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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Efficacy and safety of sofosbuvir–velpatasvir with or without ribavirin in HCV-infected Japanese patients with decompensated cirrhosis: an open-label phase 3 trial

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Abstract

Background In Japan, hepatitis C virus (HCV)-infected patients with decompensated cirrhosis currently have no treatment options. In this Phase 3 study, we evaluated sofosbuvir–velpatasvir with or without ribavirin for 12 weeks in patients with any HCV genotype and decompensated cirrhosis [Child–Pugh–Turcotte (CPT) class B or C] in Japan.

Methods Patients were randomized 1:1 to receive sofosbuvir–velpatasvir with or without ribavirin for 12 weeks. Randomization was stratified by CPT class and genotype. Sustained virologic response 12 weeks following completion of treatment (SVR12) was the primary efficacy endpoint.

Results Of the 102 patients enrolled, 57% were treatment naive, 78% and 20% had genotype 1 and 2 HCV infection, respectively, and 77% and 20% had CPT class B and C cirrhosis, respectively, at baseline. Overall, 61% of patients were female and the mean age was 66 years (range 41–83). SVR12 rates were 92% (47/51) in each group. Among patients who achieved SVR12, 26% had improved CPT class from baseline to posttreatment week 12. Most adverse events (AEs) were consistent with clinical sequelae of advanced liver disease or known toxicities of ribavirin. Four patients (8%) who received sofosbuvir–velpatasvir and seven (14%) who received sofosbuvir–velpatasvir plus ribavirin experienced a serious AE. The 3 deaths (bacterial sepsis, gastric varices hemorrhage, hepatocellular carcinoma) were attributed to liver disease progression.

Conclusion Sofosbuvir–velpatasvir for 12 weeks provides a highly effective and well-tolerated therapy for Japanese

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patients with HCV and decompensated cirrhosis. Ribavirin did not improve efficacy but increased toxicity.

Keywords Sofosbuvir · Velpatasvir · Decompensated cirrhosis · Advanced liver disease · Direct-acting antivirals

Abbreviations

AE	Adverse event
BMI	Body mass index
CI	Confidence interval
CPT	Child–Pugh–Turcotte
DAA	Direct-acting antiviral
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
LLOQ	Lower limit of quantification
MELD	Model for end-stage liver disease
NI	Nucleoside inhibitor
RAS	Resistance-associated substitution
RNA	Ribonucleic acid
SAE	Serious adverse event
SVRxx	Sustained virologic response at "xx" weeks following completion of treatment

Introduction

Globally, the treatment of HCV infection has been transformed with the development of direct-acting antiviral (DAA) agents, which target viral proteins and cellular processes essential to viral replication. These interferon-free, DAA-based regimens are generally well-tolerated and result in high rates of sustained virologic response (SVR) across most patient populations. However, some regimens containing protease inhibitors have been associated with hepatotoxicity and hepatic decompensation, particularly in patients with advanced cirrhosis thus precluding their use in some patients, including those with decompensated cirrhosis [1]. In contrast, ledipasvir/sofosbuvir and sofosbuvir/velpatasvir have demonstrated both safety and efficacy in patients with decompensated liver disease [2–4]. These studies were conducted in North America, Europe, Australia, and New Zealand. Data are lacking in Japanese patients, and there are no approved antiviral therapies currently available for this population in Japan. The current Japan Society of Hepatology (JSH) guidelines therefore do not recommend the use of DAA agents in patients with decompensated cirrhosis due to lack of safety or efficacy data in Japanese patients [5].

Of the approximately 1.0–1.5 million people chronically infected with hepatitis C virus (HCV) in Japan [5], approximately 35,000–50,000 may have decompensated cirrhosis [6, 7]. Liver transplantation is a potential nonpharmaceutical intervention; however, it is not commonly done in Japan, with only 438 liver transplants performed in 2016 [8]. Patients with decompensated cirrhosis are at high risk for development of hepatocellular carcinoma (HCC), bleeding diatheses, and fulminant infections. One-year survival rates in patients with Child–Pugh–Turcotte (CPT) class B or CPT class C cirrhosis are 80% and 45%, respectively [9, 10]. A retrospective cohort study of Japanese patients with CPT class C cirrhosis on the liver transplant registry demonstrated a mean survival time of less than 16 months and 2-year survival probability was less than 40% [11]. Without available antiviral therapy and limited options for liver transplantation in Japan [8], the prognosis for Japanese patients with chronic HCV infection and decompensated cirrhosis is poor. A safe and effective HCV treatment will address the unmet medical need for this population.

Sofosbuvir–velpatasvir (400/100 mg) is a fixed-dose combination that combines 2 DAAs. Sofosbuvir is a nucleotide analog that is a potent, pangenotypic and selective NS5B polymerase inhibitor, and velpatasvir is a potent, pangenotypic, next-generation HCV NS5A inhibitor. Sofosbuvir–velpatasvir is approved in the US, European Union, and other regions for the treatment of genotypes 1–6 chronic HCV infection in patients with and without compensated cirrhosis and for use with ribavirin in patients with decompensated cirrhosis [12, 13].

The ASTRAL-4 study evaluated 12 and 24 weeks of treatment with sofosbuvir–velpatasvir with or without ribavirin in HCV-infected patients with CPT class B decompensated cirrhosis in the US [4]. Rates of sustained virologic response 12 weeks post treatment (SVR12) were 83% in patients who received 12 weeks of sofosbuvir–velpatasvir, 94% in patients who received 12 weeks of sofosbuvir–velpatasvir plus ribavirin, and 86% in patients who received 24 weeks of sofosbuvir–velpatasvir. Notably, the numeric difference in SVR12 rates in genotype 1b and genotype 2 HCV-infected patients who received sofosbuvir–velpatasvir for 12 weeks or sofosbuvir–velpatasvir with ribavirin for 12 weeks did not differ substantially.

In this Phase 3 study, we evaluated the efficacy and safety of the fixed-dose combination tablet of sofosbuvir–velpatasvir with or without ribavirin for 12 weeks in Japanese HCV-infected patients with decompensated cirrhosis.

Methods

Patients

Eligible patients were 20 years of age and older with chronic HCV infection, quantifiable HCV RNA at screening, and CPT score 7–12, inclusive. The calculation of the

CPT score at screening used either the international normalized ratio or prothrombin activation percentage for the coagulation parameter, at the investigator's discretion (Supplemental Table 1). Patients were to have liver imaging within 4 months of baseline to exclude HCC. Patients were excluded from this study if they had a positive test result for hepatitis B surface antigen or human immunodeficiency virus, had HCC within 2 years prior to screening, any recurrence of HCC after curative treatment (e.g., successful treatment with surgical resection or radiofrequency ablation), prior treatment with an NS5A inhibitor, or creatinine clearance < 50 mL/min as calculated by the Cockcroft–Gault equation using actual body weight. Use of concomitant amiodarone was prohibited from 60 days prior to day 1 and throughout the treatment period. Full eligibility criteria are provided in the supplementary information.

Study design and randomization

This was a Phase 3, multicenter, open-label study. Via an interactive web response system, patients were randomly assigned 1:1 to sofosbuvir–velpatasvir with or without ribavirin for 12 weeks. Randomization was stratified by genotype (genotype 1 vs. non-genotype 1) and CPT class at screening (CPT class B vs C). For the purposes of randomization, a patient with nondefinitive or mixed HCV genotype results was considered non-genotype 1. Across the study population, at least 15 patients were to have non-genotype 1 HCV infection and approximately 10% of patients were to have CPT class C cirrhosis. Enrollment of patients with CPT class C cirrhosis began after an independent data monitoring committee evaluated the safety data through 4 weeks of treatment from the first 20 patients with CPT class B cirrhosis.

Sofosbuvir–velpatasvir (400/100 mg) fixed-dose combination was administered once daily. Ribavirin (REBETOL, MSD KK) was administered with food twice daily. For patients with CPT class B cirrhosis at screening dosing was based on body weight (600 mg daily in patients ≤ 60 kg, 800 mg for patients > 60–80 kg, and 1000 mg for those > 80 kg). All patients with CPT class C cirrhosis received 600 mg daily regardless of weight.

All patients provided written informed consent to participate, and the study was conducted consistent with the ethical standards, including but not limited to the International Council for Harmonisation guideline for Good Clinical Practice, the original principles embodied in the Declaration of Helsinki, and the J-GCP (Ministerial Ordinance on Good Clinical Practice for Drugs). This study was approved by an institutional review board at each study site prior to the initiation of any screening or study-specific procedures.

Study assessments

Screening assessments included HCV genotyping, *IL28B* genotyping, and standard laboratory and clinical tests. HCV genotype and subtype were determined using the Siemens VERSANT HCV Genotype INNO-LiPA2.0 Assay. *IL28B* genotype was determined by polymerase chain reaction amplification of the single-nucleotide polymorphism rs12979860, with sequence-specific forward and reverse primers and allele-specific fluorescently labeled TaqMan minor groove binder probes. Plasma HCV RNA levels were evaluated at screening; at day 1 of treatment, at weeks 2, 4, 8, and 12 during treatment, and at weeks 4, 12, and 24 after the end of treatment. HCV RNA levels were quantified using the COBAS Ampliprep/COBAS TaqMan HCV Test, v2.0 (Roche Molecular Systems, Inc., Branchburg, NJ), which has a lower limit of quantification (LLOQ) of 15 IU/mL.

Deep sequencing of the HCV NS5A and NS5B genes was performed for all patients at baseline and from those with virologic failure at the time of failure (DDL Diagnostic Laboratory, Rijswijk, Netherlands). RASs present in more than 15% of the sequence reads are reported. The resistance analysis population is comprised of patients with viral sequence data and virologic outcome data available.

Safety assessments included monitoring of adverse events (AEs) and clinical laboratory tests at all on-treatment visits; AEs were also collected up to 30 days after the last dose of study drug. Samples for clinical laboratory tests were collected at each posttreatment visit (4, 12, and 24 weeks after the last dose of study drug). All AEs and laboratory values were graded according to a standardized scale and AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 20.1.

Endpoints

The primary efficacy endpoint was SVR12, defined as HCV RNA $<$ LLOQ (i.e., < 15 IU/mL) 12 weeks after the end of treatment. Secondary efficacy endpoints included the change from baseline in the CPT and MELD scores at 12 weeks after end of treatment. CPT score for all baseline and post-baseline visits were calculated using prothrombin activation percentage for the coagulation parameter. The primary safety endpoint was discontinuation of study drugs due to AEs.

Statistical analysis

Point estimates with 2-sided 95% exact confidence intervals (CIs) for SVR12 based on the Clopper–Pearson method were provided for each treatment group. In the primary efficacy analysis, the SVR12 rate for patients in

Table 1 Baseline demographics and disease characteristics

	Sofosbuvir–velpatasvir 12 weeks $N = 51$	Sofosbuvir–velpatasvir plus ribavirin 12 weeks $N = 51$
Mean age (range) (years)	66 (43, 82)	66 (41, 83)
Female sex	33 (65)	29 (57)
Mean body mass index (range) (kg/m^2)	26.5 (20.4, 43.0)	25.8 (18.3, 58.6)
HCV genotype and subtype		
Genotype 1	41 (80)	39 (76)
Genotype 1a	1 (2)	0
Genotype 1b	40 (78)	39 (76)
Genotype 2	9 (18)	11 (22)
Genotype 2 (no confirmed subtype)	5 (10)	5 (10)
Genotype 2a	0	2 (4)[a]
Genotype 2a/2c	2 (4)	1 (2)
Genotype 2b	2 (4)	4 (8)
Genotype 3b	1 (2)	0
Mean HCV RNA (range) ($\log_{10}$ IU/mL)	5.7 (3.7–7.1)	5.8 (4.2–7.0)
IL28B CC genotype	33 (65)	37 (73)
CPT B [7–9][b]	40 (78)	39 (76)
MELD score $\leq$ 15	46 (90)	48 (94)
Ascites		
None	19 (37)	16 (31)
Mild/moderate	32 (63)	33 (65)
Severe	0	2 (4)
Encephalopathy		
None	23 (45)	22 (43)
Medication-controlled	28 (55)	29 (57)
No prior HCV treatment	27 (53)	31 (61)
Mean estimated glomerular filtration rate (range) (mL/min)[c]	93 (40, 183)	89 (42, 299)

Data presented are *n* (%) unless stated otherwise

CPT Child–Pugh–Turcotte

[a]One patient with missing HCV genotype was subsequently determined to have genotype 2a HCV infection by BLAST analysis

[b]The CPT score was calculated using prothrombin activation percentage for the coagulation parameter

[c]The estimated glomerular filtration rate was calculated using the Cockcroft–Gault equation

each treatment group was compared to the spontaneous clearance rate of 1% using a 2-sided exact 1-sample binomial test with Bonferroni alpha adjustment (each at the 0.025 significance level).

Results

Baseline characteristics and disposition

Demographics and baseline characteristics are presented in Table 1. Of 155 patients screened, a total of 102 patients were enrolled at 33 sites in Japan, of which 100 (98%) completed treatment (Supplemental Fig. 1). All 53 patients who were excluded from study participation did not meet eligibility criteria (Supplemental Table 2). Demographics and baseline characteristics of the patients enrolled were generally balanced across both treatment groups and consistent with an older population with advanced liver disease. Overall, most patients were female (61%). The mean age was 66 years (range 41–83), and 58% were $\geq$ 65 years of age. Most patients had *IL28B* CC genotype (69%) and were treatment naive (57%). Among the 44 treatment-experienced patients, only 1 had previously been treated with a DAA (simeprevir in combination with peginterferon alfa-2a and ribavirin for 23 weeks); all others had been treated with interferon alone or in combination with ribavirin.

Table 2 Virologic response during and after treatment

	Sofosbuvir–velpatasvir 12 weeks $N = 51$	Sofosbuvir–velpatasvir plus ribavirin 12 weeks $N = 51$
HCV RNA < 15 IU/mL, *n*/*n* (%)		
On treatment		
Week 2	23/51 (45)	26/51 (51)
Week 4	49/51 (96)	46/51 (90)
Week 8	51/51 (100)	49/51 (96)
Week 12	51/51 (100)	49/49 (100)
After treatment		
Week 4 (SVR4)	48/51 (94)	49/51 (96)
Week 12 (SVR12)	47/51 (92)	47/51 (92)
95% CI	81–98	81–98
Relapse after the end of treatment	4 (8)	2 (4)
Discontinued treatment due to adverse events	0	2 (4)

Overall, 80 patients (78%) had genotype 1 HCV infection [1 patient (1%) had HCV genotype 1a and 79 (77%) patients had HCV genotype 1b], 20 patients (20%) had genotype 2 HCV infection, and 1 patient (1%) had genotype 3 HCV infection. There was 1 patient who had an HCV genotype that was unable to be determined by LiPA or NS5B Sanger, but was later determined to have genotype 2a HCV infection by BLAST analysis. At baseline, 77% of patients were CPT class B (score 7–9), 20% were CPT class C (score 10–12), and 3% were CPT class A (score 6).

Efficacy

Virologic response

The SVR12 rates were 92% (47/51; 95% CI 81–98%) in each treatment group (Table 2). Both treatment groups met their primary efficacy endpoints with SVR12 rates that were statistically superior compared with the spontaneous clearance rate of 1% ($p < 0.001$).

When examined by genotype, SVR12 rates were high for patients with genotype 1 or 2 regardless if they received 12 weeks of sofosbuvir–velpatasvir or sofosbuvir–velpatasvir plus ribavirin (rates ranged from 89 to 100%, Table 3). The 1 patient with genotype 3 HCV infection in the study who was randomized to the sofosbuvir–velpatasvir group did not achieve SVR12. When examined by baseline CPT class, SVR12 rates were high in patients with CPT class B cirrhosis (≥ 95%) in both treatment groups (Table 3). Of the patients with baseline CPT class C cirrhosis, 80% (8/10) and 70% (7/10) in the sofosbuvir–velpatasvir and sofosbuvir–velpatasvir plus ribavirin groups, respectively, achieved SVR12.

Table 3 Rates of SVR12 by subgroup

	Sofosbuvir–velpatasvir 12 weeks $N = 51$	Sofosbuvir–velpatasvir plus ribavirin 12 weeks $N = 51$
Overall SVR12	47/51 (92)	47/51 (92)
Genotype		
1a	0/1 (0)	–
1b	39/40 (98)	35/39 (90)
2	8/9 (89)	12/12 (100)[a]
3	0/1 (0)	–
Baseline CPT class		
A	1/1 (100)	2/2 (100)
B	38/40 (95)	38/39 (97)
C	8/10 (80)	7/10 (70)

[a]Includes 1 patient who was initially categorized as missing HCV genotype, and subsequently determined to have genotype 2a by BLAST analysis

A total of 8 patients did not achieve SVR12, with 6 patients experiencing virologic relapse (Supplemental Table 3). No patients had virologic non-response. In the sofosbuvir–velpatasvir group, 4 of 51 patients (8%) relapsed. In the sofosbuvir–velpatasvir plus ribavirin group, 4 of 51 patients (8%) did not achieve SVR12. Of these 4 patients, 2 relapsed and 2 discontinued treatment early due to AEs and subsequently died.

Table 4 Shift of CPT class from baseline to posttreatment week 12

Posttreatment week 12 CPT class, *n* (%)	Overall *N* = 94		
	Baseline CPT class		
	CPT A (5–6) *N* = 3	CPT B (7–9) *N* = 76	CPT C (10–15) *N* = 15
CPT A (5–6)	3 (100)	19 (25)	0
CPT B (7–9)	0	55 (72)	5 (33)
CPT C (10–15)	0	2 (3)	10 (67)

CPT Child–Pugh Turcotte

Table 5 Adverse events and grade 3 and 4 laboratory abnormalities

	Sofosbuvir–velpatasvir 12 weeks *N* = 51	Sofosbuvir–velpatasvir plus ribavirin 12 weeks *N* = 51
Number (%) of patients experiencing any		
Adverse event	35 (69)	44 (86)
Grade 3 or above adverse event	2 (4)	5 (10)
Serious adverse event	4 (8)	7 (14)
Adverse event leading to discontinuation of sofosbuvir/velpatasvir	0	2 (4)
Adverse event leading to discontinuation of ribavirin	N/A	9 (18)
Adverse event leading to modification or interruption of ribavirin	N/A	18 (35)
Deaths	0	3 (6)
Common adverse events (≥ 10% either group)		
Anemia	0	20 (39)
Nasopharyngitis	7 (14)	3 (6)
Diarrhea	0	7 (14)
Laboratory abnormalities (≥ 10% either group)		
Hemoglobin < 10 g/dL	2 (4)	7 (14)
Lymphocytes, < 500/mm^3	0	5 (10)
Platelets, 25,000–50,000/mm^3	1 (2)	6 (12)
Hyperglycemia, > 250–500 mg/dL	5 (10)	9 (18)
Total bilirubin, > 2.5 × ULN	6 (12)	12 (24)

Toxicity grade must have increased at least 1 toxicity grade from baseline value (missing was considered grade 0) to be included. Patients were counted once at maximum toxicity grade for each laboratory test. Data were included up to the last dose date of any study drug + 30 days

Changes in liver function

Of all patients who achieved SVR12 in either arm, 26% (24/91) improved in CPT class and 2% (2/91) worsened in CPT class from baseline to posttreatment week 12 (Table 4). Improvement in CPT score was primarily driven by increase in albumin levels with 79% of the patients with improved CPT scores having increase in albumin (Supplemental Table 4). Similar changes were observed in MELD score with 27% (25/94) having improved MELD score and 15% (14/94) with worsening MELD score.

Analysis of resistance

Among the 100 patients included in the resistance analysis population, 41% (41/100) had baseline NS5A RASs. No patient had NS5B nucleoside inhibitor (NI) RASs.

In the sofosbuvir–velpatasvir group, 97% (33/34) of patients without baseline NS5A RASs and 82% (14/17) of patients with baseline NS5A RASs achieved SVR12. Of the 41 patients with genotype 1 HCV infection, there was 1 patient without baseline NS5A RASs and 1 patient with baseline NS5A RASs who relapsed. In the sofosbuvir–velpatasvir plus ribavirin group, 96% (24/25) of patients

without baseline NS5A RASs and 96% (23/24) of patients with baseline NS5A RASs achieved SVR12. Of the 37 patients with genotype 1 HCV infection, there was 1 patient without baseline NS5A RASs and 1 patient with baseline NS5A RASs who relapsed.

Of the 6 patients who experienced virologic relapse across both treatment groups, 4 had treatment-emergent NS5A RASs. No patient in either treatment group had NS5B NI RASs detected at baseline or relapse.

Safety

More patients treated with sofosbuvir–velpatasvir plus ribavirin experienced AEs (86%, 44/51) compared with patients treated with sofosbuvir–velpatasvir (69%, 35/51) (Table 5). No consistent, clinically significant trends were observed when looking at AE rates by CPT class, nor by age group.

Despite all the patients in the study having advanced liver disease, most AEs reported in this study were Grade 1 (mild) or Grade 2 (moderate) in severity. The most common AEs in the sofosbuvir–velpatasvir group were nasopharyngitis (14%) and in the sofosbuvir–velpatasvir plus ribavirin group they were anemia (39%) and diarrhea (14%).

Patients in the sofosbuvir–velpatasvir plus ribavirin group experienced AEs consistent with ribavirin toxicity. Eighteen of 51 patients (35%) had AEs that led to modification or interruption of ribavirin and 9 patients (18%) had AEs that led to discontinuation of ribavirin, with anemia being the most common in both instances.

Four patients (8%) in the sofosbuvir–velpatasvir group and 7 patients (14%) in the sofosbuvir–velpatasvir plus ribavirin group had serious adverse events (SAEs), and most were not considered treatment-related by the investigator (Supplemental Table 5). The only SAEs that occurred in > 1 patient were femur fracture (2 in the sofosbuvir–velpatasvir plus ribavirin group) and hepatic encephalopathy (1 in the sofosbuvir–velpatasvir group, 2 in the sofosbuvir–velpatasvir plus ribavirin group). Two of the three SAEs of hepatic encephalopathy occurred in patients with CPT class C cirrhosis.

Three patients in the study developed HCC, all of whom were diagnosed following treatment (on posttreatment day 1, posttreatment day 70 and posttreatment day 124). Two of the patients had CPT class B at baseline and one had CPT class C. The investigator did not consider these events related to study drug. There were 4 patients enrolled who had a history of HCC, none of whom experienced recurrence during the study.

Three deaths occurred during the study and all 3 patients received treatment with sofosbuvir–velpatasvir plus ribavirin. The ages of the patients who died were 51, 59 and 67 years; all 3 patients had CPT class C at baseline. Two of these patients discontinued study drugs early due to AEs not related to treatment. All 3 deaths occurred after treatment was stopped (posttreatment days 5 and 17 for the 2 patients that discontinued study drugs prematurely, and posttreatment day 158 for the patient that completed 12 weeks of study treatment). All of the deaths were due to progression of end-stage liver disease (septicemia, portal hypertension leading to gastrointestinal bleeding, and HCC) and none were considered to be related to study drugs by the investigator (Supplemental Table 6). No other patients discontinued sofosbuvir–velpatasvir in the study.

Fewer patients in the sofosbuvir–velpatasvir group had Grade 3 or 4 laboratory abnormalities compared with the sofosbuvir–velpatasvir plus ribavirin group (27 vs 53%, respectively) (Table 5). The observed laboratory abnormalities were consistent with those expected in a population with decompensated liver disease and, in the sofosbuvir–velpatasvir plus ribavirin group, consistent with the known toxicities of ribavirin. Post-baseline hemoglobin values < 10 g/dL were observed in 2 patients (4%) in the sofosbuvir–velpatasvir group and 7 patients (14%) in the sofosbuvir–velpatasvir plus ribavirin group. Additional information about laboratory abnormalities is provided in the supplementary information (Supplemental Fig. 2).

Discussion

In this Phase 3 study conducted in Japan, sofosbuvir–velpatasvir for 12 weeks was highly effective and generally safe and well-tolerated in patients with decompensated cirrhosis. The current study enrolled mostly patients with genotype 1b or 2, consistent with the Japanese population of HCV-infected patients. The identical SVR12 rates of 92% in the 2 treatment groups suggest that addition of ribavirin to sofosbuvir–velpatasvir did not improve efficacy for Japanese patients with decompensated cirrhosis. These results were comparable to those for the similar subpopulation enrolled in the ASTRAL-4 study, in which 12 weeks of treatment with sofosbuvir–velpatasvir without ribavirin resulted in SVR12 rates of 89% (16 of 18) and 100% (4 of 4) in patients with genotype 1b and 2, respectively [4]. Of note, the addition of ribavirin was most beneficial in patients with genotype 3 HCV infection in the ASTRAL-4 study, where the response was 35% higher in the group who received ribavirin (85%, 11 of 13 patients) compared to those who did not in either the sofosbuvir–velpatasvir 12 week group (50%, 7 of 14 patients) or 24 week group (50%, 6 of 12 patients).

Clinical attention to safety is appropriate in this patient population with advanced liver disease with high expected morbidity and mortality. In the current study, the AE

profile was consistent with the clinical sequelae of advanced liver disease and with the known toxicities of ribavirin. In the sofosbuvir–velpatasvir plus ribavirin group, 49% of patients needed significant modifications to their ribavirin dosing, primarily due to anemia. Overall sofosbuvir–velpatasvir was well-tolerated with the majority of AEs being Grade 1 or 2. Only 2 patients, both in the sofosbuvir–velpatasvir plus ribavirin group, discontinued sofosbuvir–velpatasvir for AEs that were not considered related to study drugs; both of these patients subsequently died due to progression of their liver disease. The safety profile observed in the current study, including the rate of deaths, was consistent with those observed in previous overseas trials of sofosbuvir–velpatasvir with and without ribavirin as well as ledipasvir–sofosbuvir with ribavirin in larger populations of patients with decompensated cirrhosis, despite the fact that the mean age of patients in the current study was 8–9 years older than in the overseas studies [2–4].

As interferon-free DAA-based regimens have only recently become available for the treatment of HCV, the clinical benefits of their use in patients with decompensated cirrhosis are being characterized. Achievement of SVR12 is associated with early improvements in liver function, as demonstrated by reductions in CPT and MELD scores through posttreatment week 12, in both the current study as well as previous studies of sofosbuvir-based regimens in this population [2–4]. In terms of long-term benefits of achieving SVR with DAA-based regimens in patients with decompensated cirrhosis, several studies have compared the survival rates of patients successfully treated with sofosbuvir-based regimens to historical matched controls from transplant waitlists and have demonstrated a decrease in mortality [14, 15]. There is also a growing body of literature demonstrating a reduction in risk of de novo HCC, consistent with observations in the interferon era [16–18].

Our study has several limitations, mostly related to characteristics of the enrolled patients. Although representative of the Japanese HCV-infected patient population, there was a lack of genotype diversity. The study included few patients with more severe cirrhosis (CPT class C) and none with baseline CPT score greater than 12. Patients who had been previously treated with DAAs were not included. Lastly, although early improvements in liver function were demonstrated through the study posttreatment period, the long-term clinical benefit of achievement of SVR in patients with decompensated liver disease can only be demonstrated through follow-up of the patients after the study.

In conclusion, treatment with sofosbuvir–velpatasvir for 12 weeks is the optimal regimen for Japanese patients with decompensated cirrhosis. The SVR12 rate was high regardless of genotype or CPT class. Addition of ribavirin to the regimen did not improve efficacy and was associated with more adverse events and laboratory abnormalities.

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Compliance with ethical standards

Conflict of interest Tetsuo Takehara has received honoraria and commercial research funding from Gilead. Naoya Sakamoto has received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, and Gilead, and has received commercial research funding from Gilead, AbbVie, Bristol-Myers Squibb, Merck Sharp & Dohme, Otsuka, and Shionogi. Shuhei Nishiguchi has received honoraria from Gilead, and has received commercial research funding from Gilead, Toray and Merck Sharp & Dohme. Yoshiyuki Ueno received commercial research funding from Gilead, Bristol-Myers Squibb, AbbVie, and Merck Sharp & Dohme. Hiroshi Yatshuhashi has received commercial research funding from Chugai. Tatsuo Kanda has received commercial research funding from AbbVie, Merck Sharp & Dohme, Chugai, and Sysmex. Minoru Sakamoto has received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, and Gilead, and has received commercial research funding from Gilead, AbbVie, Bristol-Myers Squibb, Merck Sharp & Dohme, Otsuka, and Shionogi. Akihiro Tamori has received honoraria from Gilead. Kazuaki Chayama has received honoraria from AbbVie, Merck Sharp & Dohme, Bristol-Myers Squibb, and Gilead. Gulan Zhang, Shampa De-Oertel, Hadas Dvory-Sobol, Takuma Matsuda, Luisa M. Stamm, and Diana M. Brainard are employees of and hold stock in Gilead Sciences. Yasuhito Tanaka has received honoraria from Bristol-Myers Squibb and Gilead Sciences, and has received commercial research funding from Chugai, AbbVie, Bristol-Myers Squibb, Janssen, and Gilead. Masayuki Kurosaki has served in an advisory role to AbbVie, Gilead, GlaxoSmithKline, and Otsuka, and has received honoraria from AbbVie, Bristol-Myers Squibb, Chugai, Daiichi Sankyo, Gilead, GlaxoSmithKline, Janssen, Merck Sharp & Dohme, Otsuka, and Toray. Fusao Ikeda, Tomohide Tatsumi, Yasuhiro Takikawa, and Eiji Mita, declare no conflicts of interest.

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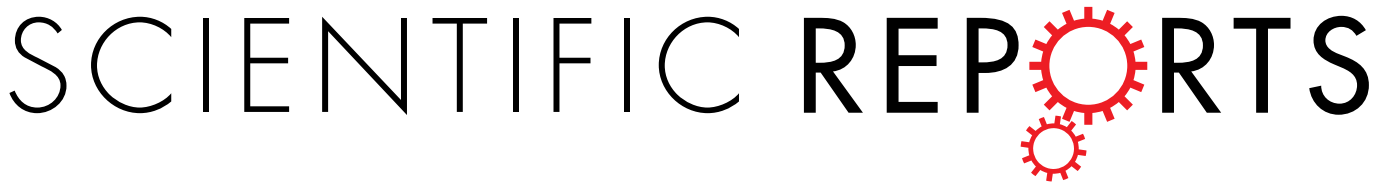

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Genome-wide association study identified new susceptible genetic variants in HLA class I region for hepatitis B virus-related hepatocellular carcinoma

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We have performed a genome-wide association study (GWAS) including 473 Japanese HBV (hepatitis B virus)-positive HCC (hepatocellular carcinoma) patients and 516 HBV carriers including chronic hepatitis and asymptomatic carrier individuals to identify new host genetic factors associated with HBV-derived HCC in Japanese and other East Asian populations. We identified 65 SNPs with P values $< 10^{-4}$ located within the HLA class I region and three SNPs were genotyped in three independent population-based replication sets. Meta-analysis confirmed the association of the three SNPs (rs2523961: OR = 1.73, $P = 7.50 \times 10^{-12}$; rs1110446: OR = 1.79, $P = 1.66 \times 10^{-13}$; and rs3094137: OR = 1.73, $P = 7.09 \times 10^{-9}$). We then performed two-field HLA genotype imputation for six HLA loci using genotyping data to

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investigate the association between HLA alleles and HCC. HLA allele association testing revealed that *HLA-A*33:03* (OR = 1.97, P = 4.58 × 10^{-4}) was significantly associated with disease progression to HCC. Conditioning analysis of each of the three SNPs on the HLA class I region abolished the association of *HLA-A*33:03* with disease progression to HCC. However, conditioning the HLA allele could not eliminate the association of the three SNPs, suggesting that additional genetic factors may exist in the HLA class I region.

Hepatitis B (HB) is a potentially life-threatening liver infection caused by hepatitis B virus (HBV), and approximately 248 million people worldwide are estimated to be chronically infected with HBV[1]. The clinical course of HBV infection is variable, including acute self-limiting infection, fulminant hepatic failure, inactive carrier state, and chronic hepatitis with progression to liver cirrhosis and hepatocellular carcinoma (HCC). Although some HBV carriers spontaneously eliminate the virus, every year 2–10% of individuals with chronic HB (CHB) develop liver cirrhosis, and a subset of these individuals suffer from liver failure or HCC[2]. Around 600,000 new HCC cases are diagnosed annually worldwide, and it is relatively common in Asia-Pacific countries and sub-Saharan Africa. More than 70% of HCC patients are diagnosed in Asia[3]. In contrast, HCC is relatively uncommon in the USA, Australia, and European countries[3,4]. The majority of HCC cases develop in patients with cirrhosis, which is most often attributable to chronic HBV infection followed by chronic hepatitis C virus infection in the Asia-Pacific region[5].

Human leucocyte antigen (HLA) proteins present self and non-self peptides to T cell receptors (TCRs) to maintain self-tolerance and adapted immunity. The HLA region resides on the short arm of chromosome 6, designated as 6p21.3. It is about 3.6 Mb in length and more than 200 functional and nonfunctional genes[6,7] are located in the region. The whole HLA region is divided into three subgroups, which are designated as class I, II, and III. The HLA class I region contains 19 HLA class I genes including 3 classical (*HLA-A*, *-B*, and *-C*), 3 non-classical (*HLA-E*, *-F*, and *-G*), and 12 non-coding genes or pseudogenes. The HLA class II region contains classical class II alpha- and beta-chain genes of *HLA-DR*, *-DQ*, and *-DP*. All HLA class I and class II molecules can present peptides to T cells, but each protein binds a different range of peptides. The presence of several different genes of each HLA class means that any one individual is equipped to present a much broader range of peptides than if only one HLA molecule of each class were expressed at the cell surface. A total of 17,695 *HLA* alleles (12,893 in class I and 4,802 in class II) were released by The IPD-IMGT/HLA database release 3.31.0 in January 2018 (https://www.ebi.ac.uk/ipd/imgt/hla/). Of the 12,893 class I alleles, 4,181, 4,950, and 3,685 alleles were registered in *HLA-A*, *-B*, and *-C* genes, respectively. Of 4,802 class II alleles, 2,146, 1,178, and 965 alleles were registered in *HLA-DRB1*, *-DQB1*, and *-DPB1* genes, respectively.

Recent genome-wide association studies (GWAS) of chronic HBV carriers with or without HCC in Chinese populations reported that one SNP (rs17401966) in *KIF1B*, two SNPs (rs9272105 and rs455804) in *HLA-DQA1/DRB1* and *GRIK1*, and two SNPs (rs7574865 and rs9275319) in *STAT4* and *HLA-DQ* were associated with disease progression to HCC[8–10]. A number of candidate genes have been investigated by genetic association studies to evaluate their roles in susceptibility to HCC. The findings from these studies, however, are inconclusive due to insufficient evidence and a lack of independent validation. All three papers referred to in this manuscript performed GWAS and replication studies using only Chinese population samples. For example, the study by Zhang *et al.*[10] used 2,310 cases and 1,789 controls of Chinese ancestry and identified one intronic SNP in *KIF1B* associated with HBV-related HCC. This result, however, was not replicated in several other populations[11,12]). These findings suggest that GWAS and subsequent replication studies should be conducted in populations other than Chinese.

In this study, we performed GWAS using Japanese CHB patients with and without HCC and a replication study using East Asian populations including Japanese, Hong Kong Chinese, and Thai.

Results

GWAS and replication study of HBV-related HCC. We conducted a GWAS using samples from 473 Japanese HBV-positive HCC patients and 516 HBV carriers including CHB and asymptomatic carrier (ASC) individuals by analyzing 447,830 autosomal SNPs. Figure 1 shows a genome-wide view of the SNP association data based on allele frequencies. There were 110 SNPs with P values < 10^{-4} in the GWAS (Supplementary Materials, Table S1). Of the 110 SNPs, 65 and 4 SNPs were located on the HLA class I and II regions, respectively. These results suggested that HBV-related HCC could be associated with SNPs located in the HLA region, although associations did not reach the genome-wide significance level. Outside the HLA region, there were 41 SNPs with P values < 10^{-4} and 4 SNPs showed P values < 10^{-5}.

In order to validate these suggestive associations, we selected seven SNPs based on the following criteria: P values < 10^{-4} in the HLA region and < 10^{-5} outside the HLA region and only SNPs with the lowest P value or highest OR were selected when multiple SNPs showed strong LD. Three independent sets of HBV-related HCC cases, CHB and ASC controls (replication-1: Japanese 153 cases and 614 controls; replication-2: Hong Kong Chinese 94 cases and 187 controls; and replication-3: Thai 185 cases and 198 controls), and the original GWAS set of 989 Japanese samples (473 cases and 516 controls) were genotyped and used in a subsequent replication analysis. Of the seven SNPs, four (rs2523961, rs1110446, and rs3094137 located on HLA class I region, and rs2295119 located on HLA class II region) were validated, and consistent associations were observed between the original GWAS set and replication sets (Table 1). For these four SNPs, no heterogeneity of association was observed between the original GWAS samples and the replication samples. Two SNPs in the HLA region (rs2523961 and rs1110446) showed a genome-wide significant association (rs2523961: OR = 1.91, P = 6.42 × 10^{-10}; and rs1110446: OR = 1.93, P = 2.52 × 10^{-10}) using the combined Japanese samples (GWAS and replication-1) (Table 1). Moreover, the meta-analysis with the combined Japanese samples and two independent sample sets (Hong Kong Chinese and

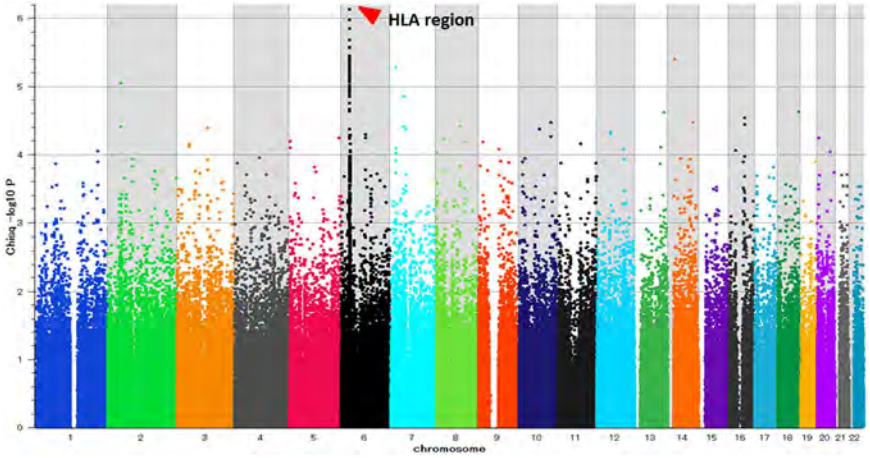

Figure 1. GWAS result. GWAS included 989 samples [473 Japanese HCC cases and 516 Japanese HBV carrier (CH and ASC) controls]. P-values were calculated using the chi-square test for allele frequencies among 447,830 SNPs.

Thai) confirmed associations for the two SNPs (rs2523961: $P = 5.81 \times 10^{-11}$; and rs1110446: $P = 9.09 \times 10^{-13}$), while the remaining two SNPs showed a marginal association (rs3094137: OR = 1.76, $P = 3.91 \times 10^{-7}$; and rs2295119: OR = 0.63, $P = 5.51 \times 10^{-7}$).

Association test for imputed HLA alleles. The two SNPs showing genome-wide significant associations were located on HLA class I region, and the marginally associated SNP was located on HLA class I and II region. To investigate the association of *HLA* alleles, we performed two-field HLA genotype imputation for six HLA loci (*HLA-A, -B, -C, -DRB1, -DQB1*, and *-DPB1*) using 989 genome-wide genotyping data used for the GWAS. Imputed *HLA* alleles were filtered (Call Threshold < 0.5) before performing association analysis for each HLA locus. The results of association tests in *HLA-A, -B, -C, -DRB1, -DQB1*, and *-DPB1* alleles are shown in Table 2 and Supplementary Materials, Table S2. To avoid false-positive results due to multiple testing for 77 HLA alleles, significance levels were set at 0.000649 (=0.05/77). A protective effect of *HLA-DPB1*02:01* (OR = 0.59, $P = 5.23 \times 10^{-6}$) was observed as previously reported[13]. We also detected that *HLA-A*33:03* was significantly associated with disease progression to HCC (OR = 1.97, $P = 4.58 \times 10^{-4}$) (Table 2).

Using GTEx-generated eQTL data[14], we checked for correlations between the three SNPs and *HLA-A* gene expression levels. The SNP rs2523961 was correlated with *HLA-A* gene expression in various tissues (muscle: $P = 6.1 \times 10^{-20}$; heart: $P = 2.3 \times 10^{-15}$, 2.1×10^{-11}; esophagus: $P = 2.8 \times 10^{-12}$, 1.8×10^{-6}; artery: $P = 4.7 \times 10^{-12}$, 3.9×10^{-11}; thyroid: $P = 1.4 \times 10^{-11}$; pancreas: $P = 3.3 \times 10^{-9}$; brain: $P = 1.9 \times 10^{-8}$, 2.2×10^{-7}; nerve: $P = 3.2 \times 10^{-8}$; testis: $P = 5.5 \times 10^{-7}$; lung: $P = 1.7 \times 10^{-5}$). The SNP rs1110446 was also associated with *HLA-A* gene expression in muscle ($P = 5.5 \times 10^{-15}$), skin ($P = 6.2 \times 10^{-11}$, 4.4×10^{-9}), artery ($P = 8.7 \times 10^{-6}$, 1.1×10^{-4}), esophagus ($P = 2.5 \times 10^{-5}$), and whole blood ($P = 5.1 \times 10^{-5}$). These results suggest that these SNPs affected *HLA-A* gene expression.

Conditioning each of the three SNPs on the HLA class I region (Supplementary Material, Fig. S1a–c) abolished the association of *HLA-A*33:03* ($P > 0.05$), but conditioning of *A*33:03* could not eliminate the association of the three SNPs (rs2523961: OR = 1.69, $P = 7.06 \times 10^{-4}$; rs1110446: OR = 1.65, $P = 9.33 \times 10^{-4}$; and rs3094137: OR = 1.54, $P = 5.68 \times 10^{-3}$) (Fig. 2). These conditional analyses suggest that additional genetic factors other than *HLA-A* allele exist in the HLA class I region. In contrast to the class I region, conditional analysis controlling for the SNP rs2295119 using *DPB1*02:01* allele suggests that *DPB1* allele could abolish the association of rs2295119 on the HLA class II region ($P > 0.05$) (Supplementary Material, Fig. S1e).

Discussion

In the current GWAS, we found a marginal association between an SNP (rs2295119) located in the *HLA-DPB* region and HBV-related HCC. Moreover, the association analysis of *HLA-DPB1* alleles and the conditional analysis with *HLA-DPB1*02:01* suggested that *DPB1*02:01* was the major protective allele in the HLA class II region. Recent GWAS also showed that SNPs located in the HLA class II region (*HLA-DQA1/DRB1*[9] and *HLA-DQ*[8]) were associated with HBV-related HCC in the Chinese population. We focused on the p-values of the HLA class II region (*HLA-DQ* and *-DR*) and six other gene regions (*KIF1B, UBE4B, PGD*, 8p12, *GRIK1* and *STAT4*) reported in previous studies and revealed the SNPs of four regions (*HLA-DQ* and *-DR*, 8p12, and *STAT4*) had p-values of less than 0.00625 (0.05/8). There were 52, 10 and 1 SNP with $P < 0.00625$ located on *HLA-DQ/DR*, 8p12, and *STAT4*, respectively, and the lowest p-value of each region was 0.00102 (rs9271894 on *HLA-DQA1*, OR = 1.46), 0.00278 (rs8084 on *HLA-DRA*, OR = 1.32), 0.00049 (rs13250548 on 8p12, OR = 0.68), and 0.0019 (rs6752770 on *STAT4*, OR = 1.44).

We also identified significant associations in the HLA class I region, especially around the *HLA-A* locus. The association test of imputed HLA alleles and conditional analyses with *HLA-A*33:03* suggested that *HLA-A*33:03* is the susceptibility allele for HCC. We performed additional conditional analyses controlling for the SNP on chromosome 6 using *A*33:03* and *DPB1*02:01* alleles. This indicated that *HLA-A* and *DPB1* alleles could

Marker	Allele (1/2)	stage	population	cases				controls				P value[b]	OR (95% CI)
				11	12	22	MAF	11	12	22	MAF		
rs2523961	A/G	GWAS	Japanese	12	174	287	0.209	11	111	394	0.129	2.57E-07	2.02 (1.54–2.66)
(class I)		Combined	Japanese	19	219	388	0.205	23	238	867	0.126	6.42E-10	1.91 (1.56–2.37)
		Replication2	Hong Kong Chinese	1	25	68	0.144	2	34	151	0.102	0.118	1.55 (0.90–2.66)
		Replication3	Thai	13	54	108	0.229	6	49	142	0.155	0.059	1.49 (0.98–2.28)
		Meta-analysis[a]										5.81E-11	
rs1110446	T/C	GWAS	Japanese	14	177	282	0.217	11	114	391	0.132	4.44E-08	2.10 (1.60–2.75)
(class I)		Combined	Japanese	21	222	383	0.211	24	245	861	0.130	2.52E-10	1.93 (1.57–2.37)
		Replication2	Hong Kong Chinese	2	22	70	0.138	1	35	151	0.099	0.138	1.52 (0.90–2.62)
		Replication3	Thai	14	66	100	0.261	5	51	142	0.154	0.002	1.93 (1.27–2.92)
		Meta-analysis[a]										9.09E-13	
rs3094137	A/G	GWAS	Japanese	9	150	314	0.178	10	97	409	0.113	9.65E-05	1.74 (1.31–2.31)
(class I)		Combined	Japanese	13	191	421	0.174	19	203	906	0.107	3.91E-07	1.76 (1.41–2.19)
		Replication2	Hong Kong Chinese	0	8	86	0.043	0	9	178	0.024	0.201	1.93 (0.71–5.21)
		Replication3	Thai	0	19	160	0.053	0	15	181	0.038	0.468	1.35 (0.60–3.03)
		Meta-analysis[a]										9.83E-05	
rs2295119	T/G	GWAS	Japanese	18	139	316	0.185	41	191	284	0.265	5.77E-06	0.59 (0.47–0.74)
(class II)		Combined	Japanese	27	179	420	0.186	78	417	635	0.254	5.51E-07	0.63 (0.53–0.76)
		Replication2	Hong Kong Chinese	2	22	70	0.138	5	54	128	0.171	0.318432	0.78 (0.47–1.28)
		Replication3	Thai	4	39	136	0.131	3	50	143	0.143	0.285443	0.76 (0.47–1.25)
		Meta-analysis[a]										4.88E-07	

Table 1. Four SNPs in the HLA region associated with disease progression to HCC. [a]Results of meta-analysis were calculated by the DerSimonian-Laird method. [b]Result of logistic regression analysis adjusted for age and sex.

abolish the association in the HLA class II region but were not sufficient to abolish the association in the HLA class I region (Fig. 2 and Supplementary Material, Fig. S1f). Therefore, not only the *HLA-A* allele but also additional genetic factor(s) likely exist in the HLA class I region. There are several genes in this region including *HLA-A*, *HCG9, HLA-J, HCG8, ZNRD1-AS1, ZNRD1, PPP1R11, RNF39, TRIM31*, and *TRIM40* (shown in Fig. 2). Although these genes include pseudogenes and poorly characterized genes, some are associated with various diseases. The zinc ribbon domain-containing 1 (ZNRD1) protein is associated with cell growth of gastric cancer cells[15], angiogenesis of leukemia cells[16], and HIV-1/AIDS disease progression[17,18]. In addition, *ZNRD1* knockdown inhibits the expression of HBV mRNA and promotes the proliferation of HepG2.2.15 cells[19], suggesting that *ZNRD1* is one of the possible additional genetic factors at the HLA class I region. The tripartite motif-containing 31 (TRIM31) protein is essential for promoting lipopolysaccharide-induced Atg5/Atg7-independent autophagy[20]. Moreover, *TRIM40* is downregulated in gastrointestinal carcinomas and chronic inflammatory lesions of the gastrointestinal tract[21].

Non-self antigens, such as virus-infected cells and cancer cells, and HLA class I molecules are generally recognized by the TCRs on CD8+ T lymphocytes, resulting in T cell activation[22]. The activated T cells divide and some of their progeny differentiate into lymphocytes capable of killing cells (cytotoxic T lymphocytes: CTLs) displaying the same peptides (such as tumor-specific peptides) on their HLA class I molecules. These CTLs target tumor-specific antigenic peptides and eliminate them. In other words, CTLs cannot eliminate cancer cells without HLA class I molecules even if the person has tumor-specific peptides. Cancer cells therefore need to escape from the immune system for patients to be identified as having cancer.

In this study, we identified a significant association between *HLA-A*33:03* and HBV-related HCC. In addition to *HLA-A*33:03*, previous studies and this study suggested that *HLA-DR*, *-DQ*, and *-DP* were associated with disease progression[8,9,13]. Functional analysis of HLA class I and II proteins could be an important step in determining the pathology of HBV-related HCC.

HLA-A	Case (2n = 892)	%	Control (2n = 998)	%	Fisher's P-value	OR	95% CI
02:01	105	11.8	113	11.3	0.7733	1.04	0.78–1.40
02:06	80	9.0	106	10.6	0.2462	0.83	0.60–1.14
02:07	38	4.3	40	4.0	0.8174	1.07	0.66–1.72
11:01	53	5.9	94	9.4	0.005757	0.61	0.42–0.87
24:02	331	37.1	393	39.4	0.3198	0.91	0.75–1.10
26:01	72	8.1	89	8.9	0.5636	0.90	0.64–1.26
26:03	18	2.0	22	2.2	0.8732	0.91	0.46–1.80
31:01	112	12.6	90	9.0	0.01384	1.45	1.07–1.97
33:03	76	8.5	45	4.5	**0.00046**	**1.97**	1.33–2.95

Table 2. Association analyses of *HLA-A* alleles.

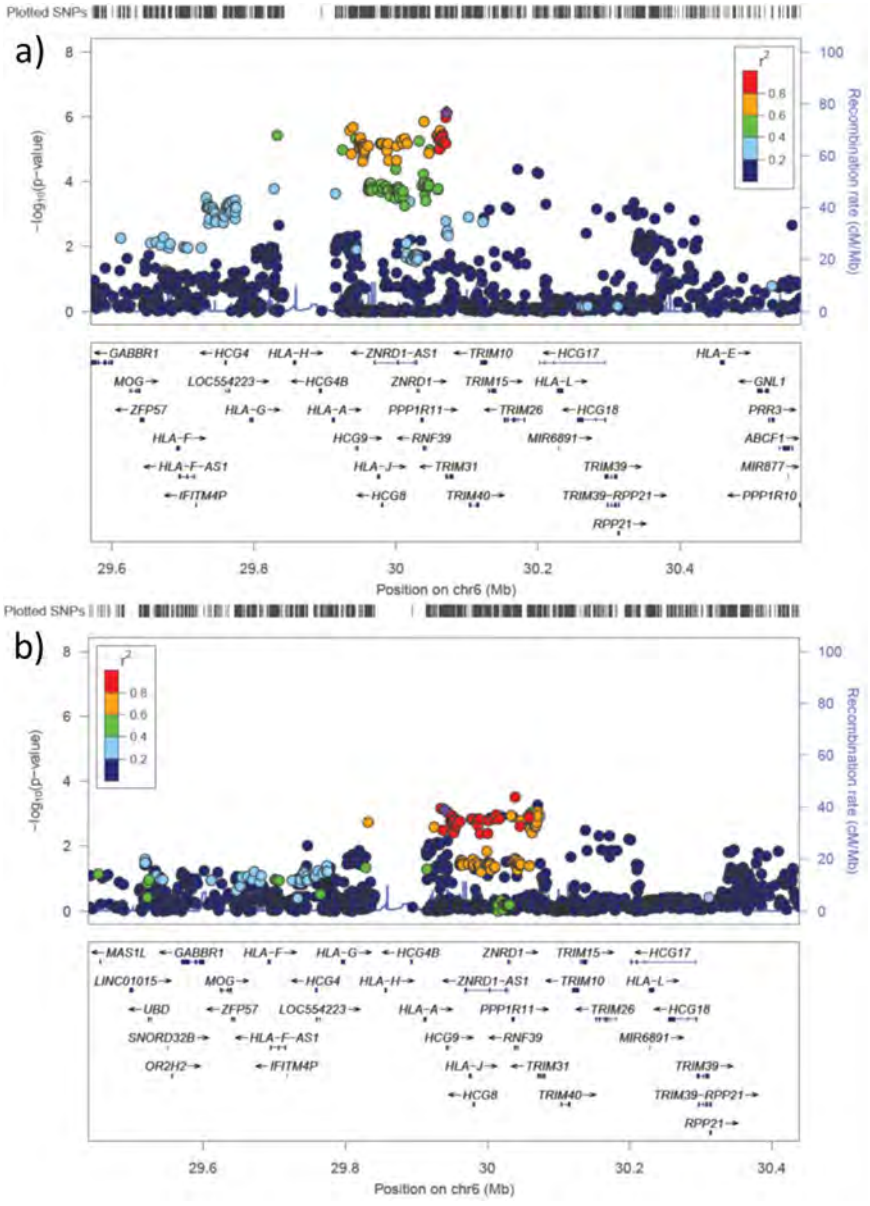

Figure 2. Association plots of the HLA class I region on chromosome 6 HLA region. (**a**) The major genetic determinant of HBV-related HCC risk to HLA class I genes. (**b**) Conditional analysis controlling for the effect of *HLA-A*33:03*.

Methods

Ethics statement. All study protocols conformed to the relevant ethical guidelines, as reflected in the *a priori* approval by the ethics committee of the University of Tokyo, and by the ethics committees of all participating universities and hospitals. All participating studies obtained informed consent from all participants in this study and all samples were anonymized.

Samples. Samples from 3,133 individuals who had HBV-derived chronic hepatitis, ASC, liver cirrhosis, or HCC and patients with other HBV-related symptoms were collected by 26 universities and hospitals (Hokkaido University Hospital, Teine Keijinkai Hospital, Iwate Medical University Hospital, Musashino Red Cross Hospital, The University of Tokyo Hospital, Saitama Medical University Hospital, Chiba University Hospital, Kitasato University Hospital, Kohnodai Hospital, Shinshu University Hospital, Kanazawa University Hospital, Nagoya City University Hospital, Kyoto Prefectural University of Medicine Hospital, National Hospital Organization Osaka National Hospital, Osaka City University Hospital, Hyogo College of Medicine, Tottori University Hospital, Ehime University Hospital, Yamaguchi University Hospital, Kawasaki Medical College Hospital, Okayama University Hospital, Nagasaki Medical Center, Kurume University Hospital, Saga University Hospital, Eguchi Hospital, and Kyusyu University Hospital). The Japanese Public Health Cancer-based Prospective (JPHC) Study samples[23] in Japan were used for the replication study. Hong Kong Chinese samples were collected at the University of Hong Kong. Thai samples were collected at Chulalongkorn University.

HBV status was measured based on serological results for HBsAg and anti-HBc with a fully automated chemiluminescent enzyme immunoassay system (Abbott ARCHITECT, Abbott Japan, Tokyo, Japan or LUMIPULSE G1200, Fujirebio, Inc., Tokyo, Japan). For clinical staging, ASC state was defined by the presence of HBsAg with normal ALT levels over 1 year (examined at least four times at 3-month intervals) and without evidence of liver cirrhosis. CH was defined by elevated ALT levels (1.5 times the upper limit of normal [35 IU/L]) persisting for over 6 months (by at least three bimonthly tests). HCC was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy, or by a combination of these.

SNP genotyping and data cleaning. For the GWAS, we genotyped 1,356 Japanese samples using the Affymetrix Axiom Genome-Wide ASI 1 Array (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's instructions and determined the genotype calls of 600,307 SNPs using the Genotyping Console v4.2.0.26 software (Supplementary Material, Fig. S2a). To increase the samples for genotyping, we used not only CHB patients with and without HCC but also patients with HBV-related other symptoms such as liver cirrhosis. All samples used for genotyping passed a Dish QC >0.82 and overall call rate $>97\%$. The average Dish QC for 1,356 samples was 0.969 (0.883–0.993) and the average call rate reached 99.42% (97.47–99.87%). All genotyped samples passed a heterozygosity check, and 25 duplicated samples were identified in identity by descent (IBD) testing. A principal component analysis (PCA) found seven outliers could be excluded by the Smirnov-Grubbs test, and we showed that all the remaining samples ($n = 1,324$) formed a single cluster with the HapMap Japanese (JPT) samples but not with the Han Chinese (CHB), Northern and Western European (CEU), and Yoruban (YRI) samples. We then applied the following thresholds for SNP quality control in data cleaning: SNP call rate of $\geq 95\%$, minor allele frequency of $\geq 3\%$ and Hardy-Weinberg equilibrium P value of ≥ 0.001. A total of 447,830 SNPs on autosomal chromosomes passed the quality control filters and were used for subsequent GWAS. For the association study of HBV-related HCC, we selected 481 HBV-related HCC patients (cases) and 538 HBV carriers (CH and ASC patients, controls) from 1,324 samples and performed IBD testing and PCA again for these samples. Twenty-three related samples and seven outliers were excluded by IBD testing and PCA (Supplementary Material, Fig. S3), respectively. We finally used 473 cases and 516 controls for GWAS. A quantile-quantile plot of the distribution of test statistics for the comparison of genotype frequencies in the cases and controls showed that the inflation factor λ was 1.016 for all tested SNPs and was 1.009 when SNPs in the HLA region were excluded (Supplementary Material, Fig. S4). All cluster plots for SNPs with P values of $<10^{-4}$ were checked visually and SNPs with ambiguous genotype calls were excluded.

In the replication stage, we selected seven SNPs with P values of $<10^{-5}$ from the results of the chi-square test in the GWAS. A TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) was used to confirm the genotypes at each SNP. We genotyped 989 and 767 Japanese samples for the validation of the GWAS and for the replication study, respectively. We further genotyped 281 Hong Kong Chinese and 383 Thai samples for the replication study (Supplementary Materials, Table S3).

Statistical analysis. The characteristics of analyzed samples are shown in Supplementary Materials, Table S3. For the GWAS and replication study, the chi-square test was applied to a two-by-two contingency table in the allele frequency model. Meta-analysis was performed using the DerSimonian-Laird method (random-effects model) in order to calculate the pooled OR and its 95% confidence interval. Fisher's exact test in a two-by-two contingency table was used to examine the association between *HLA* alleles and disease progression of HBV patients. To avoid false-positive results due to multiple testing, the resulting P-values were adjusted based on the number of observed alleles with frequencies $\geq 0.5\%$ in cases and controls. Conditional logistic regression analysis was performed for SNPs and *HLA* alleles. This analysis was performed as implemented in Plink v1.07 software[24], conditioning on *HLA-A*33:03* and *DPB1*02:01* to each of the other SNPs. Other statistical analyses were performed using the SNP & Variation Suite 7 software (Golden Helix, Bozeman, MT, USA) and statistical software R v2.6. Manhattan plot of conditioning of each SNP or HLA allele was generated by LocusZoom[25].

HLA imputation. SNP data from 989 samples were extracted from extended MHC (xMHC) regions ranging from 25759242 bp to 33534827 bp based on hg19 position. Two-field HLA genotype imputation was performed for a total of six HLA class I and class II genes using the HIBAG R package[26,27]. For *HLA-A,-B, -DRB1, -DQB1*,

and -*DPB1*, a Japanese imputation reference[26] was used for HLA genotype imputation. For *HLA-C*, the HIBAG Asian reference[27] was used for HLA genotype imputation. We applied post-imputation quality control using call-threshold (CT > 0.5); the call rate of successfully imputed samples ranged from 88.7 to 98.5% for the six HLA classes. In total, we imputed 5,650 HLA genotypes in HLA class I and class II genes.

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Study desingn and discussion: H.S., N.N., M.H., M.S., N. Sw., S.T., K.K., Y.K., H.Y., S. Ng., A. Tk., M.F., M.K., I.N., J.-H.K., K.M., K.H., S. Ns., A.M., E.T., N. Sk., K.O., K. Ymm., A. Tm., O.Y., T.K. I.S., Y.I., Y.E., S.O., S.M., M.-F.Y.,

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a p value<0.001. The factors associated with bullying were the younger age group, shorter length of service, shifting work, non-managerial position and the designation as a doctor.

Conclusion A significant proportion of healthcare workers had been bullied, and bullying exposure was shown to be associated with depression and low self-esteem. Hence, regular screening for bullying, depression and low self-esteem should be done to enable early intervention.

1551 CHANGES IN TWENTY YEARS OF THE EPIDEMIOLOGICAL STATUS OF NEEDLESTICK/SHARPS INJURIES REPORTED TO JAPAN-EPINET THROUGH A NATION-WIDE SURVEILLANCE NETWORK

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Introduction This study aimed at examining annual logs of needlestick/sharps injuries (NSIs) collected through a voluntary nation-wide surveillance network in twenty-years for preventing occupational blood-borne infections. The emphasis was placed on revealing the past and current situations of NSIs in health care settings.

Methods Japan-EPINet format was developed by the technical support of the International Healthcare Worker Safety Centre, University of Virginia in the United States in 1996. Japan-EPINet Surveillance (JES) was conducted by the Research Group for Occupational Infection Control and Prevention in Japan (JRGOICP). Data were analysed in four phases of the nation-wide surveillance network of AIDS referral hospitals out of a total of 364 registered, a total number of hospital-year was 1879. These hospitals reported employees' percutaneous injuries on a voluntary basis.

Results A total of 65,032 NSIs were reported to Japan-EPINet from 1996 to 2015. The rate of hepatitis C antibody positive cases of the total NSIs decreased from 69.9% (1,511/2,161) in 1996 to 11.5% (714/6,201) in JES2015. The proportion of NSIs due to 'recapping' decreased (28.7%, 6.9% respectively). Devices caused to NSIs by winged steel needles (25.3%, 8.6%) and vacuum tube phlebotomy needles (4.8%, 1.7%) were decreased, disposal syringe (28.5%, 26.2%) and IV catheter (6.7%, 5.2%) were fairly decreased. The proportion of Suture needle (10.3%, 16.9%) and pre-filled cartridge syringe (2.8%, 8.3%) were increased.

Discussion The changes of characteristics NSIs in Japan in twenty-year suggested that recognition of the risks of NSIs was vital for promoting the effective use of safety-engineered needle/sharp devices and point-of-use disposal containers because the rate of hepatitis C antibody positive cases among voluntary reported NSIs. The creation of the nation-wide surveillance network was effective for monitoring and evaluating NSIs and for focusing on implementation of effective countermeasures.

25 PREPARATION OF HAZARDOUS DRUGS IN BIOLOGICAL SAFETY CABIN (BSC): THE CHALLENGE OF GETTING HEALTHIER WORK ENVIRONMENTS

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Introduction Hazardous drugs are an important risk to health care workers. Some of these products may even be potentially carcinogenic.

In different Spanish hospitals it was observed that only Cytostatics drugs were prepared in biological safety cabins, leaving workers exposed to the rest of hazardous non cytostatic drugs.

Methods A bibliographical review of scientific articles and researches has been carried out, together with the laws on occupational health and recommendations of the Spanish organisms.

In the USA, research promoted the development of policies of prevention and the incorporation of these drugs in the list NIOSH.

Result After analysing the information obtained, we detected the following problems: HD's are prepared in hospitalisation rooms, where the right conditions to protect workers are non-existent; In many cases, health care workers are given only personal protective equipment to avoid exposure; Specific health control isn't performed in most cases; National legislation obliges the risk to be taken into account for the worker. Although there are no long-term epidemiological studies, protective measures should be taken.

Discussion In many hospitals in our country HD's are not prepared in biological safety cabins. Health workers are unaware that they are exposed to these risks and no specific health training or monitoring is performed. Collaborative epidemiological researches should be promoted among Public Health Units, which have information on the prevalence rate of cancer diseases, and those responsible for occupational health prevention.

250 HOW THE WORKING BACKS PROGRAMME HELPED STAFF MANAGE BACK PAIN, REMAIN IN WORK AND REDUCE ABSENTEEISM

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Introduction The Working Backs Programme (WBP) is designed for staff reporting back pain as a result of work or whose work performance is affected. It's a comprehensive approach including medical assessment, provision of information and education, a designated physiotherapy and ergonomic staff referral service and a referral pathway for further investigations and/or review. The effectiveness was evaluated by an initial audit in 2012 and subsequent audits in 2015 and 2016.

Methods Data was collected through questionnaires at initial consultation and post discharge for comparison. This included

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＜特別寄稿＞

日本肝臓学会評議員を対象としたB型肝炎ワクチンに関するアンケート調査

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要旨：B型肝炎（HB）ワクチンの在り方を検討するために，日本肝臓学会HBワクチンワーキンググループとして日本肝臓学会評議員などを対象にHBワクチンに関するアンケート調査を実施した．その結果，1)「HBワクチンの適切な接種時期（キャッチアップ）」に関しては，小学生高学年64%と最多であった．2)「ワクチン無効例に対する対策」としては，筋肉内注射や4回以上投与などが挙げられた．3)「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」が63%で最も多く，その施設の多くは職員に対するHBs抗体の定期検査を12カ月ごとに行い，HBs抗体価10 mIU/mL未満の時点でHBワクチンを追加接種していた．これらの結果を踏まえると，「追加のワクチン接種は必要ではない」とする日本環境感染学会ガイドラインについて再度議論する必要があるように思われた．

索引用語：　HBV　B型肝炎ワクチン　ワクチンブースター　HBs抗体

緒　言

わが国では，1972年に日本赤十字社の血液センターにおけるHBs抗原のスクリーニング検査が開始された．さらに，1986年に開始された母子感染防止事業に基づく出生児に対するワクチンおよび免疫グロブリン投与により，垂直感染による新たなHBVキャリア成立が阻止され，若年者におけるHBs抗原陽性率は著しく減少した．しかし，一方で性交渉に伴う水平感染によるB型急性肝炎の発症数は減少せず，近年では，肝炎が遷延し慢性化しやすいゲノタイプAのHBV感染が増加傾向にある[1)]．

2016年10月より0歳児を対象としたB型肝炎（HB）ワクチンの定期接種が開始されたが，定期接種の対象から漏れた小児への対応，性行為感染症としてのB型急性肝炎，ワクチン無反応・低反応者対策，ブースター接種の必要性，HBワクチン接種によるHBV再活性化抑制などの問題が残されている．

また，HBVワクチン接種によって免疫が得られても，HBs抗体は最初の1年で急速に低下し，それ以降はゆっくりと減少する．健常人では，ワクチン接種者の90～95%に抗体産生がみられるが，抗体産生は時間の経過とともに減弱し，8年以上経過すると約60%の人で抗体が検出されなくなる．しかし，HBVに対する免疫は保たれるため，再度ワクチンを接種する必要はないとしている[2)3)]．実際，4～23年前にワクチンが接種されてHBs抗体を獲得したにも拘わらず，時間の経過によって10 mIU/mL未満まで低下してしまった人にワクチンをブースター接種すると僅か2～4週間後に74～100%の人で抗体が再陽転化した．このデータはワクチン接種者の多くが免疫記憶を維持しており，HBVの曝露によってHBs抗体を獲得することができることを示している．以上の結果を踏まえて，米国CDC（Centers for Disease Control and Prevention）ガイドラインでは，一度十分な抗体価が得られれば，その後抗体価が低下しても曝露に際して効果的な免疫反応が得られると判断され，腎不全を含む免疫不全症例以外は，経時的な抗体価測定は不要とした[4)]．

今回，HBワクチンの在り方を検討するために，小池和彦理事長の承認の下，企画広報委員会（持田　智委員長）に依頼して，同委員会内にHBワクチン小委員会を設置し，日本肝臓学会HBワクチンワーキンググループ（WG）として日本肝臓学会評議員などを対象にHBワクチンに関するアンケート調査を実施したので，その結果を報告する．

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Table 1　B型肝炎ワクチンに関するアンケートの様式

B型肝炎ワクチンに関するアンケートのお願い

一般社団法人　日本肝臓学会
企画広報委員会　委員長　持田智
HBワクチン小委員会

2016年10月より0歳児を対象としたB型肝炎(HB)ワクチンの定期接種が開始されました。現在残された問題点として、定期接種の対象から漏れた小児への対応、性行為感染症としてのB型急性肝炎（欧米型A）及びHBV再活性化があり、これらの点に関して学会として対応を考えるべく、「HBワクチン小委員会」が発足致しました。つきましては今回、日本肝臓学会評議員の先生方のご意見を伺いたく簡単なアンケートを実施させて頂きますので、以下の質問に対する御回答をお願いします。**いずれも複数回答可です。**

1．定期接種の対象とならなかった人に対するキャッチアップとしてHBワクチンの適切な時期についてお尋ねします。
　□ 小学生高学年（他のワクチンと同時接種）
　□ 中学生　　□ 高校生
　□ キャッチアップ必要なし

2．ワクチン無効例に対する対策はどのようにされていますか？これまでの報告（八橋弘 B型肝炎ワクチンの筋肉内注射. 日本医事新報 4858:53-58, 2012）によると筋肉内注射により有意なHBs抗体価上昇が期待できます。
　（接種方法の変更）□ 筋肉内注射　　□ 皮内注射
　□ ワクチンの種類を変更　　□ 倍量投与　　□ 4回以上投与
　□ その他（　　）

3．院内で、職員に対するHBs抗体の採血は定期的にされていますか？
　□ はい　　□ いいえ
　「はい」の場合の頻度（　　）ヶ月おき

4．HBs抗体価が低下した医療従事者に対するHBワクチンのブースターはされていますか？　　□ はい　　□ いいえ
　「はい」の場合の目安
　□ HBs抗体 10 mIU/mL未満（陰性）　　□ HBs抗体100 mIU/未満

5．その他、ご意見がございしましたら、よろしくお願いします。

方　法

平成29年9月, 日本肝臓学会HBワクチンワーキンググループとして日本肝臓学会評議員など855名を対象にTable 1のようなアンケート調査を実施した. 1)定期接種の対象とならなかった人に対するキャッチアップとしてHBワクチンの適切な接種時期, 2)ワクチン無効例に対する対策, 3)院内職員に対するHBs抗体の定期検査の実施状況, 4)HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性と実際の対応について質問した.

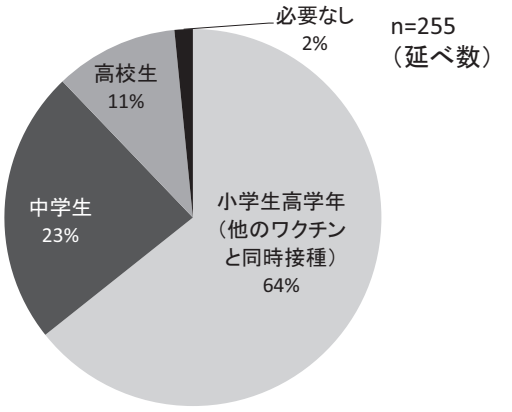

Fig. 1　HBワクチンの適切な接種時期（キャッチアップ）

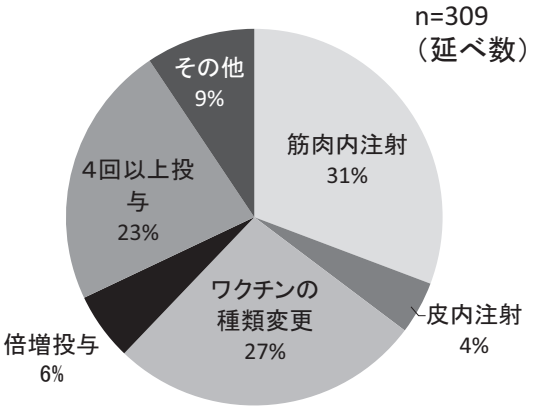

Fig. 2　ワクチン無効例対策

結　果

アンケート調査の回収率は24%（206/805）であった．1）「HBワクチンの適切な接種時期（キャッチアップ）」に関しては，小学生高学年（他のワクチンと同時接種）64%，中学生23%，高校生11%であった（Fig. 1）．2）「ワクチン無効例に対する対策」としては，筋肉内注射31%（皮内注射4%），ワクチンの種類を変更27%，4回以上投与23%，倍量投与6%であった（Fig. 2）．3）「職員に対するHBs抗体の定期検査の有無」は，「あり」62%で，検査頻度は12カ月毎の採血が91%と最多であった（Fig. 3）．4）「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」63%で，このうち93%でHBs抗体価10 mIU/mL未満の時点で実施していた（Fig. 4）．

考　察

米国CDCガイドラインの発表を受けて，日本環境感染学会ガイドラインでも「ワクチン接種シリーズ後の抗体検査で免疫獲得と確認された場合，その後の抗体検査や追加のワクチン接種は必要ではない」という勧告を出した[5]．すなわち，1）透析患者，2）HIV感染者，3）造血幹細胞移植を受けた患者，4）化学療法や免疫抑制療法を受けた患者などのハイリスクグループ以外は追加のワクチン接種は必要ではないとするガイドラインである．確かに，集団免疫（医療機関として）の観点からは，医療従事者の肝炎発症と患者への2次感染を防ぐことが目標であり，コストベネフィットを考慮した米国のガイドラインは正しいと言えよう．

一方，個人免疫の観点からは肝炎も嫌だが，将来の肝がんも防ぎたい．すなわち，HBc抗体が陽性化する感染を防ぐことにより，肝炎，肝癌，さらにはHBV再活性化すべてを予防することが可能となる．実際に福祉の国であるイギリスのガイドラインでは，抗体低下時の追加接種を推奨しており，HBs抗体価10～100 mIU/mLの人でさえ，1回追加接種したのち5年ごとに1回追加接種を推奨している[6]．特に，1）医療従事者，2）透析患者，3）パートナーや家族内にHBVキャリアがいる場合は強く推奨される．興味深いことに，今回の日本肝臓学会評議員などを対象としたアンケート調査では，「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」が63%で最も多く，その施設の多くは職員に対するHBs抗体の定期検査を12カ月ごとに行い，HBs抗体価10 mIU/mL未満の時点でHBワクチンを追加接種していた．これらの結果を踏まえると，「追加のワクチン接種は必要ではない」とする日本環境感染学会ガイドラインについて再度議論する必要があるように思われる．これは“B型肝炎”を「肝臓病」として捉えている肝臓専門医と「感染症」として捉えている感染症専門医との間にある根本的な考え方の相違に起因するものかもしれない．

これまでに医療従事者を何百人も対象とした研究や男性同性愛者やエスキモーを対象とした研究が長期間実施されており，これらの研究の成果はCDCからの勧告を支持しているが，HBc抗体が検出された症例が存在するのも事実である[7)～9)]．HBc抗体はHBVワクチンでは獲得されない抗体であり，この存在はHBV自体が体内に入り込み，免疫が反応したという根拠になる．すなわち，HBワクチン接種でHBs抗体陽性となった場合，その後のHBVへの曝露により肝炎を発症するこ

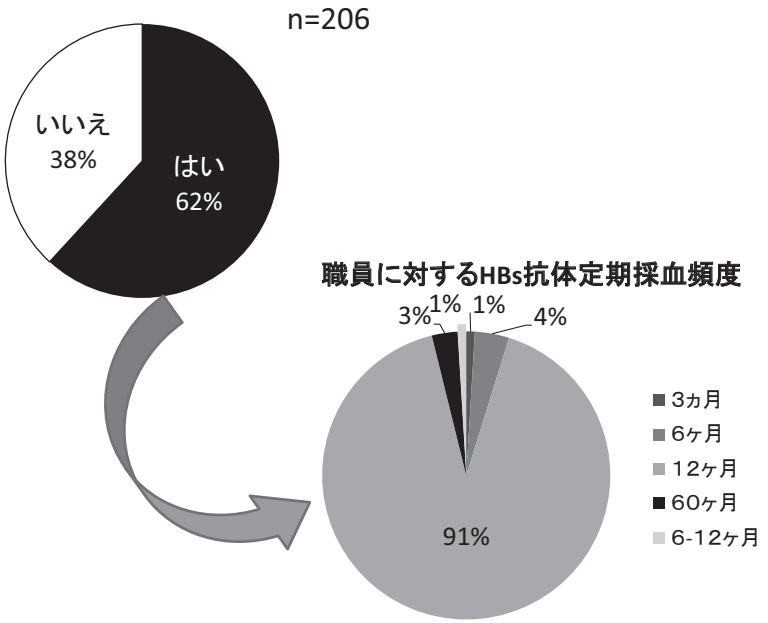

Fig. 3　職員に対する HBs 抗体の定期採血

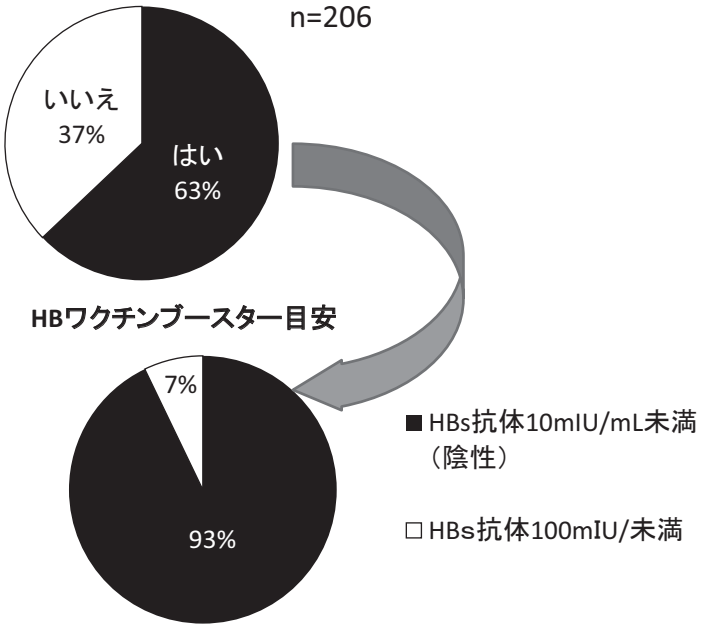

Fig. 4　医療従事者に対する HB ワクチンブースター

とはまれであるが，HBs 抗体価が低下した際には HBV への曝露後に HBV DNA が陽性となることがある[10]．このような状態はオカルト HBV 感染と称され，免疫抑制状態において HBV 再活性化を引き起こすことがある[11]．現在のところ，HB ワクチン接種後 HBs 抗体が陰転化した場合の HB ワクチン追加接種は推奨されていないが，HB ワクチン接種数年後に HBs 抗体価が低下し，急性肝炎（ALT 3,510 U/L）を発症した症例[12]や急性肝

炎発症（ALT 211 U/L）からキャリア化した症例[13]も報告されており，HBs抗体価10 mIU/mL未満に低下した場合にはHBワクチンを追加接種することも選択肢となりうる．特に，肝炎を発症しないまでも，HBc抗体が陽転化した時点で，肝臓内にはHBVはすでに侵入・感染していることになり，がん化学療法や免疫抑制剤使用時にHBV再活性化のリスクを背負うことになる．そのような予測可能な事態を肝臓専門医として容認してよいのか，今後も議論が必要と思われる．

結　語

日本肝臓学会評議員などを対象にアンケート調査を行った結果，HBワクチンに関する重要なエクスパートオピニオンが得られた．今後も，日本肝臓学会としての意見をまとめて広く情報発信する予定である．

謝辞：今回，HBワクチンの在り方を検討するための「日本肝臓学会HBワクチンワーキンググループ（企画広報委員会HBワクチン小委員会）」設立にご尽力頂きました小池和彦理事長ならびに企画広報委員会委員長の持田智先生に深く感謝申し上げます．なお，本アンケートにご協力いただきました日本肝臓学会役員及び評議員の先生方に深謝いたします．

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The Japanese Society of Gastroenterology

ORIGINAL ARTICLĒLIVER, PANCREAS, AND BILIARY TRACT

Epidemiologic features of 348 children with hepatitis C virus infection over a 30-year period: a nationwide survey in Japan

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Abstract

Background Although the epidemiology of hepatitis C virus (HCV) infection among children may be rapidly changing, few reports have characterized large nationwide cohorts of children with HCV infection. We, therefore, sought to clarify the epidemiology and natural history of HCV infection in Japanese children born over the last three decades.

Methods Sixty-five pediatric centers retrospectively and prospectively recruited consecutive, otherwise-healthy HCV-infected children born during 1986 to 2015.

Results Entry criteria were met by 348 children. Age at initial diagnosis of infection has decreased significantly in recent years. Cirrhosis and hepatocellular carcinoma were not identified. Prevalence of spontaneous clearance and of interferon treatment with/without ribavirin were 9 and 54%, respectively. Maternal transmission has increased significantly, representing over 99% of cases in the last decade. No transfusion-related cases have been seen after 1994. HCV genotype 2 has increased to become the most prevalent in Japanese children. Histopathology examination of liver specimens showed no or mild fibrosis in most children with chronic hepatitis C; none showed cirrhosis.

Conclusions This largest nationwide cohort study of Asian children with HCV infection spanned the last three decades. None of these Japanese children developed cirrhosis or hepatocellular carcinoma. Maternal transmission increased to account for 99% of cases during the last decade. Genotype 2 now is most prevalent in these children. Histopathologically, most children with chronic hepatitis C showed mild fibrosis or none.

Keywords Natural history · Maternal transmission · Genotype · Liver histopathology · Cirrhosis

Abbreviations

HCV	Hepatitis C virus
anti-HCV	Anti-HCV antibody
SVR	Sustained virologic response
IFN	Interferon
RBV	Ribavirin
CHC	Chronic hepatitis C
SD	Standard deviation
DAAs	Direct-acting antiviral agents

Introduction

Hepatitis C virus (HCV) infection is a major cause of liver disease. Recent estimates showed an increase in its worldwide prevalence over the last decade to 2.8%, amounting to

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over 185 million infections [1–3]. In Japan, estimated prevalence of HCV infection in adults has been 0.8 to 1.2% [4]. Prevalence is lower in children, estimated at 0.012% at ages 5–9 years, 0.010% at 10–14 years, and 0.022% at 15–19 years [5]. The low prevalence of HCV infection in children reflects disappearance of transmission by blood transfusions and other medical procedures, and also reduced mother-to-child (i.e., vertical or perinatal) transmission, even though this form of transmission currently is responsible for most new infections in developed countries [6–9]. Among HCV genotypes, genotype 1 is most prevalent worldwide (49.1%), followed by genotypes 3 (17.9%), 4 (16.8%), and 2 (11.0%). Genotypes 5 and 6 are responsible for the remaining infections, representing less than 5% [3]. In Japanese adults, relative prevalence of genotype 1 has declined while that of genotype 2 has increased; nonetheless, genotype 1 (65%) remains more prevalent than genotype 2 (34%) [4, 10]. Taken together, these data raise the question of possible rapid changes in the epidemiology of HCV infection among Japanese children, but few large nationwide cohort studies of children with HCV infection have been undertaken, particularly in the last decade [9, 11, 12]. To evaluate the extent of these changes, which could alter the future burden of HCV infection, we investigated epidemiologic features of a large nationwide cohort of children with HCV infection in Japan. Specifically, we aimed to clarify the epidemiology and natural history of HCV infection in Japanese children who were born over the last three decades.

Methods

Study design

This study was designed and conducted within the framework of the "Observatory for HCV Infection and Hepatitis C in Japanese Children," established in 2011 by the Hepatology Group of the Japanese Society for Pediatric Gastroenterology, Hepatology and Nutrition (JSPGHAN) with the aim of taking a census of children with HCV infection and investigating clinical aspects and outcomes of liver disease in this inadequately studied population. Sixty-five pediatric centers in Japan were involved in this survey. Over approximately 4 years, each of these centers retrospectively and prospectively collected all anti-HCV antibody (anti-HCV)-positive cases in children born from 1986 to 2015. Baseline and follow-up clinical information were obtained from patient records. Patient characteristics, clinical diagnosis at last visit, treatment, type of exposure, HCV genotype, and histopathologic features of liver biopsy specimens were determined. Features of the patients were evaluated in three groups defined by birth year: 1986–1995, 1996–2005, and 2006–2015. Some of these patients have been involved in previous studies [12–14]. The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the ethics committee of Osaka General Medical Center and other participating centers.

Patients

Inclusion criteria were age between 0 and 16 years at initial diagnosis, birth between 1986 and 2015, HCV RNA positivity in at least one serum sample, follow-up for at least 1 year after the infection was diagnosed at the observatory center, and absence of coinfection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV).

Clinical definitions were as follows. Spontaneous sustained clearance (in untreated HCV RNA-positive patients) signified disappearance of HCV RNA from at least two consecutive serum samples. Carriers were HCV RNA-positive patients with persistently normal serum alanine aminotransferase (ALT) concentrations. Chronic hepatitis was diagnosed in HCV RNA-positive patients with persistently increased ALT for more than 6 months or a liver biopsy specimen showing chronic hepatitis. Sustained virologic response (SVR) indicated HCV RNA negativity for 24 weeks following conclusion of interferon (IFN) treatment with/without ribavirin (RBV). Evidence of cirrhosis was diagnosed by liver biopsy or by clinical findings (jaundice, fatigue and/or edema), blood tests (hyperbilirubinemia, thrombocytopenia, hypoalbuminemia, and/or coagulopathy), and/or abdominal imaging including the liver using ultrasonography, computed tomography and/or magnetic resonance imaging (ascites, nodularity of the liver, and/or atrophy of the liver).

Type of HCV exposure

Putative types of HCV exposure were evaluated by concordant results of HCV genotype between mother and child and by ascertaining family history and past surgical and transfusion histories.

HCV RNA and genotype

HCV RNA was quantified in fresh or well-preserved stored sera by commercial quantitative assays such as real-time PCR (COBAS Ampliprep/COBAS TaqMan HCV test, Roche) in 90% of subjects, amplicor HCV monitor (COBAS Amplicor HCV Monitor test v 2.0, Roche) in 8% and branched DNA probe (Quantiplex HCV RNA 2.0, Bayer) in 2%. Genotype was assessed by genotyping assay using reverse transcription PCR of the core region with the genotype-specific primers in 82% of subjects and by serotyping assay in 18% according to the international classification [15, 16].

Histopathology

Histopathology of the liver was evaluated using initial liver biopsy specimens obtained from children with chronic hepatitis C (CHC) before they had received any IFN treatment with/without RBV. Liver biopsy specimens were assessed pathologically based on the New Inuyama Classification of chronic hepatitis [17], in which chronic hepatic disease is characterized according to degree of fibrosis (F) as follows: F0 (no fibrosis, equivalent to Ishak stage 0), F1 (fibrosis evident as portal expansion, equivalent to Ishak stage 1–2), F2 (bridging fibrosis, equivalent to Ishak stage 3), F3 (bridging fibrosis with lobular distortion, equivalent to Ishak stage 4), or F4 (cirrhosis, equivalent to Ishak stage 5–6) [17, 18]. Additionally, the classification assesses chronic hepatic disease activity (A) based on degree of lymphocytic infiltration and necrosis of hepatocytes as follows: A0 (no necro-inflammatory reaction), A1 (mild necro-inflammatory reaction), A2 (moderate necro-inflammatory reaction), and A3 (severe necro-inflammatory reaction) [17].

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Chi squared, Fisher's exact, ANOVA, Tukey–Kramer, and Pearson correlation tests were used as appropriate. All statistical analysis was performed using GraphPad Prism version 6.05 software (GraphPad Software, San Diego, CA, USA). Tests were two-sided. *P* values below 0.05 were considered to indicate statistical significance.

Results

During this survey, participating centers enrolled 441 consecutive anti-HCV-positive children, among whom 348 children met entry criteria. Based on birth year, they were assigned to one of three groups: group 1, including 49 children born between 1986 and 1995; group 2, including 175 born between 1996 and 2005; or group 3, including 124 born between 2006 and 2015 (Fig. 1). Ninety-three children were excluded from this study for the reasons such as unknown RNA positivity, follow-up for less than 1 year, or presence of coinfection with HIV or HBV.

Patient features

Table 1 summarizes distribution of gender, age at initial diagnosis of infection, age at last clinical visit, clinical diagnosis at last visit, and treatment in the three groups.

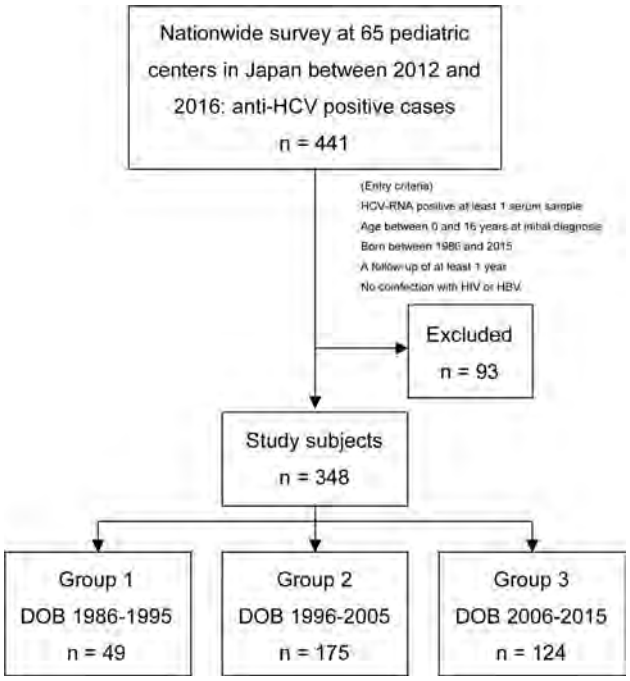

Fig. 1 Flow chart of this study. This chart summarizes entry criteria and distribution of patients into groups according to birth year. *HCV* hepatitis C virus, *anti-HCV* anti-HCV antibody, *n* number of patients, *HIV* human immunodeficiency virus, *HBV* hepatitis B virus, *DOB* date of birth

Girls accounted for 56% of patients. Age at initial diagnosis of infection had decreased significantly in recent years ($P < 0.0001$). As for clinical diagnosis at last visit, frequencies of spontaneous clearance, carrier state, chronic hepatitis, and SVR were 9, 34, 4, and 40%, respectively. Carriers had increased significantly in recent years ($P < 0.0001$), and SVR had decreased significantly ($P < 0.0001$). Cirrhosis and hepatocellular carcinoma were not identified. The overall fraction of patients who received IFN treatment with/without RBV in recent years was 54%, having decreased significantly ($P < 0.0001$).

Type of HCV exposure

Table 2 characterizes the 348 children based on putative type of exposure to HCV in the three groups. Maternal transmission, the most frequent source of infection in all groups, accounted for 90% of infections overall, with a significant increase in recent years ($P < 0.0001$), increasing to over 99% in the last decade. Transfusion was the second most frequent source of infection in the earliest decade, while no transfusion-related cases have been seen since 1994. Only 17 cases (5%) were ascribed to other putative sources of infection, horizontal transmission or unknown source.

Table 1 Demographic and clinical features of the 348 children enrolled in the study

	Total ($n = 348$)	Group 1 1986–1995 ($n = 49$)	Group 2 1996–2005 ($n = 175$)	Group 3 2006–2015 ($n = 124$)	P values[a]
Male, n (%)	154 (44)	21 (43)	79 (45)	54 (44)	0.9418
Age at diagnosis of infection, months[b,f]	37.7 ± 45.2	76.7 ± 59.6	43.0 ± 44.1	13.0 ± 16.0	<0.0001
Age at last visit, months[b,f]	130.7 ± 70.2	240.6 ± 49.6	148.9 ± 38.0	61.7 ± 28.8	<0.0001
Clinical diagnosis at last visit, n (%)					
Spontaneous clearance	30 (9)	1 (2)	13 (8)	16 (13)	0.0525
Carrier[c]	120 (34)	9 (19)	45 (26)	66 (53)	<0.0001
Chronic hepatitis	15 (4)	1 (2)	6 (3)	8 (6)	0.3134
Sustained virologic response[d]	139 (40)	33 (67)	88 (50)	18 (15)	<0.0001
During treatment	16 (5)	1 (2)	9 (5)	6 (5)	0.6488
Unknown	28 (8)	4 (8)	14 (8)	10 (8)	0.9993
Cirrhosis/HCC	0/0				
Treatment (IFN with/without RBV), n (%)[e]	188 (54)	37 (76)	118 (67)	33 (27)	<0.0001

n number of patients, *HCC* hepatocellular carcinoma, *IFN* interferon, *RBV* ribavirin

[a] Comparison among the 3 groups by Chi squared or ANOVA tests

[b] $P < 0.0001$, Group 1 vs. Group 2, Group 1 vs. Group 3, and Group 2 vs. Group 3 by Tukey–Kramer test

[c] $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[d] $P = 0.0364$, Group 1 vs. Group 2; $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[e] $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[f] Mean ± standard deviation

Table 2 Putative types of exposure to HCV infection in 348 children

	Total ($n = 348$)	Group 1 1986–1995 ($n = 49$)	Group 2 1996–2005 ($n = 175$)	Group 3 2006–2015 ($n = 124$)	P values[a]
Maternal, n (%)[b]	314 (90)	30 (61)	161 (92)	123 (99)	<0.0001
Horizontal, n (%)	2 (1)	0	2 (1)	0	0.3700
Transfusion, n (%)[c]	17 (5)	17 (35)	0	0	<0.0001
Unknown, n (%)[d]	15 (4)	2 (4)	12 (7)	1 (1)	0.0398

n number of patients

[a] Comparison among the three groups by Chi squared test

[b] $P < 0.0001$, Group 1 vs. Group 2 and Group 1 vs. Group 3; $P = 0.0054$, Group 2 vs. Group 3 by Fisher's exact test

[c] $P < 0.0001$, Group 1 vs. Group 2 and Group 1 vs. Group 3 by Fisher's exact test

[d] $P = 0.0176$, Group 2 vs. Group 3 by Fisher's exact test

HCV genotype

Table 3 characterizes 298 of the children based on the HCV genotypes in the three groups. Overall relative prevalences of genotypes 1, 2, and 3 were 42, 57, and 1%, respectively. Genotype 1 has decreased significantly in recent years ($P = 0.0427$), while genotype 2 has increased ($P = 0.0775$).

Histopathology

Table 4 summarizes the demographic and clinical features of 147 children with CHC who underwent liver biopsy between 1995 and 2015, while Table 5 presents the histopathologic features of the liver according to the New Inuyama Classification [17]. Mean age at biopsy was 8.9 ± 4.0 years. The distribution of degree of necro-

Table 3 HCV genotype in 298 children

	Total ($n = 298$)	Group 1 1986–1995 ($n = 44$)	Group 2 1996–2005 ($n = 158$)	Group 3 2006–2015 ($n = 96$)	P values[a]
Genotype 1, n (%)[b]	126 (42)	25 (57)	68 (43)	33 (34)	0.0427
Genotype 2, n (%)	169 (57)	19 (43)	89 (56)	61 (64)	0.0775
Genotype 3, n (%)	3 (1)	0	1 (1)	2 (2)	0.4095

n number of patients

[a] Comparison among the three groups by Chi squared test

[b] $P = 0.0162$, Group 1 vs. Group 3 by Fisher's exact test

Table 4 Demographic and clinical features of 147 children with chronic hepatitis C who underwent liver biopsy between 1995 and 2015

Male, n (%)	70 (48)
Age at biopsy, years[a]	8.9 ± 4.0
Duration of infection, years[a] (maternal transmission, $n = 127$)	8.4 ± 3.6
Type of exposure, n (%)	
Maternal	127 (86)
Transfusion	10 (7)
Horizontal or unknown	10 (7)
HCV genotype ($n = 131$), n (%)	
Genotype 1	63 (48)
Genotype 2	66 (50)
Genotype 3	2 (2)

n number of patients

[a] Mean ± standard deviation

Table 5 Histopathologic features of liver biopsy specimens from 147 children with chronic hepatitis C

N (%)	A0 (5)	A1 (74)	A2 (20)	A3 (1)
F0 (33)	6	34	8	0
F1 (58)	2	70	12	1
F2 (9)	0	5	9	0

n number of patients, *A0* no necro-inflammatory reaction, *A1* mild necro-inflammatory reaction, *A2* moderate necro-inflammatory reaction, *A3* severe necro-inflammatory reaction, *F0* no fibrosis, *F1* fibrosis with portal expansion, *F2* bridging fibrosis

inflammatory activity (A0, A1, A2, and A3) was 5, 74, 20, and 1%, respectively. The distribution of degree of fibrosis (F0, F1, and F2) was 33, 58, and 9%, respectively. F3 and F4 were not seen. No significant correlation was found between degree of fibrosis and age at biopsy or duration of infection (Supplementary Figs. 1 and 2). Degree of fibrosis was not related to gender, type of exposure, or genotype (Supplementary Tables 1 to 3).

Discussion

Few reports describing large nationwide cohorts of children with HCV infection are available, although recent reports concerning adults indicate that the epidemiology of HCV infection is changing dramatically worldwide [1–3, 9, 11, 12]. We investigated the epidemiologic features of Japanese children with HCV infection to clarify natural history and trends over the last three decades. Previous large nationwide cohort studies of children with HCV infection describe epidemiologic features observed about two decades before 2006 [9, 11, 12]. Our investigation represents the largest nationwide cohort study of Asian children with HCV infection over a 30-year period, including children born during the most recent decade, 2006–2015. Additionally, we included a large pediatric-age survey of HCV histopathologic features, characterizing 147 children with CHC.

Since HCV was discovered in 1989 [19, 20], the Japanese Red Cross has screened blood donors for anti-HCV with a first-generation assay beginning in 1989, or, since 1992, a second-generation assay [21]. The present study shows that because of screening, transfusion transmission has decreased dramatically, and transfusion-related cases have disappeared after 1994. Three patients had putative transfusion transmission between 1992 and 1994, most likely because risk of fibrinogen-transmitted HCV infection was yet to be eliminated in Japan during that period [22]. At present maternal transmission accounts for 99% of cases, representing nearly the sole route for pediatric-age HCV infection. Comparing group 2 (born from 1996 to 2005) with group 3 (2006–2015), ages at time of diagnosis steadily decreased. We believe that this change reflects heightened awareness of maternal transmission of HCV among Japanese obstetricians and pediatricians; nearly all pregnant women in Japan now are screened for anti-HCV.

Girls were somewhat more numerous than boys among our subjects (56%) and spontaneous clearance occurred in 9% of patients, in essential agreement with previous reports [9, 11, 23]. IFN treatment with/without RBV was given to 54% of patients. Suzuki et al. reported that pegylated IFN monotherapy and pegylated IFN combined with RBV both produced encouraging results against HCV infection and were well tolerated and reasonably safe in Japanese children and adolescents with CHC, including some enrolled in this study [13]. Interestingly, our survey identified no patients with cirrhosis. Bortolotti et al. reported that 2% of untreated children with HCV infection progressed to decompensated cirrhosis before 16 years of age [9]. We believe that none of our subjects showed cirrhosis because of racial differences, because roughly half of them received IFN therapy with/without RBV, or because of both factors.

Relative prevalence of HCV genotypes is changing worldwide. We found genotype 1 to be decreasing, as did a previous report of children with HCV infection in Italy [11]. Genotype 2 was increasing in our Japanese survey, in contrast with increases in genotypes 3 and 4 in Italy [11]. Notably, genotype 2 has become most prevalent (57%) in our pediatric survey, although a recent report concerning adults stated relative prevalences of genotypes 1 and 2 in Japan in 2011 as 65 and 34%, respectively [4]. Toyoda et al. reported that genotype 1 remains most common in adults born before 1970, although genotype 2 has become most prevalent in adults born in or after 1970. Additionally, about half of these younger infected adults had a history of intravenous drug use or tattooing (though not of blood transfusion) [24]. These results suggest that in Japan genotype 2 may have spread to young adults by drug use or tattooing and then to children by maternal transmission. Up-to-date knowledge of genotype frequencies in Japanese children will be important in considering future treatment options against HCV infection.

Histopathology examination of liver specimens from most children with CHC showed fibrosis to be absent or mild, with inflammation predominating. No cirrhosis was found. Table 6 summarizes the largest studies of liver biopsy findings in children with CHC from Europe, the US, and Japan [14, 25, 26]. Kage et al. reported that the liver showed absent or mild fibrosis in most untreated Japanese children with CHC, as well as absence of cirrhosis. However, transmission was different in that study, with transfusion accounting for 85% of cases [14]. In the present study, even though 86% of our patients who underwent liver biopsy had maternal transmission, we observed similar histopathologic features in untreated Japanese children with CHC, including absence of fibrosis in 33% of patients and absence of cirrhosis in all. In contrast, Guido et al. reported that liver histopathology showed cirrhosis in 1% of untreated children with CHC in Italy and Spain [25], while Goodman et al. found the frequency in the US to be 2% [26]. Additionally, fibrosis was absent in smaller percentages of specimens in these studies than ours (28% [25] and 14% [26] vs. 33%). Thus, Japanese children with CHC might have less risk of fibrosis and cirrhosis than chronically infected children in some Western countries. Some reports of adults with CHC have associated patient age and duration of infection with progression of fibrosis [27, 28]. In children with CHC, the present study and Goodman et al. showed no significant correlations of degree of fibrosis with age at biopsy or duration of infection, although Guido et al. found degree of fibrosis to correlate with both patient age and duration of infection [26, 29]. Additionally, Mohan et al. reported that sequential biopsy specimens demonstrated progression of fibrosis in children with CHC, aged 8.6 ± 4.1 years at the first biopsy and 14.5 ± 4.0 years at the second [30]. Accordingly, severity of fibrosis might be more closely related to age or duration of infection in adolescence and young adulthood than in childhood.

New direct-acting antiviral agents (DAAs) now are being developed at a remarkable pace. Combining DAAs targeting different stages in the viral proliferation cycle has proven highly effective, permitting development of IFN-free and largely RBV-free regimens that might be better tolerated. Such oral regimens now have shown cure rates exceeding 90% in most adult populations [31–33]. We soon should be able to treat children with HCV infection using the new DAAs [34]. The results of our study, particularly, those concerning genotype trends and histopathologic features, should be useful to pediatric hepatologists in Japan and elsewhere in considering treatment of children with HCV infection using the new DAAs.

HCV/HIV coinfection is highly prevalent in Asia [35]. Omata et al. reported that maternal transmission of HCV is affected significantly by coinfection with HIV, and safety and efficacy of recently developed DAAs and those under development in reducing maternal transmission, particularly in the presence of HIV coinfection, require further investigation [36]. In the present study, maternal transmission accounted for 99% in the last decade. We therefore should undertake curative treatment using new DAAs in young women with HCV/HIV coinfection before pregnancy in order to prevent maternal transmission.

An important limitation of this study is the retrospective nature of data from most patients, particularly those who are older. The group born from 1986 to 1995 is smaller than groups born from 1996 to 2005 or from 2006 to 2015, probably because of loss of patient record accessibility at pediatric centers following transition to adult health care. Clinical diagnosis at last visit and prevalence of treatment clearly differ between subjects born from 1986 to 2005 and

Table 6 Liver histologic findings in large studies of children with chronic hepatitis C

Author	Year	Country	Patients	Age at biopsy years, mean ± SD	Type of exposure, %		Fibrosis, %			
					Maternal	Transfusion	None	Mild	Bridging	Cirrhosis
Kage et al. [14]	1997	Japan	109	8.8 ± 4.2	11	85	96[a]		4	0
Guido et al. [25]	1998	Italy/ Spain	80	9.1 ± 4.8	60	24	28	55	16	1
Goodman et al. [26]	2008	US	121	9.8 ± 3.7	78	7	14	80	4	2
Present study	2017	Japan	147	8.9 ± 4.0	86	7	33	58	9	0

Fibrosis staging as follows: none, F0 or Ishak 0; mild, F1 or Ishak 1–2; bridging, F2-3 or Ishak 3–4; cirrhosis, F4 or Ishak 5–6

SD standard deviation

[a] Total of none and mild

those born from 2006 to 2015 because of differing length of the follow-up period.

In conclusion, we clarified the epidemiologic features and natural history of Japanese children with HCV infection over the last three decades. To our knowledge, this is the largest nationwide cohort study from Asia. Age at initial diagnosis of infection has decreased significantly. Cirrhosis and hepatocellular carcinoma did not develop. The proportion of maternal transmission significantly increased in the last decade to 99%. No transfusion-related cases have been seen since 1994. Genotype 2 has become most prevalent among Japanese children. Histopathologic examination of the liver showed fibrosis to be absent or mild in most children with CHC.

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Authors' contributions TM, TT, and HT contributed to the concept and design of the study. All authors contributed to analysis and interpretation of the data. TM and HT contributed to writing the manuscript. Thus, all authors contributed to the manuscript.

Compliance with ethical standards

Conflict of interest We have no conflict of interest.

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Short Communication

Suppression of hepatitis B surface antigen production by combination therapy with nucleotide analogues and interferon in children with genotype C hepatitis B virus infection

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Aim: Sustained suppression of hepatitis B surface antigen (HBsAg) production after interferon (IFN) treatment has not been reported for children with genotype C chronic hepatitis B virus (HBV) infection, which is prevalent in Asia. Among children with hepatitis B envelope antigen-positive genotype C chronic HBV infection, we compared the efficacy of combination therapy with nucleotide analogues and IFN-α in 11 children with 12 historical cases treated with IFN monotherapy.

Methods: The combination of lamivudine and conventional IFN-α was introduced for the first three patients; the other eight patients were treated with entecavir and pegylated IFN.

Results: Demographic factors as well as baseline HBsAg titers and HBV-DNA levels were similar between the two groups. In the combination therapy group, viral loads were suppressed in 9/11 to below 4.0 log copies/mL both at the end of the therapy (EOT) and at 6 months after EOT. In contrast, in the IFN monotherapy group, suppression of viral loads was observed in 2/12 and 3/12 at EOT and at 6 months after EOT, respectively. In the combination therapy group, HBsAg titers dropped from 4.03 at pretreatment to 2.91 log IU/mL at 6 months after EOT with 4/11 showing a drop to below 1000 IU/mL (one patient achieved HBsAg clearance). In contrast, the amount of HBsAg did not change during the corresponding periods in the IFN monotherapy group.

Conclusions: Our preliminary results suggest that combination therapy might be effective in the suppression of HBsAg production as well as HBV-DNA production for children with genotype C chronic HBV infection.

Key words: genotype C, HBeAg seroconversion, HBsAg seroconversion, interferon, nucleotide analogue

INTRODUCTION

INTERFERON (IFN) IS a standard therapy of care for children with chronic hepatitis B virus (HBV) infection.[1] However, IFN monotherapy has not been satisfactory in promoting hepatitis B surface antigen (HBsAg) clearance in children or adults in Japan.[2] Moreover, sustained suppression of HBsAg production after IFN treatment was not reported for children with chronic hepatitis B, including genotype C chronic HBV infection, which is prevalent in Asia.

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In adult patients, HBsAg loss after tenofovir plus pegylated interferon-α (PEG-IFN) therapy was recently reported and suppression of HBsAg production by combination therapy was associated with HBV genotype A.[3] Our survey of published work failed to find any reports on the efficacy of this combination therapy in children with genotype C chronic HBV infection. In this study, we investigated the efficacy of combination therapy with nucleotide analogues and IFN-α in terms of suppression of HBsAg production as well as other biochemical and virological responses, including alanine aminotransaminase (ALT) normalization, hepatitis B envelope antigen (HBeAg) seroconversion, and suppression of HBV-DNA levels.

METHODS

FROM 2010 TO 2016, 39 patients with HBeAg-positive genotype C chronic HBV infection and their guardians

visited our center. Twenty-one of the 39 patients who had a sustained elevation in ALT for more than 6 months had the therapy explained to them. Eleven of the 21 agreed to enroll in the trial therapy (combination therapy group) whereas the other 10 patients had therapy withheld. The remaining 18 had never experienced an elevation in ALT levels and were regarded as asymptomatic carriers. An elevation in ALT levels was defined as a level >60 IU/L according to Jonas *et al.*[1]

As a comparison, registered cases that had received IFN monotherapy or PEG-IFN monotherapy were searched using the medical records of children with chronic HBV infection, which were collected in a nation-wide survey.[4] We identified 82 patients with IFN monotherapy and 14 patients with PEG-IFN monotherapy. Among them, 12 patients with IFN monotherapy and four patients with PEG-IFN monotherapy met the following inclusion criteria: pretreatment HBeAg positivity, availability of laboratory data including ALT, HBsAg, HBeAg, and HBV-DNA both at baseline and at 6 months after the end of therapy (EOT), and completion of the scheduled treatment regimen as described below. On evaluation of an efficacy of combination therapy, only cases with IFN monotherapy were compared because the number of eligible cases with PEG-IFN monotherapy was too small to compare with the combination therapy group.

The effect on HBsAg production as well as circulating levels of ALT, HBeAg, and HBV-DNA were assessed prior to therapy, at EOT, and every 6 months after EOT in the 11 children with genotype C chronic HBV infection. Liver biopsy specimens were evaluated for the activity of hepatitis and the degree of fibrosis according to the classification of Desmet *et al.*[5]

Treatment regimen

Combination therapy consisted of nucleotide analogues for the first 3 months using lamivudine 3 mg/kg/day plus natural IFN-α 0.1 MU/kg body weight three times a week for 6 months in the first three patients, or entecavir 0.01 mg/kg/day plus PEG-IFN 180 μg/m^2 body surface area weekly for 6 months in the remaining eight patients. The IFN monotherapy group received natural IFN-α 0.1 MU/kg body weight three times a week for 24 weeks. The PEG-IFN monotherapy group received 180 μg/m^2 body surface area weekly for 48 weeks.

Statistical analysis

Differences in mean values and the frequency of patients' characteristics between groups were compared using the Mann–Whitney *U*-test and the Fisher's exact test, respectively. All statistical analyses were based on two-sided hypotheses tested with a significance level of $P < 0.05$.

Ethical considerations

The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the Ethics Committee of Osaka General Medical Center (Osaka, Japan).

RESULTS

Demographic data of children with HBeAg-positive genotype C chronic HBV infection

THE 11 CHILDREN who underwent the combination therapy from 2010 to 2016 consisted of seven boys and four girls with the average age of 9.2 years at treatment (Table 1). Transmission routes were mother to child in nine patients, father to child in one patient, and grandfather to child in one. Baseline factors including age at treatment, gender, transmission routes, and duration of observation were similar between the two groups. Baseline ALT values were greater in the combination therapy group than in the IFN monotherapy group, although it did not reach statistical significance. Both baseline HBsAg titers and HBV-DNA levels were in a similar range when comparing the two groups. A liver biopsy showed a mild activity of hepatitis (A1) for all patients expect one with a

Table 1 Comparison of demographic factors among children with genotype C hepatitis B virus (HBV) infection treated with interferon (IFN) monotherapy or combination therapy

	IFN monotherapy ($n = 12$)	Combination therapy ($n = 11$)	*P*-value
Age, years†	9.2 ± 4.2	9.2 ± 2.9	NS
Male sex, *n* (%)	4 (33)	7 (62)	0.22
MTCT, *n* (%)	8 (66)	9 (81)	NS
Observation, years†	4.0 ± 1.7	3.4 ± 2.1	0.45
Baseline ALT, IU/L†	155 ± 91	440 ± 375	0.06
Peak ALT, IU/L†	450 ± 605	664 ± 346	0.41
HBsAg, log IU/mL†	4.00 ± 0.30	4.23 ± 0.24	0.11
HBV-DNA, log copies/mL			
≥9	4	4	NS
8.0–8.9	4	5	
7.0–7.9	4	2	

†Mean ± standard deviation.
ALT, alanine aminotransaminase; IFN, interferon; MTCT, mother-to-child transmission; NS, not significant.

moderate degree of hepatitis (A2) (data not shown). A moderate degree of fibrosis (F2) was noted in all patients.

Natural course of children who had combination therapy withheld

Ten patients were followed for ALT, HBsAg, HBeAg, and HBV-DNA with no treatment for a median of 2.7 years. One of the 10 has had spontaneous seroconversion to HBeAb positive/HBeAg negative after 16 months of follow-up. In the remaining nine patients, HBeAg has remained positive.

Outcome of children with combination therapy or IFN monotherapy

In the combination therapy group, titers of HBeAg were rapidly decreased during the 6 months of therapy in all patients and suppressed in the negative range in eight of the 11 at EOT. Thereafter a loss of HBeAg occurred in two patients and remained positive in one patient at 6 months after EOT (Fig. 1). Hepatitis B envelope antigen seroconversion was significantly higher in the combination therapy group than in the untreated group (90.9% vs. 10.0%, $P \leq 0.001$). The seroconversion rate at 6 months after EOT was also greater in the combination therapy group than in the IFN monotherapy group ($P = 0.027$; Table 2a).

Viral loads were decreased in all patients of the combination therapy group during therapy and were suppressed in most of the patients to below 4.0 log copies/mL (LC/mL) both at EOT and at 6 months after EOT (Fig. 2a). In contrast, in the 12 patients of the IFN monotherapy group, the same degree of suppression of viral loads during the corresponding observation period was observed in only two and three patients at EOT and at 6 months after EOT, respectively (Fig. 2b). The decrease in viral loads at 6 months after EOT was more frequently seen in the combination therapy group than in the IFN monotherapy group ($P = 0.012$; Table 2a).

In the combination therapy group, HBsAg titers substantially dropped from 4.03 at pretreatment to 2.91 log IU/mL at 6 months after EOT: five of the 11 patients showed more than a 1.0-log drop in the HBsAg titers and in four of the five patients it decreased to <1000 IU/mL (Fig. 3a). Of note, one of the five patients achieved HBsAg clearance at 12 months after EOT (case 3). In contrast, the HBsAg levels did not change during the corresponding observation period in the IFN monotherapy group (Fig. 3b). The difference between the two

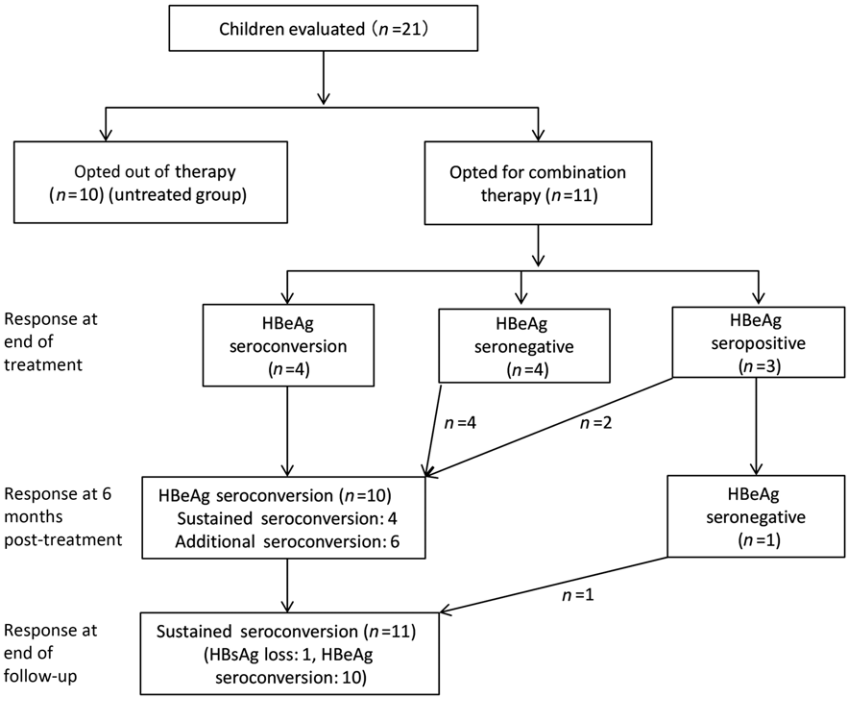

Figure 1 Flow diagram of the study of the efficacy of combination therapy with nucleotide analogues and interferon in children with genotype C hepatitis B virus infection, including summary of results. HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen.

Table 2a Comparison of efficacy between interferon (IFN) monotherapy and combination therapy groups among children with genotype C hepatitis B virus (HBV) infection

	Lamivudine plus interferon ($n = 3$)	Entecavir plus PEG-IFN ($n = 8$)	Combination therapy ($n = 11$)*	IFN monotherapy ($n = 12$)*	*P*-value*
ALT normalization	3/3	7/8	10/11	6/12	0.069
HBeAg/HBeAb seroconversion	3/3	7/8	10/11	5/12	0.027
HBV-DNA <4.0 log copy/mL	3/3	6/8	9/11	3/12	0.012
HBsAg 1.0-log drop	2/3	3/8	5/11	0/12	0.014
HBsAg <1000 IU/mL	1/3	3/8	4/11	0/12	0.037
HBsAg loss	1/3	0/8	1/11	0/12	NS

**P*-values are shown for these two groups.
ALT, alanine aminotransaminase; HBeAb, hepatitis B envelope antibody; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; NS, not significant; PEG-IFN, pegylated IFN.

Table 2b Comparison of side-effects between interferon (IFN) monotherapy and combination therapy among children with genotype C hepatitis B virus infection

	IFN monotherapy ($n = 12$)	Combination therapy ($n = 11$)	*P*-value
Leukopenia	2	1	NS
Anemia (Hb <10 g/dL)	0	0	NS
Thrombocytopenia (plt <100 000/μL)	1	1	NS
Elevated serum transaminase levels	2	1	NS
Hypothyroidism	0	0	NS
Lethargy	1	0	NS
Mental depression	0	0	NS
Hair loss	0	0	NS
Skin rash	0	0	NS

Hb, hemoglobin; NS, not significant; plt, platelets.

groups at 6 months after EOT was greater in the combination therapy group than in the IFN monotherapy group both for 1.0-log drop and for a drop below 1000 IU/mL ($P = 0.014$ and $P = 0.037$, respectively; Table 2a).

There were no differences between the first three patients treated with lamivudine plus interferon and the later eight patients with entecavir plus PEG-IFN in terms of seroconversion rate, suppression of viral loads, 1.0-log drop in HBsAg, or drop below 1000 IU/mL at 6 months after EOT (Table 2a).

Sustainability of the suppression of HBsAg production was partly shown by an 84-month follow-up in cases 2 and 3, both of which showed more than 1.0-log drop at 6 months after the end of the combination therapy (Fig. S1). Moreover, HBsAg titers decreased below 1000 IU/mL after 6 years in case 2. In the IFN monotherapy group, titers of HBsAg were available for most patients between 12 and 36 months after EOT and showed no change compared to those at 6 months after EOT (data not shown).

Outcome of children treated with PEG-IFN monotherapy

In the four patients who underwent PEG-IFN monotherapy, ALT normalization was reported in three, HBeAg seroconversion in two, and suppression of HBV-DNA in two at 6 months after EOT. The amount of HBsAg was repeatedly assessed in three of the four patients and no apparent decrement in HBsAg titers was observed in those three patients, either at EOT or 6 months after EOT.

Safety of combination therapy

A similar frequency of bone marrow suppression associated with IFN treatment was observed in the two groups; leukopenia in two and thrombocytopenia in one for the IFN monotherapy group, and one each for the combination therapy group (Table 2b). Transient elevation in serum transaminase levels was also infrequently seen in

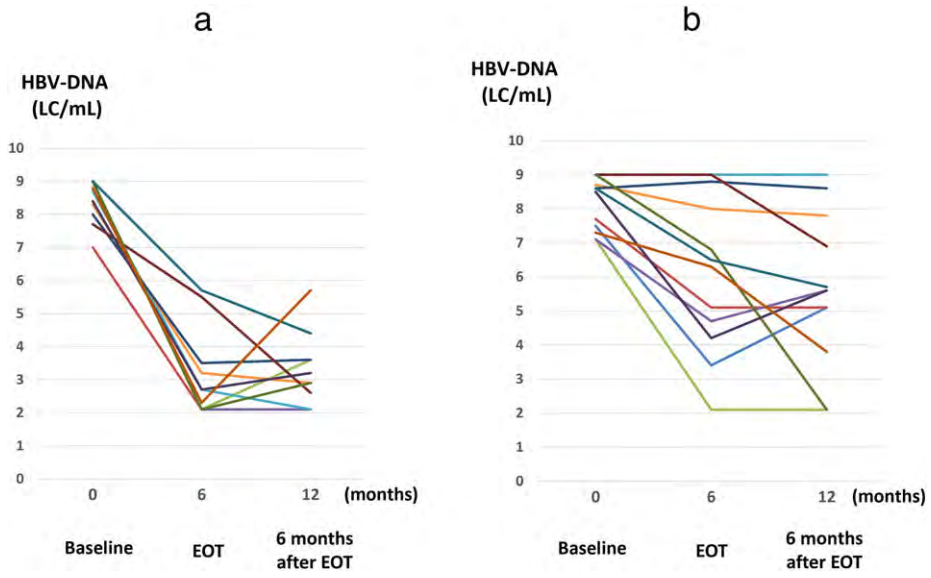

Figure 2 Hepatitis B virus (HBV)-DNA levels in two groups of children with genotype C HBV infection treated with combination therapy or interferon (IFN) monotherapy. Baseline values of each group are presented with corresponding estimations at end of treatment (EOT) and at 6 months after EOT for the combination therapy group (a) and the IFN monotherapy group (b). LC, log copies. [Color figure can be viewed at wileyonlinelibrary.com]

both groups. None of these side-effects was serious enough to warrant cessation of therapy.

DISCUSSION

IN THIS STUDY, all the 11 treated children showed a favorable response to combination therapy with IFN and nucleotide analogues. Suppression of HBeAg production occurred and serum HBV-DNA levels dropped to <4.0 LC/mL at 6 months after EOT in most patients. The mean value of HBsAg decreased from 4.03 log at baseline to 2.91 log IU/mL at 6 months among the 11 treated patients and HBsAg dropped below 1000 IU/mL in four patients. Furthermore, one of the four patients achieved

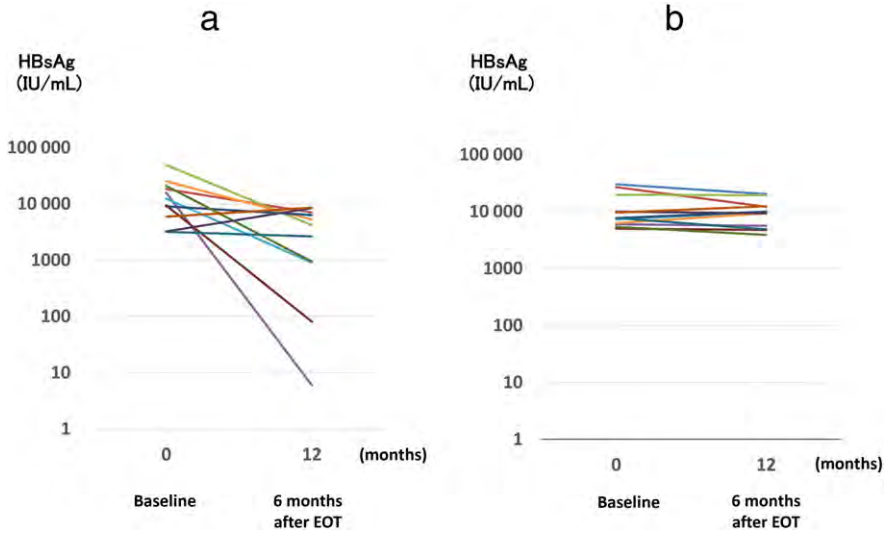

Figure 3 Hepatitis B surface antigen (HBsAg) titers (expressed as logarithms) in two groups of children with genotype C HBV infection treated with combination therapy or interferon (IFN) monotherapy. Baseline values of each group are presented with corresponding estimations at 6 months after end of treatment (EOT) for the combination therapy group (a) and the IFN monotherapy group (b). [Color figure can be viewed at wileyonlinelibrary.com]

HBsAg clearance 1 year after therapy and it was decreased below 1000 IU/mL in another patient after 6 years. The safety profile of the combination therapy group was similar to the IFN monotherapy group and no serious side-effects were observed in either group.

The first therapeutic trial in children using a similar regimen was reported by D'Antiga *et al.* in 2006.[6] They treated 23 immune-tolerant children and achieved HBeAg seroconversion in five (22%) and HBsAg loss in four (17%). All of the four patients who cleared HBsAg had genotype B HBV infection. Two of their 23 patients who had genotype C infection did not respond to the therapy. Similar combination therapy in 112 children with an ALT >1.5 times the upper limit of normal resulted in a higher response (55% vs. 27%) and more HBsAg loss (12.5% vs. 4.6%) when compared with 52 children who underwent nucleotide analogue lead-in combination therapy.[7] Twenty-eight children in an immune-tolerant phase were treated with combination therapy as reported by D'Antiga et al.[8] Eleven of the 28 become seronegative for HBeAg and five of the 11 had HBsAg clearance, but the genotype of the subjects was not examined in the latter two studies. Furthermore, these studies into the efficacy of combination therapy did not quantitatively assess the change in HBsAg production.

There have been no studies on the efficacy of combination therapy in children with genotype C chronic HBV infection. Therefore, it is unknown whether genotype C-infected children would respond to combination therapy with comparable efficacy as has been seen with genotype B in children.[6] A 20-year observation of the natural course of infection in children has shown that those with initial titers of HBsAg <1000 IU/mL were more likely to clear HBsAg than those with higher titers.[9] Accordingly, treatment-related suppression of HBsAg production <1000 IU/mL might lead to clearance of HBsAg in the near future. In this study, four of the 11 patients have achieved a suppression of HBsAg production <1000 IU/mL after the combination therapy. However, long-term observation is required to determine whether clearance of HBsAg might occur in the combination therapy group, as seen in children who showed low baseline levels of HBsAg and eventually cleared HBsAg.[9]

Our preliminary results suggest that combination therapy could be effective in suppression of HBsAg production as well as in suppression of both HBeAg and HBV-DNA production for children with chronic genotype C HBV infection. Prospective studies are needed to evaluate the efficacy of combination therapy and to clarify predictive factors of its efficacy in children with genotype C chronic HBV infection.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found online in the Supporting Information section at the end of the article.

Figure S1 Changes in hepatitis B surface antigen titers over 7 years for 11 children with genotype C hepatitis B virus infection treated with combination therapy.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Hepatitis B vaccine: Immunogenicity in an extremely low-birthweight infant

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Key words extremely low-birthweight infant, hepatitis B vaccine, hepatitis B virus, immunogenicity, mother-to-child infection.

From 2013, infants born to mothers carrying serum hepatitis B (HB) surface antigen (HBsAg) receive HB immunoglobulin at birth and HB vaccine at birth, and at 1 and 6 months of age in Japan (prevention protocol for mother-to-child HB virus infection).[1] Due to immature immune response to HB vaccine, the American Academy of Pediatrics and Japan Pediatric Society recommend that infants <2,000 g birthweight are given an additional HB vaccination at 2 months of age.[2,3] No previous case report, however, has described the trajectory of the immunogenic response for this prevention protocol, including an additional dose at 2 months of age, in extremely low-birthweight (ELBW) infants. The present case is reported with informed consent.

The present patient was born to a 29-year-old Chinese mother (gravida 0, para 0) with HBsAg. At 20 weeks of gestational age, serum HBsAg, HB envelope antigen, HB virus core-related antigen, and HB virus DNA were positive (67 878 IU/mL, 1,531.9 sample relative light units/cut-off, >7.0 log U/mL, and 9.7 log copies/mL, respectively). Both serum HB surface antibody (HBsAb) and HB envelope antibody were negative. The HB virus genotype was type C. A male newborn weighing 918 g was born at 25 weeks and 4 days of gestational age via cesarean section due to fetal distress.

He was admitted to the neonatal intensive care unit due to ELBW. Along with respiratory and circulatory treatment, i.v. immunoglobulin (IVIG; 500 mg/10 mL, Venoglobulin IH™, Japan Blood Products Organization, Tokyo, Japan) was administered soon after birth because of hypoimmune globulinemia (serum total IgG, 280 mg/dL). At 11 h after birth, a total of 200 U/mL HB immune globulin (Dried HB globulin Nichiyaku™; Nihon Pharmaceutical, Tokyo, Japan) was injected i.m. in the right and left femoral muscles (100 U/0.5 mL in each side), and HB vaccine (0.25 mL, Bimmugen™; Kaketsuken, Kumamoto, Japan) was injected s.c. in the left upper arm. No side-effects, such as redness, swelling, or induration were observed. HB vaccine was again administered at 1 and at 2 months of age. The infant was reared on breast milk and was discharged at 4 months of age. The fourth HB vaccine was injected at 6 months of age.

The HBsAb titer reached a peak at 1 month of age, and decreased to the lowest level at 4 months of age, but HBsAb was >10 mIU/mL (Fig. 1). Then, the HBsAb titer gradually increased, and after the fourth HB vaccine, it finally increased to >100 mIU/mL at 12 months of age. Serum HBsAg was negative at 12 months of age.

We herein report the HBsAb titer in an ELBW infant who received four doses of HB vaccine. In the present case, the prevention protocol for mother-to-child HB virus infection with an additional dose at 2 months of age (0, 1, 2, and 6 months of age) achieved sufficient seropositivity of HBsAb at 12 months of age. The infant had an HBsAb titer of 47 mIU/mL at the time of discharge, even with an additional vaccine at 2 months of age. Because ELBW infants are usually discharged from hospital at 3–4 months of age, and are

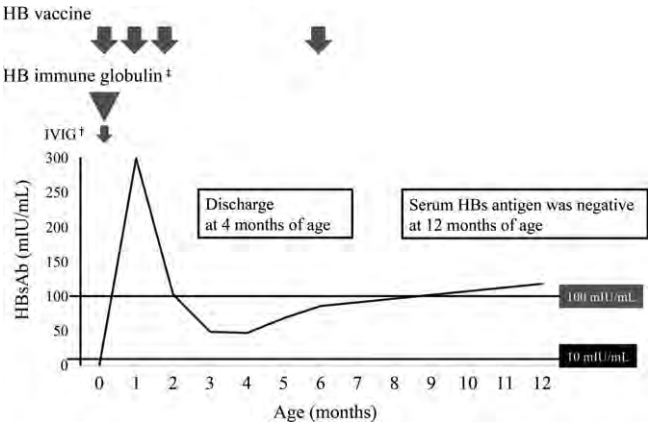

Fig. 1 Trajectory of serum hepatitis B surface antibody (HBsAb) titer. †Effect of i.v. immunoglobulin (IVIG) on HBsAb titer: the patient received 500 mg/10 mL Venoglobulin IH™ (Japan Blood Products Organization), which has an HBsAb titer of approximately 100 mIU/mL. Assuming that the circulating blood volume is 72 mL (80 mL/kg bodyweight) and the bioavailability of IVIG is 100%, IVIG treatment might have increased HBsAb titer by 14 mIU/mL. Given, however, that the half-life of Ig is 27 days,[4] the effect is limited. ‡Effect of HB immune globulin on HBsAb titer: the titer at 4 months of age (47 mIU/mL) can be explained only by the HB immune globulin at birth because the half-life of HB immune globulin is 23 days.[5]

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then in close contact with their mother who are HB virus carriers, it is important for the ELBW infant to have a sufficient HBsAb titer at that time.

The seroprotection level is usually defined as HBsAb titer ≥10 mIU/mL.[6,7] Although all infants ≥2,000 g birthweight who received three doses of HB vaccine at 0, 1, and 6 months of age at the present hospital had sufficient HBsAb (median, 210 mIU/mL; range, 21–898 mIU/mL; n = 12), in a previous study, ELBW infants who received three doses of HB vaccinations at birth and at 1–3 and at 6–8 months of age had only a 52% seropositivity rate.[6] And in another study, 98.4% of preterm infants vaccinated using another four-dose HB vaccine protocol (0, 1, 2, and 12 months of age) had a protective level.[7] Four doses of HB vaccine may be needed to obtain a sufficient rate of seropositivity in ELBW infants as recommended by the Japan Pediatric Society.

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Disclosure

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Author contributions

K.Y. and I.M. drafted the initial manuscript. K.Y. and S.I. collected the clinical data. K.Y., I.M. and K.F. interpreted the data. K.I. revised the article critically for important intellectual content. All authors contributed to the intellectual content of this manuscript and approved the final manuscript as submitted.

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厚生労働行政推進調査事業費（肝炎等克服緊急対策研究事業）

肝炎ウイルスの新たな感染防止
－残された課題・今後の対策－

平成 30 年度 総括・分担研究報告書

発行：平成 31(2019) 年 3 月

研究代表者 四柳　宏

東京大学医科学研究所先端医療研究センター 感染症分野

東京大学医科学研究所倫理審査委員会
審査結果通知書

平成３０年１２月２７日

申請者
感染症分野
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審査番号：　３０－６１
承認番号：　３０－６１－Ｂ１２２７
研究課題：　医療従事者へのB型肝炎ワクチン接種状況に関するアンケート調査
申請日：　平成３０年１２月２７日
審査委員会名：　倫理審査委員会第二委員会

上記研究計画について、平成３０年１２月２０日開催の本委員会における指摘事項の修正を確認し、下記のとおり決定しましたので、ここに通知します。

記

判定	
[承認]	~~変更の勧告~~
~~条件付き承認~~	~~否承認~~
□修正を要する	~~非該当~~
□修正不要	
理由・コメント	

整理番号	CRB-18-03-002
区分	□特定臨床研究■非特定臨床研究
	□医薬品□医療機器□再生医療等

2018年11月7日

臨床研究実施許可通知書

小児科・新生児科
髙野　智子　　様

2018年11月7日付け審査結果通知書にて承認された臨床研究について、実施を許可致します。

記

臨床研究課題名	保育の場における肝炎ウイルス感染予防の理解及び実践を図るための保育施設勤務者に対するアンケート調査

以上

大阪急性期・総合医療センター
総長

別記様式４

臨床研究倫理審査結果通知書

平成３０年１２月２８日

申請者（実施責任者）
岩淵　敦　殿

筑波大学附属病院長　　原　晃

平成３０年９月１３日付けで倫理審査申請のありました臨床研究の実施について、審査の結果、下記のとおり判定しましたので通知します。

記

１　臨床研究題目（H30-220）
「Ｂ型肝炎ワクチン定期接種化後の本邦小児におけるＢ型肝炎ウイルス感染およびワクチン接種の実態調査」

２　判　定
■　承　認
□　条件付承認
□　変更の勧告
□　不承認
□　非該当

３　理由等（判定が承認以外の場合）

研究期間　2018 年 12 月 28 日～2022 年 3 月 31 日
（ただし、臨床研究保険に加入する場合の研究開始日は、臨床研究保険補償開始日とする。）

整理番号

RK-190130-1

書式６　Ver. 1610

西暦２０１９年２月１３日

臨床研究　審査結果通知書

日本大学医学部附属板橋病院　病院長殿

日本大学医学部附属板橋病院
臨床研究倫理審査委員会
東京都板橋区大谷口上町３０番１号
委員長　武井　正美　　印

審査依頼のあった件について審査結果を下記のとおり報告いたします。

記

研究課題名	Ｂ型肝炎ワクチン定期接種化後の本邦小児におけるＢ型肝炎ウイルス感染及びワクチン接種の実態調査
審査事項 （審査資料）	■研究の実施の適否 ■臨床研究　申請書（西暦 2019年1月11日付） □臨床研究実施医療機関の概要書（西暦　年　月　日作成） □研究の継続の適否 □臨床研究　実施状況報告書（西暦　年　月　日付） □臨床研究　変更申請書（西暦　年　月　日付） □臨床研究における重篤な有害事象に関する報告書 （西暦　年　月　日付） □その他（　　）
研究期間	承認日　～　2022年3月31日
審査区分	■委員会審査（審　査　日：　2019年　2月 12日） □迅速審査　（審査終了日：　年　月　日）
審査結果	□承認　■条件付承認　□却下　□既承認事項の取り消し　□保留
指摘事項および理由・条件等	別紙(1902-07)のとおり
備考	別紙＜注意事項＞のとおり

西暦２０１９年 3 月 / 日

申請者(研究責任者)

小児・新生児病科

新生児病科外来医長　岡橋　彩　殿

申請のあった研究に関する審査事項について上記のとおり決定しましたので通知いたします。

日本大学医学部附属板橋病院　病院長　徳橋　泰明

2019 年 3 月 / 日 条件が満たされたことを確認しました。

日本大学医学部附属板橋病院　病院長

神小医第62号
平成31年3月25日

神戸大学大学院医学研究科内科系講座
小児科学分野こども急性疾患学部門
野津寛大　様

神戸こども初期急病セ
センター長　石田

神戸こども初期急病センター倫理委員会審査結果について(通知)

平成31年1月21日付けで倫理審査申請のありました「B型肝炎ワクチン定期接種化後の本邦小児におけるB型肝炎ウイルス感染およびワクチン接種の実態調査」について、倫理委員会委員長より、承認する旨の答申がありましたので通知いたします。

記

1. 答申日　平成31年3月25日

2. 参考資料　・答申書(写)

3. その他　当該研究に係る研究計画と経過、更に結果(成果)について継続的にセンターに報告し、寄附講座ホームページに掲載する等、広報に留意ください。

以上

様式２

国立感染症研究所ヒトを対象とする医学研究倫理審査結果通知書

平成３０年９月２５日

相崎　英樹　殿

国立感染症研究所長

受付番号：９２７

研究課題名：HIV感染同性愛者における急性Ａ型、Ｃ型肝炎の解析

研究者名：相崎　英樹・井戸田　一朗・三田　英治・遠藤　知之・四柳　宏・鈴木　亮介・清原　知子・杉山　隆一・村松　正道

研究期間：２０１８年承認日～２０２２年３月末日

上記課題名の研究計画・公表予定は、国立感染症研究所ヒトを対象とする医学研究倫理審査委員会において審議され、下記のとおり判定したので通知します。

記

判定	非該当	承認	条件付承認
	変更の勧告	不承認	
勧告あるいは条件・理由			