DISCUSSION

IN JAPANESE PATIENTS, virological response to triple therapy with telaprevir, PEG IFN and RBV was excellent. We have previously reported that in 20 patients with chronic HCV-1b infection with high viral load who received triple therapy for 12 weeks, HCV RNA became undetectable in 50% at 2 weeks, 79% at 4 weeks, 88% at 6 weeks, 94% at 8 weeks and 100% at 12 weeks.[26] This previous study was a randomized open-label study in which telaprevir was administrated at doses of 2250 or 1500 mg/day. Early virological response at 7 and 14 days was similar for both telaprevir doses, suggesting that virological response to triple therapy is not affected by lowering the telaprevir dose. Therefore, to expand the dataset, we retrospectively evaluated HCV RNA response and safety during 12 weeks of triple therapy including the two different telaprevir doses followed by PEG IFN and RBV for an additional 12 weeks: we analyzed 204 cases in total. However, because of the non-random nature of treatment allocation, there was a preponderance of women, elderly and anemic patients in the group receiving telaprevir 1500 mg/day. Because there were many differences in baseline characteristics between telaprevir 2250 and 1500 mg/day groups, we selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment. Therefore, there were no differences in baseline characteristics between both groups in this analysis, except for *IL28B* genotype. Although we tried to match the distribution of *IL28B* genotypes between both groups, this was not possible because of the small number of cases. Therefore, we matched the groups by the history of previous IFN-based treatment, which we considered a similarly strong predictive factor of triple therapy. Moreover, there was a significant difference in the initial dose of RBV between both groups. A significant number of patients underwent RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group because we considered that such patients were likely to experience hemoglobin decrements during triple therapy, but before November 2011, we could not reduce the initial dose of telaprevir and RBV. Nine patients (15.0%) receiving telaprevir 2250 mg/day and 32 cases (53.3%) receiving 1500 mg/day underwent RBV dose reduction at the beginning of treatment. In other words, the group receiving telaprevir 1500 mg/day had a significantly lower initial dose of telaprevir and RBV dose than did the group receiving 2250 mg/day (Table 2).

However, in the present study, HCV RNA became undetectable during the 12 weeks of treatment at similar or higher rates in the telaprevir 1500 mg/day group than in the 2250 mg/day group (Fig. 1). In the *IL28B* TT genotype, the early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group. Although we assessed baseline factors, drug adherence and drug discontinuation rates only in the *IL28B* TT genotype, there were no significant differences between both groups, except for lower telaprevir adherence up to 12 weeks and a greater number of cases of PEG IFN and RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group. Therefore, the reason for significant differences in the early virological response between both groups is unclear. However, we considered that these results did not affect the SVR rate because HCV RNA became undetectable in all patients in both groups at 8 weeks after the start of triple therapy. In all cases, *IL28B* TT cases and non-TT cases, there were no significant differences in SVR rates after triple therapy between those receiving telaprevir 2250 and 1500 mg/day (Figs 3,4). By examining the detailed course of drug administration from 12–24 weeks (Table 2), we found that the group receiving telaprevir 1500 mg/day had a lower discontinuation rate of telaprevir and higher adherence to RBV and PEG IFN up to 24 weeks in spite of the low initial RBV dose. Furthermore, hemoglobin levels showed greater reductions during triple therapy with telaprevir 2250 mg/day than with telaprevir 1500 mg/day, and the group receiving telaprevir 2250 mg/day had a significantly higher discontinuation rate of telaprevir due to anemia than did the group receiving telaprevir 1500 mg/day (Fig. 2). Therefore, telaprevir 1500 mg/day may be a safe option as part of triple therapy, while maintaining PEG IFN and RBV adherence.

Viral breakthrough or relapse can occur during telaprevir monotherapy or telaprevir plus PEG IFN dual therapy (without RBV) because of the development of mutations that confer resistance to telaprevir.[14,27–29] Furthermore, in a Japanese phase III trial of triple therapy in relapsers and non-responders who had not achieved SVR to a previously administrated IFN-based regimen, SVR rates increased as RBV adherence increased, particularly in previous non-responders.[19] In triple therapy with telaprevir, PEG IFN and RBV, we consider that telaprevir could be important for early virological response, but it could also be important for maintaining high adherence to PEG IFN and RBV, which is a key factor for achieving SVR. We speculate that triple therapy including telaprevir at the reduced dose of 1500 mg/day could maintain high levels of adherence

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to PEG IFN and RBV, and consequently achieve high SVR rates.

In this study, we investigated the independent predictors for SVR in the multivariate analysis (Table 3). As reported in previous studies, *IL28B* genotype remained the strongest predictor of SVR.[30,31] The next strongest predictive factor was sex: women had significantly lower SVR rates than did men (Fig. 3). However, when we investigated the SVR rates of the telaprevir 2250 mg/day group and 1500 mg/day group, we found that there were significant differences in SVR rates between men and women in the telaprevir 2250 mg/day group but no differences in the telaprevir 1500 mg/day group. In the previous study, we reported that female sex was one of the factors influencing decreases in hemoglobin levels during triple therapy administrated 2250 mg/day of initial telaprevir dose.[20] In the present study, the discontinuation rates of telaprevir due to anemia were significantly higher in women in the telaprevir 2250 mg/day group as compared with men (36.7% vs 3.3%, $P = 0.002$, data not shown), but there were no differences in the discontinuation rates of telaprevir due to anemia between men and women in the telaprevir 1500 mg/day group (0% vs 10%, $P = 0.237$, data not shown). Therefore, we speculate that there were significant differences in SVR rates between men and women because of high telaprevir discontinuation rates owing to anemia in women.

In conclusion, after the completion of 24 weeks of therapy, triple therapy including telaprevir at a reduced dose of 1500 mg/day was as effective as triple therapy including telaprevir 2250 mg/day at suppressing HCV RNA to undetectable levels and achieving SVR. Of note, we found that telaprevir 1500 mg/day was associated with lower levels of anemia and discontinuation of telaprevir owing to anemia, and higher PEG IFN and RBV adherence during triple therapy. These results suggest that the telaprevir 1500 mg/day regimen is an effective and safe alternative for the treatment of elderly and female Japanese patients. This study is a retrospective study. Prospective randomized controlled studies with longer follow-up periods are required to fully assess the efficacy and safety of an initial telaprevir dose of 1500 mg/day.

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VIRAL HEPATITIS

HLA-DP genes polymorphisms associate with hepatitis B surface antigen kinetics and seroclearance during nucleot(s)ide analogue therapy

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Keywords
hepatitis B surface antigen – *HLA-DP* – lamivudine – nucleot(s)ide analogue

Abbreviations
ADV, adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; GWAS, genome-wide association study; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBV, hepatitis B virus; HLA, human leucocyte antigen; HR, hazard ratio; IFN, interferon; LAM, lamivudine; NA, nucleos(t)ide analogues; SNP, single nucleotide polymorphism; VBT, virological breakthrough; VR, virological response.

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Abstract

Background & Aims: Genome-wide association studies (GWAS) recently indicated that polymorphisms in the human leucocyte antigen *(HLA)-DP* genes were associated with risk of persistent hepatitis B virus (HBV) infection and clearance of HBV, but the effect of *HLA-DP* gene polymorphisms on the effect of antiviral therapy was unknown. We here investigated whether such polymorphisms were associated with decreases in HBsAg levels and seroclearance in patients who received long-term lamivudine (LAM) treatment. *Methods:* Japanese patients (202) who were hepatitis B e antigen positive at baseline, received LAM as first-line treatment, and consented to *HLA-DP* genotyping (*HLA-DPA1* rs3077 and HLA-DPB1 rs9277535) were categorized into two cohorts, viz., a cohort who achieved virological response without rescue therapy (cohort 1) and those who did so with rescue therapy (cohort 2). *Results:* Serum HBsAg levels declined significantly between year 3 and 9 from baseline among cohort 1 patients possessing ≥2 A-alleles at rs3077 and rs9277535. The percentages of such patients in cohort 1 patients with decreases in HBsAg ≥0.5 log IU/ml were higher than those with <2 A-alleles (71.8% [28/39] vs. 38.9% [23/59]; $P = 0.004$). However, there was no significant difference in cumulative HBsAg seroclearance rates between patients with ≥2 and those with <2 A-alleles in cohort 1. In cohort 2, HBsAg seroclearance rates were higher in patients with ≥2 A-alleles than in those with <2 A-alleles ($P = 0.003$). *Conclusion:* We found an association between *HLA-DP* polymorphisms and decreases in HBsAg levels and seroclearance among HBeAg-positive patients treated with LAM.

More than 2 billion people worldwide have been exposed to hepatitis B virus (HBV) and about 350 million people are chronically infected, the majority of whom are in Asia (75%). The prevalence of HBV in Japan is 0.8%, which is lower than that in other Asian countries, such as Taiwan (>10%) and China (1–3). Recently, oral nucleot(s)ide analogues (NAs) have been used as a mainstay therapeutic strategy against chronic hepatitis B. Such antiviral agents, including lamivudine (LAM), entecavir (ETV), telbivudine, adefovir dipivoxil (ADV) and tenofovir disoproxil fumarate, inhibit viral replication. These NAs vary in both the strength and the rapidity with which they suppress HBV DNA (4–10). Sustained viral suppression by NA therapy can improve liver fibrosis and clinical outcomes of patients (11, 12). LAM was the first NA to be approved for treating chronic hepatitis B in Japan, followed by ADV and ETV.

Responses to antiviral treatments can be evaluated by monitoring serum HBV DNA levels, and hepatitis B e (HBeAg) and hepatitis B surface antigen (HBsAg) and antibody levels. Serum HBsAg levels have been recognized as one of the predictive markers of the prognosis and effect of antiviral therapy. Some studies recently reported the rates of HBsAg seroclearance and HBsAg

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kinetics after pegylated interferon (PegIFN) and/or NAs therapy (13–15). These studies demonstrated that HBsAg seroclearance is associated with HBV genotype, baseline HBsAg levels, and the decrease in HBsAg early during treatment. However, it remains unclear whether host factors are associated with treatment-related HBsAg seroclearance.

Genome-wide association studies (GWAS) have been well applied in the field of viral hepatitis, and several studies have reported that the human leucocyte antigen (*HLA*)-*DP* locus, located on chromosome 6, is associated with the risk of persistent infection with HBV (16). A few studies have reported that the *HLA-DP* locus is also associated with HBV clearance (17–20). Two single nucleotide polymorphisms (SNPs) in a region including *HLA-DPA1* and *HLA-DPB1* are strongly associated with persistent HBV infection (*HLA-DPA1* rs3077 and *HLA-DPB1* rs9277535). The minor alleles (A-alleles) of both rs3077 and rs9277535 seem to have protective effects against chronic hepatitis B (16). Although there have been two reports on the association between the *HLA-DP* locus and antiviral therapy for chronic hepatitis B, further investigation of association of *HLA-DP* SNPs with the effect of antiviral therapy is warranted (21, 22).

We therefore hypothesized that the minor alleles of *HLA-DPA1* rs3077 and *HLA-DPB1* rs9277535 may have an impact on HBsAg kinetics and seroclearance during NA therapy.

In this study, we investigated whether polymorphisms in *HLA-DP* genes are associated with reduction in HBsAg titres and seroclearance in chronic HBeAg-positive hepatitis B patients who received long-term LAM treatment and subsequently achieved favourable sustained viral responses.

Patients & methods

Study population

Over a period of 12 years (September 1995 to September 2007), 949 consecutive patients, chronically mono-infected with HBV (confirmed HBsAg positivity over a period of at least 6 months), were treated with LAM monotherapy at the Department of Hepatology, Toranomon Hospital, Metropolitan Tokyo. The indication for antiviral therapy was abnormal ALT levels accompanying the increase in HBV DNA levels (over 4 log copies/ml). However, in cases where ALT levels were normal, patients with advanced fibrosis were also administrated LAM. We selected 791 patients as study subjects after we excluded all those who had been treated with LAM for <6 months, were co-infected with hepatitis C virus, had not provided sufficient serum samples, and/or had insufficient clinical records. No patient was co-infected with human immunodeficiency virus in this cohort. Of these 791 patients, 441 were HBeAg positive and 350 were HBeAg negative at baseline.

HLA-DP SNPs were analysed in 253 of 441 patients who are HBeAg positive. Ninety eight of 253 patients achieved viral response (VR: HBV DNA <600 copies/ml) and subsequently maintained low viral load (HBV DNA <1 log copies/ml from nadir; cohort 1). Over time, 136 of these 253 individuals experienced an increase in HBV DNA (≥1 log copies/ml; e.g. because of virological breakthrough [VBT]), and as a result, 133 (98%) individuals were provided with ADV treatment (10 mg) added to LAM, as a rescue therapy. Of the 133, 104 patients achieved VR with rescue therapy and subsequently maintained a low viral load (HBV DNA <1 log copies/ml from nadir; cohort 2). Thus, in total, 202 patients were enrolled in this retrospective cohort study (Fig. 1). All of these patients are Japanese. Written informed consent was obtained from each patient. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved *a priori* by the institution's human research committee.

Clinical data collection and follow-ups

Data on patient characteristics, biochemistry, haematology, virology, histology and previous treatments were collected and registered in our institute's database at the time of patient enrolment. Prior to beginning LAM therapy, the presence of family history of HBV infection was surveyed in all patients. Data on the treatment dose and duration of previous IFN therapy were collected from our hospital's IFN therapy database or requested from other hospitals as necessary.

At least every 1–3 months, liver function and virological markers of HBV infection were measured in all patients. All serum HBsAg titres were measured from frozen serum samples collected at 6 months, 1, 3, 5 years, and once annually for 6–10 years, and then stored at −80°C. The time-point of HBsAg clearance was defined by the measurement in consecutive available serum samples before HBsAg undetected. A genotypic analysis of drug resistance was performed in cases of insufficient virological response or VBT, defined as an increase in serum HBV DNA levels ≥1 log above the nadir measured after the initial virological response. Cirrhosis was diagnosed by laparoscopy, liver biopsy, or clinical data, such as imaging modalities and portal hypertension. The primary outcome for this study was seroclearance and significant reduction in HBsAg. The endpoint of the follow-up was HBsAg clearance or last visit before March 2013.

Markers of HBV infection

Serum HBsAg titres were measured using ARCHITECT HBsAg QT assay kits (Abbott Laboratories, Tokyo, Japan), which have a lower limit of detection of 0.05 IU/ml and an upper limit of detection of 250 IU/ml. To expand the upper range from 250 to 125 000 IU/ml, serum samples exceeding the scale were

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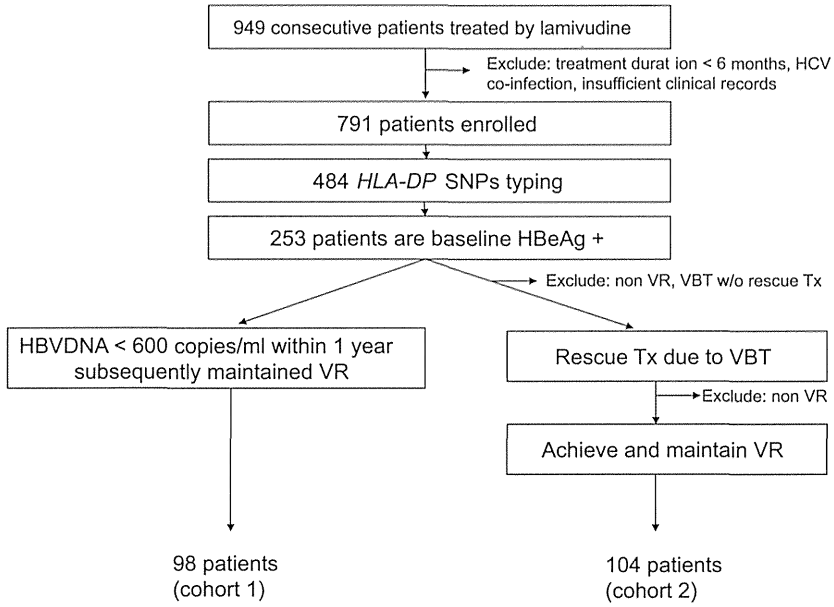

Fig. 1. Schematic of study protocol. HBeAg, hepatitis B e antigen; HCV, hepatitis C virus; HLA, human leucocyte antigen; SNP, single nucleotide polymorphism; VBT, virological breakthrough; VR, virological response.

diluted stepwise to 1:20 and 1:500 with ARCHITECT diluents as the product document described. HBeAg was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). HBV DNA was quantified using the Amplicor monitor assay (Roche Diagnostics, Tokyo, Japan), which has a dynamic range over 2.6–7.6 log copies/ml, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics), which has a dynamic range over 2.1–9.0 log copies/ml. A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to determine HBV genotypes serologically by using the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the seven major genotypes (A–G). YMDD mutants (rt M204I/V) were determined by polymerase chain reaction-based enzyme-linked minisequence assay with a commercial kit (Genome Science Laboratories, Tokyo, Japan).

HLA-DP SNPs typing

SNPs in *HLA-DPA1* (rs3077) and *HLA-DPB1* (rs9277535), located on chromosome 6, were genotyped by TaqMan assay or Invader assay, as previously described (16). *IL28B* genotype was assayed for the rs8099917 SNP using TaqMan assay or Invader assay.

Statistical analyses

Categorical data were compared between groups using chi-square or Fisher's exact tests. Continuous variables with a non-parametric distribution were analysed with Mann–Whitney *U*-tests, while those with a parametric distribution were analysed with Student's *t*-tests. Cox regression analyses were used to assess which variables were significantly associated with HBsAg clearance. All baseline factors that were found to be significantly associated with HBsAg clearance by univariate analysis were entered into a multivariate analysis. Independent baseline factors associated with clearance of HBsAg were calculated using a stepwise Cox regression analysis. Cumulative HBsAg clearance rates were analysed using the Kaplan–Meier method; differences in the resulting curves were tested using log-rank tests. Cochran–Armitage trend tests were performed for the association between HBsAg seroclearance and an increase in the number of A-alleles. Significance was defined as $P < 0.05$ for all two-tailed tests. Data analysis was performed with IBM SPSS version 19.0 software (IBM Corp., Armonk, NY, USA) and R software version 2.13 (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

Results

Patient characteristics and clinical course

Eighteen of 202 patients successfully cleared HBsAg. Of these, 11 belonged to cohort 1, and seven to cohort 2. Table 1 provides a comparison of the baseline characteristics between patients who were and were not able to successfully clear HBsAg (all patients, cohort 1 and cohort 2).

In cohort 1, baseline characteristics that were significantly associated with HBsAg clearance included HBV genotype and high HBV DNA levels; in cohort 2, such

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Table 1. Baseline and demographic characteristics of patients with and without HBsAg seroclearance

Characteristics	All patients (n = 202)	LAM VR cohort (without rescue Tx) (cohort 1)			LAM add-on rescue Tx cohort (cohort 2)		
		Persistently HBsAg positive (n = 87)	HBsAg seroclearance (n = 11)	P	Persistently HBsAg positive (n = 97)	HBsAg seroclearance (n = 7)	P
Baseline							
*Age (y) (SD)	43 (11.5)	41 (11.9)	45 (12.6)	0.553	43 (11.3)	44 (6.3)	0.590
Gender (male:female)	151:51	60:27	9:2	0.499	75:22	7:0	0.341
Family history of HBV infection	139 (69)	57 (66)	6 (60)	0.737	71 (73)	4 (57)	0.395
Previous IFN therapy	100 (49)	44 (51)	9 (82)	0.060	42 (43)	5 (71)	0.240
Pre-existing cirrhosis	42 (21)	16 (18)	1 (9)	0.685	23 (24)	2 (29)	0.676
HBV genotype				**0.016**			**1.65×10^{-6}**
A	8 (4.0)	3 (3.4)	3 (27)		0 (0)	2 (28.6)	
B	9 (4.5)	6 (6.9)	0 (0)		2 (2.1)	1 (14.3)	
C	180 (89.1)	76 (87.4)	8 (73)		92 (94.8)	4 (57.1)	
D	1 (0.5)	0 (0)	0 (0)		1 (1.0)	0 (0)	
Unclassified/missing	4 (1.9)	2 (2.3)	0 (0)		2 (2.1)	0 (0)	
Baseline HBV DNA (log copies/ml)	7.6 (6.7–8.1)	7.4 (6.4–8.0)	8.0 (7.6–8.6)	**0.036**	7.7 (6.7–8.1)	7.9 (7.2–8.0)	0.693
Baseline HBsAg level (IU/ml)	3070 (1290–10 800)	2350 (1040–6650)	5660 (773–52 500)	0.168	3370 (1720–12 100)	2300 (946–66 600)	0.948
Baseline AST level (IU/L)	86 (60–174)	105 (60–244)	229 (64–1170)	0.109	77 (60–130)	88 (49–218)	0.829
Baseline ALT level (IU/L)	149 (80–337)	173 (94–441)	480 (79–1024)	0.132	125 (71–226)	106 (96–152)	0.953
Baseline total bilirubin level (mg/dl)	0.8 (0.6–1.1)	0.8 (0.6–1.3)	0.8 (0.6–7.4)	0.409	0.8 (0.5–1.1)	0.8 (0.6–1.1)	0.799
*Platelet count (10^5/mm^3) (SD)	16.1 (5.6)	16.9 (6.4)	14.2 (3.1)	0.201	15.6 (5.3)	13.3 (2.9)	0.252
HLA-DPA1 (rs3077)				0.949			**0.001**
GG	125 (61.9)	51 (58.6)	6 (54.6)		67 (69.1)	1 (14.3)	
GA	65 (32.2)	30 (34.5)	4 (36.3)		27 (27.8)	4 (57.1)	
AA	12 (5.9)	6 (6.9)	1 (9.1)		3 (3.1)	2 (28.6)	
HLA-DPB1 (rs9277535)				0.288			**0.039**
GG	117 (57.9)	50 (57.5)	5 (45.4)		61 (62.9)	1 (14.3)	
GA	71 (35.2)	29 (33.3)	6 (54.5)		31 (32.0)	5 (71.4)	
AA	14 (6.9)	8 (9.2)	0 (0)		5 (5.1)	1 (14.3)	
Number of A-alleles ≥2 (rs3077, rs9277535)	74 (36.6)	34 (39.1)	5 (45.4)	0.750	29 (29.9)	6 (85.7)	**0.006**
Treatment duration	9.0 (7.3–11.2)	9.0 (7.2–11.8)	6.5 (2.5–9.6)	0.084	9.4 (8.0–11.2)	6.5 (3.8–11.7)	0.132

Except where marked with an asterisk (*), values are expressed as the median and 25th–75th percentile (parenthetically), or number and percentage (parenthetically). Asterisks indicate data displayed as mean values and standard deviation. Bold text indicates statistically significant P-values.

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significant characteristics included HBV genotype and *HLA-DP* SNPs. Ninety-one of 98 patients in cohort 1 sustained VR (HBV DNA <600 copies/ml) during NA treatment. Within 1 year, HBV DNA levels have increased minimally by <1 log copies/ml from the nadir in the other seven patients. Forty-four patients switched from LAM to ETV (0.5 mg/day) in cohort 1 because of favourable viral suppression. Viral suppression was subsequently continued after switching from LAM to ETV in cohort 1 patients. In cohort 2, the median duration from the start of rescue therapy to VR was 24 weeks.

Baseline characteristics and early virological response by *HLA-DP* gene polymorphisms

The genotypic distributions of rs3077 and rs9277535 genotypes were in Hardy–Weinberg equilibrium ($\chi^2 = 0.671$, $P = 0.714$ and $\chi^2 = 0.513$, $P = 0.774$ respectively). The minor allele frequencies (MAF) of rs3077 and rs9277535 were 0.220 and 0.245 respectively (minor allele = A).

Table 2 shows baseline characteristics and early virological response stratified by *HLA-DP* gene genotype. There were no differences in the distribution of baseline characteristics by rs3077, rs9277535 or the number of A-alleles at rs3077 and rs9277535. There were no differences in the early virological response (decline of HBsAg level [≥0.5 log IU/ml within 6 months], HBeAg seroclearance within 6 months, and undetectable HBV DNA [<400 copies/ml at 6 months]) by *HLA-DP* gene polymorphisms. There were no differences in the distribution of baseline characteristics, and in the early virological response by rs8099917 of *IL28B* gene (Supplementary Table).

Association between *HLA-DP* gene polymorphisms and HBsAg kinetics in cohort 1

HBsAg kinetics in cohort 1 is shown in Fig. 2A. Among patients with A-alleles ≥2 at the *HLA-DP* polymorphisms (rs3077, rs9277535), the median HBsAg change from baseline was −0.36 log IU/ml at 3 years, −0.49 at 5 years, −0.60 at 7 years and −0.73 at 9 years. Among patients with the number of A-alleles <2, the median changes were −0.06 log IU/ml at 3 years, −0.15 at 5 years, −0.23 at 7 years and −0.38 at 9 years. *HLA-DP* gene polymorphisms had a significant effect on the slopes between data collection points at 3 and 9 years. Moreover, we subanalysed HBsAg kinetics only in patients with HBV genotype C because about 90% of this cohort had genotype C. The results were similar to those of all genotypes. *HLA-DP* gene polymorphisms had a significant effect on the slopes between data collection points at 5 and 7 years (Fig. 2). The significant differences in HBsAg decline were not observed according to *IL28B* polymorphism.

We categorized the slopes of HBsAg kinetics from baseline to last visit into three groups as follows: no decline, <0.5 log IU/ml decrease or increase, slow decline, 0.5–0.99 log IU/ml decrease, and rapid decline, over 1 log IU/ml decline. The percentages of patients in which the number of A-alleles at the *HLA-DP* polymorphisms ≥2 were 30.8% (12/39) in the rapid decline group, 41.0% (16/39) in the slow decline group, and 28.2% (11/39) in the no decline group (Fig. 2C). The percentages of patients with <2 A-alleles were 22.0% (13/59) in the rapid decline group, 16.9% (10/59) in the slow decline group, and 61.0% (36/59) in the no decline group (Fig. 2C). There were significant differences in the HBsAg decline patterns according to *HLA-DP* polymorphisms ($P = 0.004$). The results were similar in HBV genotype C subpopulation. There were significant differences in the HBsAg decline patterns according to *HLA-DP* polymorphisms as shown in Fig. 2D ($P = 0.001$).

Association between *HLA-DP* gene polymorphisms and HBsAg kinetics in cohort 2

Because the timing of VBT in cohort 2 patients varied, it was difficult to analyse the kinetics of changes in HBsAg levels. Therefore, we examined HBsAg kinetics after the achievement of VR by rescue therapy (Fig. 3A). Among patients with A-alleles ≥2, the median HBsAg change from VR with rescue therapy was −0.15 log IU/ml at 1 year, −0.31 at 3 years, −0.53 at 5 years and −0.63 at 7 years, and among patients with A-alleles <2, the median changes were −0.08 log IU/ml at 1 year, −0.21 at 3 years, −0.37 at 5 years and −0.43 at 7 years. *HLA-DP* gene polymorphisms had a significant effect on the slopes of VR between 1 and 5 years. Although the tendency of HBsAg change was observed in HBV genotype C subpopulation, *HLA-DP* gene polymorphisms had only a marginally significant effect on the slopes of VR (Fig. 3B). The significant differences in HBsAg decline were not observed according to *IL28B* polymorphism. The percentages of VR patients with A-alleles ≥2 with ≥1 log IU/ml declines in HBsAg levels were significantly higher than those with <2 A-alleles (Fig. 3C). Moreover, the results were similar in genotype C subpopulation (Fig. 3D).

We evaluated whether ALT flare-up before starting ADV, HBeAg loss before starting ADV, and HBsAg levels at the start of ADV affected subsequent HBsAg seroclearance in cohort 2 because the phenomenon in which virological response and breakthrough by LAM resistance resulted might affect clinical courses. Median peek ALT levels before ADV were 234 IU/L (interquartile range, IQR: 23–385) in patients with HBsAg seroclearance, and 132 IU/L (IQR: 62–308) with persistent HBsAg positivity. There was no significant difference in peek ALT levels before ADV ($P = 0.851$). Thirty-one patients (29.8%) achieved HBeAg loss during LAM monotherapy before ADV added-on LAM. Four of 31 patients (12.9%) with HBeAg loss, and 3 of 73 patients (4.1%) without HBeAg loss achieved HBsAg

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Table 2. Baseline, demographic and on-treatment characteristics according to *HLA-DP* genotypes

Characteristics	rs3077 GG (n = 125)	MAF = 0.220 GA (n = 65)	AA (n = 12)	P	rs9277535 GG (n = 117)	MAF = 0.245 GA (n = 71)	AA (n = 14)	P	Number of A-alleles <2 (n = 128)	≥2 (n = 74)	P
Baseline											
*Age (year) (SD)	44 (12.0)	41 (10.8)	43 (8.2)	0.309	44 (12.1)	42 (10.7)	42 (10.0)	0.536	44 (11.8)	42 (10.8)	0.304
Gender (male:female)	88:37	53:12	10:2	0.191	81:36	58:13	12:2	0.101	91:37	60:14	0.132
Family history of HBV infection	79 (69)	38 (68)	7 (70)	0.807	80 (68)	48 (68)	10 (71)	0.973	86 (67)	52 (70)	0.636
Previous IFN therapy	55 (44)	37 (57)	8 (67)	0.113	51 (44)	39 (55)	10 (71)	0.076	57 (45)	43 (58)	0.080
Pre-existing cirrhosis	25 (20)	13 (20)	4 (33)	0.564	23 (20)	16 (23)	3 (21)	0.898	26 (20)	16 (22)	0.858
HBV genotype				0.772				0.673			0.767
A	4 (3.2)	3 (4.6)	1 (8.3)		2 (1.7)	5 (7.0)	1 (7.1)		4 (3.1)	4 (5.4)	
B	5 (4.0)	3 (4.6)	1 (8.3)		4 (3.4)	4 (5.6)	1 (7.1)		5 (3.9)	4 (5.4)	
C	113 (90.4)	58 (89.2)	9 (75)		107 (91.4)	61 (86.0)	12 (85.7)		116 (90.6)	64 (86.5)	
D	1 (0.8)	0 (0)	0 (0)		1 (0.9)	0 (0)	0 (0)		1 (0.8)	0	
unclassified/missing	2 (1.6)	1 (1.7)	1 (8.3)		3 (2.7)	1 (1.4)	0 (0)		2 (1.6)	2 (2.7)	
Baseline HBV DNA (log copies/ml)	7.6 (6.7–8.0)	7.5 (6.8–8.1)	7.1 (5.9–8.6)	0.892	7.6 (6.7–8.1)	7.7 (6.8–8.2)	6.8 (5.7–7.8)	0.082	7.6 (6.7–8.1)	7.5 (6.7–8.0)	0.862
Baseline HBsAg level (IU/ml)	3180 (1350–12 600)	2910 (1270–5700)	2420 (1010–10 700)	0.582	3110 (1170–12 300)	3070 (1790–7250)	1690 (1200–10 500)	0.792	3120 (1300–12 700)	2930 (1280–6030)	0.455
Baseline AST level (IU/L)	83 (58–166)	100 (63–228)	82 (62–575)	0.343	85 (57–168)	88 (64–218)	97 (58–214)	0.637	84 (58–165)	93 (63–235)	0.178
Baseline ALT level (IU/L)	137 (76–268)	188 (84–375)	138 (91–861)	0.367	145 (71–297)	151 (82–371)	150 (72–462)	0.679	142 (77–263)	173 (86–380)	0.209
Baseline total bilirubin level (mg/dl)	0.8 (0.6–1.1)	0.8 (0.6–1.2)	0.8 (0.7–1.6)	0.567	0.8 (0.6–1.1)	0.8 (0.6–1.2)	0.8 (0.6–1.2)	0.769	0.8 (0.6–1.1)	0.8 (0.6–1.3)	0.229
*Platelet count (10^5/mm^3) (SD)	16.3 (5.5)	16.1 (6.3)	14.5 (4.1)	0.535	16.4 (5.6)	15.9 (6.1)	15.0 (3.8)	0.551	15.7 (6.0)	16.4 (5.5)	0.190
Early treatment response											
Decline of HBsAg level (≥0.5 log IU/ml within 6 months)	26 (21)	19 (29)	3 (25)	0.447	24 (21)	24 (28)	3 (21)	0.375	26 (20)	22 (30)	0.170
HBeAg positive→clearance within 6 months	41 (33)	19 (29)	6 (50)	0.389	40 (34)	20 (28)	6 (43)	0.477	42 (33)	24 (32)	1.000
Undetectable HBV DNA (<400 copies/ml) at 6 months	74 (59)	43 (66)	9 (75)	0.439	72 (62)	43 (58)	11 (79)	0.464	76 (59)	50 (68)	0.287

Except where marked with an asterisk (*), values are expressed as the median and 25th–75th percentile (parenthetically), or number and percentage (parenthetically). Asterisks indicate data displayed as mean values and standard deviation. MAF; minor allele frequency. Bold text indicates statistically significant P-values.

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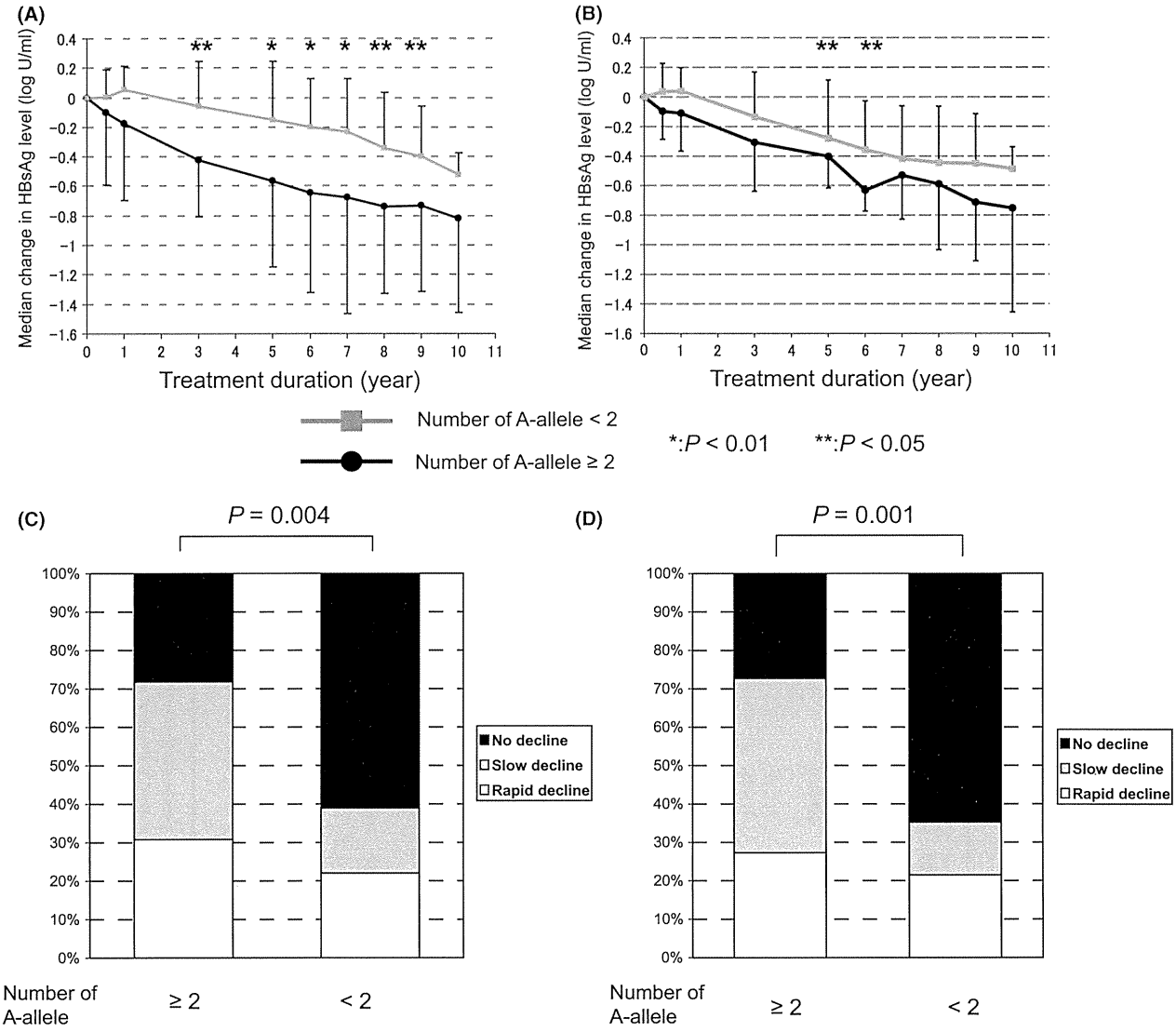

Fig. 2. (A) Median change in HBsAg level from baseline in patients with *HLA-DP* gene alleles (cohort 1; all patients). The asterisk (*) indicates a statistical significance of $P < 0.01$, and (**) indicates $P < 0.05$ as determined by the Mann–Whitney *U*-test. (B) Median change in HBsAg level from baseline in patients with *HLA-DP* gene alleles (cohort 1; HBV genotype C only). The asterisk (*) indicates a statistical significance of $P < 0.01$, and (**) indicates $P < 0.05$ as determined by the Mann–Whitney *U*-test. (C) Patterns of decrease in HBsAg in all cohort 1 patients with the number of A-alleles in rs3077 and rs9277535. No decline is defined as <0.5 log decrease or increase, slow decline as 0.5–0.99 log decline, and rapid decline as ≥1.0 log decline. (D) Patterns of decrease in HBsAg only in HBV genotype C cohort 1 patients with the number of A-alleles in rs3077 and rs9277535. No decline is defined as <0.5 log decrease or increase, slow decline as 0.5–0.99 log decline, and rapid decline as ≥1.0 log decline.

seroclearance after ADV added-on LAM. However, there was no significant difference in HBsAg seroclearance among patients with or without HBeAg loss ($P = 0.192$) before ADV because the number of patients with HBsAg seroclearance was small. There was also no significant difference in cumulative HBsAg seroclearance rates among patients with or without HBeAg loss ($P = 0.166$). Median HBsAg levels at the start of ADV were 1310 IU/ml (IQR: 6.64–44 200) in patients with HBsAg seroclearance, and 5850 IU/L (IQR: 2160–16 500) with persistent HBsAg positivity. There was no significant difference in HBsAg levels at the start of ADV ($P = 0.400$). Median peek ALT levels before ADV were 132 IU/L (IQR: 66–259) in patients with A-alleles <2, and 138 IU/L (IQR: 51–457) with A-alleles ≥2. Median HBsAg levels at the start of ADV were 5730 IU/ml (IQR: 2490–18 000) in patients with A-alleles <2, and 5450 IU/L (IQR: 1320–12 000) with A-alleles ≥2. There were no significant differences in peek ALT levels before ADV and HBsAg levels at the

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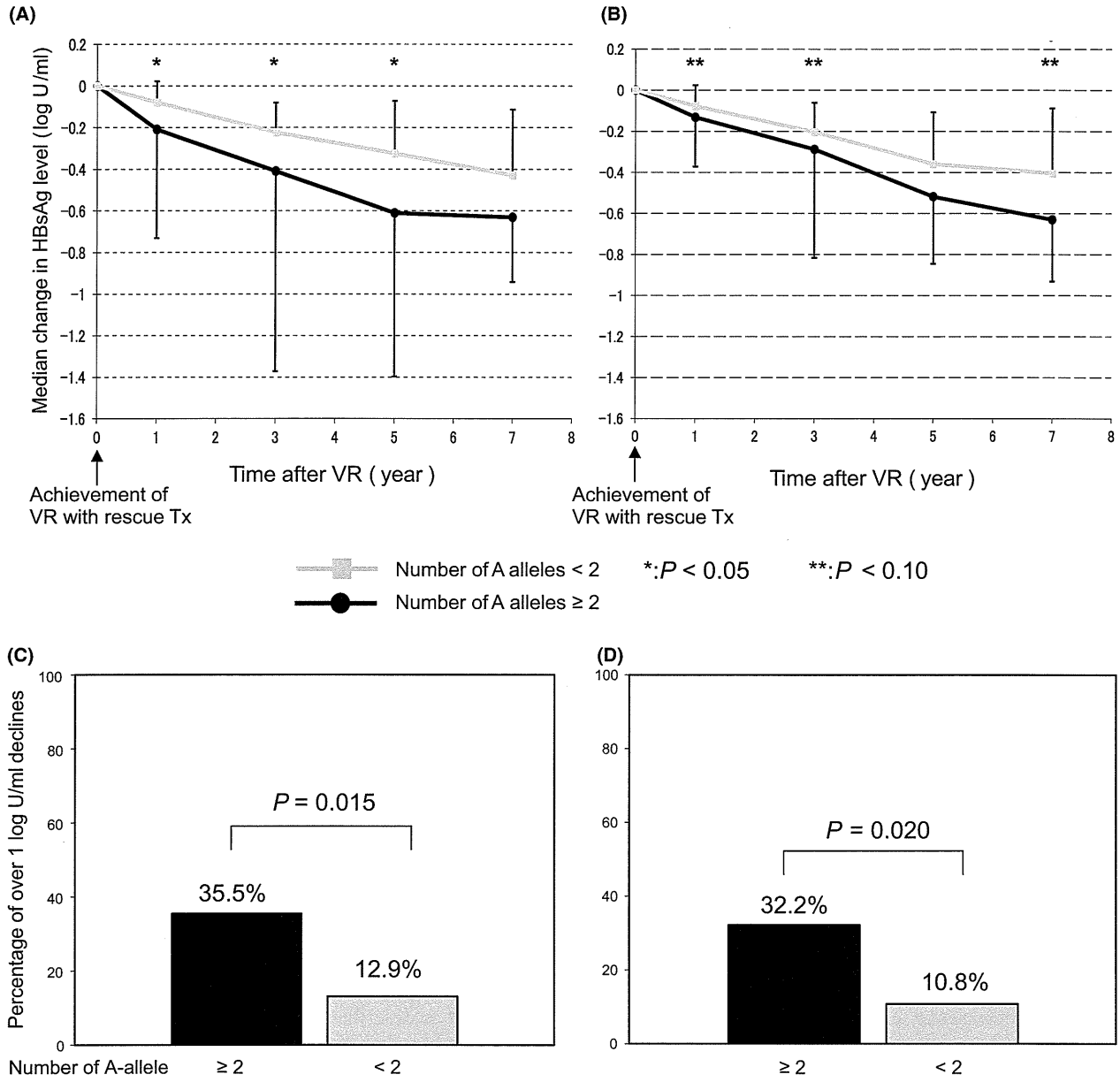

Fig. 3. (A) Median change in HBsAg level from VR in patients with *HLA-DP* gene alleles (cohort 2). The asterisk (*) indicates a statistical significance of $P < 0.05$ as determined by the Mann–Whitney *U*-test. VR, virological response. (B) Median change in HBsAg level from VR in patients with *HLA-DP* gene alleles (cohort 2; HBV genotype C only). The asterisk (**) indicates a marginal significance of $P < 0.10$ as determined by the Mann–Whitney *U*-test. VR, virological response. (C) Decreases of ≥1 log U/ml of HBsAg levels over time in all cohort 2 patients with the number of A-alleles in rs3077 and rs9277535. (D) Decreases of ≥1 log U/ml of HBsAg levels over time only in HBV genotype C cohort 2 patients with the number of A-alleles in rs3077 and rs9277535.

start of ADV according to the number of A-alleles (ALT; $P = 0.625$, HBsAg; $P = 0.320$).

Association between *HLA-DP* polymorphism and HBsAg seroclearance

We performed a detailed analysis of the association between *HLA-DP* gene polymorphisms and HBsAg seroclearance in patients treated with LAM. Cumulative HBsAg clearance rates from baseline in cohort 1 patients were as follows: 2.6% at 3 y, 5.3% at 5 y, and 14.4% at 7 y in patients with A-alleles ≥2; 1.7% at 3 y, 5.5% at 5 y, 7.4% at 7 y, and 12.4% at 9 y in patients with <2 A-alleles (Fig. 3A). There was no significant difference in HBsAg seroclearance rates between these two patient groups.

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Cumulative HBsAg clearance rates from the achievement of VR with rescue therapy in cohort 2 patients were as follows: 2.9% at 1 y, 14.3% at 3 y, and 17.6% at 5 y in patients with A-alleles ≥2; 0% at 3 y and 1.7% at 5 y for patients with <2 A-alleles (Fig. 3B). HBsAg seroclearance rates from VR were significantly higher in cohort 2 patients with ≥2 A-alleles than in those with fewer A-alleles.

Multivariate Cox regression analysis identified four significant baseline characteristics related to HBsAg clearance: previous IFN therapy, infection with HBV genotype A, high total bilirubin levels and A-alleles ≥2 in the combined cohort (Table 3; model 1). However, in subanalysis among patients with genotype C, A-alleles ≥2 was not significantly associated with HBsAg clearance (Table 3; model 2). It seems to be that the reason is because the rates of HBsAg seroclearance was relatively low in genotype C subpopulation (Table 1). IL28B polymorphism was not associated with HBsAg seroclearance.

Number of A-alleles at rs3077 and rs9277535 and HBsAg seroclearance

We performed a detailed analysis of the association between the number of A-alleles and HBsAg clearance. Figure 4C shows the percentage of HBsAg seroclearance over time in all patients (cohort 1 + 2), stratified by the number of A-alleles. The percentage of HBsAg seroclearance over time was positively correlated with the number of A-alleles (P for trend = 0.009).

Discussion

We found that *HLA-DP* gene polymorphisms are associated with HBsAg kinetics in HBeAg-positive chronic hepatitis B patients who began treatment with LAM and continued with long-term NA therapy. *HLA-DP* gene polymorphisms were significantly associated with HBsAg seroclearance, and particularly in patients who received add-on rescue therapy. HBsAg kinetics and seroclearance during NA therapy were positively affected by a higher number (≥2) of A-alleles, i.e. the minor alleles at rs3077 and rs9277535.

Kamatani *et al.* first reported the association between the *HLA-DP* locus and chronic HBV infection, after GWAS in Japanese and Thai samples (16). Similar results have been reported in Chinese, Korean, German and other Japanese populations (17, 19, 20, 23, 24). The *HLA-DP* locus appears to be associated with natural HBV clearance. Kamatani *et al.* identified that two SNPs, viz., rs3077 and rs9277535, from a region including *HLA-DPA1* and *HLA-DPB1*, were strongly associated with chronic hepatitis B (16). Therefore, we here analysed the association between these two SNPs and HBsAg kinetics and seroclearance during NA therapy. Previous studies showed that the minor alleles (A) of rs3077 and rs9277535 protected against chronic HBV infection. We could also demonstrate that HBsAg levels decreased faster in patients with than those without A-alleles, as we had hypothesized. Although the reason for this finding is unclear, O'Brien *et al.* reported that the expression of *HLA-DPA1* and *HLA-DPB1* mRNA in normal human liver tissue increased in healthy donors with the presence of the minor allele of rs3077 and rs9277535 (18). They also showed that the order of expression levels of *HLA-DPA1* and *HLA-DPB1* was AA > AG > GG in both rs3077 and rs9277535, while the odds ratio for chronic HBV infection followed the opposite order. These findings support our finding that a larger number of A-alleles at rs3077 and rs9277535 were associated with a higher percentage of HBsAg seroclearance in patients receiving long-term NA therapy, as shown in Fig. 4C. Greater expression of *HLA-DPA1* and *HLA-DPB1* may facilitate HBsAg level decrease and seroclearance during NA therapy. Furthermore, previous studies reported that genetic variants in the antigen-binding region of *HLA-DQ* were also associated with persistent HBV infection (19, 23). Future studies should investigate the association between the combination of genetic variants at the *HLA-DP* and *HLA-DQ* loci and HBsAg kinetics.

In this study, besides the *HLA-DP* polymorphisms, HBsAg seroclearance was likely to occur in patients who had HBV genotype A, high bilirubin levels at baseline, and had previously undergone IFN therapy (Table 3). It has previously been reported that HBV genotype A is associated with HBsAg seroclearance during NA therapy (15, 25, 26). High ALT flares sometimes result in bilirubin flares and high virological responses have been reported in response to robust IFN therapy-induced ALT flares (27, 28). Moreover, Wursthorn *et al.* indicated that both antiviral potential of NAs and antiviral T-cell reactivity are associated with HBsAg clearance in response to telbivudine treatment (25). Although the treatment duration and timing of previous IFN were not associated with HBsAg seroclearance during LAM treatment as described in our previous paper (15), these results imply that both direct antiviral potential and host immune response are needed to achieve HBsAg seroclearance.

There were several limitations to our study. First, because LAM has a high potential for drug resistant mutations, many patients receiving LAM had experienced VBT and required rescue therapy (add-on ADV). Consequently, the study population available for this study had to be divided into a VR without rescue therapy cohort (cohort 1) and a LAM add-on rescue therapy cohort (cohort 2) to ensure a uniform treatment response, resulting in small cohort sizes. Second, because *HLA-DP* SNP analysis could not be conducted in all patients received LAM, there may have been a selection bias. However, the allele frequencies of rs3077 in *HLA-DPA1* and rs9277535 in *HLA-DPB1* in this study were similar to those observed in previous studies. Third, we were not able to collect immunological data

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Table 3. Baseline factors associated with HBsAg clearance, as determined by univariate and multivariate analysis (cohort 1 + 2)

	Univariate		Multivariate (Model 1)		Multivariate (Model 2)	
Variable	HBsAg clearance rate ratio (95% CI)	*P*	HBsAg clearance rate ratio (95% CI)	*P*	HBsAg clearance rate ratio (95% CI)	*P*
Age (per year)	1.01 (0.97–1.06)	0.493				
Gender (F)	0.41 (0.09–1.78)	0.234				
Family history of HBV infection	0.61 (0.23–1.61)	0.318				
Previous IFN therapy	**3.47 (1.14–10.5)**	**0.028**	**3.14 (1.02–9.65)**	**0.045**	**5.51 (1.13–26.8)**	**0.035**
Pre-existing cirrhosis	0.91 (0.60–1.38)	0.645				
HBV genotype (A)	**16.0 (5.63–45.4)**	**1.88×10^{-7}**	**21.6 (7.05–66.3)**	**7.63×10^{-8}**		
HBV DNA (per log copies/ml)	1.47 (0.92–2.34)	0.104				
HBsAg (per log IU/ml)	1.71 (0.86–3.38)	0.123				
AST (per IU/L)	**1.001 (1.000–1.002)**	**0.018**				
ALT (per IU/L)	**1.001 (1.000–1.001)**	**0.044**				
Total bilirubin (per mg/dl)	**1.21 (1.03–1.43)**	**0.018**	**1.23 (1.02–1.48)**	**0.029**	**1.30 (1.05–1.61)**	**0.015**
Platelet count (per $1.0 \times 10^4/mm^3$)	0.93 (0.84–1.02)	0.132				
rs3077 (non-GG)	**2.69 (1.04–6.93)**	**0.041**				
rs9277535 (non-GG)	**2.78 (1.04–7.42)**	**0.041**				
Number of A-alleles among rs3077 and rs9277535 (≥2)	**2.81 (1.09–7.25)**	**0.033**	**2.88 (1.09–7.58)**	**0.044**		
IL28B rs8099917 (non-TT)	0.31 (0.04–2.36)	0.313				
Treatment group (without rescue Tx)	1.35 (0.84–2.17)	0.213				

Bold text indicates statistically significant *P*-values; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; CI, confidence interval; IFN, interferon; Tx, treatment.
Model 1; including all patients. Model 2; including only patients with genotype C.

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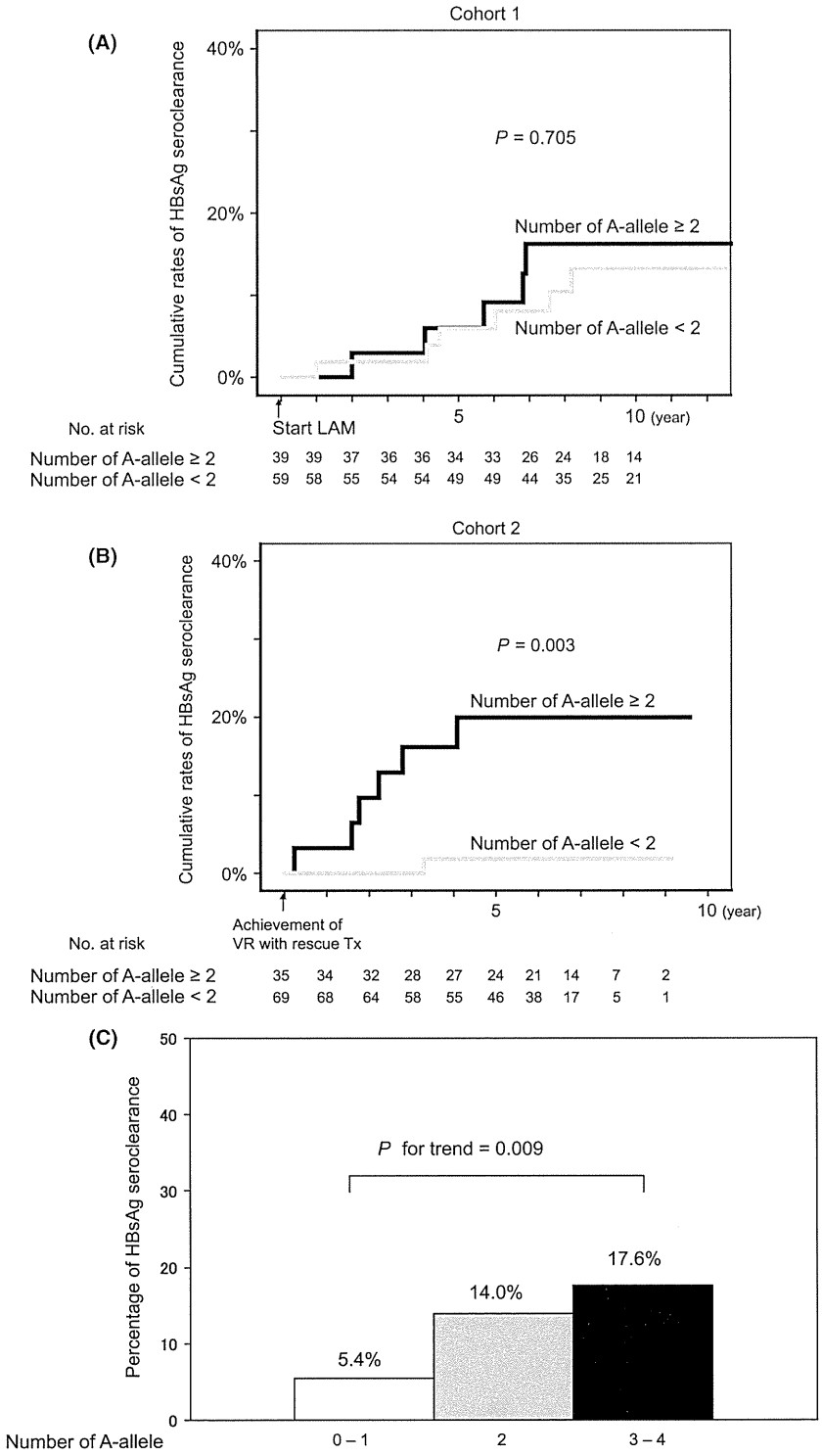

Fig. 4. (A) Kaplan–Meier life table showing cumulative HBsAg clearance rates by the number of A-alleles in rs3077 and rs9277535 (cohort 1). (B) Kaplan–Meier life table showing cumulative HBsAg clearance rates after achievement of VR with rescue therapy by the number of A-alleles (cohort 2). (C) Percentage of HBsAg seroclearance over time in all patients with the number of A-alleles in rs3077 and rs9277535.

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on our subjects. Fourth, we could not find association between the *HLA-DP* polymorphisms and HBsAg kinetics and seroclearance in HBeAg-negative patients receiving long-term LAM in our institute (data not shown). The reason for the results in HBeAg-negative patients remains unclear, but may be necessary to repeat this analysis in a larger population. Finally, our results should be validated by further studies investigating a large study population receiving long-term ETV or tenofovir with high antiviral potency and a high genetic barrier.

In our study, we observed an association between *HLA-DP* polymorphisms and declines in HBsAg levels and seroclearance among HBeAg-positive patients treated with LAM and who subsequently achieved favourable VR. HBsAg levels declined faster in patients with two or more A-alleles (minor alleles) at rs3077 and rs9277535, than those with fewer A-alleles. Although *HLA-DP* polymorphisms may not markedly affect the decision of the treatment choice, it will be helpful to identify the mechanism of HBsAg seroclearance among HBV-infected patients in future. Moreover, future studies should validate these findings in high antiviral treatment regimens among large cohorts of patients with chronic hepatitis B.

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Conflict of interest: These authors disclose the following: Dr. Kumada reports having received investigator, lecture, and consulting fees from Bristol-Myers Squibb, Dainippon Sumitomo Pharma Co., MSD KK, and Toray Co. Dr. Ikeda reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co. The rest of the authors do not have any disclosures to report.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline, demographic and on-treatment characteristics according to *IL28B* genotypes

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第Ⅰ章

HCV 研究の最先端

5 HCV 感染実験系における代謝変化

Cellular Metabolome during Hepatitis C Virus Infection

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はじめに

肝臓は腸で吸収されたさまざまな栄養素を代謝，貯蔵するなど生命の維持に必要な多くの働きを行っていることから，体内の化学工場，貯蔵庫に例えられている．そこにC型肝炎ウイルス（HCV）が感染すると，インスリン抵抗性や脂肪肝などの糖質や脂質の代謝異常も引き起こすことが明らかになってきている．抗ウイルス療法によりウイルスを駆除すると脂肪化が改善すること，genotype 3型のウイルスで脂肪化が顕著なことなどから，これらの代謝異常の発生にはウイルス感染に伴う炎症よりも，ウイルスそのものの宿主細胞への直接作用が深く関わっているものと考えられている．

これまで細胞の働きを理解しようとするとき，DNA配列の網羅的解析（ゲノミクス）や蛋白質の網羅的解析（プロテオミクス）が行われてきた．しかしながら，実際の細胞内ではホメオスタシスによりゲノムレベルでの変動が表現型に一致しないことも多い．その点で，代謝産物は表現型にもっとも近いため，表現型での変化が観察しやすいという特徴があり，代謝産物の網羅的解析（メタボロミクス）が注目されている．本稿では，メタボロミクス解析の結果も含めて，HCV感染が宿主代謝に与える影響について述べたい．実験にはHCV感染Huh7細胞[1]以外に，HCVのどの蛋白が影響するかを調べるため，coreからNS2までを恒常的に発現する細胞株とNS3からNS5Bまでを発現するサブゲノムレプリコン細胞株を用いた．

Ⅰ HCV 感染が宿主糖質代謝に与える影響

糖質代謝は，おもに解糖系と糖新生により調整されている．解糖系は，グルコースを利用してエネルギーであるATPを産生するとともに，ほとんどのATPがつくられるクエン酸回路お

Key words：HCV，メタボロミクス，代謝異常，エネルギー代謝，脂質代謝，糖代謝

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よび酸化的リン酸化に基質を供給している．さらに，解糖系は多くの生合成経路への前駆体となる中間体をつくる役割を担っている．一方，糖新生は糖原性アミノ酸と脂肪分解によるグリセロールなどからグルコースを合成する過程である．

HCV感染が糖代謝に与える影響については，遺伝子・蛋白質レベルの解析から，解糖系が亢進する，あるいは糖新生が亢進するという，相反する報告が複数存在している．HCVcc[1]を感染させメタボロミクス解析を行ったところ，糖代謝の中間代謝産物のglucose 6-phosphate（G6P），fructose 6-phosphate（F6P），fructose-1,6-biphosphate（FBP），phosphoenolpyruvic acid（PEP），pyruvic acidが顕著に増加していた．プロモーターアッセイでは，糖新生の律速酵素であるphosphoenolpyruvate carboxylase（PEPCK）のプロモーター活性は低下した．これらの変化はcore-NS2発現細胞株でも認められた．解糖系の律速酵素であるglucokinase（GK），phosphofructokinase（PFK），pyruvate kinase（PK）のmRNAは増加傾向を，糖新生の律速酵素であるpyruvate carboxylase（PBC），PEPCK，FBPase，G6Paseは減少傾向を示した．さらに，isoleucine，leucine，phenylalanine，tryptophan，tyrosineなどのケト原性アミノ酸には変化がなかったものの，糖新生に利用されるaspartic acid，glutamine，glutamic acid，glycine，proline，serine，threonineなどの糖原性アミノ酸の低下が認められ，core-NS2発現細胞株でも同様であった．以上の結果から，HCV感染では解糖系が亢進するものと考えられ，この変化はHCV構造蛋白質による可能性が示唆された．

Ⅱ HCV感染が宿主脂質代謝に与える影響

脂質代謝に関しては，HCV感染に伴い，コレステロール，中性脂肪，sphingosineおよびsphinganineなどのスフィンゴ脂質が増加していた．コレステロールやスフィンゴ脂質はいずれも生体膜の主要脂質成分として知られており，生活環の多くのステップで細胞の小胞体，ゴルジ体，細胞膜といった生体膜脂質を利用しているHCVの増殖にとって好都合な状況となっていると考えられる．とくに，スフィンゴ脂質とコレステロールは脂質ラフトの構成成分であることから，HCVの感染，複製，粒子形成にも役立っているものと考えられる[2),3)]．

HCVによる肝細胞の脂肪化の機序はHCVコア遺伝子トランスジェニックマウスを用いた研究などで明らかになっている[4)]．HCVコア蛋白は脂質合成系の転写因子sterol regulatory element binding protein-1の増加を介して，脂肪酸合成酵素の活性を上げて，脂肪酸合成を亢進させている．一方，ミトコンドリアに局在化したコア蛋白は脂肪酸のベータ酸化を抑制し，脂肪酸の消費を低下させている．また，microsomal triglyceride transfer proteinを低下させるため，超低比重リポ蛋白（VLDL）分泌を抑制し，細胞外への脂肪酸の放出を抑制している．さらに，HCVが誘発するインスリン抵抗性による高インスリン血漿は脂肪細胞からの肝細胞への遊離脂肪酸の取り込みを増加させている．

脂質はリン脂質，コレステロール，脂肪酸，中性脂肪，コレステロールエステルに大別され，生体膜の主要成分として生体構造を司るとともに，エネルギー代謝の中心を担っており，生命維持のうえでも重要な役割を果たしている．食物として取り入れられた脂質は脂質代謝の流れに乗って全身の必要な臓器に移行する．この脂質輸送の中心を担っているのがリポ蛋白である．前述のようにHCV感染による肝脂肪化の原因の一つとして，VLDL分泌低下が報告されている．一方，VLDLはHCVの感染性に必須と報告されており，筆者らはこれらの矛盾

点を解明するためHCV感染に伴う肝細胞の脂肪化の分子メカニズムについて，リポ蛋白に注目し解析した．HCV感染後，培養上清中のVLDLの割合が増加し，低比重リポ蛋白（LDL）の割合が低下することを見出し，また，その原因としてHCV感染細胞でのVLDL分解酵素であるhepatic lipaseの発現低下を見出した．HCV感染に伴う，肝細胞内での脂質蓄積と培養上清中のVLDL割合の増加は，ウイルスにとって合目的な作用といえる．

Ⅲ HCV感染が宿主エネルギー代謝に与える影響

クエン酸回路は，解糖や脂肪酸のβ酸化によって生成するアセチルCoAを取り込み酸化されることによって，ATPや電子伝達系で用いられるNADHなどが生じ，効率の良いエネルギー生産を行っている．また，アミノ酸などの生合成に関わる物質を生産するという役割も担っている．HCV感染細胞のメタボロミクス解析では，citrate，succinate，malate，aconitic acid，fumarateなどのクエン酸回路の中間代謝産物は低下し，これらの変化は構造蛋白発現細胞でも観察された．

その酵素であるaconitase，ketoglutarate dehydrogenase，succinate dehydrogenase，fumaraseなどのmRNAも低下傾向を認めた．さらに，ATP，GTP，phosphocreatineなどのエネルギー供与体も減少しており，これらの変化はレプリコン細胞でも観察された．

クエン酸回路や電子伝達系を有し，生体内でエネルギー供給するATPを産生する主要な場所がミトコンドリアである．そこで，HCVが感染した細胞を電子顕微鏡で観察したところ，ミトコンドリアのクリステ構造が破壊されていた．さらに，蛍光抗体法による観察では，HCVコア蛋白局在部位でミトコンドリア機能低下がみられた．Moriyaらもコア発現トランスジェニックマウスにおいてミトコンドリアに障害が生じていることを見出している[4]．このようなHCVによるミトコンドリア障害はエネルギー産生低下をもたらす可能性がある．

次に，われわれはHCV複製による細胞におけるATP消費量の変化を調べた[5]．レプリコン細胞から複製複合体を含む画分を分離し，ATPを添加しその減少量を比較したところ，オリジナルの細胞に比べてATP消費量が亢進していた．ATPが結合するとビーナスとCFPが近接しFRET蛍光を発する，ATP濃度測定プローブATeamを用いて，生細胞内のATPの量と局在の解析を目指した．ATeamをレプリコンRNAのNS5Aの下流に挿入することにより，FRET強度からATPの量を評価するとともに，ドット状の蛍光から複製複合体の局在を識別した．レプリコン細胞では複製複合体で強いFRETシグナルを観察した．われわれは，レプリコン細胞から複製複合体を粗精製し[2]，そこに含まれるHCVのゲノム複製に関与する宿主因子をプロテオミクスの手法を利用して探索し，creatine kinase B（CKB）を見出した[6]．CKBは，エネルギーを多く必要とする組織，あるいは急速に必要とする組織でのATPの供給およびATPレベルの維持に重要であるとされており，CKBはNS4Aとの結合を介してHCV複製複合体にリクルートされ，ATPを供給することでHCV複製活性の維持に重要であると考えられた[7]．

Ⅳ HCV感染が宿主核酸合成に与える影響

HCV感染細胞では，adenosine monophosphate，adenosine diphosphate，guanosine monophosphate，guanosine diphosphate，guanosine triphosphateなどプリンヌクレオチド，およびuridine diphosphate，uridine triphosphate，cytidine monophosphate，cytidine di-

phosphate, cytidine triphosphate, thymidine triphosphate, deoxycytidine triphosphate などピリミジンヌクレオチドなどの核酸合成は低下していた.

おわりに

メタボロミクス解析の結果, HCV の感染により, 核酸合成, TCA 回路, エネルギー産生系, 糖新生は減少し, 一方, 解糖系, 脂質合成は顕著に亢進していた. 本研究から C 型肝炎患者の脂肪肝は説明できるが, インスリン抵抗性については相反する結果となった. 脂質, 糖質, 蛋白質, エネルギー代謝は代謝経路のなかで多くの結節点をもち, 互いに影響を与え合っているものと考えられており, HCV 感染細胞内での解糖系の亢進は著しく低下したエネルギー代謝を補完している可能性がある. このような解析は C 型肝炎患者の病態の基礎的な理解につながると期待できる.

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