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.

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Chronic Hepatitis B Prevalence among Children and Mothers: Results from a Nationwide, Population-Based Survey in Lao People's Democratic Republic

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Abstract

Background: Hepatitis B is regarded as a serious public health issue in Lao People's Democratic Republic (Lao PDR), a Southeast Asian country. However, disease epidemiology among the general population is not well known, and thus a nationwide cross-sectional survey for hepatitis B surface antigen (HBsAg) prevalence in children and their mothers was conducted.

Methods and findings: We applied three-stage cluster sampling using probability proportionate to size. After randomly selecting child (5 to 9 years old) and mother (15 to 45 years old) pairs from the selected villages, questionnaires and HBsAg rapid tests were conducted. Data from 965 child and mother pairs were analyzed. Multivariate logistic regression analyses were used to investigate the independent association of individual background characteristics for the odds of being HBsAg positive. In total, 17 children and 27 mothers were HBsAg positive. HBsAg prevalence was estimated to be 1.7% (95% confidence interval: 0.8%-2.6%) in children, and 2.9% (95% confidence interval: 1.7%–4.2%) in their mothers after taking sampling design and weight of each sample into account. Mother's infection status was positively associated with HBsAg positivity in children ($p<0.001$), whereas other potential risk factors, such as ethnicity, proximity to health centers, and history of surgery, were not. There were no significant associations between mother's HBsAg status and history of surgery, and other sociodemographic factors.

Conclusions: Despite the slow implementation of the hepatitis B vaccination program, HBsAg prevalence among children and their mothers was not high in Lao PDR compared to reports from neighboring countries. The reasons for the differences in prevalence among these countries are unclear. We recommend that prevalence surveys be conducted in populations born before and after the implementation of a hepatitis B vaccination program to better understand the epidemiology of hepatitis B.

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Introduction

More than two billion people have been infected with hepatitis B worldwide, and among these individuals, more than 350 million suffer from chronic hepatitis B virus (HBV) infection [1,2,3]. Infection with HBV results in 600,000 to 1.2 million deaths per year due to chronic hepatitis, cirrhosis, and hepatocellular carcinoma [2,4]. HBV is responsible for 60% to 80% of the world's hepatocellular carcinoma cases, one of the major three causes of death in Africa, Asia, and the Pacific Rim, and accordingly, has been categorized as a Group 1 carcinogenic agent to humans by the International Agency for Research on Cancer [5].

The prevalence of hepatitis B differs throughout the world. Southeast Asian countries have been estimated to have a chronic HBV infection rate of more than 8% before the introduction of hepatitis B vaccination [6]. The Western Pacific region of the World Health Organization (WHO), to which most of the Southeast Asian countries belong, is assumed to have a high prevalence of hepatitis B [7]. Specifically, the prevalence is estimated to be 9% to 12% among women of childbearing age [8] and 8% to 10% among children in pre-vaccine era [9]. The WHO

estimates that the region has 28% of the global population, while it accounts for almost half of all chronic hepatitis B infections worldwide [10].

Hepatitis B vaccination, especially within 24 hours after childbirth, is considered the most effective and efficient preventive measure against hepatitis B infection [3,11]. Based on these assumptions, the WHO set goals to lower the prevalence of chronic hepatitis B among children over 5 years of age to 2% by 2012 and 1% by 2017. To achieve these goals, the WHO plans to increase immunization coverage to 65% for the birth dose and 80% for the third dose of the hepatitis B vaccine [7].

Lao People's Democratic Republic (Lao PDR) is a Southeast Asian country, located in the center of the Indochina peninsula. The country is landlocked and surrounded by China, Vietnam, Cambodia, Thailand, and Myanmar. The neighboring countries report relatively high hepatitis B prevalence compared to other parts of the world. For example, a survey from two provinces in Cambodia reported a hepatitis B surface antigen (HBsAg) prevalence of 7.7% (95% CI: 6.2%–9.3%) among healthy volunteer adults [12]. Another population-based survey in a province in rural Vietnam found that 18.8% (95% CI: 15.7%–21.9%) of adults and 12.5% (95% CI: 9.7%–15.3%) of infants were HBsAg positive at the time of the survey [13]. Thus, Lao PDR has been regarded as one of the hyperendemic countries for hepatitis B for quite some time and is ranked as a priority country by the WHO [7,9] despite a lack of data on the prevalence in a representative population. Pre-vaccine era prevalence was estimated as 11.8% [4], 8–10% [9], or 8% or more [6] for Lao PDR and Indochina countries. In response to this situation, Lao PDR has implemented the hepatitis B vaccine into the routine immunization program since 2002 (at 6, 10, and 14 weeks after birth), as well as birth dosing since 2004. The birth dosing was initiated at referral hospitals in the capital city, and then gradually expanded into rural hospitals (2006), and eventually home deliveries (2010). However, since then, no direct investigation has been conducted, and thus a nationwide survey is warranted [7,9]. The routine immunization coverage is reported as 56% for BCG, 50% for the third DPT, 50% for the third hepatitis B, 40% for measles, and 46% for oral polio vaccine in 2007, when a proportion of target children were born [14].

The primary objective of the present study was to estimate the chronic HBV infection rates by measuring the seroprevalence of HBsAg among children aged 5 to 9 years, and their mothers aged 15 to 45 years.

Methods

Ethical considerations

The survey protocol was reviewed and approved by the Ethical Committee of the Ministry of Health, Lao PDR, and the institutional review board of the National Center for Global Health and Medicine, Japan (NCGM-G-001130-00). Access to selected households was granted by the Ministry of Health, and the provincial and district government authorities.

After obtaining approval to conduct the survey from local authorities, surveyors explained the purpose of the survey to village leaders, selected participants, and their caregivers, assured them that all information would be strictly confidential and that no names would be gathered, and that there would be no benefit or penalties for agreeing or refusing to participate. Written informed consent was obtained from each mother on behalf of her child for each pair. Written informed consent was obtained from legal representatives (next of kin, caregivers, or guardians) when mothers were illiterate. The respondents' names were not recorded on the questionnaire sheets.

Study population

The target population was children aged 5 to 9 years (date of birth: January 2, 2002 to January 1, 2007) and their mothers aged 15 to 45 years (date of birth: January 2, 1966 to January 1, 1997) living in the selected cluster at the time of the survey. The reasons for this selection criteria are: 1) the national and regional hepatitis control policy target is to reduce chronic hepatitis B prevalence among children aged 5 years or older [7]; 2) Lao PDR does not have reliable HBsAg prevalence data among healthy adults, and mothers of childbearing age are considered the major source of hepatitis B infection for children; and 3) our pilot survey revealed that between 20 and 25 mother and child pairs can be practically sampled from each village.

Calculation of sample size

The equation used to calculate the required sample size is as follows [15,16]:

$$n = Z^2 \times p(1-p)DEFF \times 2/(d^2 \times RR)$$

where n = sample size
Z = significance level for 95% confidence
p = expected prevalence
$DEFF$ = design effect
d = precision
RR = response rate

The sample size (n) of 961 was calculated on the basis of an expected HBsAg seroprevalence (p) of 5%, a 5% level of significance (Z), precision (d) of ±2.0%, design effect ($DEFF$) of 2.0, two strata, and response rate (RR) of 95%. For field practicability, we requested 24 survey teams to sample 21 child and mother pairs from each cluster, with the aim of gathering 1,008 pairs in total.

Survey design and sampling

The survey applied a stratified three-stage random cluster sampling design, a type of probability sampling recommended by the WHO [15,17]. The survey was carried out by 24 survey teams (two members per team). Team members were recruited from the same districts that were under investigation to implement the survey more smoothly. The survey teams consisted of epidemiology, surveillance, or laboratory staff. The survey teams were supervised by 11 national personnel (six from the National Immunization Program and five from the National Center for Laboratory and Epidemiology, Ministry of Health) as well as 13 provincial officers.

For stratified multistage cluster sampling, immunization coverage by district and population data were obtained from the National Immunization Program, the Ministry of Health, and the Department of Statistics, Lao PDR. For post-survey weight adjustment, the survey teams obtained the latest population data from village leaders or health volunteers.

All 143 districts in Lao PDR were stratified into two strata, one having high (more than 76%) and the other having low (76% or less) immunization coverage for the third diphtheria, pertussis, tetanus, and hepatitis B (DPT-HepB)vaccines as reported in 2010. For the first stage, we selected 12 districts from each stratum using probability proportionate to size (PPS) sampling based on the population census of 2005. For the second stage, we selected two villages from each selected district by PPS sampling, and 48

villages were randomly sampled in total. In the instances in which the selected village lacked a sufficient number of children or the survey team could not approach the selected village due to safety or security reasons, the nearest village on the way back to the district center was selected. For each selected village, surveyors obtained a list of households, including age and sex, primarily from the poverty reduction program data with the assistance of the village leader, women's union, and/or healthcare volunteer. From these lists, 21 mothers aged 15 to 45 years old with children aged 5 to 9 years were randomly selected using a paper-based lottery system. When a mother had multiple children aged 5 to 9 years old, the youngest child was chosen for the survey. Special attention was paid to ensure that the child's biological mother was surveyed, as adoption is common in rural Lao PDR.

The survey was carried out from January 25th to February 4th, 2012. Each survey team successfully approached their assigned villages, with the exception of one village, which could not be visited because of road difficulties. An alternative village was chosen according to the predetermined selection criteria. In total, 1,008 children and 1,008 mothers were sampled. The overall response rate for HBsAg was 100%; however, 43 pairs were excluded from the analysis due to age ineligibility. That is, one child was over 9 years of age and 33 were less than 5 years of age. Furthermore, three mothers were over 45 years of age and six were less than 15 years of age. This happened as 43 mothers confused calendar age with traditional age. In rural areas, newborns start at one year old and a year is added to their age for each passing of a Lunar New Year. The surveyors asked participants for their age in years and their date of birth, and checked that they matched. A total of 965 pairs were included for analysis.

Questionnaires

A brief face-to-face questionnaire was administered to the sampled mother. The questionnaire consisted of 25 questions in four domains of inquiry: sociodemographic background of the family (i.e., ethnicity, family head's occupation, and mother's education level), family history of liver diseases, including mother, demographic characteristics of the child (i.e., age, sex, and place of birth), and immunization records. Additionally, questions were asked regarding exposure to potential risk factors for acquiring hepatitis B infection (e.g., history of blood transfusion, surgical operation, and sharing of toothbrush). The questionnaire was developed in English, translated into Lao, back-translated into English, and then compared and revised by bilingual staff members. A small pilot test was conducted prior to the data collection.

Testing for HBsAg

We used a simple and rapid test (Alere Determine HBsAg test card; Alere Medical Co. Ltd., Chiba, Japan) rather than the traditional ELISA test, as it was better suited to use in the field [14]. The sensitivity and specificity of the test were reported as high in two Asian countries [18,19]. In Vietnam, the Determine HBsAg test validity was measured based on comparison with HBsAg EIA. Results were 100% in both sensitivity and specificity in 328 samples [18]. In China, the Determine HBsAg performance was evaluated in comparison with HBsAg EIA for 671 samples. The sensitivity was reported to be 98.9% and specificity 100% [19]. The Determine HBsAg examination kit is one of the most reliable point-of-care HBsAg tests, and is recommended by the WHO [15]. HBsAg testing was performed according to the manufacturer's instructions. Blood was collected from a finger prick using a safety lancet (BD Safety Lancet, Becton Dickinson, NJ, USA) and glass capillary tube, and the blood was applied onto the sample pad of the rapid test kit. After applying the chase buffer, surveyors assessed the results after at least 15 minutes, but no longer than 24 hours. When no control bar appeared after 15 minutes, the test results were considered invalid, and the test was repeated. Blood spots were collected onto filter paper for further testing. A 2-day training session was organized for surveyors and supervisors on the use of the rapid test and the completion of the questionnaire. To ensure the safety of the blood collection procedure, surveyors always used a new pair of latex gloves. Surveyors were instructed to place all capillary tubes and lancets into safety boxes immediately after use.

Data entry and statistical analysis

All of the completed questionnaires were brought to a centralized location and the data were entered into a Microsoft Excel 2007 spreadsheet. Data were double-entered and cross-checked. Logistic regression tests and odds ratios were used to examine the relationship between the independent variables and HBsAg results. Multivariate logistic regression was used to investigate the independent association of different household and individual characteristics with the odds of being HBsAg positive. All estimates and standard errors were calculated by taking the multistage clustered sampling design and the weight of each sample into account to give representative, unbiased results. A p value <0.05 was considered statistically significant.

In our regression analyses, we adjusted for potential confounders by using the following variables: third DPT-HepB immunization coverage at the location of current residence, mother's age, ethnic group, mother's education level, family head's occupation, and mother's HBsAg status. For multivariate logistic regression analyses, multicollinearity was tested by calculating the variance inflation factors for each independent variable, and a value of more than 10 was considered as having multicollinearity.

All statistical analyses were carried out using STATA version 12 (Stata Corp., College Station, TX). Means and proportions were calculated using STATA's 'svy' function, with each sample weighted according to estimated population size.

Results

Socioeconomic backgrounds

The baseline characteristics of the 965 mothers and their children are summarized in Table 1. The mean age of the mothers was 29.1 years (95% CI: 26.2–33.1), and the mean age of the children was 5.8 years (95% CI: 5.4–6.3). Of the sampled children, 474 (49.4%) were male and 486 (50.6%) were female (five were unknown).

HBsAg prevalence among the general population

Of the 965 pairs included in the study, 17 children and 27 mothers were positive for HBsAg. Six child and mother pairs were HBsAg positive. The estimated prevalence was 1.7% for children (95% CI: 0.8%–2.6%) and 2.9% for mothers (95% CI: 1.7%–4.2%) after taking the sampling design and weight of each sample into account. HBsAg prevalence did not change significantly between DPT-HepB3 high and low coverage districts in both children and mothers (Table 2).

Potential risk factors

To determine whether background characteristics affect HBsAg status, we conducted multivariate logistic regression analysis in children and their mothers. In children, the mother's HBsAg status was positively associated with hepatitis B infection (Table 3),

Table 1. HBsAg prevalence among children (5 to 9 years old) and mothers (15 to 45 years old) in Lao PDR by selected background characteristics.

		n	%	Children's HBsAg (+)	%	95% CI	Mothers' HBsAg (+)	%	95% CI
Mothers' age(n = 965)	15–19	4	0.41	0	0.00		0	0.00	
	20–24	85	8.80	1	1.18	0.00–3.52	3	3.53	0.00–7.53
	25–29	294	30.47	7	2.38	0.63–4.13	8	2.72	0.85–4.59
	30–34	275	28.50	6	2.18	0.44–3.92	9	3.27	1.16–5.39
	35–39	176	18.24	3	1.70	0.00–3.64	3	1.70	0.00–3.64
	40–45	131	13.58	0	0.00		4	3.05	0.07–6.04
Ethnicity (n = 963)	Low land Lao	651	67.60	9	1.38	0.48–2.28	19	2.92	1.62–4.22
	Mid land Lao	248	25.75	6	2.42	0.49–4.34	5	2.02	0.25–3.78
	High land Lao	64	6.65	2	3.13	0.00–7.51	3	4.69	0.00–10.01
[1]Transportation (n = 939)	on foot	298	31.74	1	0.34	0.00–1.00	6	2.01	0.41–3.62
	bicycle	14	1.49	0	0.00		0	0.00	
	motor bike	364	38.76	7	1.92	0.51–3.34	10	2.75	1.06–4.43
	car	183	19.49	5	2.73	0.35–5.12	6	3.28	0.67–5.88
	hand tractor	66	7.03	3	4.55	0.00–9.71	4	6.06	0.15–11.97
	other	14	1.49	0	0.00		0	0.00	
[2]Time (n = 901)	< 5 minutes	31	3.44	0	0.00		1	3.23	0.00–9.81
	5 to 15 minutes	274	30.41	3	1.09	0.15–2.33	6	2.19	0.45–3.93
	15 to 30 minutes	231	25.64	5	2.16	0.27–4.06	11	4.76	2.00–7.53
	30 to 60 minutes	209	23.20	5	2.39	0.30–4.48	4	1.91	0.04–3.79
	> 60 minutes	156	17.31	3	1.56	0.00–4.68	4	2.56	0.06–5.07
[3]Education (n = 962)	did not finish primary school	307	31.91	7	2.28	0.60–3.96	12	3.91	1.73–6.09
	primary school	374	38.88	5	1.34	0.17–2.51	10	2.67	1.03–4.32
	junior high	185	19.23	3	1.62	0.00–3.46	2	1.08	0.00–2.59
	high school	73	7.59	0	0.00		1	1.37	0.00–4.10
	college/univ	20	2.08	1	5.00	0.00–15.47	2	10.00	0.00–24.41
	other or unknown	3	0.31	1	33.33	0.00–100.00	0	0.00	
[4]Occupation (n = 963)	farmer	683	70.92	13	1.90	0.88–2.93	19	2.78	1.55–4.02
	fisherman	5	0.52	0	0.00		0	0.00	
	laborer	92	9.55	1	1.09	0.00–3.25	5	5.43	0.71–10.16
	public officer	88	9.14	1	1.14	0.00–3.40	3	6.25	1.70–10.80
	factory employee	8	0.83	0	0.00		0	0.00	
	general employee	16	1.66	1	6.25	0.00–19.57	0	0.00	
	merchant	63	6.54	1	1.59	0.00–4.76	0	0.00	
	others	8	0.83	0	0.00		0	0.00	
Mother's surgery (n = 962)	yes	95	9.88	2	2.11	0.00–5.05	3	3.16	0.00–6.74
	no	867	90.12	15	1.73	0.86–2.60	24	2.77	1.67–3.86
Child's sex (n = 960)	male	474	49.38	9	1.89	0.67–3.13			
	female	486	50.63	7	1.44	0.38–2.50			
Place of delivery (n = 961)	province hospital	207	21.54	4	1.93	0.04–3.82	6	2.90	0.59–5.20
	district hospital	105	10.93	2	1.90	0.00–4.56	5	4.76	0.62–8.90
	health center	10	1.04	0	0.00		0	0.00	
	private clinic	11	1.14	0	0.00		1	9.09	0.00–29.35
	at home	569	59.21	8	1.41	0.44–2.38	14	2.46	1.18–3.74
	in the forest	56	5.83	3	5.36	0.00–11.44	1	1.79	0.00–5.36
	other health facility	3	0.32	0	0.00		0	0.00	
Child's surgery (n = 960)	yes	22	2.29	0	0.00				

Table 1. Cont.

	n	%	Children's HBsAg (+)	%	95% CI	Mothers' HBsAg (+)	%	95% CI
no	938	97.71	16	1.71	0.88–2.54			

[1]Transportation to the nearest health facility, [2] Time to the nearest health facility, [3] Mothers' completed education, [4] Family head's occupation.
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whereas the other potential risk factors were not associated according to the adjusted odds ratio. We did not obtain information regarding the type of delivery, and we did not find significant differences in HBsAg prevalence associated with delivery settings. No independent factor was positively associated with HBsAg positivity in mothers, according to the adjusted odds ratio (Table 4).

Immunization status

Written immunization records were available for 213 out of 965 children (22.1%). One hundred ninety eight children were vaccinated with three doses of hepatitis B vaccine, and 34 children were immunized on the day of birth or the following day. Five out of 213 children with immunization records were HBsAg positive (2.35%; 95% CI: 0.30–4.40%), while 12 of 752 without immunization records were HBsAg positive (1.60%; 95% CI: 0.70–2.49%). The differences between the two groups were not significant ($p = 0.46$).

Discussion

HBsAg prevalence among the general population

The estimated HBsAg prevalence in the general population was much lower in both children and adults than that of previous reports from neighboring countries and Lao PDR. For example, HBsAg prevalence in adults in Cambodia, Thailand, and Vietnam was reported to be 7.7% (95% CI: 6.2%–9.3%) [12], 6 to 10% [15,20], and 18.8% (95% CI: 15.7%–21.9%) [13], respectively. Data on HBsAg prevalence amongst children was relatively scarce, and reported to be 3.5% (95% CI: 2.4%–4.8%) in Cambodia [21], and 18.4% (95% CI: 13.4%–23.4%) in Vietnam [13]. In Lao PDR, studies in blood donors, hospitalized patients, and Lao migrant workers tested in Thailand showed HBsAg prevalence of 8.73% (95% CI: 8.69%–8.77%) [22], 17.99% (95% CI: 17.81%–18.17%) [23], and 6.86% (95% CI: 6.80%–6.92%) [24] based on the given numerators and denominators in the articles, respectively.

Since the study objective was to estimate the nationwide HBsAg prevalence among the general population of Lao PDR, and thus the study design is a cross sectional survey, it is difficult to explain the reasons for the unexpectedly low prevalence. There are several potential explanations for this observation. The survey methodology used was very different from that used for blood donors, patients, and migrant workers. We used probability sampling and thus the results are representative of the whole population, whereas studies of blood donors, hospitalized patients, and migrant workers used non-probability sampling and therefore the results are restricted to these populations. The primary objective of our survey was to estimate HBsAg prevalence among the general population, so probability sampling was the most appropriate choice. Demographic conditions among the sampled population are determined by survey methodology, and therefore the results showed discrepancy. The WHO strongly recommends probability sampling for hepatitis B prevalence survey [7,15,17]. Although Lao PDR has the lowest population density of the Indochina peninsula countries [25], the precise effects on hepatitis B prevalence of the reduced frequency of human to human contact due to the country's relatively low population density and less developed infrastructure remain unclear.

The number of HBsAg positives varied from 0 to 4 per cluster. Since the sampling design of the survey aimed to estimate the prevalence in the whole country, it is difficult to determine whether these differences reflect the local endemic status.

Potential risk factors

Our survey revealed that no potential risk factors were significantly associated with the children's infection status, with the exception of the mothers' hepatitis B infection status. HBsAg prevalence surveys in other countries revealed that history of surgery [26,27], level of education [26], and ethnicity [28] were independently associated with hepatitis B infection. The reason why we could not find any potential risk factors positively associated with hepatitis B infection among children is not clear. However, it should be noted that the primary objective of the present study was to assess HBsAg prevalence, and not its risk factors. Additionally, some reports found that HIV positive individuals are positively associated with hepatitis B virus infection

Table 2. HBsAg prevalence among children (5 to 9 years old) and mothers (15 to 45 years old).

	Children's HBsAg (+)	%	95% CI	Standard error	Design effect	Mothers' HBsAg (+)	%	95% CI	Standard error	Design effect
High coverage districts (n = 486)	6	1.14	0.23–2.04	0.44	0.82	18	3.79	1.79–5.79	0.97	1.24
Low coverage districts (n = 479)	11	2.39	0.75–4.03	0.79	1.27	9	1.88	0.49–3.37	0.69	1.22
Total (n = 965)	17	1.72	0.81–2.63	0.44	1.10	27	2.93	1.65–4.20	0.61	1.28

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Table 3. Unadjusted and adjusted odds ratio for being HBsAg positive among children from five to nine years old in Lao PDR by selected background characteristics.

		Unadjusted odds ratio	95% CI	p	Adjusted odds ratio	95% CI	p
DPT3 coverage	high	1(reference)					
	low	2.13	0.73–6.21	0.16	3.47	0.77–15.64	0.10
Mothers' age	15 to 29	1(reference)					
	30 to 45	0.70	0.28–1.78	0.44	0.87	0.31–2.47	0.79
Ethnicity	Low land Lao	1(reference)					
	others	1.90	0.67–5.40	0.22	1.41	0.26–7.72	0.68
Education	none	1(reference)					
	finished primary school or upper	1.50	0.67–3.36	0.30	1.03	0.27–3.89	0.96
Occupation	white collar	1(reference)					
	blue collar	1.15	0.37–3.64	0.80	0.60	0.18–1.96	0.38
Sex	male	1(reference)					
	female	0.75	0.21–2.62	0.63	0.65	0.21–2.08	0.46
Birth place	health facility	1(reference)					
	non-health facility	0.98	0.39–2.49	0.97	0.79	0.28–2.21	0.64
Mothers' HBsAg	negative	1(reference)					
	positive	24.02	9.45–61.07	0.00	28.13	10.21–77.53	0.00

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[29,30]; however, we did not investigate HIV due to limited budget.

WHO's regional target

The interim target of the WHO is to reduce HBsAg prevalence to less than 2% in children aged at least 5 years old by 2012 [7,31]. The point prevalence is used for monitoring the control of hepatitis B. The Regional Office for the Western pacific recommended that the country conduct a national HBsAg prevalence survey to verify whether the country has reached the regional prevalence target [9]. Following these criteria, Lao PDR had already achieved its goal. However, it is unlikely that Lao PDR achieved the target through the immunization program alone because the country has the lowest immunization coverage of all countries in the region [7,9]. Considering the relatively lower HBsAg seroprevalence among the mothers compared to those reported in previous studies, it is likely that Lao PDR had a lower prevalence even before the introduction of the hepatitis B immunization program. Therefore, the final target of reducing HBsAg prevalence to less than 1% in children aged at least 5 years could be difficult to achieve if the country simply continues its current immunization policy.

A nationwide prevalence survey targeting the general population is ideally conducted before implementing the immunization

Table 4. Unadjusted and adjusted odds ratio for being HBsAg positive among mothers from 15 to 45 years old in Lao PDR by selected background characteristics.

		Unadjusted odds ratio	95% CI	p	Adjusted odds ratio	95% CI	p
DPT3 coverage	high	1(reference)					
	low	0.50	0.20–1.28	0.14	0.47	0.19–1.16	0.10
Mothers' age	15 to 29	1(reference)					
	30 to 45	1.03	0.43–2.51	0.94	0.94	0.39–2.25	0.88
Ethnicity	Low land Lao	1(reference)					
	others	0.80	0.30–2.17	0.65	0.68	0.25–1.85	0.44
Education	none	1(reference)					
	finished primary school or upper	1.68	0.70–4.01	0.23	2.04	0.89–4.68	0.09
Occupation	white collar	1(reference)					
	blue collar	1.71	0.53–5.55	0.35	1.93	0.68–5.50	0.21
History of surgery	no	1(reference)					
	yes	1.28	0.39–4.25	0.67	1.30	0.35–4.78	0.68

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strategy to evaluate hepatitis B epidemiology. However, we were able to understand the epidemiology to some degree, even after implementation of immunization policy, because adults usually represent the pre-vaccination era [15,17].

Strengths of the study

The present study is the first nationwide survey on the prevalence of hepatitis B in the general population both before and after the implementation of a hepatitis B immunization policy in Lao PDR and other Southeast Asian countries. We applied multistage stratified cluster sampling to better represent the general population. The design effect of prevalence was calculated between 0.8 and 1.3, which was acceptable as we set it around 2.0 before the survey.

The background characteristics of our sampled population were similar to those of another nationwide population-based study, the Lao PDR Reproductive Health Survey (LRHS) [32] conducted in 2005. For example, the locations of current residence (north, central, and south) were 33.3%, 41.7%, and 25.0% in our survey, and 38.6%, 38.9%, and 22.5% in the LRHS. The levels of mothers' completed education (none, primary school, secondary school or more) were 31.9%, 38.9%, and 29.2% in our survey, and 28.8%, 43.7%, and 27.5% in the LRHS. The LRHS applied the multistage stratified cluster sampling method and surveyed more than 13,000 women all over the country. A direct comparison of the populations sampled by the two different surveys is difficult to perform as the primary objectives were different. Despite this, our sampled population is considered to likely represent the general population in Lao PDR.

Limitations of the study

There are several limitations in our study that should be addressed. First, the population data is based on the census conducted in 2005. After 2005, the population distribution may have changed and some of the villages could have merged, thereby creating bias in the findings. Fortunately, we did not survey any villages that disappeared or merged.

Second, floating or marginal populations are likely to be missed from the residential lists, and these populations could be a source of HIV and hepatitis B virus infections [33]. In future seroprevalence surveys, these subpopulations should be accounted for by using specific approaches, such as oversampling.

Third, population immunity levels were difficult to measure or estimate. The possession of immunization certificates was low, because many participants had already finished their scheduled vaccinations before 12 months of age, and relevant documents were lost. In the present study, we did not have enough data from health centers due to time and budget limitations. Since we did not examine immunization markers, such as HBsAb, herd immunity levels are unknown.

Lastly, adult men were not included in the survey. Serological studies in the past indicated that men have higher HBsAg rates than women [8,21,28]. In Lao PDR, male blood donors presented with 9.7% HBsAg positive prevalence, while the prevalence in females was 6.2% [22]. When considering the disease burden of hepatitis B virus infections, it is better to include both sexes [26].

To the best of our knowledge, this is the first nationwide, population-based serological survey on chronic hepatitis B virus infections both before and after implementation of hepatitis B immunization in Southeast Asia, where disease burden is high. As such, our results provide valuable information on a hepatitis B immunization program and a useful baseline against which to compare future assessments in this region.

National immunization policy should be based on the disease epidemiology [3]. However, in Southeast Asia, understanding of the epidemiology of hepatitis B remains unsatisfactory. Even when a country implements a hepatitis B immunization program for children and the prevalence of disease reaches the target (i.e., less than 2% among children aged 5 years or older), we cannot conclude that the immunization program alone contributed to reduced disease prevalence without comparing it to the disease prevalence in the pre-vaccine generation, i.e., adults. Nationwide surveys assessing disease prevalence in the generations before and after the implementation of a vaccination program will provide valuable information for understanding hepatitis B epidemiology. Therefore, we recommend surveying hepatitis B seroprevalence in both generations.

Conclusions

We determined the nationwide HBsAg prevalence among children (1.7%; 95% CI: 0.8%–2.6%) and their mothers (2.9%; 95% CI: 1.6%–4.2%) in Lao PDR. This is the first report to estimate the nationwide prevalence of chronic hepatitis B in pre- and post-hepatitis B immunization generations in Southeast Asia, where hepatitis B infections are a substantial burden. The estimated prevalence was below that of previous studies, suggesting that our understanding of this disease's epidemiology is lacking and warrants further investigation. We recommend that the prevalence among the pre- and post-vaccine eras should be investigated when conducting hepatitis B seroprevalence surveys.

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Author Contributions

Conceived and designed the experiments: AX MH KI TW MS. Performed the experiments: KK TK PV CP KP DP BS VSO TS. Analyzed the data: KK TK MH. Contributed reagents/materials/analysis tools: KI TW MS. Wrote the paper: AX PV MH. Revised the manuscript: KK TK PV CP KP DP BS VSO KI TW MS. Arranged laboratory for diagnosis: PV KI TW MS.

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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.

In the Japanese population, both haplotypes *DPA1*01:03-DPB1*04:01* and *DPA1*01:03-DPB1*04:02* showed significant associations with protection against HBV infection ($P = 1.17 \times 10^{-5}$; OR = 0.32; 95% CI, 0.18–0.56 for *DPA1*01:03-DPB1*04:01* and $P = 1.95 \times 10^{-7}$; OR = 0.37; 95% CI, 0.24–0.55 for *DPA1*01:03-DPB1*04:02*). In the Korean subjects, a significant association of *DPA1*01:03-DPB1*04:02* was also demonstrated; however, no association was observed for *DPA1*01:03-DPB1*04:01*. Because the observed number of each haplotype was small, none of the other haplotypes showed a significant association with protection against HBV infection.

In order to identify trans-ethnic DPA1-DPB1 haplotypes associated with HBV infection, a meta-analysis was performed. A meta-analysis further revealed that the *DPA1*01:03-DPB1*02:01* haplotype was significantly associated with protection against HBV infection ($P = 1.45 \times 10^{-5}$; OR = 0.69; 95% CI, 0.58–0.82) (Fig. S1C).

Discussion

Among 2.2 billion individuals worldwide who are infected with HBV, 15% of these are chronic carriers. Of chronic carriers, 10–15% develops LC, liver failure and HCC, and the remaining individuals eventually achieve a state of nonreplicative infection, resulting in HBsAg negative and anti-HBc positive, i.e. HBV-resolved individuals. To identify host genetic factors associated with HBV-related disease progression may lead HBV patients to discriminate individuals who need treatment.

The *HLA-DPA1* and *HLA-DPB1* genes were identified as host genetic factors significantly associated with CHB infection, mainly in Asian populations [7–12], and not in European populations [13]. In the previous association analyses of *HLA-DPB1* alleles with HBV infection, one risk allele *HLA-DPB1*05:01* (OR = 1.52; 95% CI, 1.31–1.76), and two protective alleles, *HLA-DPB1*04:01* (OR = 0.53; 95% CI, 0.34–0.80) and *HLA-DPB1*04:02* (OR = 0.47; 95% CI, 0.34–.64), were identified in the Japanese population [7]. In this study, we further identified a new risk allele *HLA-DPB1*09:01* (OR = 1.94; 95% CI, 1.45–2.62) for HBV infection and a new protective allele *HLA-DPB1*02:01* (OR = 0.71; 95% CI, 0.56–0.89) in the Japanese population, in addition to the previously reported alleles (Table S2) [7]. The discrepancy in the association of *HLA-DPB1*09:01* allele with risk for HBV infection in a previous study [7] results from the elevated frequency of *HLA-DPB1*09:01* in the controls (12.2%), which is higher than our controls (8.7%). In this study, healthy subjects were recruited as controls. In contrast, individuals that were registered in BioBank Japan as subjects with diseases other than CHB were recruited as controls in the previous study [7], which may have included patients with diseases with which *HLA-DPB1*09:01* is associated. Although no significant association of *HLA-DPB1*09:01* with risk for HBV infection was observed in the Korean subjects, *HLA-DPB1*09:01* appears to have a susceptible effect on HBV infection, as it showed the same direction of association. When the association analyses in Japanese and Korean subjects were combined in meta-analysis, the association was statistically significant ($P = 1.36 \times 10^{-6}$; OR = 1.97; 95% CI, 1.50–2.59). Thus, *HLA-DPB1*09:01* may be a Northeast Asian-specific allele associated with risk for HBV infection.

Moreover, a significant association of *HLA-DPB1*13:01* with risk of HBV infection (OR = 2.17; 95% CI, 1.40–3.47) was identified in the Thai subjects. However, the frequency of *HLA-DPB1*13:01* in Thai healthy controls (11.5% in the present study) reportedly varies, ranging from 15.4% to 29.5%, due to the population diversity [15–17]. Therefore, a replication analysis is required to confirm the association of *HLA-DPB1*13:01* with HBV infection in the Thai subjects. There were four other marginally associated *HLA-DPB1* alleles with low allele frequencies below 5% in HBV patients and healthy controls, including *HLA-DPB1*28:01*, *-DPB1*31:01*, *-DPB1*100:01*, and *-DPB1*105:01*, in the Hong Kong and Thai subjects. Because these infrequent alleles may have resulted from false positive associations, the association needs to be validated in a large number of subjects.

*HLA-DPB1*02:01* showed a significant association with protection against HBV infection in both Japanese and Hong Kong populations (Table S2); however, the *HLA-DPB1*02:01* allele was not associated with HBV infection in the previous study [7]. Although *HLA-DPB1*02:01* showed no association in either Korean or Thai populations, a significant association of *HLA-DPB1*02:01* with protection against HBV infection among four Asian populations was detected in meta-analysis ($P = 5.22 \times 10^{-6}$; OR = 0.68; 95% CI, 0.58–0.81) (Fig. S1B). We therefore conclude that the present finding is not a false positive.

A recent report showed that *HLA-DPB1*02:01:02*, **02:02*, **03:01:01*, **04:01:01*, **05:01*, **09:01*, and **14:01* were significantly associated with response to booster HB vaccination in Taiwan neonatally vaccinated adolescents [18]. The *HLA-DPB1*02:01:02*, **02:02*, **03:01:01*, **04:01:01*, and **14:01* were significantly more frequent in recipients whose post-booster titers of antibodies against HBV surface antigen (anti-HBs) were detectable, on the other hand, *HLA-DPB1*05:01* and **09:01* were significantly more frequent in recipients who were undetectable. Moreover, the *HLA-DPB1*05:01* and **09:01* significantly increase the likelihoods of undetectable pre-booster anti-HBs titers. These results seem consistent with our findings, in which *HLA-DPB1*05:01* and **09:01* are associated with susceptibility to chronic hepatitis B infection.

We also identified a protective effect of *HLA-DPB1*02:01* allele on disease progression in Asian populations. Previous studies identified the association of HLA class II genes including *HLA-DQ* and *HLA-DR* with development of HBV related hepatocellular carcinoma in the Chinese population [6,19,20]. In this study using Japanese and Korean samples, we identified significant associations between *HLA-DPB1*02:01* and disease progression in CHB patients ($P = 4.26 \times 10^{-5}$; OR = 0.45; 95% CI, 0.30–0.67, for Japanese and $P = 8.74 \times 10^{-4}$; OR = 0.47; 95% CI, 0.29–0.75 for Korean) (Table S4). Although the association of *HLA-DPB1*02:01* with disease progression was weaker after adjustment for age and gender in Korean subjects ($P = 2.54 \times 10^{-2}$; OR = 0.55; 95% CI, 0.33–0.93), the same direction of association was observed (i.e. protective effect on disease progression) (Table 2). The protective effects of *HLA-DPB1*02:01* on disease progression showed a significant association after adjustment for age and gender in the Japanese population ($P = 1.77 \times 10^{-4}$; OR = 0.47; 95% CI, 0.32–0.70); moreover, a significant association between *HLA-DPB1*02:01* was observed among four Asian populations, under which population was adjusted by using dummy variables in a multivariate logistic regression analysis ($P = 1.55 \times 10^{-7}$; OR = 0.50; 95% CI, 0.39–0.65) (Table 2).

The *HLA-DPA1* and *HLA-DPB1* belong to the HLA class II alpha and beta chain paralogues, which make a heterodimer consisting of an alpha and a beta chain on the surface of antigen presenting cells. This HLA class II molecule plays a central role in the immune system by presenting peptides derived from extracellular proteins. We identified two susceptible haplotypes (*DPA1*02:02-DPB1*05:01* and *DPA1*02:01-DPB1*09:01*) and three protective haplotypes (*DPA1*01:03-DPB1*04:01*, *DPA1*01:03-DPB1*04:02*, and *HLA-DPA1*01:03-DPB1*02:01*) to chronic hepatitis B infection, which may result in different binding

affinities between HLA-DP subtypes and extracellular antigens. Although functional analyses of HLA-DP subtypes to identify HBV-related peptides are not fully completed, identification of susceptible and protective haplotypes as host genetic factors would lead us to understand the pathogenesis of HBV infection including viral factors.

In summary, we identified a new risk allele *HLA-DPB1*09:01*, which was specifically observed in Northeast Asian populations, Japanese and Korean. Moreover, a new protective allele *HLA-DPB1*02:01* was identified among four Asian populations: Japanese, Korean, Hong Kong and Thai. The protective allele *HLA-DPB1*02:01* was associated with both chronic HBV infection and disease progression in chronic HBV patients. Identification of a total of five alleles, including two risk alleles (*DPB1*09:01* and *DPB1*05:01*) and three protective alleles (*DPB1*04:01*, *DPB1*04:02* and *DPB1*02:01*), would enable HBV-infected individuals to be classified into groups according to the treatment requirements. Moreover, the risk and protective alleles for HBV infection and disease progression, identified in this study by means of trans-ethnic association analyses, would be key host factors to recognize HBV-derived antigen peptides. The present results may lead to subsequent functional studies into HLA-DP molecules and viral factors in order to understand the pathogenesis of HBV infection and development of hepatocellular carcinoma.

Materials and Methods

Ethics Statement

All study protocols conform to the relevant ethical guidelines, as reflected in the *a priori* approval by the ethics committee of National Center for Global Health and Medicine, and by the ethics committees of all participating universities and hospitals, including The University of Tokyo, Japanese Red Cross Kanto-Koshinetsu Block Blood Center, The University of Hong Kong, Chulalongkorn University, Yonsei University College of Medicine, Nagoya City University Graduate School of Medical Sciences, Musashino Red Cross Hospital, Tokyo Medical and Dental University, Teine Keijinkai Hospital, Hokkaido University Graduate School of Medicine, Kurume University School of Medicine, Okayama University Graduate School of Medicine, Yamaguchi University Graduate School of Medicine, Tottori University, Kyoto Prefectural University of Medicine, Osaka City University Graduate School of Medicine, Nagoya Daini Red Cross Hospital, Ehime University Graduate School of Medicine, Kanazawa University Graduate School of Medicine, National Hospital Organization Osaka National Hospital, Iwate Medical University, Kawasaki Medical College, Shinshu University School of Medicine, Saitama Medical University, Kitasato University School of Medicine, Saga Medical School, and University of Tsukuba.

Written informed consent was obtained from each patient who participated in this study and all samples were anonymized. For Japanese healthy controls, 419 individuals were de-identified with information about gender, and all were recruited after obtaining verbal informed consent in Tokyo prior to 1990. For the 419 Japanese healthy individuals, written informed consent was not obtained because the blood sampling was conducted before the "Ethical Guidelines for Human Genome and Genetic Sequencing Research" were established in Japan. Under the condition that DNA sample is permanently de-linked from the individual, this study was approved by the Research Ethics Committee of National Center for Global Health and Medicine.

Characteristics of studied subjects

All of the 3,167 genomic DNA samples were collected from individuals with HBV, HBV-resolved individuals (HBsAg-negative and anti-HBc-positive) and healthy controls at 26 multi-center hospitals throughout Japan, Korea, Hong Kong, and Thailand (Table 1). In a total of 1,291 Japanese and 586 Korean samples, 1,191 Japanese individuals and all 586 Korean individuals were included in our previous study [9]. With regard to additional Japanese individuals, we collected samples from 48 healthy controls at Kohnodai Hospital, and 52 HBV patients at Okayama University Hospital and Ehime University Hospital, including 26 individuals with LC and 26 individuals with HCC. A total of 661 Hong Kong samples and 629 Thai samples were collected at Queen Mary Hospital and Chulalongkorn University, respectively.

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 liver cirrhosis. Chronic hepatitis (CH) was defined by elevated ALT levels (>1.5 times the upper limit of normal [35 IU/L]) persisting over 6 months (by at least 3 bimonthly tests). Acute exacerbation (AE) of chronic hepatitis B was defined as an elevation of ALT to more than 10 times the upper limit of normal (ULN, 58 IU/L) and bilirubin to at least three times ULN (15 μmol/L). LC was diagnosed principally by ultrasonography (coarse liver architecture, nodular liver surface, blunt liver edges and hypersplenism), platelet counts<100,000/cm^3, or a combination thereof. Histological confirmation by fine-needle biopsy of the liver was performed as required. 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 agreement (anonymization in a de-identified manner) in this study. Some of the unrelated and anonymized Japanese healthy controls were purchased 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.

Genotyping of *HLA-DPA1* and *HLA-DPB1* alleles

High resolution (4-digit) genotyping of *HLA-DPA1* and *-DPB1* alleles was performed for HBV patients, resolved individuals, and healthy controls in Japan, Korea, Hong Kong, and Thailand. LABType SSO HLA DPA1/DPB1 kit (One Lambda, CA) and a Luminex Multi-Analyte Profiling system (xMAP; Luminex, Austin, TX) were used for genotyping, in according with the manufacturer's protocol. Because of the small quantity of genomic DNA in some Korean samples, we performed whole genome amplification for a total of 486 samples using GenomiPhi v2 DNA Amplification kit (GE Healthcare Life Sciences, UK), in accordance with the manufacturer's instruction.

A total of 2,895 samples were successfully genotyped and characteristics of these samples are summarized in Table S1.

Statistical analysis

Fisher's exact test in two-by-two cross tables was used to examine the associations between *HLA-DP* allele and chronic HBV infection or disease progression in chronic HBV patients,

using statistical software R2.9. To avoid false-positive results due to multiple testing, significance levels were adjusted based on the number of observed alleles at each locus in each population. For *HLA-DPA1* alleles, the number of observed alleles was 3 in Japanese, 4 in Korean, 5 in Hong Kong, and 5 in Thai subjects. Therefore, the significant levels for α were set at $\alpha = 0.05/3$ in Japanese, $\alpha = 0.05/4$ in Korean, $\alpha = 0.05/5$ in Hong Kong, and $\alpha = 0.05/5$ in Thai subjects. In the same way, significant levels for *HLA-DPB1* alleles were $\alpha = 0.05/10$, $0.05/11$, $0.05/12$, and $0.05/16$, respectively. Multivariate logistic regression analysis adjusted for age and sex (used as independent variables) was applied to assess associations between the number of *DPB1*02:01* alleles (i.e., 0, 1, or 2) and disease progression in CHB patients. To examine the effect of *DPB1*02:01* allele on disease progression in all populations, population was further adjusted by using three dummy variables (i.e., (c1, c2, c3) = (0, 0, 0) for Japanese, (1, 0, 0) for Korean, (0, 1, 0) for Hong Kong, and (0, 0, 1) for Thai) in a multivariate logistic regression analysis. We obtained the following regression equation: $\text{logit}(p) = -3.905 + 0.083 * \text{age} + (-0.929) * \text{sex} + (-0.684) * \textit{DPB1*02:01} + 1.814 * c1 + (-0.478) * c2 + 0.782 * c3$. Significance levels in the analysis of disease progression in CHB patients were set as $\alpha = 0.05/10$ in Japanese, $\alpha = 0.05/11$ in Korean, $\alpha = 0.05/15$ in Hong Kong, and $\alpha = 0.05/15$ in Thai subjects. The phase of each individual (i.e., a combination of two *DPA1-DPB1* haplotypes) was estimated using PHASE software [21], assuming samples are selected randomly from a general population. In comparison of the estimated *DPA1-DPB1* haplotype frequencies, significant levels were set as $\alpha = 0.05/14$ in Japanese, $\alpha = 0.05/17$ in Korean, $\alpha = 0.05/17$ in Hong Kong, and $\alpha = 0.05/18$ in Thai subjects. Meta-analysis was performed using the DerSimonian-Laird method (random-effects model) in order to calculate pooled OR and its 95% confidence interval (95% CI). We applied meta-analysis for alleles with frequency>1% in all four Asian populations. The significance levels in meta-analysis were adjusted by the total number of statistical tests; $\alpha = 0.05/20$ for *DPA1* alleles, $\alpha = 0.05/57$ for *DPB1* alleles, and $\alpha = 0.05/74$ for *DPA1-DPB1* haplotypes.

Supporting Information

Figure S1 Comparison of odds ratios in association analyses for *HLA-DP* with chronic HBV infection among four Asian populations: (A) *HLA-DPA1* alleles; (B) *HLA-DPB1* alleles; and (C) *HLA DPA1-DPB1* haplotypes. Meta-analysis was performed using the DerSimonian-Laird method (random-effects model) to calculate pooled OR and its 95% confidence interval (95% CI). Bold depicts a statistically significant association after correction of significance level.
(DOCX)

Table S1 Individuals with successfully genotyped for *HLA-DPA1* and *HLA-DPB1*.
(DOCX)

Table S2 Frequencies of HLA-DP alleles in HBV patients and healthy controls among Asian populations.
(XLSX)

Table S3 Frequencies of HLA-DP alleles in HBV patients and resolved individuals among Asian populations.
(XLSX)

Table S4 Associations of HLA-DPB1 alleles with disease progression in CHB patients among Asian populations.
(XLSX)

Table S5 Estimated frequencies of HLA DPA1-DPB1 haplotypes in HBV patients and healthy controls among Asian populations.
(XLSX)

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Author Contributions

Conceived and designed the experiments: NN HS MS KT M. Mizokami. Performed the experiments: NN HS KK Y. Mawatari M. Kawashima M. Minami. Analyzed the data: NN HS M. Kawashima JO. Contributed reagents/materials/analysis tools: W-KS M-FY NP YP SHA K-HH K. Matsuura YT M. Kurosaki YA NI J-HK SH TI KY IS Y. Murawaki YI AT EO YH MH SK EM KS KH ET SM MW YE NM K. Murata M. Korenaga KT M. Mizokami. Wrote the paper: NN HS JO KT M. Mizokami.

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Risk Factors for Long-Term Persistence of Serum Hepatitis B Surface Antigen Following Acute Hepatitis B Virus Infection in Japanese Adults

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The proportion of patients who progress to chronicity following acute hepatitis B (AHB) varies widely worldwide. Moreover, the association between viral persistence after AHB and hepatitis B virus (HBV) genotypes in adults remains unclear. A nationwide multicenter study was conducted throughout Japan to evaluate the influence of clinical and virological factors on chronic outcomes in patients with AHB. For comparing factors between AHB patients with viral persistence and those with self-limited infection, 212 AHB patients without human immunodeficiency virus (HIV) coinfection were observed in 38 liver centers until serum hepatitis B surface antigen (HBsAg) disappeared or a minimum of 6 months in cases where HBsAg persisted. The time to disappearance of HBsAg was significantly longer for genotype A patients than that of patients infected with non-A genotypes. When chronicity was defined as the persistence of HBsAg positivity for more than 6 or 12 months, the rate of progression to chronicity was higher in patients with genotype A, although many cases caused by genotype A were prolonged cases of AHB, rather than chronic infection. Multivariate logistic regression analysis revealed only genotype A was independently associated with viral persistence following AHB. A higher peak level of HBV DNA and a lower peak of alanine aminotransferase (ALT) levels were characteristics of AHB caused by genotype A. Treatment with nucleotide analogs (NAs) did not prevent progression to chronic infection following AHB overall. Subanalysis suggested early NA initiation may enhance the viral clearance. *Conclusion:* Genotype A was an independent risk factor for progression to chronic infection following AHB. Our data will be useful in elucidating the association between viral persistence after AHB, host genetic factors, and treatment with NAs in future studies. (HEPATOLOGY 2014;59:89-97)

Abbreviations: AHB, acute hepatitis B; ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to HBsAg; HBeAg, hepatitis B e-antigen; CLIA, chemiluminescent enzyme immunoassay; EIA, enzyme immunoassay; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IgM, immunoglobulin M; anti-HBe, antibody to HBeAg; NAs, nucleotide analogs; RPHA, reverse passive hemagglutination.

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Hepatitis B virus (HBV) infection is one of the most common persistent viral infections in humans. Approximately 2 billion people worldwide have been exposed to HBV and 350 million of them remain persistently infected.[1] The incidence of HBV infection and the patterns of transmission vary greatly worldwide among different population subgroups.[2] In Western countries, chronic HBV infection is relatively rare and acquired primarily in adulthood by way of sexual transmission or the use of injectable drugs. Meanwhile, in Asia and most of Africa, the majority of infections are the result of transmission from an infected mother to her newborn. However, very few studies of acute hepatitis B (AHB) in adults have been reported.

HBV genomic sequences vary worldwide and have been classified into at least eight genotypes (A through H) based on an intergroup divergence of 8% or more over the complete nucleotide sequence.[3,4] These genotypes have distinct geographic distributions.[5-7] In particular, genotype A is predominant in Northwestern Europe, the United States, Central Africa, and India.[8, 9] The Japanese have been infected with genotypes B and C since prehistoric times.[10] Recently, many lines of evidence have revealed among the Japanese an increase in acute infection with HBV genotype A following sexual transmission.[11,12] As a result of this increasing transmission of genotype A, the distribution of HBV genotypes in Japan clearly differs among patients with acute and chronic infections.[13] Moreover, recent studies suggest that acute infection with HBV genotype A may be associated with an increased risk of progression to persistent infection.[15] Indeed, the prevalence of HBV genotype A in chronic hepatitis B patients doubled in Japan between 2000-2001 and 2005-2006 (1.7% versus 3.5%).[11]

Human immunodeficiency virus (HIV)-1 infection results in an immunodeficient state, with the virus sharing routes of transmission with HBV. HIV-related immunodepletion influences the natural history of HBV infection, and epidemiological studies have revealed that HIV-positive patients are more likely to have a prolonged acute illness following HBV infection and lower rates of hepatitis B e-antigen (HBeAg) clearance.[16] Therefore, in this study patients with coinfection of HIV were excluded to examine the influence of HBV genotype directly without the confounding influence of HIV.

From 2005 to 2010, a multicenter cohort study was conducted throughout Japan on 212 patients with AHB. The aim of this cohort study was to assess the influence of clinical and virological factors, including HBV genotypes and treatment with nucleotide analogs (NAs), on AHB patients who became persistently infected.

Patients and Methods

Patients With AHB. The multiple-source cohort included 212 randomly selected AHB patients without coinfection of HIV. From 2005 through 2010, the study participants were recruited from 38 liver centers throughout Japan. The cohort included patients who were admitted to the hospital because of AHB and who visited the hospital every month after being discharged. The diagnosis of AHB was contingent on the rapid onset of clinical symptoms accompanied by elevated serum alanine aminotransferase (ALT) levels, the detection of serum hepatitis B surface antigen (HBsAg), and a high-titer antibody to hepatitis B core antigen (anti-HBc) of the immunoglobulin M (IgM) class. Patients with initial high-titer anti-HBc (>10.0 S/CO) were diagnosed as having an exacerbation of chronic hepatitis B and were excluded. If the patient had been tested previously, the absence of serum HBsAg and anti-HBc before admission was verified from the medical record to discriminate a new infection from an acute exacerbation of a persistent infection. Patients with acute hepatitis A, hepatitis C, and drug- or alcohol-induced acute hepatitis were also excluded; hepatitis D virus infection was not determined because of its extreme rarity in Japan. The study protocol conformed to the 1975 Declaration of

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

Table 1. Characteristics of Patients With Genotype A or a Non-A Genotype Acutely Infected With Hepatitis B Virus

Features	Genotype A (n = 107)	Non-A Genotypes (n = 105)*	*P* Value
Age (years)	36.3 ± 12.0	40.7 ± 14.3	0.032
Male sex	102 (95.3)	75 (71.4)	<0.001
HBeAg positive	104 (97.2)	79 (75.2)	<0.001
ALT (IU/L)	1210 ± 646	2225 ± 2851	0.045
Total bilirubin (mg/dL)	9.9 ± 9.4	7.5 ± 6.7	0.115
HBV DNA (log copies/mL)	7.0 ± 1.5	5.8 ± 1.5	<0.0001
Duration until disappearance of HBsAg (month)	6.7 ± 8.5	3.4 ± 6.5	<0.0001
Persistence of HBsAg positivity more than 6 months	25 (23.4)	9 (8.6)	0.003
Persistence of HBsAg positivity more than 12 months	8 (7.5)	1† (0.9)	0.018
Sexual transmission	81/84 (96.4)‡	71/79 (89.9)§	0.095
Treatment with NAs	61 (57.0)	42 (40.0)	0.013

Data are presented as n (%), mean ± standard deviation. HBV, hepatitis B virus; HBeAg, hepatitis B e-antigen; ALT, alanine aminotransferase; NAs, nucleotide analogs.

*Non-A genotypes include genotypes B, C, D, F and H (n = 25, 77, 1, 1, and 1, respectively).

†One patient had genotype C.

‡Transmission routes were unknown for 23 patients.

§Transmission routes were unknown for 26 patients.

Helsinki and was approved by the Ethics Committees of the institutions involved. Every patient gave informed consent for this study.

Serological Markers of HBV Infection. HBsAg, HBeAg, antibodies to HBsAg (anti-HBs), HBeAg (anti-HBe), and HBcAg, and anti-HBc of the IgM class were tested by a chemiluminescent enzyme immunoassay (CLIA) by ARCHITECT (Abbott Japan, Tokyo, Japan). HBV DNA measurements were performed using a real-time polymerase chain reaction (PCR) assay (Cobas TaqMan HBV Auto; Roche Diagnostics, Tokyo, Japan).

Genotyping of HBV. The six major HBV genotypes (A through F) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV GENOTYPE EIA; Institute of Immunology, Tokyo, Japan). This method is based on the pattern of detection by monoclonal antibodies of a combination of epitopes on preS2-region products, which is specific for each genotype.[17,18] Samples for which EIA could not determine the genotype were examined by direct sequencing of the pre-S2/S gene, followed by phylogenetic analysis.

Treatment With NAs. Treatments with NAs were performed using lamivudine or entecavir for more than 3 months. The individual clinicians determined if NAs were administered to patients, and when the treatment was to be started. The time to onset of treatment with NAs was measured in days from onset of AHB.

Statistical Analysis. Categorical variables were compared between groups by the chi-squared test and noncategorical variables by the Mann-Whitney *U* test. A *P* value less than 0.05 was considered significant. Multivariate analysis was performed using a backward stepwise logistic regression model to determine independent factors for viral persistence following AHB. Variables in the multivariate analysis were selected based on variables that were marginally significant with $P < 0.1$ in univariate analysis. Maintenance of HBsAg positivity was analyzed using the Kaplan-Meier method and significance was tested with the log-rank test. STATA Software (StataCorp, College Station, TX) v. 11.0 was used for analyses.

Results

Comparison of Characteristics Between Genotype A and Non-A Genotype AHB Patients. A total of 107 AHB patients (50.5%) were infected with genotype A while 105 AHB patients (49.5%) were infected with non-A genotypes, including genotypes B (25 [11.8%]), C (76 [35.8%]), D (1 [0.5%]), F (1 [0.5%]), and H (1 [0.5%]). Compared to those infected with non-A genotypes, genotype A patients were significantly younger (36.3 ± 12.0 versus 40.7 ± 14.3 years, $P = 0.032$), predominantly men (95.3% versus 71.4%, $P < 0.001$), and more frequently positive for HBeAg (97.2% versus 75.2%, $P < 0.001$). Moreover, genotype A patients had a lower peak ALT levels (1,210 ± 646 versus 2,225 ± 2,851 IU/L, $P = 0.045$) and a higher peak level of HBV DNA (6.7 ± 8.5 versus 3.4 ± 6.5 log copies/mL, $P < 0.0001$). A significantly higher percentage of genotype A patients were treated with NAs (57% versus 40%, $P = 0.013$). These data are summarized in Table 1.

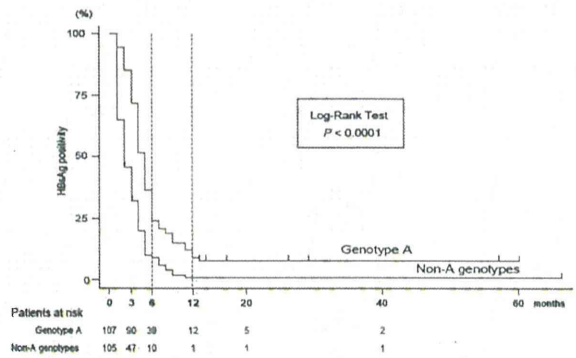

Fig. 1. Comparison of the cumulative proportion of AHB patients maintaining HBsAg positivity between genotype A and non-A genotypes, analyzed using the Kaplan-Meier test. $P < 0.0001$, genotype A: red line, non-A genotypes: blue line.

Cumulative Maintenance of HBsAg Positivity During Follow-up in Patients With Genotype A and Non-A Genotypes. In the patients infected with genotype A and non-A genotypes, the mean durations of HBsAg positivity maintenance were 6.7 ± 8.5 and 3.4 ± 6.5 months, respectively ($P < 0.0001$; Table 1, Fig. 1). For 6 months after AHB onset, the number of patients with genotype A and non-A genotypes maintaining HBsAg positivity were 39/107 (36.4%) and 10/105 (9.5%), respectively ($P < 0.001$). However, in many patients HBsAg disappeared between 7 and 12 months after AHB onset; that is, HBsAg disappeared in 31/107 (29.0%) of patients with genotype A and in 9/105 (8.6%) of patients with non-A genotypes during this time period. However, in some patients HBsAg never disappeared after persisting for more than 12 months following AHB onset. When chronicity after AHB was defined as the persistence of HBsAg for more than 12 months, chronicity developed in 7.5% (8/107) of patients with genotype A and in 0.9% (1/105) of patients with non-A genotypes ($P = 0.018$).

Comparison of Characteristics Between Patients in Whom HBsAg Persisted More Than 6 or 12 Months and Those With Self-Limited AHB Infection. Table 2 compares the demographic and clinical characteristics between patients in whom HBsAg disappeared within 6 months and those in whom HBsAg persisted for more than 6 months from AHB. The peak ALT levels ($1,882 \pm 2,331$ versus $1,018 \pm 696$ IU/L, $P = 0.0024$) and peak HBV DNA levels (6.3 ± 1.6 versus 7.4 ± 1.6 mg/dL, $P = 0.0004$) were significantly higher and lower in the former group than in the latter group, respectively. Moreover, marked differences were present in the distribution of genotypes between the two groups. The percentage of the HBV genotype A (46.1% versus 73.5%, $P = 0.003$) was significantly higher among patients in whom HBsAg was persistent for more than 6 months. In addition, we compared the demographic and clinical characteristics between patients in whom HBsAg disappeared within 12 months and those in whom HBsAg persisted for more than 12 months from AHB. Peak ALT ($1,787 \pm 2,118$ versus 775 ± 513 IU/L, $P = 0.0089$) and peak total bilirubin (8.7 ± 8.2 versus 3.8 ± 6.6 mg/dL, $P = 0.0039$) levels were significantly higher in the former group than in the latter group. In contrast, the peak HBV DNA levels (6.4 ± 1.6 versus 7.9 ± 1.4 mg/dL, $P = 0.0046$) were significantly lower

Table 2. Comparison Between Patients With Chronicity Following Acute Hepatitis B and Those With Self-Limited Acute Infections Determined by the Persistence of HBsAg for More Than 6 or 12 Months

Features	Disappearance of HBsAg Within 6 Months (n = 178)	Persistence of HBsAg for More Than 6 Months From AHB (n = 34)	P Value	Disappearance of HBsAg Within 12 Months (n = 203)	persistence of HBsAg for More Than 12 Months From AHB (n = 9)	P Value
Age (years)	38.2 ± 13.1	40.0 ± 14.5	0.454	38.1 ± 13.2	46.7 ± 14.0	0.061
Male sex	147 (82.6)	30 (88.2)	0.416	169 (83.3)	8 (88.9)	0.677
HBeAg positive	150 (84.3)	32 (94.1)	0.131	175 (86.2)	8 (88.9)	0.815
ALT (IU/L)	1882 ± 2331	1018 ± 696	0.0024	1787 ± 2118	775 ± 513	0.0089
Total bilirubin (mg/dL)	8.6 ± 7.5	8.7 ± 11.3	0.137	8.7 ± 8.2	3.8 ± 6.6	0.0039
HBV DNA (log copies/mL)	6.3 ± 1.6	7.4 ± 1.6	0.0004	6.4 ± 1.6	7.9 ± 1.4	0.0046
HBV genotype						
Non-A	96 (53.9)	9 (26.5)		104 (51.2)	1 (11.1)	
A	82 (46.1)	25 (73.5)	0.003	99 (48.8)	8 (88.9)	0.018
Sexual transmission	128/137 (93.4)*	24/26 (92.3)†	0.711	146/157 (93.0)‡	6/6 (100.0)§	0.356
NAs treatment (+)	82 (46.1)	21 (61.8)	0.093	98 (48.3)	8 (88.9)	0.017

Data are presented as n (%) and mean ± SD. HBsAg, hepatitis B surface antigen; AHB, acute hepatitis B, HBeAg, hepatitis B e-antigen; ALT, alanine aminotransferase; HBV, hepatitis B virus; NAs, nucleotide analogs.

*Transmission routes of 41 patients were unknown.

†Transmission routes of 8 patients were unknown.

‡Transmission routes of 46 patients were unknown.

§Transmission routes of 3 patients were unknown.