References

1. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009;41:1105–9.
2. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399–401.
3. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet. 2009;41:1100–4.
4. Ng HH, Bird A. DNA methylation and chromatin modification. Curr Opin Genet Dev. 1999;9:158–63.
5. Kotenko SV, Gallagher G, Baurin VV, et al. IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol. 2003;4:69–77.
6. Sheppard P, Kindsvogel W, Xu W, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol. 2003;4:63–8.
7. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C replication. J Virol. 2005;79:3851–4.
8. Ank N, Iversen MB, Bartholdy C, et al. An important role for type III interferon (IFN-lambda/IL-28) in TLR-induced antiviral activity. J Immunol. 2008;180:2474–85.
9. Davis GL, Wong JB, McHutchison JG, et al. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology. 2003;38:645–52.
10. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C. Nature. 2009;46:798–802.
11. Thompson AJ, Muir AJ, Sulkowski MS, et al. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology. 2010;139:120–9.
12. Kawai T, Akira S. Toll-like receptor and RIG-I-like receptor signaling. Ann NY Acad Sci. 2008;1143:1–20.
13. Loo YM, Owen DM, Li K, et al. Viral and therapeutic control of IFN-β promoter stimulator 1 during hepatitis C virus infection. Proc Natl Acad Sci USA. 2006;103:6001–6.
14. Meylan E, Curran J, Hofmann K, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature. 2005;437:1167–72.
15. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001;2:675–80.
16. Siren J, Pirhonen J, Julkunen I, et al. IFN-α regulates TLR-dependent gene expression of IFN-α, IFN-β, IL-28, and IL-29. J Immunol. 2005;174:1932–7.
17. Ito K, Higami K, Masaki N, et al. The rs8099917 polymorphism, determined by a suitable genotyping method, is a better predictor for response to pegylated interferon-α/ribavirin therapy in Japanese patients than other SNPs associated with IL28B. J Clin Microbiol. 2011;49:1853–60.
18. Sugiyama M, Kimura T, Naito S, et al. Development of interferon lambda 3 specific quantification assay for its mRNA and serum/plasma specimens. Hepatol Res. 2012;42:1089–99.
19. Takahashi H, Ono N, Eguchi Y, et al. Evaluation of acoustic radiation force impulse elastography for fibrosis staging of chronic liver disease: a pilot study. Liver Int. 2010;30:538–45.
20. Lauterbach H, Bathke B, Gilles S, et al. Mouse CD8α$^+$ DCs and human BDCA3$^+$ DCs are major producers of IFN-λ in response to poly IC. J Exp Med. 2009;207:2703–17.
21. Dzionek A, Fuchs A, Schmidt P, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol. 2000;165:6037–46.
22. McHutchison JG, Manns M, Patel K, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology. 2002;123:1061–9.
23. Saito H, Ito K, Sugiyama M, et al. Factors responsible for the discrepancy between IL28B polymorphism prediction and the viral clearance to peginterferon plus ribavirin therapy in Japanese chronic hepatitis C patients. Hepatol Res. 2012;42:958–65.
24. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology. 2010;52:421–9.
25. Thomas E, Gonzalez VD, Li Q, et al. HCV infection induces a unique hepatic innate responses associated with robust production of type III interferons. Gastroenterology. 2012;142:978–88.
26. Fukuhara T, Taketomi A, Motomura T, et al. Variants in IL28B in liver recipients and donors correlate with response to Peg-interferon and ribavirin therapy for recurrent hepatitis C. Gastroenterology. 2010;139:1577–85.
27. Honda M, Sakai A, Yamashita T, et al. Hepatic interferon-stimulated genes expression is associated with genetic variation in interleukin 28B and the outcome of interferon therapy for chronic hepatitis C. Gastroenterology. 2010;139:499–509.
28. Yin Z, Dai J, Deng J, et al. Type III IFNs are produced by and stimulate human plasmacytoid dendritic cells. J Immunol. 2012;189:2735–45.
29. Heil F, Hemmi H, Hochrein H, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 2004;303:1526–9.
30. Diebold SS, Kaisho T, Hemmi H, et al. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004;303:1529–31.
31. Lund JM, Alexopoulou L, Sato A, et al. Recognition of single-stranded RNA viruses by toll-like receptor 7. Proc Natl Acad Sci USA. 2004;101:5598–603.
32. Hemmi H, Kaisho T, Takeuchi O, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 2002;3:196–200.
33. Gorden KB, Gorski KS, Gibson SJ, et al. Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. J Immunol. 2005;174:1259–68.
34. Chang S, Kodys K, Szabo G. Impaired expression and function of toll-like receptor 7 in hepatitis C virus infection in human hepatoma cells. Hepatology. 2010;51:35–42.
35. Dellgren C, Gad HH, Hamming OJ, et al. Human interferon-λ3 is a potent member of the type III interferon family. Genes Immun. 2009;10:125–31.
36. Osterlund PI, Pietilä TE, Veckman V, et al. IFN regulatory factor family members differentially regulate the expression of type III IFN (IFN-λ) genes. J Immunol. 2007;179:3434–42.
37. O'Brien TR. Interferon-alfa, interferon-lambda and hepatitis C. Nat Genet. 2009;41:1048–50.
38. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet. 2001;358:958–65.

39. Picard C, Puel A, Bonnet M, et al. Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science. 2003;299:2076–9.
40. Urban TJ, Thompson AJ, Bradrick SS, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. Hepatology. 2010;52:1888–96.
41. Sugiyama M, Tanaka Y, Wakita T, et al. Genetic variation of the IL28B promoter affecting gene expression. PLoS ONE. 2011;6:e26620.

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IL28B Polymorphisms and Clinical Implications for Hepatitis C Virus Infection in Uzbekistan

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Abstract

Aims: Genome-wide association studies highlighted single nucleotide polymorphisms (SNPs) within the IFNL3/IL28B locus predict the treatment outcome for patients with HCV. Furthermore, SNPs in newly discovered IFNL4 are shown to have population-specific correlation with spontaneous clearance of HCV. The aim of this study was to examine the prevalence and clinical significance of the outlined SNPs in a population from Central Asia, a multi-ethnic region with a developing economy and a high prevalence of HCV infection.

Methods: One hundred and thirty-five chronic HCV patients from Uzbekistan were enrolled. DNA specimens were extracted from peripheral blood mononuclear cells and the IFNL3 SNPs (rs8099917, rs12979860) were genotyped by the Invader Plus assay, the TaqMan assay, and by direct sequence analysis. The IFL4 region (ss469415590) was sequenced.

Results: Of the 135 patients that completed 24 or 48 weeks of treatment with Peg-IFN-α plus RBV, 87.4% were of Central Asian (CA) ancestry and 12.6% were of Eastern European (EE) ancestry. A non-virological response was observed in 21.2% of CA and in 35.3% of EE, respectively ($p<0.32$). The rs12979860 was strongly associated with treatment response (OR, 5.2; 95% CI, 1.9–14.6; $p<0.004$) in the overall sample; however, SNP rs8099917 was the most predictive of outcome for CA group (OR, 6.9; 95% CI, 2.6–18.0; $p<0.002$). The allele frequency of IFNL4 SNP, ss469415590, was identical with that of rs12979860 in all samples.

Conclusions: SNPs in IFNL3 and IFNL4 can be used to predict HCV treatment outcome in a population of Central Asian ancestry.

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Introduction

Chronic Hepatitis C virus (HCV) infection is a global healthcare problem, with thestimated number of people positive for anti-hepatitis C virus antibodies increasing from >122 million to >185 million between 1990 and 2005 [1]. Central and Eastern Asia, North Africa, and the Middle East are thought to have the highest prevalence of anti-HCV antibodies (>3.5%) [1]. Although successful implementation of direct-acting antiviral therapy was recently reported in Western countries, combined treatment with pegylated interferon-alpha (PEG-IFN-α) plus ribavirin (RBV) is still the most effective treatment for patients with chronic hepatitis C in central Asia [2,3]. However, this treatment is both costly and associated with significant adverse side effects, resulting in poor compliance. Furthermore, approximately half of treated patients fail to achieve a sustained virological response (SVR). Of the various host (age, sex, race, fibrosis stage) and viral (genotype, viral load) factors (reviewed in ref 2, 4) associated with the effectiveness of IFN-based therapy, the recently discovered genetic polymorphisms (SNPs) of *interleukin 28B (IL28B)* has been reported. The SNPs had the most significant predictive value for treatment outcomes in several countries [4–6]. The polymorphism in *IL28B* forms a cluster of single nucleotide polymorphisms (SNPs) that appear to delineate a genetic haplotype within a very low recombination fragment containing the *IL28B* gene. Among all the SNPs within this cluster, rs12979860 and rs8099917 are the strongest markers of the haplotype, and consistently predict treatment outcomes for patients receiving IFN-based regimens [7–10]. Recently, a study examining a cohort of African Americans identified a novel *interferon lambda 4 (IFNL4)* gene located in an immediate proximity to the *IL28B*, and suggested that it was associated with HCV clearance [11]. The IFNL4 SNP improved the prediction rate of IFN-based regimens in African Americans, and more recently in Caucasians and Japanese [12–20].

Table 1. Summary of results of genotyping by three different methods.

Total n = 135		No.(%) of cases with genotype by:			
SNP	Genotype	Direct sequencing	Invader	TaqMan	Concordance
rs12979860	CC	57	57	57	
	CT	64	64	64	1
	TT	14	14	14	
rs8099917	TT	90	89	89	
	TG	40	40	40	0.992
	GG	5	6	6	

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Uzbekistan is one of the most populous countries in Central Asia. The HCV infection prevalence in the general population is very high, at >6.4%, and is >20% in "high-risk" groups [21]. The most prevalent HCV genotypes are HCV-1b followed by 3a [22,23]. Because the population of Uzbekistan comprises individuals from many different genetic backgrounds, the aim of this study was to examine the prevalence and clinical relevance of IL28B and IFNL4 polymorphisms in the context of the ethnic ancestry background of populations in this country.

Methods

Study population

Outpatients with chronic HCV infection treated with PEG-IFN-α plus RBV at the Institute of Virology Ministry of Public Health of Uzbekistan between May, 2009 and December, 2011 were enrolled in the study. The study protocol was approved by the Institutional Review Board and Institute of Virology Ministry of Public Health of Uzbekistan. Written informed consent was obtained from all patients. This study conforms to the provisions of the Declaration of Helsinki (as revised in Seoul, Korea, October 2008). The patients and their physicians completed a written questionnaire, which was used to collect socioeconomic, demographic, clinical, and laboratory data. The data were then subjected to a per-protocol analysis. The diagnosis of HCV infection was based on the detection of anti-HCV antibodies. The viral load was determined using the AmpliSens HCV-Monitor-FL (InterLabService Ltd., Moscow) Real-Time PCR kit, which has a detection range limits of 300–10^8 IU/mL (equivalent to 1×10^3–3×10^8, HCV RNA copies/mL).

Patients consisting of 135 subjects received the full treatment course (see below). Data derived from the patients that received at least 80% of the prescribed drug dose were used for the outcome association study. Patients with end-stage kidney disease, hepatocellular carcinoma, or decompensated liver cirrhosis (as defined by a Child-Pugh score greater than 6) were excluded. The ethnic background of each individual was assessed according to the patient questionnaire; patients of Uzbek, Kyrgyz, Kazakh and Tajik ethnicities were included into the Central Asian ancestry (CA) group, patients of Russian and Tatar ethnicities were included into the Eastern Europe (EE) group Other ethnic minorities were excluded from the study.

Table 2. Summary of population completed antiviral treatment for chronic HCV.

	Central Asian			East European			Overall		
	VR	NVR	p	VR	NVR	p	VR	NVR	p
n.	93	25		11	6		104	31	
Age (mean years old ± SE)	39.7±1.5	39.7±3.2	0.437	45.2±3.6	39.3±5	0.358	40.6±1.3	37.9±2.9	0.346
Baseline HCV viral load (mean ×106±SE)	3.1±8.9	4.2±2.3	0.609	1.6±6	1.2±5	0.663	2.9±6.7	3.4±1.7	0.727
HCV genotype (1/non-1)	77/16	22/3	0.759	11	6		88/16	28/3	0.673
Treatment duration (mean months±SE)	7.6±0.2	8±0.5		8.3±0.8	6.0±1.2		7.7±0.5	7.6±0.8	
Drug configuration (IFN/Peg IFN)	38/55	14/11	0.176	7/4	5/1	0.6	59/45	12/19	0.101
IL28B (rs8099917)									
MA: n(%)	71(76.4)	8(32)	<0.001	8(72.7)	3(50)	0.6	79 (76)	11 (35.5)	<0.001
HE&MI: n(%)	22(23.6)	17(68)		3(27.3)	3(50)		25 (24)	20 (64.5)	
IL28B (rs12979860)									
MA: n(%)	47(50.5)	4(16)	0.003	5(45.5)	1(16.6)	0.333	52 (50)	5 (16.1)	<0.001
HE&MI: n(%)	46(49.5)	21(84)		6(54.5)	5(83.4)		52 (50)	26 (83.9)	
IFNL4 (ss469415590)									
MA: n(%)	47(50.5)	4(16)	0.003	5(45.5)	1(16.6)	0.333	52 (50)	5 (16.1)	<0.001
HE&MI: n(%)	46(49.5)	21(84)		6(54.5)	5(83.4)		52 (50)	26 (83.9)	

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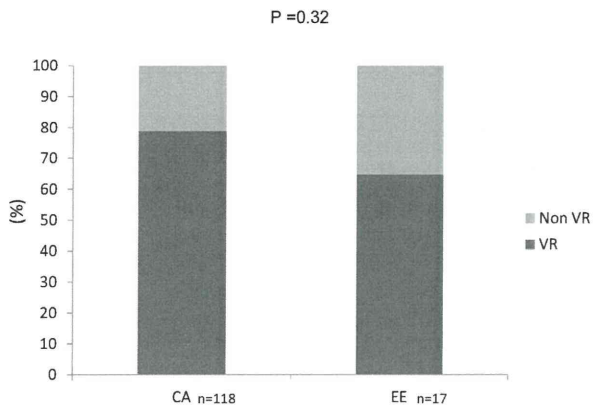

Figure 1. Different HCV treatment outcomes in groups of individuals of central Asian (CA) or eastern European (EE) ancestry. Treatment outcome was measured in terms of virological and non-virological response (VR and NVR, respectively) (see text for details).
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Treatment for hepatitis C

Patients were treated with a weekly dose of PEG-IFN-α (1.5 mcg/kg) coupled with a daily dose RBV (1000 mg/day for patients up to 75 kg, and 1,250 mg/day for those over 75 kg). The viral load was determined by real-time reverse transcription-polymerase chain reaction (RT-PCR) prior to the start of treatment. On-treatment viral kinetics were evaluated at Weeks 4 and 12. To evaluate the power of the SNP genotype as a predictor of responses to antiviral treatment, all patients were classified into one of two groups: (I) non-responders (including those who still had detectable HCV RNA levels at Weeks 4 and 12 or at the post-treatment follow-up [24 weeks after treatment]); and (II) responders (including those with no detectable HCV RNA both during and/or after treatment).

Treatment was stopped if a patient failed to achieve a 2log (or greater) reduction in viral load after 12 weeks.

IL28B genotyping

Whole blood was collected from all participants and centrifuged to separate the buffy coat. Genomic DNA was extracted from the buffy coat (containing peripheral blood mononuclear cells) using a QIAamp DNA Mini Kit (QIAGEN, Venlo, Netherlands).

All patients were genotyped for the SNPs rs8099917, rs12979860, rs8103142, and rs11881222 using a probe-based assay as previously described [24]. Two different probe-based assays, Invader Plus and the TaqMan probe assay, were used, and their sensitivity and specificity were compared with those of direct sequencing. For direct sequencing, the region of genomic DNA around rs12979860 was amplified using primers t63_L (5′-GGAAGGAGCAGTTGCG-3′), t63_R (5′-GGCTGTGGGT-CCTGT-3′), t64_L (5′-GACAGGAACGGGTGTATG-3′), and t64_R (5′-AGCTCTGATGTTGGGAAAG-3′).

Table 3. SNPs showed statistical significance in predicting treatment outcome in studied population.

Ethnic origin	ss469415590 TT	rs8099917 TT	rs12979860 CC
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Central Asian	5.364 (1.7–16.8) *	6.858 (2.6–18.0) *	5.364 (1.7–16.8) *
East EU	4.167 (0.4–48.4)	2.667 (0.3–21.3)	4.167 (0.4–48.4)
Overall	5.2 (1.9–14.6) *	5.745 (2.4–13.6) *	5.2 (1.9–14.6) *

*(p≤0.05).
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Statistical analysis

Data were analyzed using SPSS 17.0 (SPSS for Windows, Chicago, IL). Categorical variables were expressed as numbers and percentages and continuous variables with a normal distribution were expressed as the mean and standard deviation. The Chi-squared and Fisher's exact tests were used where appropriate, and $p<0.05$ was considered statistically significant. Statistical odds ratios (OR) for treatment prediction were derived by logistic regression analysis.

Results

Comparison of the genotyping assays

Genotyping of *IL28B* was performed using the Invader Plus and TaqMan probe-based assays [24], and by direct sequencing. There was 100% concordance between the two assays, and there was 99.2% agreement between the two assays and direct sequencing (i.e., a discrepancy of 0.8%) (**Table 1**). Therefore, we used the broadly-prevalent TaqMan probe assay to examine the association between SNPs and treatment responses in the present study.

Association between SNPs and treatment responses

The characteristics of each patient group are summarized in Table 2. One hundred thirty five patients (87.5% CA, 12.5% EE) completed either 24 or 48 weeks of treatment with Peg-IFN-α plus RBV. There was no significant difference between the groups in terms of age, gender, HCV viral load, and viral genotype (**Table 2**). There was no statistically significant difference between the percentages of CA and EE that showed a NVR (21.2% and 35.2%, respectively; $p<0.32$) (**Fig. 1**). However, there was a significant difference in the prevalence of SNPs within *IL28B* and *IFNL4* between VR and NVR in each ethnic. To evaluate the clinical applicability of individual SNPs, we calculated the predictive ORs for each SNP between VR and NVR in each ethnic (**Table 3**). All of the identified SNPs (favorable genotype) predicted positive response to treatment outcome in the overall study population and in the CA population, but not in the EE population. Interestingly, the polymorphism identified in the newly discovered *IFNL4* gene, ss469415590 [11], showed a strong linkage with the rs12979860 SNP around *IFNL3* in the overall study population; therefore, each had equal predictive value (**Table 3**). The most informative marker to predict VR of HCV treatment outcome was rs8099917 (OR, 5.75; 95% CI, 2.4–13.6, $p<0.001$), followed by rs12979860/ss469415590 (OR, 5.2;95% CI, 1.9–14.6; $p=0.002$). The predictive values of the SNPs are shown here for the entire studied population inclusive all HCV genotypes. There was no significant difference in predictive power (OR) of the SNPs when population was analyzed in the context of different HCV genotypes (1 vs non-1), however statistical power of the analysis was lower, most likely due to the smaller size of the non-1 genotype infected patients in this study.

Genetic differences between ethnic groups

The alleles associated with all of the tested SNPs were in the Hardy-Weinberg equilibrium. The rs12979860, rs8103142, and rs1188122 SNPs showed high linkage disequilibrium (LD) in both

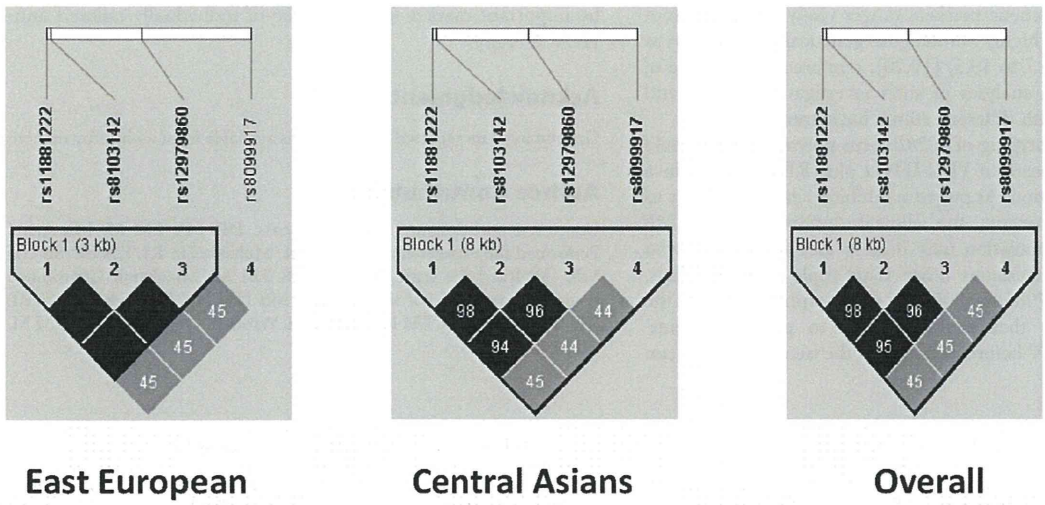

Figure 2. Linkage disequilibrium diagram showing clustering of the studied SNPs. The diagram was generated using HaploView software (available through the HapMap project).
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ethnic groups (**Fig. 2**). There was no difference in the frequency of rs8099917 alleles between the CA and EE populations; however, a minor allele of the rs12979860 SNP was observed more frequently in the EE group (0.32 *vs.* 0.44; $p<0.002$) (**Fig. 3**). The rs8099917 SNP had a higher predictive value than rs12979860/ss469415590 in the CA population (**Table 3**), whereas the reverse tended to be true in the EE population, although the differences were not statistically significant.

Discussion

The aim of this study was to examine the prevalence and clinical significance of SNPs within the *IFNL3/IL28B* and *IFNL4* alleles in a population of HCV-infected patients in Central Asia. We also evaluated the ability of these SNPs to predict responses to anti-HCV treatments in this population. We found that rs12979860 and rs8099917 were informative markers of treatment response in

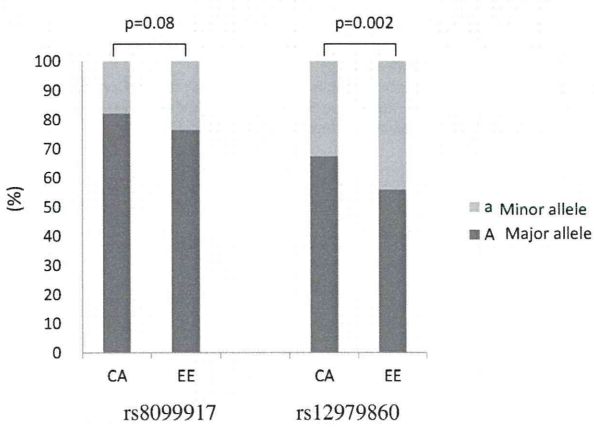

Figure 3. Allele frequencies of the tested SNPs (n = 135). The capital letter "A" represents ancestral ("major") alleles and lower case letter "a" represents mutant alleles ("minor"). CA: population with central Asian ancestry; EE: population with eastern European ancestry.
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Uzbekistan with different ethnicity. The rs8099917 genotype TT was the most common in the overall study population (67.8%), followed by the rs12979860 genotype CC (49%). This is the first report showing the distribution and linkage between the recently described *IFNL4* ss469415590 SNP [11] and the *IL28B* rs12979860 SNP in HCV-infected individuals in Uzbekistan.

According to the Human Haplotype Mapping project, only 15–19% of Caucasians carry the rs8099917 G allele. Notably, the GG genotype of rs8099917 was identified in 3.6% of patients in the present study, a lower prevalence than that observed in other countries [7–10]. These results agree with those of a previous study showing the variability of allele frequencies (2–31%) between different ethnic groups [25].

The rs8099917 SNP was a better predictor of treatment outcome in subjects of CA ancestry than the rs12979860/ss469415590 SNPs (**Table 3**). However, the reverse tended to be true for patients with EE ancestry. This is in agreement with the results of a previous study that examined populations of Western European ancestry [7,10]. A greater number of individuals of Eastern European ancestry must be examined to confirm the trend observed in the present study. Finally, although previous reports show that combined polymorphisms may show increased predictive value in terms of a SVR [26], no significant improvements were noted for the populations examined herein. Interestingly, the degree of LD between rs12979860 and the two SNPs within the IL28-encoding gene identified herein was slightly different in the two populations studied (Fig. 2), i.e., a strong LD was observed among patients of EE ancestry. One possible explanation for this is the smaller size of this patient population. Thus, we need to confirm our findings in a larger cohort. Another interesting observation is that, differently from a previously described Japanese population [9], we found a very low LD between rs8099917 and SNPs within the IL28B-encoding region; nevertheless both the favorable genotype of rs12979860 and rs8099917 were independent predictors of treatment outcome, suggesting the possibility of different mechanisms of involvement of the genetic regions around IL28B.

Predictive power of the SNPs, particularly of the IFNL4 ss469415590 variation reported here was in the range of that reported among Caucasians with HCV/1b [14,15]. The fact that

predictive power of genetic markers ranges vastly across different reports even within a highly homologous genetically population as Japanese (OR from 4.7 to 19.5) [19,20], reinforces importance of replication and meta-analyses of such investigations across and within populations with different ethnic background.

In conclusion, genotyping of *IL28B* locus polymorphisms could help to predict responses to PEG-IFN-α plus RBV therapy in a Central Asian population. As protease inhibitors gain popularity as a form of HCV therapy, the clinical application of *IL28B* genotyping to this population may help to identify patients who might benefit from therapies other than triple therapy. Thus, genotyping the rs12979860/rs8099917 polymorphisms are still the best known markers that could be used to predict patients' responses to IFN/RBV before initiation of the treatment. This can be important marker for the choice of individually tailored anti-HCV therapy.

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Genotyping data reported in this study is available from authors by request.

Author Contributions

Conceived and designed the experiments: DK MS EM M. Mizokami. Performed the experiments: DK MS M. Mukaide DS RL KN SD SS GU MR. Analyzed the data: DK MS NN EM M. Mizokami. Contributed reagents/materials/analysis tools: DK MS NM NN M. Mukaide DS RL KN SD SS GU MR EM M. Mizokami. Wrote the paper: DK MS EM M. Mizokami.

References

1. Hanafiah KM, Groeger J, Flaxman AD, Wiersma ST (2013) Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to hepatitis C virus seroprevalence. Hepatology 57: 1333–1342.
2. Beinhardt S, Rutter K, Stättermayer AF, Ferenci P (2013) Revisiting the predictors of a sustained virologic response in the era of direct-acting antiviral therapy for hepatitis C virus. Clin Infect Dis 56: 118–122.
3. Ghany MG, Strader DB, Thomas DL, Seeff LB (2009) Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 49: 1335–1374.
4. Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M (2010) lambda-Interferons and the single nucleotide polymorphisms: A milestone to tailor-made therapy for chronic hepatitis C. Hepatol Res 40: 449–460.
5. Hayes CN, Imamura M, Aikata H, Chayama K (2012) Genetics of IL28B and HCV-response to infection and treatment. Nat Rev Gastroenterol Hepatol 9: 406–417.
6. Hayes CN, Kobayashi M, Akuta N, Suzuki F, Kumada H, et al. (2011) HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. Gut 60: 261–267.
7. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna K V, et al. (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461: 399–401.
8. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, et al. (2009) IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41: 1100–1104.
9. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, et al. (2009) Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41: 1105–1109.
10. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, et al. (2010) Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 138: 1337–1338.
11. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, et al. (2013) A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet 45: 164–171.
12. Clark PJ, Thompson AJ, McHutchison JG (2011) IL28B genomic-based treatment paradigms for patients with chronic hepatitis C infection: the future of personalized HCV therapies. Am J Gastroenterol 106: 38–45.
13. Clark PJ, Thompson AJ (2012) Host genomics and HCV treatment response. J Gastroenterol Hepatol 27: 212–222.
14. Stättermayer a F, Strassl R, Maieron a, Rutter K, Stauber R, et al. (2014) Polymorphisms of interferon-λ4 and IL28B - effects on treatment response to interferon/ribavirin in patients with chronic hepatitis C. Aliment Pharmacol Ther 39: 104–111.
15. Covolo L, Bibert S, Donato F, Bochud P-Y, Lagging M, et al. (2013) The novel ss469415590 variant predicts virological response to therapy in patients with chronic hepatitis C virus type 1 infection. Aliment Pharmacol Ther: 1–9.
16. Franco S, Aparicio E, Parera M, Clotet B, Tural C, et al. (2013) IFNL4 ss469415590 variant is a better predictor than ILF3 (IL28B) rs12979860 of pegylated interferon-alpha/ribavirin therapy failure in hepatitis C virus/HIV-1 coinfected patients. AIDS: 131–136.
17. Krämer B, Nischalke HD, Boesecke C, Ingiliz P, Voigt E, et al. (2013) Variation in IFNL4 genotype and response to interferon-based therapy of hepatitis C in HIV-positive patients with acute and chronic hepatitis C. AIDS 6: 27–29.
18. Bibert S, Roger T, Calandra T, Bochud M, Cerny A, et al. (2013) IL28B expression depends on a novel TT/-G polymorphism which improves HCV learance prediction. J Exp Med 210: 1109–1116.
19. Nozawa Y, Umemura T, Katsuyama Y, Shibata S, Kimura T, et al. (2014) Genetic polymorphism in IFNL4 and response to pegylated interferon-α and ribavirin in Japanese chronic hepatitis C patients. Tissue Antigens 83: 45–48.
20. Fujino H, Imamura M, Nagaoki Y, Kawakami Y, Abe H, et al. (2013) Predictive value of the IFNL4 polymorphism on outcome of telaprevir, peginterferon, and ribavirin therapy for older patients with genotype 1b chronic hepatitis C. J Gastroenterol.
21. Ruzibakiev R, Kato H, Ueda R, Yuldasheva N, Hegay T, et al. (2001) Risk factors and seroprevalence of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus infection in uzbekistan. Intervirology 44: 327–332.
22. Kurbanov F, Tanaka Y, Avazova D, Khan A, Sugauchi F, et al. (2008) Detection of hepatitis C virus natural recombinant RF1_2k/1b strain among intravenous drug users in Uzbekistan. Hepatol Res 38: 457–464.
23. Kurbanov F, Tanaka Y, Sugauchi F, Kato H, Ruzibakiev R, et al. (2003) Hepatitis C virus molecular epidemiology in Uzbekistan. J Med Virol 69: 367–375.
24. Ito K, Higami K, Masaki N, Sugiyama M, Mukaide M, et al. (2011) The rs8099917 polymorphism, when determined by a suitable genotyping method, is a better predictor for response to pegylated alpha interferon/ribavirin therapy in Japanese patients than other single nucleotide polymorphisms associated with interleukin-28B. J Clin Microbiol 49: 1853–1860.
25. Lindh M, Lagging M, Norkrans G, Hellstrand K (2010) A model explaining the correlations between IL28B-related genotypes, hepatitis C virus genotypes, and viral RNA levels. Gastroenterology 139: 1794–1796.
26. Fischer J, Böhm S, Scholz M, Müller T, Witt H, et al. (2012) Combined effects of different IL28B gene variants on the outcome of dual combination therapy in chronic HCV type 1 infection. Hepatology 55: 1700–1710.

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New Susceptibility and Resistance HLA-DP Alleles to HBV-Related Diseases Identified by a Trans-Ethnic Association Study in Asia

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Abstract

Previous studies have revealed the association between SNPs located on human leukocyte antigen (*HLA*) class II genes, including *HLA-DP* and *HLA-DQ*, and chronic hepatitis B virus (HBV) infection, mainly in Asian populations. *HLA-DP* alleles or haplotypes associated with chronic HBV infection or disease progression have not been fully identified in Asian populations. We performed trans-ethnic association analyses of *HLA-DPA1*, *HLA-DPB1* alleles and haplotypes with hepatitis B virus infection and disease progression among Asian populations comprising Japanese, Korean, Hong Kong, and Thai subjects. To assess the association between *HLA-DP* and chronic HBV infection and disease progression, we conducted high-resolution (4-digit) *HLA-DPA1* and *HLA-DPB1* genotyping in a total of 3,167 samples, including HBV patients, HBV-resolved individuals and healthy controls. Trans-ethnic association analyses among Asian populations identified a new risk allele *HLA-DPB1*09:01* ($P = 1.36 \times 10^{-6}$; OR = 1.97; 95% CI, 1.50–2.59) and a new protective allele *DPB1*02:01* ($P = 5.22 \times 10^{-6}$; OR = 0.68; 95% CI, 0.58–0.81) to chronic HBV infection, in addition to the previously reported alleles. Moreover, *DPB1*02:01* was also associated with a decreased risk of disease progression in chronic HBV patients among Asian populations ($P = 1.55 \times 10^{-7}$; OR = 0.50; 95% CI, 0.39–0.65). Trans-ethnic association analyses identified Asian-specific associations of *HLA-DP* alleles and haplotypes with HBV infection or disease progression. The present findings will serve as a base for future functional studies of HLA-DP molecules in order to understand the pathogenesis of HBV infection and the development of hepatocellular carcinoma.

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Introduction

Hepatitis B virus (HBV) infection is a major global health problem, resulting in 0.5–1.0 million deaths per year [1]. The prevalence of chronic HBV infection varies. About 75% of the chronic carriers in the world live in Southeast Asia and East Pacific [2]. Due to the introduction of vaccination programs, the prevalence of HBV infection in many countries has gradually been decreasing with consequent decreases in HBV-related hepatocellular carcinoma (HCC) [3]. Although some HBV carriers spontaneously eliminate the virus, about 10–15% of carriers develop liver cirrhosis (LC), liver failure and HCC [4]. Moreover, the progression of liver disease was revealed to be associated with the presence of several distinct mutations in HBV infections [5]. Genetic variations in *STAT4* and *HLA-DQ* genes were recently identified as host genetic factors in a large-scale genome-wide association study (GWAS) for HBV-related HCC in China [6].

With regard to the genes associated with susceptibility to chronic HBV infection, *HLA-DP* and *HLA-DQ* genes were identified by GWAS in Japanese and Thai populations in 2009 [7] and 2011 [8], respectively. In addition, our previous GWAS confirmed and identified the association of SNP markers located on *HLA-DPA1* (rs3077) and *HLA-DPB1* (rs9277535) genes with susceptibility to chronic hepatitis B (CHB) and HBV clearance in Japanese and Korean subjects[9]. The significant associations of *HLA-DP* with CHB and HBV clearance have mainly been detected in Asian populations, such as Japanese [8,9], Thai [7], Chinese [10–12], and Korean [9]. In 2012, the association between *HLA-DPA1* gene SNPs and persistent HBV infection was replicated in a Germany non-Asian population for the first time; however, this showed no association with HBV infection [13]. These results seem to be explained by the fact that allele frequencies of both rs3077 (0.155, 0.587 and 0.743 for C allele, on HapMap CEU, JPT, and YRI) and rs9277535 (0.261, 0.558 and 0.103 for G allele, on HapMap CEU, JPT, and YRI) are markedly different between populations. Moreover, the previous study showed that HBsAg seropositivity rates were higher in Thailand and China (5–12%) than in North America and Europe (0.2–0.5%) [2]. These results suggest that comparative analyses of *HLA-DP* alleles and haplotypes in Asian populations would clarify key host factors of the susceptible and protective *HLA-DP* alleles and haplotypes for CHB and HBV clearance. Here, we performed trans-ethnic analyses of *HLA-DP* alleles and haplotypes in Asian populations comprising Japanese, Korean, Hong Kong and Thai individuals. The findings from this study will serve as a base for future functional studies of HLA-DP molecules.

Results

Characteristics of studied subjects

The characteristics of a total of 3,167 samples, including Japanese, Korean, Hong Kong and Thai subjects, are shown in Table 1. Each population included three groups of HBV patients, resolved individuals and healthy controls. The clinical definitions of HBV patients and resolved individuals are summarized in Materials and Methods. Some of the Japanese and all of the Korean samples overlapped with the subjects in our previous study [9,14].

We performed genotyping for *HLA-DPA1* and *HLA-DPB1* in all 3,167 samples, and a total of 2,895 samples were successfully genotyped. The characteristics of successfully genotyped samples are shown in Table S1.

Association of *HLA-DPA1* and *HLA-DPB1* alleles in Asian populations

As for a general Asian population, including 464 Japanese, 140 Korean, 156 Hong Kong, and 122 Thai subjects, five *HLA-DPA1* alleles and twenty-four *HLA-DPB1* alleles were observed (Table S2). The frequencies of *HLA-DPA1* and *HLA-DPB1* alleles were similar between Japanese and Korean subjects. On the other hand, the number of alleles with frequencies of 1–2% was larger in Hong Kong and Thai populations, despite the small sample size. Although the frequencies of *HLA-DP* alleles varied in Asian populations, *HLA-DPB1*05:01* was the most prevalent with over 30% in all populations.

The associations of *HLA-DPA1* and *HLA-DPB1* alleles with chronic HBV infection (i.e., comparison between HBV patients and healthy controls) are shown in Table S2. To avoid false positives caused by multiple testing, the significance levels were corrected based on the numbers of *HLA-DPA1* and *HLA-DPB1*

Table 1. Number of individuals in this study.

Population	Japanese	Korean	Hong Kong	Thai
Total number of samples	1,291	586	661	629
HBV patients	489	340	281	390
IC	114	-	-	-
CH	147	175	187	198
AE	21	-	-	-
LC	38	-	-	-
HCC	169	165	94	192
Mean age (y)	57.1	44.7	57.9	52.0
(min-max)	(20–84)	(18–74)	(32–86)	(21–84)
Gender (M/F)	338/151	265/75	239/42	289/101
Resolved individuals*	335	106	190	113
HCV (−)	249	106	190	113
HCV (+)	86	-	-	-
Mean age (y)	59.7	43.1	40.0	48.2
(min-max)	(18–87)	(12–66)	(18–60)	(39–66)
Gender (M/F)	173/162	61/45	113/77	83/30
Healthy controls	467	140	190	126
Mean age (y)	39.0**	33.7	26.2	46.6
(min-max)	(23–64)	(1–59)	(16–60)	(38–79)
Gender (M/F)	370/97	67/73	87/103	73/53

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

* Resolved individuals were HBsAg negative and HBcAb positive.

** 419 of 467 healthy controls were de-identified, without information on age.

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alleles in the focal population. Briefly, the significance level was set at 0.05/(# of observed alleles at each locus) in each population (see Materials and Methods). With regard to high-risk alleles of *HLA-DPA1*, the most prevalent allele *HLA-DPA1*02:02* was significantly associated with susceptibility to HBV infection in Japanese ($P = 3.45 \times 10^{-4}$; OR = 1.39; 95% CI, 1.16–1.68) and Korean subjects ($P = 2.66 \times 10^{-5}$; OR = 1.89; 95% CI, 1.39–2.58), whereas this association was not observed in Hong Kong or Thai subjects. The association of *HLA-DPA1*02:01* with susceptibility to HBV infection was significant only in Japanese ($P = 2.61 \times 10^{-7}$; OR = 1.88; 95% CI, 1.46–2.41). The significant association of *HLA-DPA1*01:03* with protection against HBV infection was commonly observed among four Asian populations (Table S2). The pooled OR and 95% CI were 0.51 and 0.41–0.63, respectively in a meta-analysis ($P = 3.15 \times 10^{-10}$) (Fig. S1A).

As shown in Table S2, *HLA-DPB1* shows higher degree of polymorphism than *HLA-DPA1*. The most common allele in Asian populations, *HLA-DPB1*05:01*, was significantly associated with HBV susceptibility in both Japanese and Korean subjects. Although *HLA-DPB1*05:01* showed no significant association in the Hong Kong and Thai populations, the same direction of association (i.e., HBV susceptibility) was observed. Meta-analysis of the four populations revealed a significant association between *HLA-DPB1*05:01* and susceptibility to HBV infection ($P = 1.51 \times 10^{-4}$; OR = 1.45; 95% CI, 1.19–1.75) (Fig. S1B). The frequency of *HLA-DPB1*09:01* was significantly elevated in Japanese HBV patients (15.7%) as compared with healthy controls (8.7%) ($P = 3.70 \times 10^{-6}$; OR = 1.94; 95% CI, 1.45–2.62), and this association was most significant (i.e., the smallest P value) in the Japanese population. Because of lower allele frequencies of *HLA-DPB1*09:01* or lack of statistical power in the other populations, no significant associations were observed. A common allele in Thai subjects, *HLA-DPB1*13:01*, was significantly associated with susceptibility to HBV infection ($P = 2.49 \times 10^{-4}$; OR = 2.17; 95% CI, 1.40–3.47) with the same direction of associations in Japanese and Hong Kong (OR = 1.52 and 1.40, respectively).

*HLA-DPB1*04:02* was identified as the most protective allele for HBV infection in Japanese ($P = 1.59 \times 10^{-7}$; OR = 0.37; 95% CI, 0.24–0.55) and Korean subjects ($P = 1.27 \times 10^{-7}$; OR = 0.19; 95% CI, 0.10–0.38). Both *HLA-DPB1*02:01* and *HLA-DPB1*04:01* were also significantly associated with protection in the Japanese population, and the former was significantly associated with protection in Hong Kong subjects ($P = 9.17 \times 10^{-4}$; OR = 0.49; 95% CI, 0.32–0.76). This common allele among four Asian populations, *HLA-DPB1*02:01*, showed a significant association with protection against HBV infection ($P = 5.22 \times 10^{-6}$; OR = 0.68; 95% CI, 0.58–0.81) in a meta-analysis (Fig. S1B).

The frequencies of associated *HLA-DP* alleles in a comparison of HBV patients with healthy controls (Table S2) or with HBV-resolved individuals (Table S3) were similar in all four Asian populations. In the Japanese population, the associations of susceptible and protective *HLA-DPB1* alleles to chronic HBV infection seem weaker in the comparison of HBV patients with HBV-resolved individuals than in the comparison of HBV patients with healthy controls. Moreover, the results of association analyses showed no difference in the comparison of HBV patients with HBV-resolved individuals, including or excluding HCV positive individuals (Table S3). In contrast, the association became stronger in the comparison of HBV patients with HBV-resolved individuals among the Korean subjects. The protective allele *HLA-DPB1*04:01* was also identified to have a strong association with HBV clearance in Hong Kong subjects (Table S3). Moreover, in Hong Kong subjects, the *HLA-DPB1*05:01* associated with the risk for HBV infection showed lower frequency in HBV-resolved individuals (42.9%) than in the healthy controls (48.1%), which accounts for a strong association in the comparison of HBV patients with HBV-resolved individuals ($P = 6.24 \times 10^{-3}$; OR = 1.64; 95% CI, 1.14–2.36). Although the number of samples was insufficient, *HLA-DP*100:01* showed a significant association with protection against HBV infection in the Hong Kong population ($P = 3.05 \times 10^{-6}$; OR = 0.03; 95% CI, 0.0007–0.20).

Table 2. Association of number of *DPB1*02:01* alleles (i.e., 0, 1 or 2) with disease progression in CHB patients assessed by multivariate logistic regression analysis adjusted for age and sex.

Population	P value	OR (95% CI)
Japanese	0.000177	0.47 (0.32–0.70)
Korean	0.025358	0.55 (0.33–0.93)
Hong Kong	0.040842	0.46 (0.22–0.97)
Thai	0.087782	0.58 (0.31–1.08)
All*	1.55×10^{-7}	0.50 (0.39–0.65)

*Population was adjusted using dummy variables.

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As for disease progression in CHB patients among Asian populations, a protective effect of *HLA-DPB1*02:01* on disease progression was observed in the Japanese ($P = 4.26 \times 10^{-5}$; OR = 0.45; 95% CI, 0.30–0.67) and Korean populations ($P = 8.74 \times 10^{-4}$; OR = 0.47; 95% CI, 0.29–0.75) (Table S4). Multivariate logistic regression analysis adjusted for age and sex revealed that the number of *DPB1*02:01* alleles (i.e., 0, 1, or 2) was significantly associated with disease progression in CHB patients in Japanese ($P = 1.77 \times 10^{-4}$; OR = 0.47; 95% CI, 0.32–0.70) (Table 2). Moreover, protective effects of *DPB1*02:01* on disease progression in Asian populations ($P = 1.55 \times 10^{-7}$; OR = 0.50; 95% CI, 0.39–0.65) were detected in a multivariate logistic regression analysis adjusted for age, gender, and population (Table 2).

Associations of *DPA1-DPB1* haplotypes in Asian populations

The estimated frequencies of *HLA DPA1-DPB1* haplotypes are shown in Table S5. The most frequent haplotype among the four Asian populations was *DPA1*02:02-DPB1*05:01*. The number of haplotypes with low frequencies of 1–2% was 10 in both Japanese and Korean subjects, whereas more haplotypes appeared with frequencies of 1–2% in Hong Kong and Thai subjects. The associations of *DPA1-DPB1* haplotypes with HBV infection are shown in Table S5. In the Japanese population, *DPA1*02:01-DPB1*09:01* showed the most significant association with susceptibility to HBV infection ($P = 3.38 \times 10^{-6}$; OR = 1.95; 95% CI, 1.46–2.64). The most common haplotype in the four Asian populations, *DPA1*02:02-DPB1*05:01*, was found to be significantly associated with susceptibility to HBV infection in the Japanese and Korean subjects ($P = 7.40 \times 10^{-4}$; OR = 1.37; 95% CI, 1.14–1.66 for Japanese, and $P = 4.50 \times 10^{-6}$; OR = 2.02; 95% CI, 1.48–2.78 for Korean). In the Thai subjects, *HLA-DPB1*13:01* was the most significant risk allele for HBV infection (Table S2); however, no significant associations were found for the three different haplotypes bearing *HLA-DPB1*13:01*: *DPA1*02:01-DPB1*13:01*, *DPA1*02:02-DPB1*13:01*, and *DPA1*04:01-DPB1*13:01*,indicating that the association of *HLA-DPB1*13:01* with susceptibility to HBV infection did not result from a specific *DPA1-DPB1* haplotype or combination with a specific *DPA1* allele.