

Figure 1. Results of genome-wide association studies. a) HBV carriers and healthy controls, and b) HBV carriers and HBV-resolved individuals were compared. *P* values were calculated by chi-squared test for allele frequencies. Dots with arrows on chromosome 6 show strong associations with protective effects against persistent HB infection and with HBV clearance. doi:10.1371/journal.pone.0039175.g001

Clearance of Hepatitis B virus in Japanese and Korean Individuals

We also conducted a GWAS to identify the host genetic factors related to clearance of HBV in the above 181 Japanese HBV carriers and 185 Japanese HBV-resolved individuals using a genome-wide SNP typing array (Affymetrix Genome-Wide Human SNP Array 6.0 for 900 K SNPs). The same two SNPs (rs3077 and rs9277542) showed strong associations in the allele frequency model ($P=9.24 \times 10^{-7}$ and $P=3.15 \times 10^{-5}$) with clearance of HBV (Figure 1b).

The above 32 SNPs, including the two associated SNPs (rs3077 and rs9277542), were selected for a replication study in two independent sets of HBV carriers and HBV resolved individuals (replication-1:256 Japanese HBV carriers and 150 Japanese HBV resolved individuals; and replication-2:344 Korean HBV carriers and 106 Korean HBV resolved individuals; Table 1). All 32 SNPs were genotyped using the DigiTag2 assay and 29 of 32 SNPs were successfully genotyped (Table S3). The associations of the original SNPs were replicated in both replication sets [replication-1 (Japanese): rs3077, $P=3.32 \times 10^{-2}$, OR = 0.72 and rs9277542, $P=1.25 \times 10^{-2}$, OR = 0.68; replication-2 (Korean): rs3077, $P=2.35 \times 10^{-7}$, OR = 0.41 and rs9277542, $P=4.97 \times 10^{-6}$, OR = 0.46; Table 3]. Meta-analysis using random effects model showed $P_{meta}=1.56 \times 10^{-1}$ for rs3077 (OR = 0.51, 95% CI = 0.36–0.72), and 5.91×10^{-7} for rs9277542 (OR = 0.55, 95% CI = 0.43–0.69). While there was evidence of heterogeneity between these studies for rs3077 ($P_{het}=0.03$) and no evidence for rs9277542 ($P_{het}=0.19$), significant associations with HBV clearance were observed with Mantel-Haenszel $P_{meta}=3.28 \times 10^{-12}$ for rs3077 and 1.42×10^{-10} for rs9277542, when using CMH fixed-effects model. Among the remaining 27 SNPs in the replication study, two SNPs (rs9276431 and rs7768538), located in a genetic region including *HLA-DQ* gene, were marginally replicated in the two sets of HBV carriers and HBV resolved individuals with Mantel-Haenszel *P* values of 2.10×10^{-5} (OR = 0.59) and 1.10×10^{-3} (OR = 0.56), respectively (Table S3), when using CMH fixed-effect model. Due to the existing heterogeneity among three groups (GWAS, Replication-1 and Replication-2) ($P_{het}=0.03$ for rs9276431 and 0.04 for rs7768538), weak associations were

observed with $P_{meta}=0.03$ for rs9276431 and 0.02 for rs7768538 by the random effects model meta-analysis.

Meta-analysis across 6 independent studies, including 5 additional published data, showed $P_{meta}=1.48 \times 10^{-9}$, OR = 0.60 for rs3077, $P_{meta}=1.08 \times 10^{-17}$, OR = 0.66 for rs9277535 and $P_{meta}=5.14 \times 10^{-5}$, OR = 0.55 for rs9277542 (Table S4). As shown in Table S4, the OR for the rs9277535 and rs9277542 were similar among the 6 independent studies, and heterogeneity was negligible ($P_{het}=0.03$ for rs9277535 and 0.14 for rs9277542). However, significant level of heterogeneity for rs3077 was observed with $P_{het}=9.57 \times 10^{-6}$ across 5 independent studies, including our study.

URLs

The results of the present GWAS are registered at a public database: https://gwas.lifesciencedb.jp/cgi-bin/gwasdb/gwas_top.cgi.

Discussion

The recent genome-wide association study showed that the SNPs located in a genetic region including *HLA-DPA1* and *HLA-DPBI* genes were associated with chronic HBV infection in the Japanese and Thai population [10,11]. In this study, we confirmed a significant association between SNPs (rs3077 and rs9277542) located in the same genetic region as *HLA-DPA1* and *HLA-DPBI* and protective effects against CHB in Korean and Japanese individuals. Meta-analysis using the random effects model across 6 independent studies including our study suggested that, widely in East Asian populations, variants in antigen binding sites of *HLA-DP* contribute to protective effects against persistent HBV infection (Table S2).

On GWAS and replication analysis with Japanese and Korean individuals, we identified associations between the same SNPs (rs3077 and rs9277542) in the *HLA-DPA1* and *HLA-DPBI* genes and HBV clearance; however, no new candidate SNPs from the GWAS were detected on replication analysis (Table S3). When the data of reference#18 was excluded from the meta-analysis across 6 independent studies, heterogeneity among 4 studies was estimated to be $P_{het}=0.15$ and significant association of rs3077 with HBV clearance was observed with $P_{meta}=5.88 \times 10^{-21}$, OR = 0.56 (Table S4). In our study, a negligible level of heterogeneity for rs3077 was also observed ($P_{het}=0.03$) on meta-analysis by adding replication-1 (Table 3). Despite the heterogeneity in replication-1, a marginal association was observed for rs3077 with the same downward trend in the odds ratio ($P=3.32 \times 10^{-2}$, OR = 0.72). Moreover, meta-analysis using GWAS and replication-2 showed significant association of $P_{meta}=1.89 \times 10^{-12}$, OR = 0.43 for rs3077 with no evidence of heterogeneity ($P_{het}=0.75$). Although the reason why heterogeneity was observed in replication-1 is unclear, one possible reason is the clinical heterogeneity due to different kits being used for antibody testing. The associations of *HLA-DPA1*/*-DPBI* with CHB and HBV clearance showed the same level of significance in the comparison of HBV patients with HBV resolved individuals (OR = 0.43 for rs3077 and 0.49 for rs9277542) as the one with healthy controls (OR = 0.46 for rs3077 and 0.50 for rs9277542), when the replication-1 was excluded in the analysis (Table 2 and Table 3). The results of meta-analysis across 6 independent studies including our study also showed the same or slightly weaker associations in the

Table 1. Number of study samples.

population		GWAS	Replication-1	Replication-2
		Japanese	Japanese	Korean
HBV carriers	Total	181	256	344
	IC	20	94	–
	CH	67	101	177
	LC	3	10	–
	HCC	91	51	167
Healthy controls		184	236	151
Resolved individuals		185	150	106

Abbreviation: IC, Inactive Carrier; CH, Chronic Hepatitis; LC, Liver Cirrhosis; HCC, Hepatocellular Carcinoma.

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Table 2. Results of replication study for protective effects against CHB.

dbSNP rsID	Position		Nearest Gene	MAF ^a (allele)	Allele (1/2)	Stage (population)	HBV carriers			Healthy controls			OR ^b				
	Chr	Build 36.3					11	12	22	11	12	22	HWE ^p	95% CI	P-value ^c	P _{het} ^d	
rs3077	6	33141000	HLA-DPA1	0.44	T/C	GWAS	11	12	22	11	12	22	0.919	0.42	1.14×10 ⁻⁷		
							(7.2)	(28.2)	(64.6)	(15.3)	(48.1)	(36.6)		(0.30–0.58)			
							Replication-1	26	95	134	46	125	65	0.309	0.48	2.70×10 ⁻⁸	
							(Japanese)	(10.2)	(37.3)	(52.5)	(19.5)	(53.0)	(27.5)		(0.37–0.62)		
							Replication-2	23	81	111	31	74	40	0.767	0.47	2.08×10 ⁻⁶	
							(Korean)	(10.7)	(37.7)	(51.6)	(21.4)	(51.0)	(27.6)		(0.35–0.65)		
Meta-analysis ^e									0.46	4.40×10 ⁻¹⁹	0.80						
										(0.39–0.54)							
rs9277542	6	33163225	HLA-DPB1	0.45	T/C	GWAS	18	53	110	29	102	52	0.073	0.42	5.32×10 ⁻⁸		
							(9.9)	(29.3)	(60.8)	(15.8)	(55.7)	(28.4)		(0.31–0.58)			
							Replication-1	30	106	118	54	114	67	0.681	0.54	3.33×10 ⁻⁶	
							(Japanese)	(11.8)	(41.7)	(46.5)	(23.0)	(48.5)	(28.5)		(0.42–0.70)		
							Replication-2	30	87	94	35	72	36	0.933	0.54	8.29×10 ⁻⁵	
							(Korean)	(14.2)	(41.2)	(44.5)	(24.5)	(50.3)	(25.2)		(0.40–0.74)		
Meta-analysis ^e									0.50	1.28×10 ⁻¹⁵	0.40						
										(0.43–0.60)							

^aMinor allele frequency and minor allele in 198 healthy Japanese (ref#19).

^bOdds ratio of minor allele from two-by-two allele frequency table.

^cP value of Pearson's chi-square test for allelic model.

^dHeterogeneity was tested using general variance-based method.

^eMeta-analysis was tested using the random effects model.

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comparison of HBV patients with HBV resolved individuals (OR = 0.56 for rs3077, 0.66 for rs9277535 and 0.55 for rs9277542) than in the one with healthy controls (OR = 0.55 for rs3077, 0.61 for rs9277535 and 0.51 for rs9277542), which was the opposite result as we expected (Table S2 and Table S4). These results may suggest that other unknown immune system(s) exist to eliminate the HBV in the HBV resolved individuals.

Among the HLA class II loci (*HLA-DPA1*, *HLA-DPB1* and *HLA-DQB2*), which were associated with CHB and HBV clearance, a weak linkage disequilibrium ($r^2 < 0.1$) was observed between *HLA-DQB2* locus and *HLA-DPA1*/*-DPB1* loci in Japanese and Korean populations (Figure S2). We also found that similar linkage disequilibrium blocks (r^2) were observed among three subgroups (HBV carriers, HBV resolved individuals and Healthy controls). Moreover, logistic regression analysis of *HLA-DP* (rs3077 and rs9277542) with use of *HLA-DQ* (rs9276431 and rs768538) as covariates showed that the same level of significant associations of *HLA-DP* with CHB and HBV clearance as shown in the single-point association analysis, while no associations of *HLA-DQ* with $P_{log} > 0.05$ were detected both in Japanese and in Korean (Table S5). These results show that *HLA-DP* is the main genetic factor for susceptibility to CHB and HBV clearance, and the associations of *HLA-DQB2* would result from linkage disequilibrium of *HLA-DPA1*/*-DPB1*.

In this study, we confirmed the significant associations between *HLA-DPA1* and *HLA-DPB1*, and protective effects against CHB and HBV clearance in Japanese and Korean individuals. These results suggest that the associations between the *HLA-DP* locus, CHB and HBV clearance are widely replicated in East Asian populations, including Chinese, Thai, Japanese and Korean individuals; however, there have been no similar GWAS performed in Caucasian and African populations. Moreover,

there were no significant SNPs associated with HCC development in this study, thus suggesting that it is necessary to increase the sample size. To clarify the pathogenesis of CHB or the mechanisms of HBV clearance, further studies are necessary, including a functional study of the *HLA-DP* molecule, identification of novel host genetic factors other than *HLA-DP*, and variation analysis of HBV.

Materials and Methods

Ethics Statement

All study protocols conform to the relevant ethical guidelines, as reflected in the *a priori* approval by the ethics committees of all participating universities and hospitals. The written informed consent was obtained from each patient who participated in this study and all samples were anonymized.

Genomic DNA Samples and Clinical Data

All of the 1,793 Japanese and Korean samples, including individuals with CHB, healthy controls and HBV-resolved individuals (HBsAg-negative and anti-HBc-positive), were collected at 20 multi-center hospitals (liver units with hepatologists) throughout Japan and Korea. The 19 hospitals in Japan were grouped into the following 8 areas: Hokkaido area (Hokkaido University Hospital, Teine Keijinkai Hospital), Tohoku area (Iwate Medical University Hospital), Kanto area (Musashino Red Cross Hospital, Saitama Medical University, Kitasato University Hospital, University of Tokyo), Koshin area (Shinshu University Hospital, Kanazawa University Hospital), Tokai area (Nagoya City University Hospital, Nagoya Daini Red Cross Hospital), Kinki area (Kyoto Prefectural University of Medicine Hospital, National Hospital Organization Osaka National Hospital, Osaka

Table 3. Results of replication study for clearance of hepatitis B virus.

dbSNP rsID	Position			MAF ^a (allele)	Allele (1/2)	Stage (population)	HBV carriers			Resolved individuals			OR ^b 95% CI	P-value ^c	P _{het} ^d
	Chr	Build	36.3 Nearest Gene				11	12	22	11	12	22			
rs3077	6	33141000	HLA-DPA1	0.44 (T)	T/C	GWAS	11	12	22	11	12	22	0.44	9.24 × 10 ⁻⁷	
						(Japanese)	(7.2)	(28.2)	(64.6)	(15.7)	(44.3)	(40.0)	(0.32–0.61)		
						Replication-1	26	95	134	20	64	60	0.72	3.32 × 10 ⁻²	
						(Japanese)	(10.2)	(37.3)	(52.5)	(13.9)	(44.4)	(41.7)	(0.53–0.97)		
						Replication-2	23	81	111	29	48	28	0.41	2.35 × 10 ⁻⁷	
						(Korean)	(10.7)	(37.7)	(51.6)	(27.6)	(45.7)	(26.7)	(0.29–0.58)		
Meta-analysis ^e												0.51	1.56 × 10 ⁻⁴	0.03	
												(0.36–0.72)			
Meta-analysis ^e												0.43	1.89 × 10 ⁻¹²	0.75	
												(0.34–0.54)			
rs9277542	6	33163225	HLA-DPB1	0.45 (T)	T/C	GWAS	18	53	110	28	88	69	0.51	3.15 × 10 ⁻⁵	
						(Japanese)	(9.9)	(29.3)	(60.8)	(15.1)	(47.6)	(37.3)	(0.37–0.70)		
						Replication-1	30	106	118	28	62	52	0.68	1.25 × 10 ⁻²	
						(Japanese)	(11.8)	(41.7)	(46.5)	(19.7)	(43.7)	(36.6)	(0.51–0.92)		
						Replication-2	30	87	94	30	53	22	0.46	4.97 × 10 ⁻⁶	
						(Korean)	(14.2)	(41.2)	(44.5)	(28.6)	(50.5)	(21.0)	(0.33–0.64)		
Meta-analysis ^e												0.55	5.91 × 10 ⁻⁷	0.19	
												(0.43–0.69)			
Meta-analysis ^e												0.49	9.69 × 10 ⁻¹⁰	0.65	
												(0.39–0.61)			

^aMinor allele frequency and minor allele in 198 healthy Japanese (ref#19).

^bOdds ratio of minor allele from two-by-two allele frequency table.

^cP value of Pearson's chi-square test for allelic model.

^dHeterogeneity was tested using general variance-based method.

^eMeta-analysis was tested using the random effects model.

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City University), Chugoku/Shikoku area (Tottori University Hospital, Ehime University Hospital, Yamaguchi University Hospital, Kawasaki Medical College Hospital) and Kyushu area (Kurume University Hospital). Korean samples were collected at Yonsei University College of Medicine.

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 f or G1200; Fujirebio, Inc., Tokyo, Japan). For clinical staging, inactive carrier (IC) 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 portal hypertension. Chronic hepatitis (CH) was defined by elevated ALT levels (>1.5 times the upper limit of normal [35 IU/L]) persisting over 6 months (at least by 3 bimonthly tests). Liver cirrhosis (LC) was diagnosed principally by ultrasonography (coarse liver architecture, nodular liver surface, blunt liver edges and hypersplenism), platelet counts <100,000/cm³, or a combination thereof. Histological confirmation by fine-needle biopsy of the liver was performed as required. Hepatocellular carcinoma (HCC) was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy or a combination thereof.

The Japanese control samples from HBV-resolved subjects (HBsAg-negative and anti-HBc-positive) at Nagoya City University-affiliated healthcare center were used by comprehensive agree-

ment (anonymization in an unlinkable manner) in this study. Some of the unrelated Japanese healthy controls were obtained from the Japan Health Science Research Resources Bank (Osaka, Japan). One microgram of purified genomic DNA was dissolved in 100 µl of TE buffer (pH 8.0) (Wako, Osaka, Japan), followed by storage at -20°C until use.

SNP Genotyping and Data Cleaning

For GWAS, we genotyped a total of 550 individuals, including 181 Japanese HBV carriers, 184 Japanese healthy controls and 185 spontaneously HBV-resolved Japanese individuals (HBsAg-negative and anti-HBc-positive), using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc., Santa Clara, CA), in accordance with the manufacturer's instructions. The average QC call rate for 550 samples reached 98.47% (95.00–99.92%), which had an average sample call rate of 98.91% (93.55–99.74%) by determining the genotype calls of over 900 K SNPs using the Genotyping Console v4.1 software (with Birdseed v1 algorithm) provided by the manufacturer [19]. We then applied the following thresholds for SNP quality control in data cleaning: SNP call rate ≥95% and MAF ≥1% for three groups (HBV carriers, healthy controls and HBV-resolved individuals), and HWE P-value ≥0.001 for healthy controls [20]. Here, SNP call rate is defined for each SNP as the number of successfully genotyped samples divided by the number of total samples genotyped. A total of 597,789 SNPs and 590,278 SNPs on autosomal chromosomes

passed the quality control filters in the genome-wide association analysis using HBV carriers and healthy controls, and using HBV carriers and HBV-resolved individuals, respectively (Figure 1). All cluster plots for the SNPs showing $P < 0.0001$ on association analyses in the allele frequency model were confirmed by visual inspection, and SNPs with ambiguous cluster plots were excluded.

In the following replication stage, we selected a set of 32 SNPs with $P < 0.0001$ in the GWAS using HBV carriers and HBV-resolved individuals. SNP genotyping in two independent sets of 256 Japanese HBV carriers, 236 Japanese healthy controls and 150 Japanese HBV-resolved individuals (Table 1, replication-1), and 344 Korean HBV carriers, 151 Korean healthy controls and 106 Korean HBV-resolved individuals (Table 1, replication-2) was completed for the selected 32 SNPs using the DigiTag2 assay [21,22] and custom TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) on the LightCycler 480 Real-Time PCR System (Roche, Mannheim, Germany).

Statistical Analysis

The observed associations between SNPs and the protective effects on chronic hepatitis B or clearance of hepatitis virus B were assessed by chi-squared test with a two-by-two contingency table in allele frequency model. SNPs on chromosome X were removed because gender was not matched among HBV carriers, healthy controls and HBV-resolved individuals. A total of 597,789 SNPs and 590,278 SNPs passed the quality control filters in the GWAS stage; therefore, significance levels after Bonferroni correction for multiple testing were $P = 8.36 \times 10^{-8}$ ($0.05/597,789$) and $P = 8.47 \times 10^{-8}$ ($0.05/590,278$), respectively. For the replication study, 29 of 32 SNPs were successfully genotyped; therefore, we applied $P = 0.0017$ ($0.05/29$) as a significance level, and none of the 29 markers genotyped in the replication stage showed deviations from the Hardy-Weinberg equilibrium in healthy controls ($P > 0.01$).

The genetic inflation factor λ was estimated by applying the Cochran-Armitage test on all SNPs and was found to be 1.056 and 1.030 in the GWAS using HBV carriers and healthy controls, and using HBV carriers and HBV-resolved individuals, respectively (Figure S3). These results suggest that the population substructure should not have any substantial effect on statistical analysis. In addition, the principal component analysis in a total of 550 individuals in the GWAS stage together with the HapMap samples also revealed that the effect of population stratification was negligible (Figure S4).

Based on the genotype data of a total of 1,793 samples including 1,192 Japanese samples and 601 Korean samples in both GWAS and replication stages, haplotype blocks were estimated using the Gabriel's algorithm using the Hapview software (v4.2) (Figure S2). In the logistic regression analysis, two SNPs (rs9276431 and rs7768538) within the HLA-DQ locus were individually involved as a covariate (Table S5). Statistical analyses were performed using the SNP & Variation Suite 7 software (Golden Helix, MT, USA).

Supporting Information

Figure S1 GWAS using samples from HBV carriers with LC or HCC, and HBV carriers without LC and HCC. P values were calculated using chi-squared test for allele frequencies. (PPTX)

Figure S2 Estimation of linkage disequilibrium blocks in HBV patients, HBV resolved individuals and healthy controls in Japanese and Korean. The LD blocks (r^2) were analyzed using the Gabriel's algorithm. (PPTX)

Figure S3 Quantile-quantile plot for test statistics (allele-based chi-squared tests) for GWAS results. Dots represent P values of each SNP that passed the quality control filters. Inflation factor λ was estimated to be: a) 1.056 in the analysis with HBV carriers and healthy controls; and b) 1.030 with HBV carriers and HBV-resolved individuals. (PPTX)

Figure S4 Principal component analysis on a total of 550 individuals in GWAS, together with HapMap samples (CEU, YRI and JPT). (PPTX)

Table S1 Results for 29 SNPs selected in replication study using samples of HBV carriers and healthy controls. ^a P values by chi-squared test for allelic model. ^bOdds ratio of minor allele from two-by-two allele frequency table. ^cMeta-analysis was tested using additive, two-tailed CMH fixed-effects model. (XLSX)

Table S2 Results of meta-analysis for protective effects against persistent HB infection across 6 independent studies, including this study. ^aMinor allele frequency and minor allele in 198 healthy Japanese (ref#19). ^bOdds ratio of minor allele from two-by-two allele frequency table. ^c P value of Pearson's chi-squared test for allelic model. ^dHeterogeneity was tested using general variance-based method. ^eMeta-analysis was tested using the random effects model. (XLSX)

Table S3 Results for 29 SNPs selected in replication study using samples from HBV carriers and HBV-resolved individuals. ^a P values by chi-squared test for allelic model. ^bOdds ratio of minor allele from two-by-two allele frequency table. ^cMeta-analysis was tested using additive, two-tailed CMH fixed-effects model. (XLSX)

Table S4 Results of meta-analysis for clearance of HBV across 6 independent studies, including this study. ^aMinor allele frequency and minor allele in 198 healthy Japanese (ref#19). ^bOdds ratio of minor allele from two-by-two allele frequency table. ^c P value of Pearson's chi-squared test for allelic model. ^dHeterogeneity was tested using general variance-based method. ^eMeta-analysis was tested using the random effects model. (XLSX)

Table S5 Logistic regression analysis of HLA-DP (rs3077 and rs9277542) and HLA-DQ (rs9276431 and rs7768538) with susceptibility to CHB and HBV clearance using the HLA-DQ genotypes individually as a covariate. (XLSX)

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Author Contributions

Conceived and designed the experiments: NN HS YT. Performed the experiments: HS Y. Mawatari M. Sageshima YO. Analyzed the data: NN MK AK. Contributed reagents/materials/analysis tools: KM M. Sugiyama SHA JYP SH JHK KS M. Kurosaki YA SM MW ET MH SK EO YI EM AT Y. Murawaki YH IS M. Korenaga KH TI NI KHH YT MM. Wrote the paper: NN M. Kawashima YT KT MM.

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No association for Chinese HBV-related hepatocellular carcinoma susceptibility SNP in other East Asian populations

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Abstract

Background: A recent genome-wide association study (GWAS) using chronic HBV (hepatitis B virus) carriers with and without hepatocellular carcinoma (HCC) in five independent Chinese populations found that one SNP (rs17401966) in *KIF1B* was associated with susceptibility to HCC. In the present study, a total of 580 HBV-derived HCC cases and 1351 individuals with chronic hepatitis B (CHB) or asymptomatic carrier (ASC) were used for replication studies in order to evaluate the reported association with HBV-derived HCC in other East Asian populations.

Results: We did not detect any associations between rs17401966 and HCC in the Japanese cohorts (replication 1: OR = 1.09, 95 % CI = 0.82-1.43; replication 2: OR = 0.79, 95 % CI = 0.54-1.15), in the Korean cohort (replication 3: OR = 0.95, 95 % CI = 0.66-1.36), or in the Hong Kong Chinese cohort (replication 4: OR = 1.17, 95 % CI = 0.79-1.75). Meta-analysis using these cohorts also did not show any associations with $P = 0.97$.

Conclusions: None of the replication cohorts showed associations between rs17401966 and HBV-derived HCC. This may be due to differences in the genetic diversity among the Japanese, Korean and Chinese populations. Other reasons could be the high complexity of multivariate interactions between the genomic information and the phenotype that is manifesting. A much wider range of investigations is needed in order to elucidate the differences in HCC susceptibility among these Asian populations.

Keywords: Hepatitis B, hepatocellular carcinoma, candidate SNP, replication study, genome-wide association study

Background

Hepatitis B (HB) is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV), and approximately 360 million people worldwide are thought to be chronically infected with HBV. 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 cirrhosis and

hepatocellular carcinoma (HCC). Although some HBV carriers spontaneously eliminate the virus, 2-10 % of individuals with chronic HB (CHB) develop liver cirrhosis every year, and a subset of these individuals suffer from liver failure or HCC. Around 600,000 new HCC cases are diagnosed annually worldwide, with HCC being relatively common in Asia-Pacific countries and sub-Saharan Africa; more than 70 % of HCC patients are diagnosed in Asia (with 55 % in China) [1]. However, HCC is relatively uncommon in the USA, Europe and Australia [1,2]. The majority of HCC develops in patients with cirrhosis, which is most often attributable

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to chronic HBV infection followed by chronic HCV in the Asia-Pacific region [3].

A recent genome-wide association study (GWAS) using Japanese CHB cases and controls confirmed that 11 SNPs in a region including *HLA-DPA1* and *-DPB1* were associated with CHB [4]. Moreover, a GWAS using chronic HBV carriers with and without HCC in five independent Chinese populations reported that one SNP (rs17401966) in *KIF1B* was associated with HCC susceptibility [5]. In the present study, we performed replication studies using Japanese, Korean and Hong Kong Chinese cases and controls in order to evaluate the reported association with HBV-derived HCC in other East Asian populations.

Results

We performed SNP genotyping of rs17401966 located in the *KIF1B* gene for the purpose of replication analysis of the previous GWAS report [5]. Four distinct cohorts were used for these replication analyses (Table 1). We first examined two independent Japanese case-control samples including 179 cases and 769 controls from Biobank Japan (replication 1), and 142 cases and 251 controls from various hospitals (replication 2). We did not detect any associations between rs17401966 and HCC in the Japanese cohorts (replication 1: OR = 1.09; 95 % CI = 0.82-1.43, replication 2: OR = 0.79; 95 % CI = 0.54-1.15). We further examined Korean case-control samples comprising 164 cases and 144 controls (replication 3) and Hongkongese 94 HCC cases and 187 CHB controls (replication 4), but again did not detect any association (replication 3: OR = 0.95; 95 % CI = 0.66-1.36, replication 4: OR = 1.17; 95 % CI = 0.79-1.75). Logistic regression analysis adjusted for age and gender also did not show any association ($P_{\log} = 0.65, 0.27, 0.11, 0.56$ for each replication

panel). Moreover, we conducted meta-analysis to combine these studies, also not detect any association ($P_{\text{meta}} = 0.97$).

Discussion and conclusions

Zhang et al. [5] reported that SNP rs17401966 was significantly associated with HBV-related HCC (joint OR = 0.61). They conducted a GWAS using 348 cases and 359 controls in a population in Guangxi in southern China, and selected 45 SNPs for the replication study based on the results ($P < 10^{-4}$). In the first replication study, they used 276 cases and 266 controls from Beijing in northern China, and 5 SNPs showed the same direction of association as in the GWAS ($P < 0.05$). They performed a further replication study (of 507 cases and 215 controls) in Jiangsu in eastern China and only one SNP showed the same trend ($P = 3.9 \times 10^{-5}$). Guangdong and Shanghai samples from southern and eastern China were used for further replication studies. The association yielded a p-value of 1.7×10^{-18} on meta-analysis.

We performed four replication analyses using Japanese, Korean and Hong Kong Chinese samples (Table 1). Although sample size of each cohort is smaller than that of the previous GWAS, we conducted meta-analysis of all our study. The result did not show any association between rs17401966 and HBV-derived HCC ($P_{\text{meta}} = 0.97$).

This may be due to differences in genetic diversity among Japanese, Korean and Chinese populations. A maximum-likelihood tree of 126 populations based on 19,934 SNPs showed that Japanese and Korean populations form a monophyletic clade with a 100 % bootstrap value [6]. However, Chinese populations form a paraphyletic clade with two other populations. This indicates that Japanese and Korean populations are genetically closer to one another than the Chinese population.

Table 1 Association between rs17401966 and HBV-derived HCC

cohort	sample size (cases/controls)	cases			controls			HWE p	OR (95 % CI)	P^a	P_{het}^b
		GG	AG	AA	GG	AG	AA				
replication 1 (Japan 1)	179/769	13 (7.2)	61 (34.1)	105 (58.7)	45 (5.9)	261 (33.9)	463 (60.2)	0.599	1.09 (0.82-1.43)	0.578	
replication 2 (Japan 2)	142/251	5 (3.5)	46 (32.4)	91 (64.1)	14 (5.6)	91 (36.2)	146 (58.2)	1	0.79 (0.54-1.15)	0.212	
replication 3 (Korea)	164/144	17 (10.4)	59 (36.0)	88 (53.6)	15 (10.4)	55 (38.2)	74 (51.4)	0.616	0.95 (0.66-1.36)	0.790	
replication 4 (Hong Kong)	94/187	10 (10.6)	39 (41.5)	44 (46.8)	13 (6.9)	80 (42.8)	94 (50.3)	0.767	1.17 (0.79-1.75)	0.432	
Meta-analysis ^c									0.996 (0.84-1.18)	0.965	0.423

^aP value of fisher's exact test for allele model.

^bResult of Breslow-Day test.

^cResults of meta-analysis were calculated by the Mantel-Haenzel method.

We did not find any association with Hong Kong Chinese cohort ($P = 0.43$). Moreover, a study using 357 HCC cases and 354 HBV-positive non-HCC controls in Hong Kong Chinese did not show any significant difference ($P = 0.91$) [7]. Previous population studies have revealed that various Han Chinese populations show varying degrees of admixture between a northern Altaic cluster and a southern cluster of Sino-Tibetan/Tai-Kadai populations in southern China and northern Thailand [6]. Although Hong Kong is located close to the Guangdong (cohort 3 of Zhang et al study), there is great heterogeneity for rs17401966 between Hong Kong cohorts (our study and Chan's study [7]) and Guangdong cohort (our study versus Zhang's study: $P_{\text{het}} = 0.0066$; Chan's study versus Zhang's study: $P_{\text{het}} = 0.035$). This result suggests the existence of other confounding factors, which can differentiate the previous study in China and this study.

One of the possible reasons could be the high complexity of multivariate interactions between the genomic information and the phenotype that is manifesting. HCC development is a multiple process which links to causative factors such as age, gender, environmental toxins, alcohol and drug abuse, higher HBV DNA levels, and HBV genotype variations [8]. The eight HBV genotypes display distinct geographical and ethnic distributions. Genotypes B and C are prevalent in Asia. Specific variations in HBV have been associated with cirrhosis and HCC. These variations include in particular mutations in pre-core region (Pre-C), in basal core promoter (BCP) and in ORF encoding Pre-S1/Pre-S2/S and Pre-C/C. Because there is an overlap between Pre-C or BCP mutations and genotypes, these mutations appear to be more common in genotype C as compared to other genotypes [9].

Aflatoxins are a group of 20 related metabolites and Aflatoxin B1 is the most potent naturally occurring chemical liver carcinogen known. Aflatoxin exposures multiplicatively increase the risk of HCC in people chronically infected with HBV, which illustrates the deleterious impact that even low toxin levels in the diet can have on human health [10–12]. Liu and Wu estimated population risk for aflatoxin-induced HCC around the world [13]. Most cases occur in sub-Saharan Africa, Southeast Asia and China, where populations suffer from both high HBV prevalence and largely uncontrolled exposure to aflatoxin in food. But we could not obtain the information of these confounding factors from both of the previous GWAS study and this study. A much wider range of investigations is thus needed in order to elucidate the differences in HCC susceptibility among these Asian populations.

Methods

Samples

Case and control samples used in this study were collected from Japan, Korea and Hong Kong listed in supplementary

Additional file 1: Table S1. A total of 179 cases and 769 control subjects were analyzed in the first replication study. DNA samples from both CHB controls and HBV-related HCC cases used in this study were obtained from the BioBank Japan at the Institute of Medical Science, the University of Tokyo [14]. Among the BioBank Japan samples, we selected HBsAg-seropositive CHB patients with elevated serum aminotransferase levels for more than six months, according to the guidelines for diagnosis and treatment of chronic hepatitis from The Japan Society of Hepatology (<http://www.jsh.or.jp/medical/guidelines/index.html>). The mean (and standard deviation; SD) age was 62.0 (9.4) years for the cases and 54.7 (13.5) years for the controls. The second Japanese replication sample sets for the cases ($n = 142$) and controls ($n = 251$) study were obtained from 16 hospitals. The case samples for the second replication included 142 HCC patients and the controls included 135 CHB patients and 116 asymptomatic carriers (ASC). The mean (SD) age was 61.3 (10.2) years for the cases and 56.2 (10.9) years for the controls. The Korean replication samples were collected from Yonsei University College of Medicine. The third replication set was composed of 165 HCC patients and 144 CHB patients. The mean (SD) age was 52.2 (8.9) and 37.3 (11.3) years for the cases and controls, respectively. The samples in Hong Kong were collected from the University of Hong Kong, Queen Mary Hospital. The fourth replication set was composed of 94 HCC patients and 187 CHB patients. The mean (SD) age was 58.0 (10.5) and 56.9 (8.3) years for the cases and controls, respectively. All participants provided written informed consent. This research project was approved by the Research Ethics Committees at the Institute of Medical Science and the Graduate School of Medicine, the University of Tokyo, Yonsei University College of Medicine, the University of Hong Kong, National Center for Global Health and Medicine, Hokkaido University Graduate School of Medicine, Teine Keijinkai Hospital, Iwate Medical University, Saitama Medical University, Kitasato University School of Medicine, Musashino Red Cross Hospital, Kanazawa University Graduate School of Medicine, Shinshu University School of Medicine, Nagoya City University Graduate School of Medical Sciences, Kyoto Prefectural University of Medicine, National Hospital Organization Osaka National Hospital, Kawasaki Medical College, Tottori University, Ehime University Graduate School of Medicine, and Kurume University School of Medicine.

SNP Genotyping

For the first replication samples, we genotyped rs17401966 using PCR-based Invader assay (Third Wave Technologies, Madison, WI) [15], and for the second, third and fourth replication samples, we used TaqMan genotyping assay (Applied Biosystems, Carlsbad, CA). In the TaqMan SNP

genotyping assay, PCR amplification was performed in a 5- μ l reaction mixture containing 1 μ l of genomic DNA, 2.5 μ l of KAPA PROBE FAST qPCR Master Mix (Kapa Biosystems, Woburn, MA), and 40 x TaqMan SNP Genotyping Assay probe (ABI) for this SNP. QPCR thermal cycling was performed as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The SNP call rate of each replication panel was 100 %, 100 %, 99.7 % and 99.6 %.

Statistical analysis

We performed Hardy-Weinberg equilibrium test for the case and control samples in each replication study. Fisher's exact test was applied to two-by-two contingency tables for three different genetic models; allele frequency, dominant and recessive model. Odds ratios and confidence intervals were calculated using the major alleles as references. Meta-analysis was conducted using the Mantel-Haenszel method. Heterogeneity among studies was examined by using the Breslow-Day test. Genotype-phenotype association for the SNP rs17401966 was assessed using logistic regression analysis adjusted for age and gender in plink 1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>).

Additional file

Additional file 1: Table S1. Samples used in this study.

Abbreviations

HB: Hepatitis b; HBV: Hepatitis b virus; HCC: Hepatocellular carcinoma; CHB: Chronic hepatitis b; HCV: Hepatitis c virus; GWAS: Genome-wide association study; ASC: Asymptomatic carrier.

Competing interests

The authors declare that they have no competing interests.

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Virological response and safety of 24-week telaprevir alone in Japanese patients infected with hepatitis C virus subtype 1b

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SUMMARY. Hepatitis C virus (HCV) subtype 1b, which infects approximately 70% of Japanese carriers, is likely to be more eradicable by a telaprevir regimen than subtype 1a because of the higher genetic barrier of Val³⁶ and Arg¹⁵⁵ substitutions. The aims of this exploratory study were to evaluate the virological response and safety of 24-week oral administration of telaprevir alone in chronic HCV subtype 1b infection. Fifteen treatment-naïve patients were treated with telaprevir 750 mg every 8 h for 24 weeks. All patients were Japanese whose median age was 58.0 years (range: 45–68), and six patients (40%) were men. Median baseline HCV RNA level was 6.80 log₁₀ IU/mL (range: 3.55–7.10). The HCV RNA levels decreased to undetectable in five patients (33%) within 8 weeks. Three patients (20%) with negative HCV RNA by Week 4 achieved end of treatment response. One patient

(7%) who achieved sustained virological response had a low baseline viraemia of 3.55 log₁₀ IU/mL. Most of the adverse events including anaemia and skin disorders were mild to moderate. Developed variants were T54A and A156V/T/F/Y with or without secondary substitutions rather than V36M ± R155K. Telaprevir alone for 24 weeks in Japanese patients with HCV subtype 1b resulted in a sustained viral response rate of 7% (1/15) and was well tolerated for 24 weeks. These results will support the implementation of further studies on oral combination of telaprevir with other direct-acting antiviral agents in patients infected with HCV subtype 1b.

Keywords: hepatitis C virus, monotherapy, subtype 1b, telaprevir.

INTRODUCTION

The World Health Organization (WHO) estimates that approximately 170 million people are infected with hepatitis C virus (HCV) [1]. In Japan, it is estimated that more than 1.5 million people are chronically infected with hepatitis C.

Telaprevir is a novel peptidomimetic HCV NS3-4A protease inhibitor. The mechanism of inhibition involves the formation of a stable, reversible, covalent bond between the ketocarbonyl of telaprevir and the active site serine of NS3

protease. Recently, telaprevir was approved for patients with HCV genotype 1 infection in the United States (US), Canada, European Union (EU) and Japan. The Phase 3 studies showed that patients who received telaprevir in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV) achieved significantly higher rates of sustained viral response (SVR) compared to those who received PEG-IFN and RBV alone, regardless of their prior treatment experience [2–4]. The Japanese Phase 3 studies of the telaprevir-based triple regimen also showed high SVR rates [5,6]. The most common side effects in the telaprevir-based triple regimen were anaemia, rash and IFN-induced systemic symptoms.

The epidemiology of HCV in Japan takes on a different aspect from US and EU; that is, the majority of patients are aged more than 55 years [7]. Accordingly, the RBV dose reduction rate and the frequency of discontinuation of telaprevir in Japan are higher than those in US and EU [2–6]. Taking such problems with telaprevir in combination with PEG-IFN and RBV into consideration, IFN-free

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DAA, direct-acting antiviral agent; EU, European Union; HCV, Hepatitis C virus; LDL, low-density lipoprotein; LOQ, lower limit of quantification; PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained viral response; T-bil, total bilirubin.

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regimens may become very useful options and satisfy important unmet medical needs especially for intolerant patients with IFN-based regimens. Clinical trials of IFN-free therapy for patients with chronic hepatitis C would provide us with meaningful knowledge for the future development of HCV therapy. Interestingly, HCV subtype 1b, which infects approximately 70% of Japanese HCV carriers [8], is likely to be more eradicable by telaprevir regimens than subtype 1a because of the higher genetic barrier of Val³⁶ and Arg¹⁵⁵ substitutions [9,10]. When treating with direct-acting antiviral agent (DAA), HCV subtypes of genotype 1 are now an important factor that affects treatment response. The main aim of this exploratory study is to evaluate the virological response and safety of telaprevir as monotherapy for 24 weeks in Japanese patients infected with HCV subtype 1b.

PATIENTS AND METHODS

Study design and organization

This Phase 2, single-arm, open-label study was conducted from January 2008 to February 2009 at Sapporo Kosei General Hospital, Musashino Red Cross Hospital, Toranomon Hospital and Hiroshima University Hospital. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices. Before starting the study, the protocol and informed consent forms were reviewed and approved by the institutional review board in each site. All patients provided written informed consent following sufficient explanation before participating in the

study. All the patients received 750 mg telaprevir orally every 8 h (q8h) (2250 mg/day) after a meal for 24 weeks. Telaprevir was given as a 250-mg tablet. This study is registered in ClinicalTrials.gov NCT 00621296.

Patients

Participants enrolled in this study were treatment-naïve, male or female chronic hepatitis C patients with the characteristics shown in Table 1 who met the inclusion criteria and did not conflict the exclusion criteria described previously [11], except the age and HCV RNA levels at the time of enrolment; age from 20 to 70 years and HCV RNA levels were not defined.

Virological responses

Virological response to telaprevir was evaluated based on the HCV RNA kinetics in patients. Serum HCV RNA levels were measured using the COBAS TaqMan HCV test (Roche Diagnostics Co., Ltd., Tokyo, Japan). The linear dynamic range was 1.2–7.8 log₁₀ IU/mL. A qualitative result below the lower limit of quantification (LOQ) was also determined as positive (1.0) and negative (0.5). Measurements were obtained on Week 4 before the first dose, Days 1 (prior to the first dosing) and 3, Weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 of the treatment period, and Weeks 2, 4, 8, 12, 16, 20, and 24 of the follow-up period. Day 1 was defined as the date of starting telaprevir treatment.

Table 1 Patient characteristics, treatment duration and viral response

	Sex	Age	BMI (kg/m ²)	Baseline HCV RNA (log ₁₀ IU/mL)	Treatment duration (day)	HCV RNA Nadir (log ₁₀ IU/mL)	Virological response
1	M	67	25.2	5.85	169 (complete)	Undetectable	Relapse
2	M	59	24.5	3.55	169 (complete)	Undetectable	SVR
3	F	45	18.7	6.80	44*	2.8	Breakthrough
4	F	68	20.9	7.05	43 [†]	<1.2 detectable	Partial responder
5	F	48	21.5	6.45	169 (complete)	Undetectable	Breakthrough
6	F	57	20.9	4.75	43*	1.8	Breakthrough
7	F	51	19.9	5.95	170 (complete)	Undetectable	Partial responder
8	F	58	19.2	6.85	105*	1.5	Breakthrough
9	M	62	20.4	6.25	14 [†]	1.4	Partial responder
10	M	58	24.5	7.10	39*	3.1	Breakthrough
11	M	63	16.2	7.00	74*	<1.2 detectable	Breakthrough
12	F	53	25.0	7.10	169 (complete)	Undetectable	Relapse
13	F	60	19.7	5.00	10 [‡]	<1.2 detectable	Breakthrough
14	F	55	23.8	6.95	78*	<1.2 detectable	Breakthrough
15	M	50	27.5	6.90	26 [‡]	1.3	Partial responder

HCV, Hepatitis C virus; SVR, sustained viral response. Subjects discontinued telaprevir because of *viral breakthrough, [†]AE and [‡]other reasons.

Sustained viral response was defined as an undetectable HCV RNA level at 24 weeks after the end of treatment. Relapse was defined as the reappearance of serum HCV RNA during the follow-up period from the state of undetectable serum HCV RNA at the end of treatment. Breakthrough was defined as the state when the viral level increased by 2 \log_{10} IU/mL from nadir or a level of more than 3 \log_{10} IU/mL after reaching undetectable levels during treatment. Partial responders were subjects whose HCV RNA level dropped by at least 2 \log_{10} IU/mL during treatment but was still detected at the end of treatment.

Sequence analysis at HCV NS3 protease domain

HCV RNA was isolated from serum samples collected on the same day for the measurement of HCV RNA levels. A DNA fragment of 543 bases long (181 amino acids) from the NS3 protease domain was amplified by nested RT-PCR and cloned. At least 39 clones per specimen were sequenced bidirectionally. The limit of detection for the sequencing analysis was 3.0 \log_{10} IU/mL.

Safety assessments

Safety of telaprevir was assessed by clinical laboratory tests, vital signs, abdominal ultrasonography and AEs. Twelve-lead electrocardiogram (ECG) examinations were performed once during the screening period. These safety parameters were reported at regular intervals from 4 weeks before the first dosing to the end of the follow-up period.

Statistical analysis

Statistical analyses were performed using the statistical software SAS Version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Reported AEs were classified according to MedDRA/J version 12.0 (MedDRA Japanese Maintenance Organization, Tokyo, Japan).

RESULTS

Baseline characteristics

Fifteen treatment-naïve patients infected with HCV subtype 1b were enrolled in this study. Baseline characteristics of patients are shown in Table 1. All patients were Japanese whose median age was 58.0 years (range: 45–68); 6 (40.0%) patients were men. Patients over 54 years of age accounted for 66.7% (10 of 15). Median baseline HCV RNA level was 6.80 \log_{10} IU/mL (range: 3.55–7.10). The median BMI was 20.9 kg/m^2 (range: 16.2–27.5).

Virological response

Telaprevir alone caused a rapid decrease in HCV RNA levels after the initiation of treatment in all patients. The average changes were $-3.24 \log_{10}$ IU/mL on Day 3 and $-4.24 \log_{10}$ IU/mL on Week 1 (Fig. 1). The average of maximum reduction in each patient was 5.01 \log_{10} IU/mL. The HCV RNA levels became undetectable in 1, 3, 3 and 5 patients at Weeks 1, 4, 6 and 8, respectively. Three patients with negative HCV RNA after 4 weeks achieved end of treatment response (ETR), of whom one patient achieved a SVR. The patient who achieved SVR had the lowest baseline viral load (3.55 \log_{10} IU/mL) among all the patients.

Ten of 15 patients discontinued the telaprevir treatment because of the following reasons: six patients because of viral breakthrough, two patients because of AEs, one patient because of own drug discontinuation and one patient who met the exclusion criteria after administration.

Safety

AEs observed in two or more patients in this study are shown in Table 2. During the study, 14 of 15 patients experienced 80 AEs in total and 62 events were judged as adverse drug reactions. The common AEs that occurred in

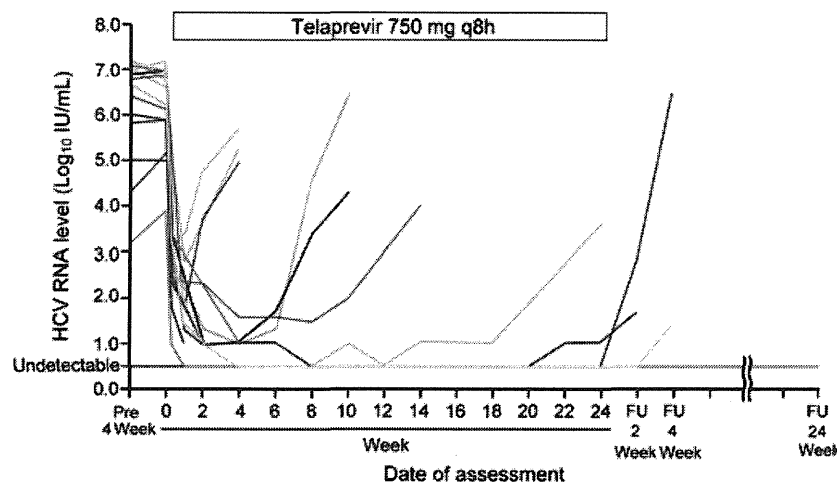


Fig. 1 HCV RNA kinetics during and after treatment with telaprevir monotherapy.

Table 2 Incidence of adverse events that occurred in two or more patients

	N = 15			
	Mild n (%)	Moderate n (%)	Severe n (%)	Total n (%)
Rash	5 (33.3)	3 (20.0)	0 (0.0)	8 (53.3)
Anaemia	7 (46.7)	0 (0.0)	0 (0.0)	7 (46.7)
Low-density lipoprotein increased	6 (40.0)	0 (0.0)	0 (0.0)	6 (40.0)
Blood uric acid increased	4 (26.7)	0 (0.0)	0 (0.0)	4 (26.7)
Pruritus	3 (20.0)	1 (6.7)	0 (0.0)	4 (26.7)
Anorexia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Dysgeusia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Headache	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Diarrhoea	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Pyrexia	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Thirst	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Nasopharyngitis	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood creatinine increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood triglycerides increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Platelet count decreased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Dizziness	1 (6.7)	1 (6.7)	0 (0.0)	2 (13.3)

MedDRA (Ver.12.0).

more than 25% of patients were rash (53.5%), anaemia (46.7%), low-density lipoprotein (LDL) increases (40.0%), blood uric acid increase (26.7%) and pruritus (26.7%). Two patients discontinued telaprevir treatment because of AEs (herpes zoster or rash pruritic). Except for the herpes zoster whose severity was judged as severe and serious, all the

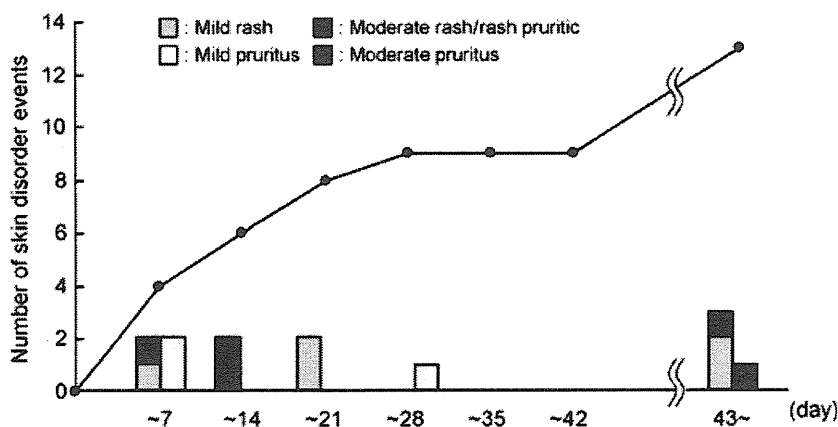
events were mild to moderate. Fifty of the 80 AEs were observed within the first 4 weeks.

In relation to skin AEs, rash, pruritus and rash pruritic were observed in 8, 4 and 1 patients, respectively. The onset day of these events is described in Fig. 2. The range of the onset day was Day 1 to Day 113, and the median was Day 15. Rash in three patients, pruritus in one patient and rash pruritic in one patient were moderate, and the others were mild. One patient discontinued telaprevir at Week 6 because of moderate rash pruritic. Most of the skin AEs were treated with oral antihistamines or topical steroids.

A decrease in haemoglobin levels was observed in all patients (Fig. 3a). Seven of 15 patients developed anaemia during and after the treatment. All anaemia events were mild and no patient needed discontinuation of telaprevir. Uric acid and LDL cholesterol increased during the treatment (Fig. 3b,c), but these changes were mild and no patient needed any medication for these AEs. There were no substantial increases in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (T-bil).

Sequence analysis at HCV NS3 protease domain

Amino acid substitutions in the NS3 protease domain were examined in 39 clones or more in each sample. Before Week 8, V36A/G, T54A and A156T/V as single substitutions, and T54A + R155K and A156T/V + V158I as multiple substitutions were observed. Among two patients who discontinued telaprevir within 2 weeks, all clones but three in one patient were wild-type variants after withdrawal of telaprevir. In three patients who discontinued at Weeks 5–7 because of viral breakthrough, predominant clones possessed A156V/T substitutions after the nadir of viral load. Predominant variants observed during and after telaprevir monotherapy in the eight patients who received telaprevir beyond 8 weeks are shown in Fig. 4 together with HCV RNA levels. In the two patients who showed the lowest HCV RNA level of on Week 4, the predominant clones detected after

**Fig. 2** Rash and pruritus occurrence.

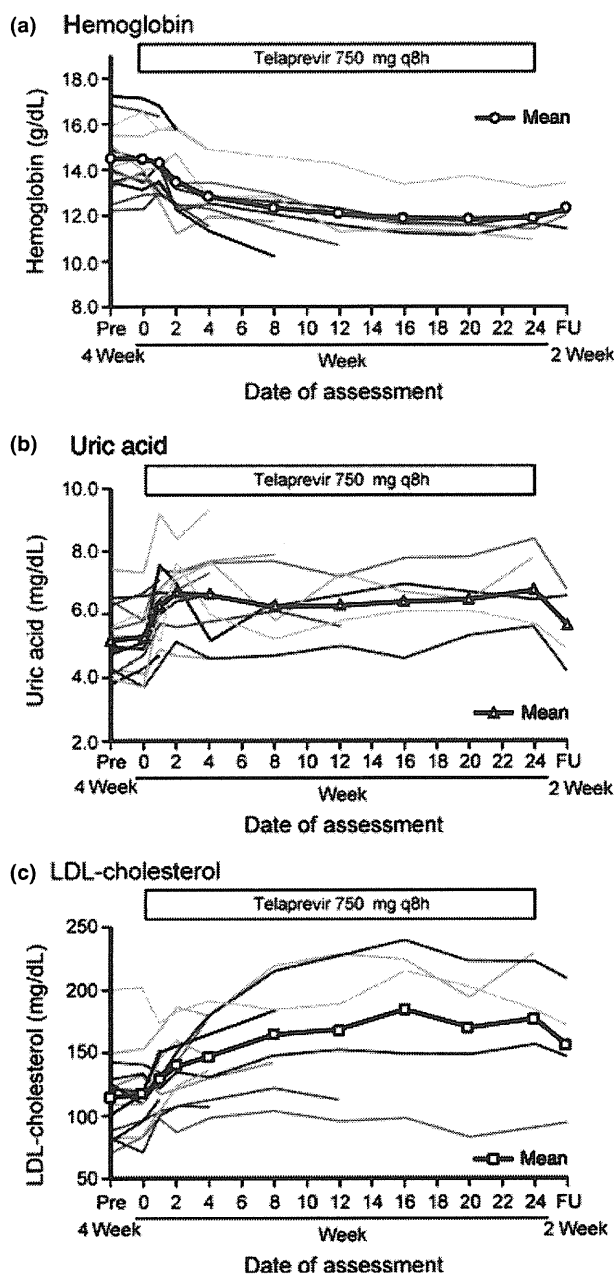


Fig. 3 Changes in (a) hemoglobin, (b) uric acid, (c) LDL-cholesterol.

viral breakthrough were A156F and T54A. One other patient with nadir HCV RNA level on Week 8 had a predominant clone of T54A + I132L after viral breakthrough. Among the five patients who completed the telaprevir treatment for 24 weeks as scheduled, two patients were HCV RNA positive at the end of treatment. One of these two patients had an A156F substitution at the end of treatment, and a A156Y substitution was also detected on Week 1 of the follow-up period. In the two patients who relapsed during the follow-up period, the predominant clone was T54A which shifted to the wild-type variant in one patient.

DISCUSSION

Although higher SVR rates and shorter duration of treatment were achieved by telaprevir in combination with PEG-IFN and RBV in US, EU and Japan [2–6], the DAA combination regimens also increased the frequency and severity of side effects usually observed in the PEG-IFN and RBV therapy. As most patients in Japan are aged people, IFN-free regimens are in urgent need because these patients are intolerant to IFN-based therapies [12–14].

In this exploratory study, one of 15 patients on telaprevir monotherapy was able to achieve SVR. A low viral load of $<4 \log_{10}$ IU/mL in this patient probably contributed to the achievement of SVR, and Suzuki *et al.* [15] published this case report in detail. Although the SVR rate obtained in the study was not beneficial enough, the telaprevir monotherapy could decrease HCV RNA levels dramatically in all cases. The severity of skin-related AEs during telaprevir monotherapy was milder than those of cases developing in the co-administration with PEG-IFN and RBV [5,6,16–18]. All the events were mild to moderate and manageable with antihistamines or topical steroids. Similarly to the skin-related events, decreases in haemoglobin levels were mild, and the incidence of anaemia was 46.7%. As all the anaemia events were mild, there was no need for discontinuation of telaprevir or use of any medications. Severe skin rash and anaemia observed in the therapy with telaprevir in combination with PEG-IFN and RBV are probably ascribable to the synergistic effect of these three drugs. Although the mechanism of uric acid and LDL cholesterol elevation during treatment with telaprevir has been established, these changes disappeared at the end of telaprevir dosing. Telaprevir was generally well tolerated in all the patients.

Amino acid substitutions in the HCV NS3 protease domain were monitored during the study. The relationship between these substitutions and resistance to NS3-4A protease inhibitors has been well documented by *in vitro*, *in vivo* and clinical studies [19–22]. In the eight patients who received the telaprevir monotherapy beyond 8 weeks, the predominant breakthrough variants were T54A and A156F, which were not observed at the earlier time points (Fig. 4). Furthermore, in the clones accounting for more than 10% of each specimen, the secondary substitution of V158I and I132L was identified along with the primary resistant-associated substitution of A156T/V and T54A, respectively, and a novel substitution of A156Y was also observed. This study confirms the higher genetic barrier of HCV subtype 1b against the V36M ± R15K substitutions. Our results clearly indicate that the prolonged telaprevir monotherapy leads to the development of various variants. As the replication fitness of drug-resistant variants tends to be lower than that of wild type, the former are likely to be overtaken by the wild-type virus under drug-free conditions within 3–7 months [11,23,24]. As Ozeki *et al.* [25] reported that four patients with favourable IL28B SNP who failed to eradicate HCV with telaprevir monotherapy were

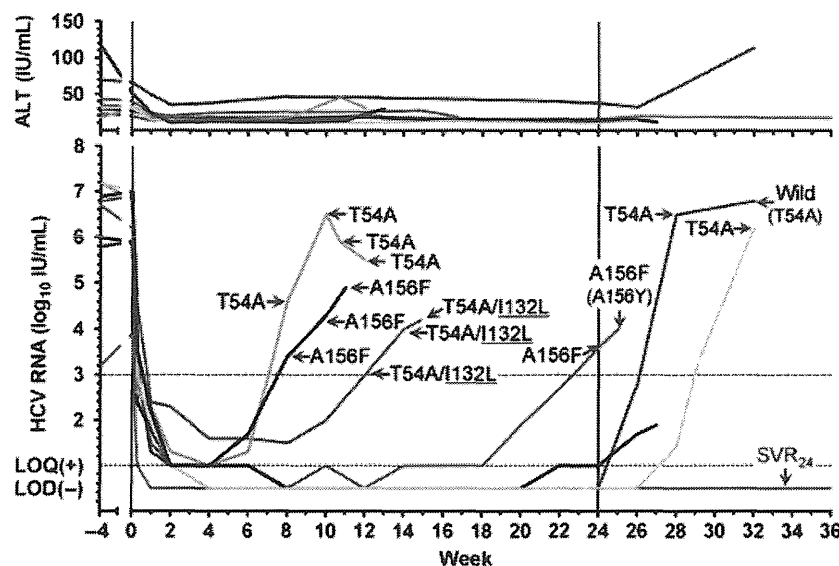


Fig. 4 Viral kinetics and predominant variants during and after telaprevir monotherapy beyond 8 weeks. Besides predominant clones, minority clones which account for 10% and more in a specimen are also summarized by brace notation. Putative secondary resistant-associated mutation is indicated by underline.

responsive to sequential therapy with PEG-IFN and RBV, the substitutions in the NS3 protease domain by the telaprevir treatment are not correlated with resistance to PEG-IFN and/or RBV directly as described previously [23,24]. Sequential therapy with PEG-IFN and RBV after relapse or viral breakthrough on telaprevir monotherapy might be a therapeutic option in some cases, including the case of low haemoglobin. By taking the error-prone nature of HCV replication into account, successful eradication with IFN-free DAA(s) regimens probably depends on how efficiently DAA can suppress various DAA-resistant variants that pre-exist and are selected under DAA pressure. The telaprevir-based combination therapy with other DAA(s) such as NS5A or NS5B polymerase inhibitors may be useful for successful treatment. Using a human chimeric liver mouse model for HCV infection, Ohara *et al.* [26] reported that the combination of telaprevir with a high-dose nucleoside analogue could successfully eradicate HCV infection. Recently, it was reported that the dual therapy with daclatasvir, an NS5A replication complex inhibitor, and asunaprevir, NS3-4A protease inhibitor, had high SVR rates in difficult-to-treat patients with subtype 1b and null responders [27,28]. These successful results are also

helpful for us to consider telaprevir-based IFN-free regimens in combination with other DAAs against HCV.

In conclusion, telaprevir monotherapy was well tolerated and provided potent but temporary antiviral activity in Japanese patients with subtype 1b HCV, with an SVR rate of 7%. Most AEs were mild to moderate and much milder than those recorded in patients on combinations with PEG-IFN and RBV. As the essential characteristics of DAAs including telaprevir are substantially masked in the co-administration with other antivirals, the knowledge obtained from the long-term telaprevir monotherapy is most likely to contribute to the future HCV treatment with DAA-based regimens.

ACKNOWLEDGEMENT AND DISCLOSURES

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インフォームド・コンセント
のための
図説シリーズ

肝炎ウイルス -B型・C型

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Ⓔ 医薬ジャーナル社

informed consent

II. C型肝炎

3. C型肝炎の経過と予後

C型肝炎ウイルスの感染

C型肝炎ウイルスは血液や体液を介して感染するとされ、食べ物などから経口感染することはありません。主に過去の輸血や血液製剤の投与などがきっかけとなっていることが多く、その他、鍼治療、刺青、注射の回し打ちなどが原因として挙げられます。性行為感染や母子感染は比較的少ないといわれています。しかし、感染者の中には感染の原因がはっきりとしない場合も多くみられます。C型肝炎は自覚症状に乏しいため感染している本人も気づかないことも多いことから、身に覚えのない人でも一度は感染しているかどうかの検査をした方がよいといわれています。

C型肝炎ウイルス感染の危険性がある人

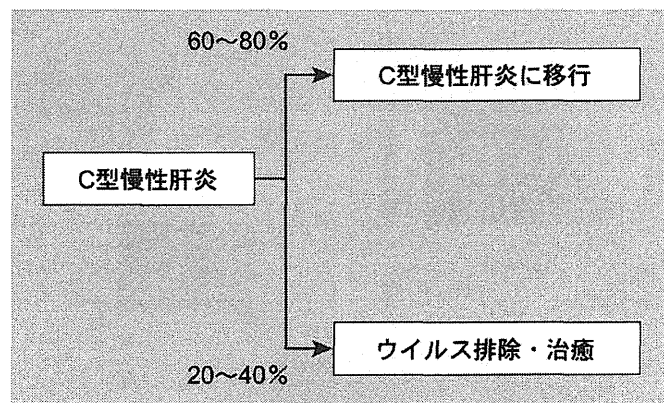
- ・ 1992年以前に輸血や大きな手術を受けたことがある人
- ・ 過去に肝機能異常を指摘されたことがある人
- ・ 1994年以前にフィブリノゲン製剤を投与されたことがある人
- ・ 透析患者
- ・ 頻回の経皮的曝露の危険がある人（鍼、刺青、麻薬常習など）
- ・ HIV感染者
- ・ C型肝炎ウイルス感染者の家族歴を有する人*

*ただし、母児感染は4～10%、夫婦間感染は0～0.6%/年とされ低率です。

(筆者作成)

C型急性肝炎

C型肝炎ウイルスに感染すると、15～150日の潜伏期間のあと急性肝炎を発症し、ALTなどの肝機能の数値が上昇します。急性肝炎は自覚症状がないことが多いですが、時に倦怠感、食欲不振、黄疸がみられることがあります。C型急性肝炎は60～80%の高い確率で慢性肝炎に移行するといわれています。C型肝炎ウイルスの感染が長引き慢性化が心配される場合、インターフェロン治療が行われることがあり、感染から6カ月以内に開始されると90%近い確率でウイルスが消えるとされていますが、現在のところ保険適用はありません。



C型肝炎の慢性化率

(筆者作成)



C型肝炎ウイルス感染から6カ月以内にインターフェロン治療を行うと、90%近い確率でウイルスが消えるとされています。