	82.1	206	85.5	0.74	0.578
KIR2DS4/HLA-C2	5	12.8	40	16.6	0.79	0.551
KIR3DL1/HLA-Bw4	23	59.0	124	51.5	1.34	0.383
KIR3DS1/HLA-Bw4	12	30.8	50	20.7	1.72	0.162
Abbreviations: HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; HCC, hepatocellular carcinoma; OR, odds ratio						

Clinical characteristics and KIR/HLA genotyping of patients receiving NUC treatment (Study 3)

Twenty of the 134 patients having been treated with NUCs achieved freedom from NUC therapy by the end of 2018. In terms of background characteristics, there were no significant differences between NUC free and NUC continue patients apart from lower frequencies of HBsAg and HBeAg positivity, no progression to cirrhosis, and no development of HCC in the NUC free group (Table 4). The HLA-Bw6 positive rate was significantly lower in the NUC free group than in the NUC continue group (60.0% vs. 89.5%, OR 0.18, P = 0.001). The KIR3DL1/HLA-Bw4 pair was also significantly less frequent in the NUC free group (25.0% vs. 52.6%, OR 0.30, P = 0.042) (Table 5).

Table 4
Demographic and clinical characteristics of patients receiving NUC treatment

	Total (n = 134)	Patients achieving NUC freedom (n = 20)	Patients requiring NUC continuation (n = 114)	P-value
Age, y	62 (50–70)	65 (54–71)	61 (50–70)	0.545
Male, n (%)	77 (57.5)	10 (50.0)	67 (59.3)	0.464
AST, U/L	22 (18–27)	19 (16–25)	23 (18–27)	0.087
ALT, U/L	17 (13–25)	17 (12–21)	18 (13–25)	0.399
PLT, $\times 10^9$/L	18.7 (14.4–22.4)	19.4 (14.8–22.1)	18.6 (14.4–22.7)	0.903
AFP, ng/mL	2.4 (1.8–3.7)	2.7 (1.8–3.4)	2.3 (1.8-4.0)	0.622
DCP, mAU/mL	20.0 (16.5–24.5)	19.0 (15.8–21.0)	20.0 (17.0–25.0)	0.391
FIB-4	1.7 (1.1–2.7)	1.6 (1.1–2.4)	1.7 (1.1–2.7)	0.851
APRI	0.30 (0.20–0.43)	0.26 (0.18–0.40)	0.31 (0.21–0.43)	0.321
M2BPGi, -/1+/2+	87/38/9	13/6/1	74/32/8	0.939
HBsAg positive, n (%)	117 (87.3)	11 (55.0)	106 (93.0)	< 0.001
HBeAg positive, n (%)	109 (81.3)	0 (0.0)	25 (21.9)	0.025
HBV DNA, LIU/mL	0.0 (0.0-1.3)	0 (0.0-1.9)	0.0 (0.0-1.3)	0.158
CH/LC, n	107/27	20/0	86/28	0.014
HCC (+), n (%)	31 (23.1)	0 (0.0)	31 (27.2)	0.004

Abbreviations: NUC, nucleot(s)ide analogue; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; FIB-4, fibrosis index-4; APRI, aspartate aminotransferase to platelet ratio index; M2BPGi, MAC-2 binding protein glycosylation isomer; HBV, hepatitis B virus; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma

Table 5
Frequency of HLA alleles, KIR genes, and KIR/HLA pairs in patients receiving NUC treatment

Genetic factor	Patients achieving NUC freedom (n = 20)		Patients requiring NUC continuation (n = 114)		OR	P-value
	n	%	n	%		
HLA						
HLA-Bw4	9	45.0	65	57.0	0.62	0.319
HLA-Bw6	12	60.0	102	89.5	0.18	0.001
HLA-C1	19	95.0	113	99.1	0.17	0.687
HLA-C2	6	30.0	17	14.9	2.45	0.099
KIR						
KIR2DL1	20	100.0	114	100.0		
KIR2DL2	1	5.0	19	16.7	0.26	0.312
KIR2DL3	20	100.0	114	100.0		
KIR2DL4	20	100.0	114	100.0		
KIR2DL5	7	35.0	49	43.0	0.71	0.504
KIR2DS1	5	25.0	46	40.4	0.49	0.192
KIR2DS2	3	15.0	21	18.4	0.78	0.959
KIR2DS3	2	10.0	23	20.2	0.44	0.444
KIR2DS4	15	75.0	101	88.6	0.39	0.100
KIR2DS5	7	35.0	36	31.6	1.17	0.762
KIR3DL1	16	80.0	106	93.0	0.30	0.061
KIR3DL2	20	100.0	114	100.0		
KIR3DL3	20	100.0	114	100.0		
KIR3DS1	7	35.0	48	42.1	0.74	0.551
KIR/HLA pairs						
KIR2DL1/HLA-C2	6	30.0	17	14.9	2.45	0.099

Abbreviations: HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; NUC, nucleot(s)ide analogue; OR, odds ratio

Genetic factor	Patients achieving NUC freedom (n = 20)		Patients requiring NUC continuation (n = 114)		OR	P-value
KIR2DS1/HLA-C2	0	0.0	5	4.4	0.00	0.753
KIR2DL2/HLA-C1	1	5.0	19	16.7	0.26	0.312
KIR2DS2/HLA-C1	3	15.0	21	18.4	0.78	0.959
KIR2DL3/HLA-C1	18	90.0	114	100.0	0.00	0.959
KIR2DS4/HLA-C1	14	70.0	100	87.7	0.33	0.087
KIR2DS4/HLA-C2	6	30.0	17	14.9	2.45	0.099
KIR3DL1/HLA-Bw4	5	25.0	60	52.6	0.30	0.042
KIR3DS1/HLA-Bw4	5	25.0	30	26.3	0.93	0.902

Abbreviations: HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; NUC, nucleot(s)ide analogue; OR, odds ratio

Discussion

This single-center, cross-sectional study retrospectively examined whether specific KIR/HLA pairs were associated with progression to liver cirrhosis, HCC development, and freedom from NUC treatment in chronic HBV patients. Our results showed that: 1) patients with the KIR2DS4/HLA-C2 pair had a significantly increased risk of liver cirrhosis, 2) patients with KIR2DS3 had a significantly higher risk of HCC, with no remarkable involvement of any KIR/HLA pair, and 3) patients with the KIR3DL1/HLA-Bw4 pair were significantly more likely to achieve freedom from NUCs. These genotype differences might have affected the clinical course of HBV by stimulating or escaping immune surveillance and other intrahepatic inflammatory processes.

Many factors are involved in the progression to liver cirrhosis in HBV patients, such as the viral factors of HBV DNA, HBsAg level, and HBV genotype, the host factors of gender and age, and such environmental factors as alcohol intake [18]. Among those, age is a strong predictor of significant fibrosis progression [18]; indeed, the patients with cirrhosis in the present study were significantly older than those without. Regarding host genetic factors, HLA class II has been widely analyzed and linked to disease progression [19]. KIR and HLA class I combination was reportedly associated with HCC development in Chinese while

its combination was not related to liver cirrhosis or HCC development in Gambians [16, 17]. However, no association studies have considered KIR-HLA combinations in the Japanese to date. This investigation identified a significant association of the KIR2DS4/HLA-C2 pair with disease progression to liver cirrhosis. The pair is present in the minority of Japanese, and may stimulate NK cells to produce more inflammation in the liver towards progression to liver cirrhosis. Longitudinal studies with more subjects are needed to confirm the involvement of this KIR/HLA pair in HBV infection.

Regarding host genetic factors for HCC development in HBV patients, a number of candidate genes specifically for HLA class II regions have been investigated by genetic association studies to evaluate their role in HCC susceptibility [19, 20]. A genome-wide association study very recently proposed HLA-A*33:03 as a susceptibility allele for HCC in HBV [21]. The present investigation on the impact of KIRs and HLA-Bw or HLA-C on HCC development detected no remarkable KIR/HLA pairs, although a significant association of KIR2DS3 with HCC onset was found. The ligand to KIR2DS3 has not been identified to date. However, KIR2DS3 has been related to colorectal cancer [22], spontaneous HCV clearance failure [23], and fatal outcome of Ebola virus infection [24]. Moreover, KIR2DS3 was seen to be expressed at low level on the NK cell surface with unknown function [25], and might therefore represent a genetic marker for a closely related linked gene with biological effects. Further research is required to confirm this hypothesis.

Lastly, HBV patients who receive NUCs are expected to continue treatment indefinitely since there is currently no way to completely eliminate the virus from the liver, with discontinuation sometimes leading to virological relapse and hepatic flares. However, some patients are able to control infection without ongoing treatment. We observed that the KIR3DL1/HLA-Bw4 pair frequency was significantly higher in the NUC continue group despite the KIR3DL1 and HLA-Bw4 expression rates being comparable between the groups. This KIR/HLA pair may be a new biomarker for predicting drug continuation or freedom in HBV patients under NUC treatment. Other ligands to KIR3DL1 include HLA-A23, HLA-A24, and HLA-A32 on the HLA-A locus, which have very recently been reported to exhibit a similar biological function to HLA-Bw4, and have thus been termed HLA-A^{Bw4} [26]. Associations of the HLA-A genotype with HBV should be addressed.

There are several limitations to this study. First, as the number of patients with liver cirrhosis, HCC, and NUC freedom were too small for a definitive conclusion, a larger validation analysis with more subjects is needed to confirm our results. Second, this cross-sectional study was retrospectively designed; prospective, longitudinal follow-up analysis is required. Third, this investigation analyzed HLA-Bw4/Bw6 and HLA-C1/C2, but not other HLA class I genotypes. Additional classical HLA class I genotypes, such as HLA-A, and non-classical HLA class I genotypes should be addressed in future studies.

In conclusions, the present study revealed significant associations for the KIR2DS4/HLA-C2 pair with progression to liver cirrhosis, KIR2DS3 with HCC development, and the KIR3DL1/HLA-Bw4 pair with freedom from NUC in chronically infected HBV patients. KIR/HLA pairs may therefore play a role in the clinical course of HBV infection. Further study is warranted to establish the clinical validity of these associations.

Materials And Methods

Patients

A total of 324 patients of at least 20 years of age who regularly visited Shinshu University Hospital in Matsumoto, Japan, for HBV infection management in the year 2018 (January 1, 2018, to December 31, 2018) were retrospectively targeted. Patients exhibiting other causes of chronic liver disease, such as alcoholic liver disease, non-alcoholic liver disease, primary biliary cholangitis, or autoimmune hepatitis, were not considered. The study participant selection flowchart is depicted in Figure 1. After the exclusion of 44 cases (2 with acute hepatitis B virus infection and 42 without stored DNA or serum), 280 patients with chronic HBV infection were enrolled in the analysis. The racial background of all patients was uniformly Japanese.

Total bilirubin, alanine aminotransferase, and other relevant biochemical tests were performed using standard methods. HBsAg, HBeAb, and MAC-2 binding protein glycosylation isomer (M2BPGi; Sysmex Co., Kobe, Japan) were measured with a HISCL-5000 (Sysmex Co., Kobe, Japan) system using stocked serum. M2BPGi readings of <1.00 COI, ≥1.00 COI and <3.00 COI, and ≥3.00 COI were judged as negative, 1+, and 2+, respectively.

Determination of liver cirrhosis

Patients with liver cirrhosis were judged as those with histologically proven liver cirrhosis and/or characteristic clinical signs of advanced liver disease by imaging studies, including ultrasonography, computed tomography, and magnetic resonance imaging, in the year 2018.

Determination of HCC

HCC patients were defined as having HCC in the prior quarter century (i.e., between 1993 and 2018) or complicating HCC in 2018. HCC was diagnosed by imaging characteristics, arterial hypervascularity, and venous or delayed phase washout by contrast-enhanced dynamic computed tomography and/or magnetic resonance imaging when a nodular lesion was detected by ultrasonography or a tumor marker was elevated.

Definition of NUC freedom

Among the 133 patients with prior or ongoing NUC treatment, 20 patients had successfully discontinued therapy by the end of 2018 and were defined as NUC free.

HLA class I and KIR genotyping

Whole-genomic DNA was extracted from whole blood samples from all participants using QuickGene-800 assays (Fujifilm, Tokyo, Japan). HLA-Bw4, HLA-C1, and HLA-C2 genotyping [27] as well as KIR genotyping [28] were performed using the polymerase chain reaction with sequence-specific primers. HLA

and KIR typing were used to stratify patients into groups according to predicted KIR-ligand interactions and binding affinities. The KIR/HLA pairs of interest were KIR2DL1/2DS1/2DS4-HLA-C2, 2DL2/3/2DS2-HLA-C1, and 3DL1/3DS1-HLA-Bw4. All genotyping was blinded to clinical variables.

Statistical analysis

Statistical analysis and data visualization were carried out using StatFlex ver. 7.0.11 software (Artech Co., Ltd., Osaka, Japan). Continuous variables were compared using the Mann–Whitney U test. Categorical variables were evaluated by Pearson's chi-squared test, Fisher's exact test, or Yates' continuity correction, as appropriate. A P-value of <0.05 was considered statistically significant.

Abbreviations

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NUC, nucleot(s)ide analogue; cccDNA, covalently closed circular DNA; NK, natural killer; KIR, killer immunoglobulin-like receptor; HLA, human leukocyte antigen; M2BPGi, MAC-2 binding protein glycosylation isomer; OR, odds ratio

Declarations

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Authors' contributions

SJ is responsible for conceptualization, original draft preparation, formal analysis, data curation, and methodology. MO are responsible for sample analysis. SJ, HK, SW, YY, AS, TY, ET, and TU are responsible for data curation. SJ and TU are responsible for supervision.

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Conflict of interest

All authors have nothing to disclose regarding funding from industries or other conflicts of interest with respect to this manuscript.

Ethical statement

This investigation was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Shinshu University School of Medicine (protocol code 302 approved on October 10, 2010, and 527 approved on August 10, 2015). Informed consent was obtained from all participants involved in this study.

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Figures

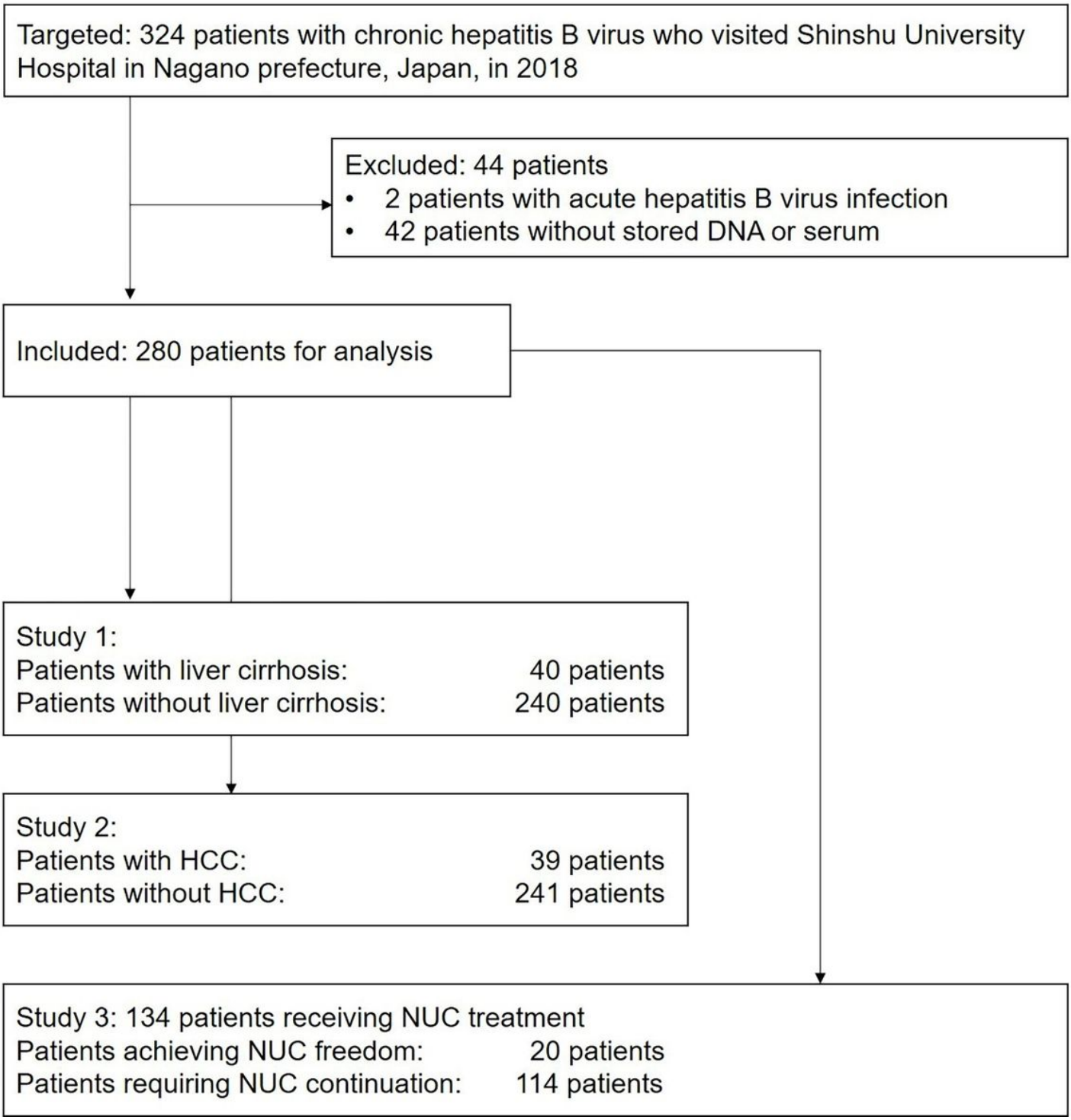

Figure 1

Study participant selection flowchart Abbreviations: HCC, hepatocellular carcinoma; NUC, nucleot(s)ide analogue