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ORIGINAL ARTICLE

Full genome characterization and phylogenetic analysis of hepatitis B virus in gibbons and a caretaker in Central Kalimantan, Indonesia

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Abstract Hepatitis B virus (HBV) from gibbons was characterized, and the possibility of horizontal transmission between gibbons and humans was examined in a gibbon rehabilitation center in Central Kalimantan, Indonesia. Ten gibbons that were positive for the hepatitis B surface antigen (HBsAg) on arrival and 13 caretakers for those gibbons were included in this study. The duration of stay at the rehabilitation center ranged from 1 to 10 years. Serological and molecular analyses were performed. Six gibbons were positive for HBsAg, whereas HBV DNA was detected in all ten of the gibbons sampled. On the other hand, HBsAg was detected in only 1 of the 13 caretakers. HBV samples from seven gibbons and from the one infected human were chosen for complete genome sequencing. A phylogenetic analysis revealed that the cluster of gibbon strains in this study was distinct from strains previously reported from other countries. In the pre-S1 region, we found a unique amino acid residue substitution (P89K), three insertions between T87 and L88 in the genomes of three gibbons, and a 33-nucleotide deletion at the start of pre-S1 that is common in non-human primates. The caretaker sample was identified as HBV subgenotype B3, the most common type in Indonesia. For the complete HBV sequences, the similarity between gibbons in this study and other non-human primate and human HBV isolates was 90–91.9 % and 85.5–89.6 %, respectively. In conclusion, the gibbon HBV genotype was influenced by geographic location and species. To the best of our knowledge, this is the first report characterizing the HBV genes and genomes of indigenous gibbons in Indonesia.

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Introduction

Hepatitis B virus (HBV) is present worldwide, and chronic HBV infection frequently results in cirrhosis and hepatocellular carcinoma (HCC). HBV is the smallest mammalian DNA virus, with a genome size of approximately 3,200 nucleotides that contains four open reading frames for the P, C, S, and X genes. HBV variants have been classified into at least nine genotypes (A through H and J) for humans [1, 2], and genomic differences distinguish strains infecting humans from those infecting non-human primates, including chimpanzees, orangutans, gorillas, and gibbons [3, 4]. Human HBV genotypes have a distinct geographical distribution and differ in the clinical manifestations they induce [5, 6], but it is unclear whether this is also true of non-human HBV genotypes [4].

HBV can be found in non-human primates of the families *Hominidae* (chimpanzee, gorilla, and orangutan) and *Hylobatidae* (gibbon), which are distributed across Africa (chimpanzee and gorilla) and Southeast Asia (orangutan and gibbon), which are endemic areas of human HBV [7–9]. The prevalence of asymptomatic HBV carriers is 23–33 % in gibbons and 15 % in orangutans [4]. The genome organization of non-human primate HBVs is

nearly identical to that of human HBVs [4]. Because of this close similarity, cross-transmission of HBV between species has been speculated to occur. There have been many studies on the cross-transmission of human HBVs to non-human primates [10, 11], but a cross-transmission of HBVs from non-human primates to humans has not yet been reported [3, 4, 11].

A recent study has shown that a novel HBV strain (genotype "J", HBV/J) discovered in an elderly Japanese male patient with HCC who was involved in military action in Borneo (Kalimantan) during World War II is phylogenetically intermediate to human and gibbon/orangutan strains [12]. Although it is not certain, HBV/J in gibbon/orangutan or human inhabitants is likely to have originated in Borneo (Kalimantan).

We have investigated HBV infection of gibbons and possible horizontal transmission between gibbons and humans and confirmed the presence of HBV/J in Kalimantan, Indonesia.

Materials and methods

Study population

A total of 142 captive gibbons were kept at Kalaweit Gibbon Conservation Center and Sanctuary (Kalaweit), Central Kalimantan, Indonesia, in 2012. Ten out of 15 gibbons that tested positive for hepatitis B surface antigen (HBsAg) in the screening upon arrival at Kalaweit were randomly selected for this study. All of them were born in the wild. The population consisted of six males and four females ranging from 3 to 17 years old and of two species, *Hylobates albibarbis* (Bornean white-bearded gibbons, n = 7) and *Hylobates mulleri* (Müller's Bornean gibbons, n = 3), originally found in Kalimantan. The Kalaweit animal caretakers (n = 13; mean age, 28 years) included in this study consisted of nine males and four females.

Demographic data for the gibbons and humans were collected from each animal caretaker and veterinary coordinator in Kalaweit. Written informed consent was obtained from all caretakers, and a research permit was obtained from the Ministry of Forestry in Indonesia. The study protocol was reviewed and approved by the Ethics Committees of Kobe University in Japan and Veterinary Medicine of Airlangga University in Indonesia.

Sample collection

Gibbon blood samples were obtained by venepuncture during a brief period of anesthesia with ketamine, part of the routine health-care programme, in May (5 samples) and November (5 samples) 2012. Human blood was taken from animal caretakers in October 2013. In total, serum samples were collected from 10 gibbons and 13 animal caretakers.

Serological test

All serum samples were tested for HBsAg by reverse passive hemagglutination (R-PHA) (Mycell II HBsAg; Institute of Immunology, Tokyo, Japan), for antibodies to HBsAg (anti-HBs) by passive hemagglutination (PHA) (Mycell II anti-HBs; Institute of Immunology), and for antibodies to hepatitis B core antigen (anti-HBc) by PHA (Mycell anti-rHBc; Institute of Immunology) according to the manufacturer's instructions. All sera were confirmed to be HBsAg positive using an enzyme-linked immunosorbent assay (ELISA) (Hepalisa HBsAg PT INDEC DIAGNOSTICS, Jakarta, Indonesia). In addition, serological markers related to active hepatitis, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and α-fetoproteins (AFP), were examined.

Detection of HBV DNA and viral load in gibbons

The sera were stored at −20 °C until the assay was carried out. Sample DNA was extracted from 200 μL of serum using a DNA extraction kit (QIAamp DNA Blood Mini Kit; QIAGEN, Tokyo, Japan). The presence of HBV DNA and the viral load were assessed by real-time PCR using an ABI PRISM 7300 Analyzer (Applied Biosystems, Foster City, CA). HBV was amplified using a previously described primer and probe set [13].

Nucleotide sequence analysis

After being assayed for their HBV serological status, all serum samples were subjected to a HBV genetic analysis. The complete HBV genome sequences were determined by a method reported previously [14]. In brief, the complete genome of HBV was first amplified as two overlapping fragments, a 3,200-bp amplicon (fragment A) and a 462-bp amplicon (fragment B) covering the remaining region. Fragment A was then subjected to nested PCR to amplify 11 overlapping fragments. The amplified fragments were sequenced directly using a Big Dye Deoxy Termintor Cycle Sequencing Kit with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Phylogenetic analysis

Reference sequences were retrieved from the DDBJ, EMBL, and GenBank databases. Sequences were aligned using the Clustal X multiple sequence alignment program. Phylogenetic trees were constructed using the neighbor-joining method, and bootstrap resampling was performed

1,000 times. These analyses were carried out using the Molecular Evolutionary Genetics Analysis (MEGA) program. Subgenotypes were assigned as described previously [3, 15, 16].

Nucleotide sequence accession numbers

The nucleotide sequence data reported in this paper were deposited in the DDBJ, EMBL, and GenBank databases under accession nos. AB823656 through AB823662 for the gibbon HBV sequences and AB976562 for the caretaker sequence.

Results

Seroprevalence of HBV in gibbons and humans

To determine the prevalence of current HBV infection in gibbons and animal caretakers in Kalaweit, 23 serum samples (10 gibbons and 13 caretakers) were tested for the presence of HBsAg, anti-HBs, and anti-HBc. A total of six gibbons were chronic carriers, as defined by the presence of HBV DNA and HBsAg in the absence of antibody to protein S, and the remaining four gibbons showed previous infection. All 10 gibbons were positive for at least one marker of HBV infection (Table 1), and one of the 13 animal caretakers (7.7 %) was positive for HBsAg. One gibbon was negative for anti-HBc in the presence of HBsAg (Table 1). ALT, AST, and AFP levels were within the normal range for all gibbons. Anti-HBs antibodies were found in 10 of the 13 caretakers, and 8 of those 10 were positive for anti-HBc.

HBV DNA detection in gibbons and caretakers

HBV DNA was detected in all samples regardless of HBsAg status. The HBV viral load was higher in HBsAg-positive samples (mean, 7.0; 6.4–7.7 log copies/mL) than in HBsAg-negative samples (mean, 4.1; 3.0–4.4 log copies/mL) (Table 1). Four HBsAg-negative gibbons had occult HBV-like infection (Table 1). HBV DNA was also detected in one caretaker with HBsAg.

Gibbon and human HBV nucleotide and amino acid sequences

The complete HBV genomes from seven gibbons comprised 3,182–3,191 nucleotides and showed a genetic organization similar to that of the human viruses. We compared the HBV sequences of all seven gibbons and the human obtained in this study with representative sequences in GenBank, including nine human HBV genotypes, orangutan, gibbon, chimpanzee, and gorilla HBV sequences. The nucleotide sequence of the GB1 isolate (accession no. AB823656) in this study showed similarities ranging from 85.5 to 91.9 % with known complete HBV genome sequences. The highest degree of similarity was found for GB1 and the HBV genomes of viruses isolated from Thai gibbon (accession no. EU155829), orangutan (accession no. AF193863), and chimpanzee (accession no. AF222323) (91.9 %, 91.2 %, and 90.6 % similarity, respectively). Interestingly, the GB1 isolate had higher similarity, not only with primate HBV but also with the human HBV genotypes C and J (92.9 % and 94.1 %), in the pre-C/C region than in the full-length sequence.

Complete HBV genome sequences

We compared the HBV sequences from the seven gibbons with each of those from other primates, as well as those of human HBV genotypes A through H and J. We observed 91.6–99.1 % sequence identity for pairwise comparisons with the Kalaweit gibbon HBV sequences. The gibbon HBV sequences from this study showed 90.7–92.3 % sequence identity to those from orangutan origin previously found in Kalimantan [15].

Pre-S/S gene

We aligned the nucleotide sequences of the pre-S/S region to identify nucleotide and amino acid differences between the HBV isolates from gibbons and the human. Moreover, we compared the gibbon and human HBV sequences with those of previously reported HBV strains. In comparison to the pre-S/S genes of isolates from humans and other species, the isolates from the seven gibbons in this study had a deletion of 33 nucleotides, representing 11 codons at the 5′ end of the pre-S1 region, consistent with previous results [2, 6, 11] (Fig. 1). For the GB1, GB7, and GB8 HBV isolates, we discovered insertions of Ala (A), Leu (L), and Arg (R) between Thr^{87} (T) and L^{88}, and a substitution of Lys^{89} (K) (Fig. 1). On closer investigation, the only gibbon-specific HBV amino acids we found in the pre-S1 region were His^{57} (H), L^{88}, Ser^{88} (S), and H^{100} (Fig. 1). Those in the pre-S2 region included L^{10}, Phe^{20} (F), Tyr^{21} (Y), and L^{35}, and those in the S region included Val^{4} (V), Met^{28} (M), Glu^{44} (Z), L^{53}, A^{114}, M^{118}, L^{154}, T^{210}, S^{216}, Trp^{221} (W), and Ile^{222} (I). The amino acids in the *a* determinant region were highly conserved among gibbons, with the exception of an A^{131} observed in the GB9 isolate.

Pre-C/C gene

We observed less sequence divergence among isolates in the pre-C/C region than in the pre-S/S gene. Three gibbon

Table 1 Demographic data, HBV serological markers, and viral load in gibbons

ID	Age (yrs)	Sex	Species	Duration in the center (yrs)	HBsAg	Anti-HBs	Anti-HBc	HBV viral load (log copies/mL)
GB1	15	M	*Hylobates albibarbis*	5	+	−	+	7.7
GB2	15	M	*Hylobates mulleri*	6	−	−	+	4.4
GB3	10	F	*Hylobates albibarbis*	9	−	+	+	3.9
GB4	16	F	*Hylobates mulleri*	8	−	−	+	4.1
GB5	17	M	*Hylobates mulleri*	10	+	−	+	6.5
GB6	8	M	*Hylobates albibarbis*	1	+	−	+	7.0
GB7	8	F	*Hylobates albibarbis*	2	+	−	+	7.5
GB8	4	M	*Hylobates albibarbis*	3	−	+	+	3.9
GB9	3	F	*Hylobates albibarbis*	1	+	−	+	6.4
GB10	4	M	*Hylobates albibarbis*	3	+	−	−	7.1

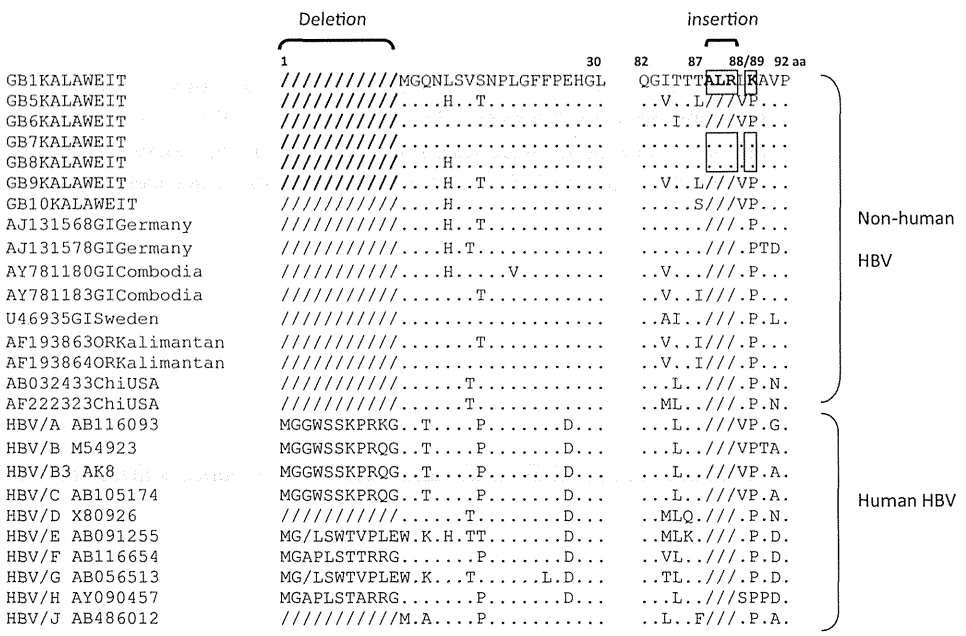

Fig. 1 Amino acid sequence alignment of the pre-S1 region of seven gibbon HBV strains (G1, G5, G6, G7, G8, G9, and G10) and an isolate from a human caretaker with HBV sequences from five gibbons, two orangutans, and two chimpanzees, as well as nine human HBV sequences from databases. The sequence of the G1 isolate is indicated at the top. Dots represent amino acids shared by G1, and a dash indicates the deletion of an amino acid

HBV sequences had a G-to-T mutation, and one had a G-to-A mutation at position 1896; two had a T-to-C mutation at position 1753. The gibbon HBV sequences did not have the double mutations at positions 1762 and 1764 that are commonly observed in humans. The core protein amino acids were highly conserved among the gibbon HBV sequences from this study. The only gibbon HBV amino acid substitutions were Leu^{11} (L) in the pre-C region and Ser^{67} (S) and Pro^{127} (P) in the C region.

Phylogenetic analysis of gibbon and human HBV

Of the 11 serum samples (10 gibbons and one human) obtained in Kalaweit, we successfully determined the complete HBV genome sequences for eight samples. We constructed phylogenetic trees of the complete nucleotide sequence, the pre-S/S region, and the pre-C/C region (Fig. 2–4). The phylogenetic analysis included the eight gibbon HBV strains and 52 HBV strains from DDBJ, EMBL, and GenBank. The gibbon HBV strains were classified as Kalaweit gibbon HBV, and the HBV from the human caretaker as human HBV genotype B3 (HBV/B3).

The Kalaweit gibbon HBV strains formed a distinct cluster, separate from the previously reported gibbon HBV strains from Thailand, Cambodia, and Germany [3, 16–18]. A high bootstrap value (95 %) supported the clustering of the gibbon HBV sequences from Kalimantan in the phylogenetic analysis of the complete genome. The subgenotype of the caretaker, HBV B3, was the most common type observed in Indonesia. Furthermore, the gibbon HBV

isolates (GB1, GB5, GB6, GB7, GB8, GB9, and GB10) were more distantly related to the HBV sequences from orangutans previously found in Kalimantan (accession no. AF193863, AF193864) than they were to gibbon HBV strains from other regions, except Thailand. Although one gibbon caretaker was found to be a chronic HBV carrier in this study, we did not find any evidence for zoonotic disease transmission. The data for the complete genome and pre-S/S gene support separate clusters for the human HBV isolates and the non-human primates (Figs. 2 and 3). The Kalaweit gibbon HBV strains showed a closer relationship to other HBV genotypes in the pre-C/C gene (Fig. 4).

Discussion

We performed a complete genome sequence analysis and found that HBV in *Hylobates albibarbis* and *Hylobates mulleri* originally found in Kalimantan clustered into a single group (Fig. 2). To the best of our knowledge, this is the first report of HBV genotypes for indigenous gibbons in Indonesia. On the other hand, the HBV subgenotype of a gibbon caretaker in Kalaweit was B3, the most common type observed in Indonesia [19, 20], supporting previous work showing that human HBV genotypes have a distinct geographical distribution. HBV cross-transmission between gibbons and humans was not supported by our phylogenetic analysis. The Kalaweit gibbon HBV strains had variation in the pre-S region, including a unique amino acid residue substitution (P89 K), three insertions (found in three gibbons), and a 33-nucleotide deletion that is common in non-human primates (Fig. 1). All ten gibbons in this study were HBsAg positive as determined by routine screening of new gibbons arriving at Kalaweit; they were already infected with gibbon HBV before encountering caretakers. Immunization was recommended for the protection of Kalaweit caretakers, and approximately 40 % of caretakers were immunized with the hepatitis B vaccine. In addition, none were susceptible.

Previous reports indicate that the origin of HBV infection in humans and other primates is unresolved [3, 10]. We suspect that the new HBV/J recently found in a Japanese HCC patient infected individuals in Kalimantan, because it is more closely related to human than to non-human HBV strains [12], with a 33-nucleotide deletion in the pre-S1 region unique to non-human primate HBV. This discovery is particularly important for the inhabitants of Kalimantan, because the HBV/J strain is associated with HCC, an advanced stage of chronic hepatitis B. Hence, it is necessary to monitor the spread of HBV/J in Kalimantan among human and non-human primates. We did not find HBV/J in this study. Interestingly, the HBV/J strain was

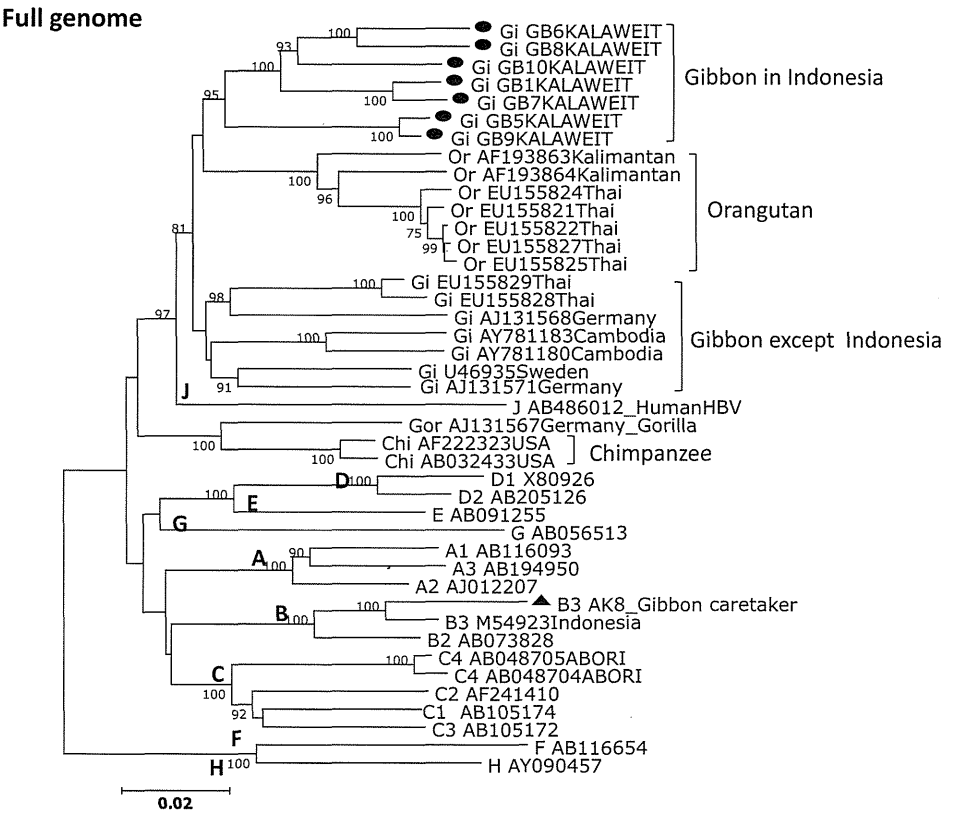

Fig. 2 Phylogenetic tree based on complete genome sequences of HBV strains isolated from seven gibbons and a human in Kalimantan along with 34 reference strains. The numbers at the nodes indicate the bootstrap reliability. The lengths of the horizontal bars indicate the number of nucleotide substitutions per site. The country of origin and accession numbers are provided for isolates from the databases

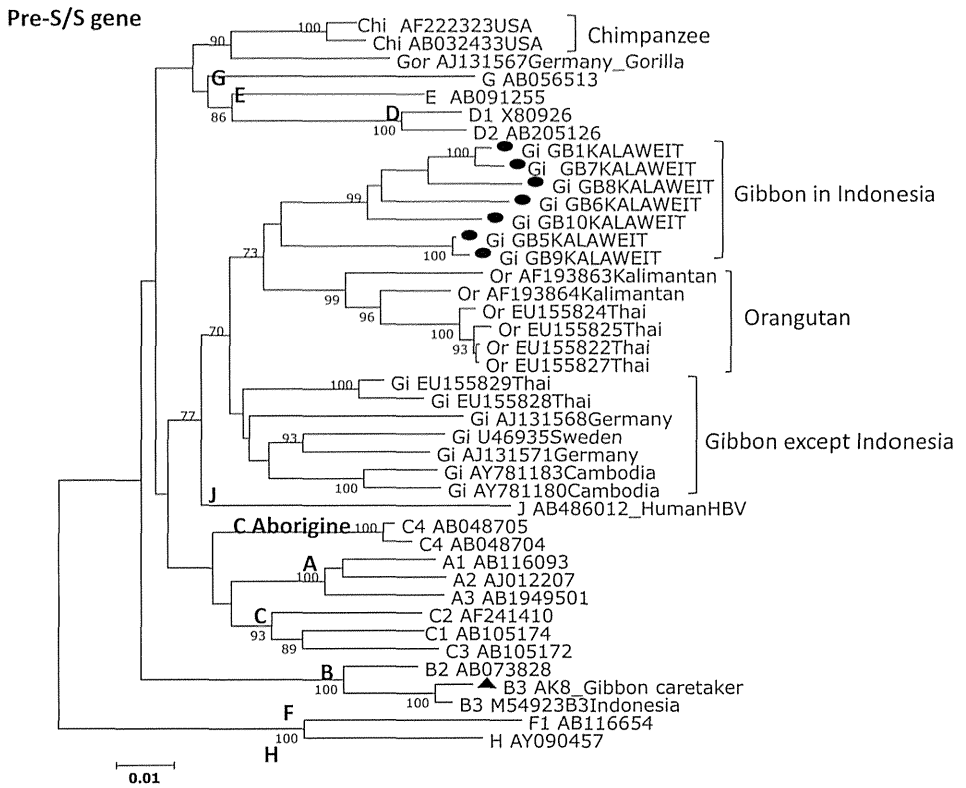

Fig. 3 Phylogenetic tree based on pre-S/S gene sequences of HBV strains isolated from seven gibbons and a human in Kalimantan along with 34 reference strains. The numbers at the nodes indicate the bootstrap reliability. The lengths of the horizontal bars indicate the number of nucleotide substitutions per site. The country of origin and accession numbers are provided for isolates from the databases

more closely related to the gibbon strains in this study in the core region than in the complete genome and S region, both of which were found to be distinct. We also did not find the HBV/J genotype in our studies of HBV among asymptomatic carriers and chronic HBV patients in East Kalimantan (Utsumi et al., unpublished data), suggesting that this strain is not widespread in Indonesia. This also suggests that cross-infection between humans and non-human primates is extremely rare. Indonesia is an endemic area for HBV infection. Several studies on orangutan genetics have been carried out in Kalimantan [15, 21]. There are no reports of HBV transmission between human and non-human primates in Indonesia. However, the discovery of HBV/J suggests that cross-transmission is possible, and the genome organization of non-human primate HBVs is nearly identical to that of human HBVs. Considering the discovery of HBV/J in Kalimantan, it is necessary to accumulate additional molecular epidemiological data on HBV infection in human and non-human primates inland of Kalimantan.

The prevalence of HBV carriers in primates is approximately 20–30 % or more [4, 22]. Because we could only obtain ten serum samples from gibbons at Kalaweit, the prevalence of HBV is not clear. Of the ten gibbons, six were still positive for HBsAg since arriving at Kalaweit (the duration of stay ranged from 1 to 10 years) (Table 1) and were considered chronically infected. Interestingly, in the four remaining HBsAg-negative gibbons, we detected HBV DNA; this pattern was consistent with a human HBV infection called occult HBV infection, characterized by the absence of HBsAg and the presence of HBV DNA. Occult HBV infection has not yet been reported in non-human primates. Human occult HBV infection is possibly caused by changes in antigenicity [23]. In this study, we did not observe a specific mutation in the *a* determinant region that could influence antigenicity, so further research is needed to confirm this occult-HBV-infection-like phenomenon. In addition, even though the viral load was low in HBsAg-negative gibbons, it was detectable and sufficient for sequencing; it could be a source of virus spread in the gibbon population. We need to be aware that these infections have been detected in captive animals, which may have been exposed to other HBV-infected hosts during captivity. The C^{1753}, A^{1896}, and T^{1896} mutations in the core region, which have been associated to advanced liver disease [24], were found in three gibbons. The precise clinical course of gibbons infected with HBV is not fully understood. In this study, HBsAg-positive gibbons had higher HBV-DNA levels compared with HBsAg-negative ones, and anti-HBs was detected only in gibbons that were HBsAg negative and had low HBV-

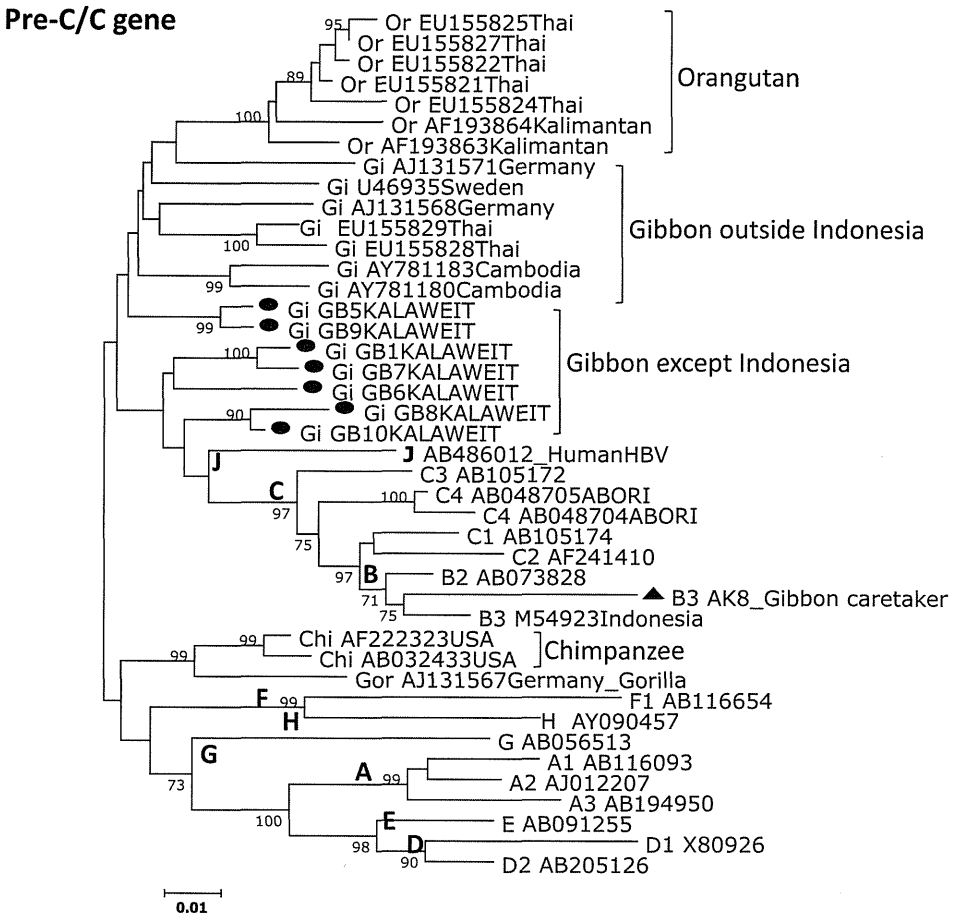

Fig. 4 Phylogenetic tree based on pre-C/C gene sequences of HBV strains isolated from seven gibbons and a human in Kalimantan along with 34 reference strains. The numbers at the nodes indicate the bootstrap reliability. The lengths of the horizontal bars indicate the number of nucleotide substitutions per site. The country of origin and accession numbers are provided for isolates from the databases

DNA levels (Table 1). This suggests that HBs seroconversion occurs after HBsAg seroclearance. Due to the similarity between the genetic organization of human and non-human primate HBV, the clinical manifestations may be similar to hepatitis induced by human HBV [12]. This requires further investigation.

In conclusion, a phylogenetic analysis of complete genome sequences revealed that gibbon HBV strains in Central Kalimantan formed a distinct cluster, separate from hepadnaviruses of other hosts. A unique amino acid residue (P89 K), three insertions, and other point variations in the pre-S region contribute to this distinct HBV genotype, and the geographic location and host species influenced the gibbon HBV genotype [25]. To the best of our knowledge, this is the first report characterizing the HBV genes and genomes of indigenous gibbons in Indonesia.

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Conflict of interest The authors declare that they have no conflict of interest.

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Acute Hepatitis due to Hepatitis A Virus Subgenotype IA as an Imported Infectious Disease from Indonesia

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ABSTRACT

A 25-year-old Japanese man was admitted with general malaise and fever, which had developed 12 days after coming back to Japan from Indonesia. Blood examination revealed elevated transaminase levels and positivity for the IgM anti-HAV antibody; therefore, he was diagnosed with acute hepatitis A. HAV-RNA was detected in his serum and phylogenetically classified as subgenotype IA. The partial genome in the VP1/P2A region was consistent with the strain recently isolated from Surabaya, which indicated that he had been infected during his stay in Indonesia. Thus, HAV vaccination is recommended before visiting HAV-endemic countries for a long period of time.

INTRODUCTION

Hepatitis A virus (HAV) is a non-enveloped RNA virus with an icosahedral symmetry that belongs to the genus Hepatovirus of the Picornaviridae family (1), and has been classified into 7 genotypes (I to VII) that exhibit distinct geographic distributions (2-4). HAV causes acute hepatitis in humans worldwide, and is transmitted by a fecal-oral route in areas with poor sanitation and weak public-health infrastructures, either from person-to-person contact or through contaminated food or water. The HAV infection is endemic in developing countries including Indonesia, with the majority of individuals in these countries being exposed to HAV during early childhood (5, 6). In contrast, the exposure rate of the adult population in developed countries including Japan to HAV has decreased due to improvements in hygiene. On the other hand, the incidence of the so-called imported hepatitis A, which is carried by a person infected during a visit to HAV endemic regions, has increased (7, 8).

In the present study, we described a 25-year-old Japanese man who developed acute hepatitis due to HAV subgenotype IA (HAV/IA) 12 days after coming back to Japan from Indonesia. The HAV strain that was isolated from the patient, who was presumed to have contracted HAV infection while visiting Indonesia, was consistent with the strain recently isolated from Surabaya. Additionally, it was closest to the Indonesian HAV/IA strain obtained from Genbank, with an identity of 99.1%. These results supported the Indonesian origin of the imported strain.

CLINICAL CASE

A 25-year-old Japanese man from Hyogo prefecture developed a high fever, general malaise, and anorexia from the end of January 2014, and visited a nearby Internal Medicine Clinic 3 days after the onset of these clinical symptoms. A blood examination revealed markedly elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. The patient was transferred to a general hospital in the same area of Hyogo the next day with a clinical diagnosis of acute hepatitis. The patient began living in Surabaya, Indonesia from September 2012 for his work, and had returned to Japan for a short visit 12 days before the onset of clinical symptoms. He had no history of liver disease or hepatitis and had been immunized with the hepatitis B vaccine, but not with the hepatitis A vaccine. Alcohol intake was reported at 330 mL beer on a weekly basis for 5 years. He had not eaten raw fish or shellfish one month before the onset of clinical symptoms, but often bought and

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consumed food from local food stalls in Surabaya, Indonesia. The patient denied having had sexual activities during his stay in Surabaya.

Table I. Laboratory data on admission

Parameters	Results	Normal range
White blood cell count (/mm^3)	7000	4000-9000
Hemoglobin (g/dL)	17.1	13.5-17.0
Platelet count (x 10^4/mm^3)	13.8	15.0-35.0
Prothrombin time activity (second)	13.0	9.6-12.7
Prothrombin time activity (%)	87	80-125
Albumin (g/dL)	4.4	3.8-5.1
Total bilirubin (mg/dL)	5.2	0.2-1.2
Aspartate aminotransferase (IU/L)	1650	10-30
Alanine aminotransferase (IU/L)	2838	3-30
Alkaline phosphatase (IU/L)	283	165-320
IgM anti-hepatitis A virus antibody (S/CO)	Positive (9.65)	Negative
IgM anti-hepatitis B core antibody	Negative	Negative
Hepatitis B surface antigen	Negative	Negative
Anti-hepatitis C virus antibody	Negative	Negative

On admission, a persistent fever and jaundice were noted. His consciousness was clear, and vital signs were as follows: blood pressure 120/70 mmHg, heart rate 72/min, temperature 38.2℃. Laboratory data on admission are shown in Table I. Serum levels of aspartate aminotransferase (AST; 1,650 IU/L), alanine aminotransferase (ALT; 2,838 IU/L), and total bilirubin (T-Bil; 5.2 mg/dL) were markedly elevated. Tests for the IgM anti-hepatitis B virus core antibody, hepatitis B surface antigen, and hepatitis C virus antibody were all negative. However, the serum IgM anti-hepatitis A virus antibody was positive, and he was consequently diagnosed with acute hepatitis A. HIV test was not performed. The patient was suspected of having imported hepatitis A because he lived in Indonesia, and we genetically analyzed HAV using serum obtained after discharge (24 days and 31 days after the onset of clinical symptoms). HAV RNA was detected by RT-PCR amplifications using HAV-specific primers in VP1/P2A from the samples obtained after his discharge (after 24 days, but not 31 days).[2] The PCR products were then directly sequenced (isolate name KSI25, DDBJ/EMBL/GenBank accession No. AB917146). A phylogenetic tree of the HAV genome constructed using 215 nucleotide sequences in the VP1/P2A region (Fig.1) indicated KSI25 belongs to HAV/IA. KSI25 was 100% identical to a strain recently identified in Surabaya (isolate name SSI19, DDBJ/EMBL/GenBank accession No. AB918714), and 99.1% to previously reported Indonesian strains (AB839696, AB839697, AB839693) from Java Island, in which Surabaya city belongs to (Fig. 1) (5).

The patient was given bed rest during a 4-day hospital stay, recovered rapidly without sequelae, and was subsequently discharged. AST/ALT serum levels gradually decreased and reached normal ranges one month after the onset of clinical symptoms (Fig. 2).

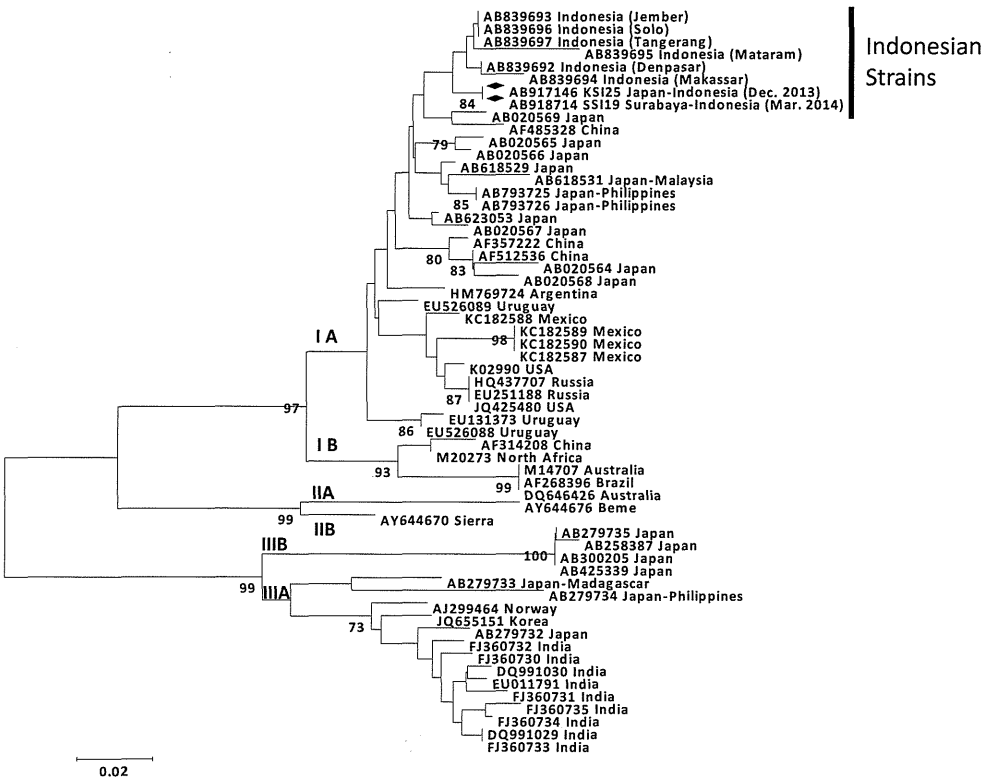

Figure 1. Phylogenetic tree of the VP1/P2A region of the hepatitis A virus (HAV) strain isolated from the Japanese patient and 57 reference strains described herein. Numbers in the tree indicate bootstrap reliabilities. The lengths of the horizontal bars indicate the number of nucleotide substitutions per site. Isolates from the database are indicated by their accession number, and relevant country names have been added to each HAV strain.

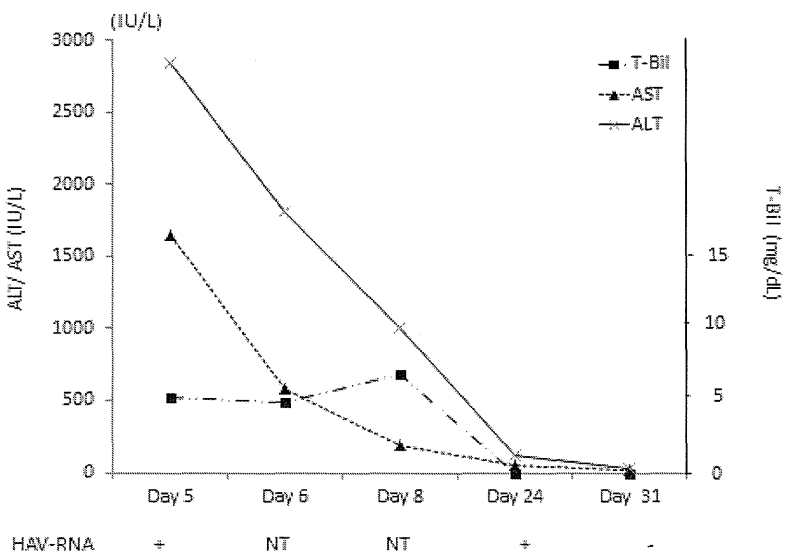

Figure 2. Clinical course of the case. Day shows the period after the onset of clinical symptoms. NT= Not tested.

DISCUSSION

We determined and analyzed the HAV genomic sequence of a HAV/IA isolate that was recovered from a Japanese patient who lived in Surabaya and developed acute hepatitis A 12 days after returning to Japan. The HAV isolate from the patient was consistent with the strain recently isolated from Surabaya, and was closest to the Indonesian HAV/IA strains obtained from Genbank, with an identity of 99.1%. This result supports the Indonesian origin of the imported strain. The period of one month from suspected infection to the onset of clinical symptoms, which represents the average incubation period of HAV infection, was also supportive to this diagnosis. As well as HAV, HEV infection is also endemic in Indonesia (6). HEV outbreak, however, usually occur after flood in the limited areas, and the prevalence is as low as 0.5% in Surabaya (7).

HAV/IA is a common subgenotype worldwide. Furthermore, Indonesia is a well-known endemic region of HAV, and no other HAV subgenotypes has ever reported (5, 8, 11). The incubation phase lasts between 2 and 7 weeks (mean 28-30 days). Thus, we speculated that our patient was infected with HAV/IA in Indonesia in December 2013, just before he left for Japan. Viremia terminates shortly after hepatitis develops, although feces remain infectious for another 1 - 2 weeks (12). However, in this case, HAV RNA was detectable until 24 days after the onset of clinical symptoms and a decrease in serum ALT/AST levels, which suggested that this patient was a source of infection until HAV cleared. A previous study also reported that some cases had HAV viremia after seroconversion to the HAV antibody (13). Hereafter, HAV/IA infection should be considered also as an imported infectious disease. Physicians are recommended to take precautionary measures against the spread of HAV infection when encountering patients with acute hepatitis A.

In 2003, the overall anti-HAV prevalence in Japan was 12%. Fifty percent of individuals over 50 years of age had anti-HAV whereas only 2% of people younger than 50 years of age had immunity (14). Moreover, in recent years, the incidence of hepatitis A in Japan has dramatically decreased, and therefore there might be a decrease in the proportion of persons who have immunity against HAV (15). HAV infection is preventable by vaccination, but vaccine is unavailable for hepatitis E. Regardless of vaccine availability, the improvement of public sanitation and health education for improved hygiene practices is the first line of defense against hepatitis A and E, as well as other enterically-transmitted diseases in developing world including Indonesia. Several countries, including Argentina, China, Israel and United States of America (USA) have introduced the hepatitis A vaccine in routine childhood immunizations. While the two-dose regimen of inactivated hepatitis A vaccine is common in many countries, a single-dose vaccination is used in some countries (http://www.who.int/mediacentre/factsheets/fs328/en/). Some countries also recommended the vaccine for people at increased risk of hepatitis A such as Japan. The current incidence of hepatitis A in Japan might be too low to warrant the introduction of a universal vaccination policy (14). In USA, recommendations now call for routine hepatitis A vaccination for all children beginning at the age of 1 year (12-23 months). In Japan, on the other hand, those more than 16 years old are considered to be vaccinated two-dose with 6 month interval not routinely but on request.

The present patient had not received vaccination for HAV prior to traveling to Indonesia. Therefore, travelers to countries where HAV infection is endemic should receive vaccine before travel, unless they have previously been vaccinated or infected with HAV. For the travelers in a hurry, even single dose can produce 99.3% efficacy for anti-HAV immunization after 4 weeks of vaccination, as previous study in Japan found. Similarly it described that a single dose of hepatitis A vaccine is sufficient to produce rapid immunity within 2 weeks (16).

In conclusion, attention should be paid to HAV/IA infection as an imported infectious disease because HAV IA infection is widespread. The results of the present study indicate that HAV vaccination is recommended prior to visiting HAV endemic countries, especially for long periods of time.

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Interleukin-28B Polymorphisms and Response of Chronic Hepatitis C Patients from Indonesia to Pegylated Interferon/Ribavirin Treatment

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This study demonstrated that Indonesian patients with chronic hepatitis C (mostly ethnic Java people) mostly were infected with hepatitis C virus (HCV) genotype 1; however, they carried mainly the major genotypes of interleukin 28B (IL-28B) single nucleotide polymorphisms (SNPs) (rs12979860 CC, rs11881222 TT, rs8103142 AA, and rs8099917 TT), and they mostly achieved sustained virological responses to pegylated interferon/ribavirin treatment.

Plasma and peripheral blood mononuclear cells (PBMC) were collected from chronic hepatitis C (CHC) patients in Dr. Saiful Anwar General Hospital, Malang, East Java Province, or a private clinic in Sanglah, Denpasar, Bali Province, Indonesia. All the patients were treated with pegylated interferon (PEG-IFN)-alfa-2a (Pegasys) and ribavirin (RBV). Patients with hepatitis C virus (HCV) genotype 1 were treated for 48 weeks, and patients with HCV genotype 2 or 3 were treated for 24 weeks. The maternal and paternal ethnicities of each patient were carefully documented for three previous generations. Plasma and PBMC were examined at the Institute of Tropical Disease, Airlangga University, Surabaya, East Java Province. Data on the pretreatment HCV viral loads and HCV genotypes of the patients were obtained from their medical records. Ethical clearance for this study was obtained from the Ethics Committee of Dr. Saiful Anwar General Hospital.

HCV RNA was extracted from 140 μl plasma using a commercially available kit (QIAmp viral RNA kit; Qiagen, Tokyo, Japan). To amplify the NS5B region of the HCV genome, the extracted RNA was reverse transcribed and amplified using SuperScript One-Step reverse transcription-PCR (RT-PCR) (Invitrogen, Tokyo, Japan) and a set of primers. PCR amplifications using outer primers (nucleotides [nt] 7999 to 8825) and inner primers (8159 to 8630) were performed as previously described (1) using Hot Star *Taq* master mix (Qiagen). The amplified fragments were sequenced by a direct sequencing method with the BigDye Terminator v1.1 cycle sequencing kit and an ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA, USA). Based on the sequence similarity to the reported sequences from the international DNA databases (DDBJ, EMBL, and GenBank) using the program Genetyx-Win version 9.0 (Genetyx Corporation, Tokyo, Japan), each HCV isolate was assigned an HCV subtype (2). The HCV genotypes/subtypes were reexamined to confirm the HCV genotype/subtype data obtained from the medical records.

The quantification of plasma HCV RNA titers was performed with the TaqMan gene expression master mix using the Applied Biosystems 7300 real-time PCR machine. The HCV 5′ noncoding region (NCR) was amplified with a primer and probe set, as described previously (3). The lowest detectable titer with this kit was 3.0 $\log_{10}$ RNA copies/ml. This assay was used to measure the HCV viral load posttreatment or during treatment (after 12 weeks of treatment) to determine the virological response to PEG-IFN/RBV based on the pretreatment HCV viral load data obtained from the medical records.

Host DNA was extracted from each PBMC sample using a QIAmp DNA kit (Qiagen, Tokyo, Japan) following the manufacturer's guidelines. To determine the interleukin 28B (IL-28B) single nucleotide polymorphisms (SNPs), PCRs amplified a short fragment containing rs12979860, rs11881222, rs8103142, and rs8099917 using specific primer pairs (4, 5). PCR amplification was performed as previously described (4, 5) using Hot Star *Taq* master mix (Qiagen). The amplified fragments were sequenced with a BigDye kit on an ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA, USA).

The data were analyzed by the chi-square test or Fisher's exact test for categorical variables. A P value of <0.05 was considered significant.

A total of 34 samples were collected from 19 (55.9%) women and 15 (44.1%) men (32 to 76 years of age; mean ± standard deviation, 58.8 ± 10.90 years) with CHC. The majority of these patients were ethnic Java people (82.4%), and the other 6 patients were of Batak-Lampung (Sumatera) (2.9%), Java-Madura (2.9%), Gorontalo (Sulawesi) (2.9%), Japan-Toraja (Sulawesi) (2.9%), or Bali (5.9%) ethnicity. Among the 34 patients, 28 (82.4%) completed the entire course of the PEG-IFN/RBV treat-

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TABLE 1 Demographic and clinical characteristics of HCV-infected patients according to their virological response

Factor	All patients	Patients with EVR/SVR	Patients with NVR/TVR	*P*
Age > 40 yr	31/34 (91.2)[a]	24/25 (96.0)	7/9 (77.7)	0.16
Gender, male	15/34 (44.1)	12/25 (48.0)	3/9 (33.3)	0.70
Race, Javanese	28/34 (82.4)	21/25 (84.0)	7/9 (77.7)	0.64
Pretreatment ALT level (IU/liter)	103.5 ± 90.50	110.50 ± 104.80	89.50 ± 55.14	0.53
HCV genotype 1	24/34 (70.6)	16/25 (64)	8/9 (88.9)	0.23
Pretreatment Metavir score, F3 to F4	3/24 (12.5)	1/15 (6.7)	2/9 (22.2)	0.25

[a] Data indicate either the number of patients with the indicated characteristic/total number of patients (%) or the mean ± standard deviation.

ment and were followed up for 24 weeks. The other 6 patients were treated for >12 weeks.

In this study, virological responses to PEG-IFN/RBV were classified as being in one of two groups, (i) nonvirological response (NVR)/transient virological response (TVR) (poor response) or (ii) early virological response (EVR)/sustained virological response (SVR) (good response). Overall, 25 (73.5%) patients achieved an EVR/SVR, while 9 (26.5%) had an NVR/TVR. No significant differences in age, gender, race, pretreatment alanine aminotransferase (ALT) level, severe fibrosis (stage F3 or F4), or HCV genotype were observed among the virological responses (all $P > 0.05$) (Table 1). All these factors may not have influenced the virological response; however, this has been attributed to the small number of patients with poorly distributed factors, i.e., a large number of HCV genotype 1 patients together with a small number of patients with a TVR or NVR. Most of the patients (24/34, 70.6%) were infected with HCV genotype 1. The confirmation assay showed that HCV subtype 1b (56%, 5/9) was predominant among all positive PCR products obtained, followed by HCV subtypes 1a (22%, 2/9) and subtypes 1c and 2a (11%, 1/9 each). These results were consistent with the findings of previous studies in Indonesia, in which HCV subtype 1b was predominant in HCV-associated liver disease patients (6). Patients infected with HCV genotype 1, as documented, were mostly slow responders and required longer treatment durations than patients infected with genotype 2 or 3 (7). In a study performed in the United States and Europe, 42% to 52% of patients with HCV genotype 1 achieved an SVR (8). However, the response rate was markedly higher in China when patients were treated with the corresponding regimen (9). Our study on the Indonesian population showed that the proportion of patients with HCV genotype 1 was higher (64.0%) in the EVR/SVR group. As has long been suspected, host genetic factors may be the key determinants for CHC treatment success.

The results showed that most of the patients (94%) carried the major genotypes (rs12979860 CC, rs11881222 TT, rs8103142 AA, and rs8099917 TT). The frequencies of the major genotypes of the four SNPs were higher in the EVR/SVR group (75.0% to 75.8%) than in the NVR/TVR group (24.2% to 25.0%) (Table 2); however, these differences were not statistically significant ($P > 0.05$ for each SNP), which may have been due to the rare event of the heterozygous/minor genotype of IL-28B SNPs. The majority of patients (64.0%) who achieved an EVR/SVR were infected with HCV genotype 1, and most of them (93.8%) carried the major genotypes of the four SNPs of IL-28B. Homozygosity for the major allele of SNPs associated with IL-28B was correlated with a better response to PEG-IFN/RBV treatment, and minor allele-positive patients were found to be poor responders (10). Of the limited number of patients with HCV genotype 2 or 3 infection, most (90.0%, 9/10) achieved an EVR/SVR, and all of them carried the major genotypes of the four SNPs. In contrast to the data on HCV genotype 1 infection, several studies have not demonstrated any clear association between IL-28B polymorphisms and SVR in patients with HCV genotype 2 or 3 infection (11, 12). The role of predictive factors such as IL-28B polymorphisms in patients with HCV genotype 2 or 3 infection may not be as important as that in the former group (12).

One patient with the heterozygous genotypes of the four SNPs showed an NVR, while another patient with the major genotypes of rs12979860, rs11881222, and rs8099917 and the heterozygous genotype of rs8103142 achieved an SVR. Akkarathamrongsin et al. (1) reported that most patients with heterozygous and minor homozygous genotypes of rs8103142 and rs11881222 (70% and

TABLE 2 Virological responses according to IL-28B polymorphisms

Virological response (*n*) or data type	No. (%) of IL-28B polymorphism[a]:							
	rs12979860		rs11881222		rs8103142		rs8099917	
	CC[b]	CT[c]	TT[b]	TC[c]	AA[b]	AG[c]	TT[b]	TG[c]
NVR/TVR (9)	8 (24.2)	1 (100)	8 (24.2)	1 (100)	8 (25.0)	1 (50)	8 (24.2)	1 (100)
SVR/EVR (25)	25 (75.8)	0	25 (75.8)	0	24 (75.0)	1 (50)	25 (75.8)	0
Total	33 (100)	1 (100)	33 (100)	1 (100)	32 (100)	2 (100)	33 (100)	1 (100)
P value	0.265		0.265		0.465		0.265	
Odds ratio (95% CI[d])	NA[e]		NA		3.00 (0.17–53.71)		NA	

[a] Minor types of IL-28B polymorphisms are not indicated, since there was no sample obtained.
[b] Major types.
[c] Heterozygous types.
[d] CI, confidence interval.
[e] NA, not available.

100%, respectively) were nonresponders (4). However, another study found an NVR in a patient with the heterozygous genotypes of rs8099917 and rs12979860 and with the major genotypes of rs11881222 and rs8103142 (10). Therefore, rs8099917 and rs12979860 may have stronger influences on treatment than those of rs11881222 and rs8103142.

Based on IL-28B polymorphisms, the frequencies of HCV clearance vary markedly across ethnic groups. The protective allele of rs12979860 was reported to be predominant in an East Asian population (13), and that of rs8099917 was predominant in European and Japanese ancestries (14, 15). The several other SNPs within the IL-28B gene (including rs11881222 and rs8103142) showed strong linkage disequilibrium with rs12979860 and rs8099917 (15). Among the 25 patients who achieved an EVR/SVR, 84.0% (21/25) were Javanese, all of whom carried the major genotypes of the four SNPs of IL-28B. The remaining 4 patients with an EVR/SVR were people of Java-Madura ($n = 1$), Bali ($n = 1$), or Batak-Lampung ($n = 1$) ethnicity with the major genotypes of the four SNPs, and the other 1 Balinese patient carried the major genotypes of rs12979860, rs11881222, and rs8099917 and the heterozygous genotype of rs8103142. Further studies using more samples from patients from other ethnic groups are warranted because Indonesia has hundreds of ethnic groups.

This study showed that although these patients (mostly Javanese) were infected mostly with HCV genotype 1, most of them achieved good responses (EVR/SVR) to the PEG-IFN/RBV treatment. The major types of IL-28B polymorphisms may have contributed to these results. Further study in other ethnic groups of Indonesians is now underway in our laboratory.

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REVIEW

Molecular epidemiology of hepatitis B virus in Asia

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Abstract

Although safe and effective vaccines against hepatitis B virus (HBV) have been available for three decades, HBV infection remains the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) worldwide, especially in Asian countries. HBV has been classified into at least 9 genotypes according to the molecular evolutionary analysis of the genomic DNA sequence and shown to have a distinct geographical distribution. Novel HBV genotypes/subgenotypes have been reported, especially from Southeast Asian countries. The clinical characteristics and therapeutic effectiveness of interferon (IFN) and nucleos(t)ide analogues vary among different HBV genotypes. Mutations at T1653C in subgenotype C2 from Japan and South Korea, C/A1753T and C1858T in subgenotype C1 from Vietnam, and C1638T and T1753V in subgenotype B3 from Indonesia were reported to be associated with advanced liver diseases including HCC. Genotype distribution in Japan has been changed by an increasing ratio of subgenotype A2 in chronic hepatitis B. While a large number of epidemiological and clinical studies have been reported from Asian countries, most of the studies were conducted in developed countries such as Taiwan, China, South Korea and Japan. In this review, the most recent publications on the geographical distribution of genetic variants of HBV and related issues such as disease progression and therapy in Asia are updated and summarized.

Key words: Hepatitis B virus; Genotype; Subgenotype; Molecular epidemiology; Asia; Pathogenicity; Drug resistance

Core tip: Chronic hepatitis B virus (HBV) infection usually progresses to liver cirrhosis and hepatocellular carcinoma. The variation of the HBV genotype is related to the geographical distribution. Also, the clinical characteristics and therapeutic effectiveness of interferon and nucleos(t)ide analogue vary among different HBV genotypes. A large number of epidemiological and clinical studies have been reported from Asian countries. However, most of the studies were conducted in developed countries such as Taiwan, China, South Korea and Japan. In this review, epidemiologically and clinically important aspects of HBV genotypes/subgenotypes found in East and Southeast Asian countries are updated and summarized.

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INTRODUCTION

Although safe and effective vaccines against Hepatitis B virus (HBV) have been available for more than three decades, HBV infection remains a burden to global public health, resulting in 600000 to 1 million deaths per year worldwide[1]. Two billion people are estimated to be exposed to HBV infection once in their life and it causes a wide spectrum of liver disease, including acute or fulminant hepatitis, inactive carrier state, reactivation, chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC)[2]. More than 420 million individuals in the world are estimated to have chronic HBV infections; 15%-40% of them are at risk of death due to liver failure or HCC[3]. The prevalence of HBV infection varies markedly in different geographical areas of the world. Overall, approximately 45% of the global population live in areas of high HBV prevalence, such as sub-Saharan Africa, the Pacific and particularly Asia[4].

HBV has been classified into at least 9 genotypes (A through H and J) and shown to have a distinct geographical distribution[5,6]. In Asia, HBV genotypes B and C are prevalent, with genotype C having been shown to cause more serious liver diseases than genotype B. High prevalence of HBV mutants with various forms, such as the pre-S mutants, basal core promoter (BCP) mutants, YMDD motif mutants and vaccine escape mutants[7,8], were seen in Asia and these were found to be related to severe liver diseases and resistance to treatment and prevention. This article provides an overview of the molecular-based epidemiology of HBV in Asian countries.

HBV GENOME

HBV contains a partially double-stranded DNA genome of approximately 3200 base pairs. HBV replicates *via* a RNA intermediate anti-genome sequence, which encodes a potentially error-prone polymerase without proof-reading activity. The error frequencies are similar to those of retroviruses and other RNA viruses. The HBV genome encodes viral proteins through four open and partially overlapping reading frames: surface (*S*), core (*C*), polymerase (*P*) and *X* genes. This unusual genomic structure can compress a large amount of information into short sequences but implies a constrained evolution for the virus. This constraint can be reflected on the calculated rate of substitution, 10^{-5} per site per year, slower than the rate displayed by the retroviruses of around 10^{-3} per site per year[9].

EPIDEMIOLOGY OF HBV INFECTION

The prevalence of chronic HBV infection varies greatly in different parts of the world and can be categorized as high (≥ 8%), intermediate (2%-7%) and low (< 2%) endemicity. Table 1 shows the prevalence of hepatitis B surface antigen (HBsAg)-positive individuals in the general population of Southeast Asia and East Asia. HBV infection is highly endemic in Myanmar[10]; has intermediate to high endemicity in Indonesia[11-13], Cambodia[10,14], Thailand[15,16], the Philippines[17-19], Vietnam[20,21] and Laos[10,24]; low to high endemicity in Malaysia[22,23] and China[25-27]; and intermediate endemicity in Singapore[28,29], Brunei[30,31] and South Korea[32,33]. Japan is the only country with low endemicity of HBV infection in Asia[22].

Table 1 Prevalence of hepatitis B surface antigen in the general Asian population

Country	HBsAg positivity (%)	Ref.
Southeast Asia		
Brunei	4.7	Sebastian *et al*[30]
	6.0	Alexander *et al*[31]
Cambodia	7.7	OI *et al*[14]
	10.8	Sa-Nguanmoo *et al*[10]
Indonesia	3.5-9.1	Hasan[11]
	4.9	Achwan *et al*[12]
	2.1-10.5	Lusida *et al*[13]
Laos	6.9	Jutavijittum *et al*[24]
	8.7	Sa-Nguanmoo *et al*[10]
Malaysia	3.0-5.0	Merican *et al*[22]
	0.5-1.8	Yousuf *et al*[23]
Myanmar	9.7	Sa-Nguanmoo *et al*[10]
Philippines	10.0	Lingao *et al*[17]
	2.0-16.0	Lansang *et al*[18]
	16.7	Wong *et al*[19]
Singapore	3.6-4.0	James *et al*[28]
	2.7-4.0	Ang *et al*[29]
Thailand	4.0	Suwannakarn *et al*[15]
	13.8	Louisirirotchanakul *et al*[16]
Vietnam	11.4	Viet *et al*[20]
	7.5	Reekie *et al*[21]
East Asia		
China	2.4	Ting-Lu *et al*[25]
	1.0	Liu *et al*[26]
	10.6	Chen *et al*[27]
Japan	0.8	Merican *et al*[22]
South Korea	3.0-4.0, 6.0	Kim *et al*[32]
	6.0	Hyun *et al*[33]

HBsAg: Hepatitis B surface antigen.

HBV infection is highly endemic in developing regions with a large population such as Southeast Asia and China, where at least 8% of the population are HBV chronic carriers. For example, in Indonesia, which consists of thousands of islands with many ethnicities, the endemicity of HBV infection greatly varies even within the country. The wide range of the HBV prevalence is largely related to differences in age at the time of infection[3].

HBV GENOTYPES/SUBGENOTYPES AND THEIR GEOGRAPHICAL DISTRIBUTIONS

HBV is currently grouped into at least 9 genotypes (A through H and J, with I still being controversial)[6,34,35], based on a full genome diversity of more than 8% at the nucleotide (nt) level, and phylogenetic analyses have shown that most of the genotypes can be further divided into subgenotypes differing by at least 4% of their full genome sequences. The prevalence of each HBV genotype and subgenotype varies in different geographical regions and is strongly associated with ethnicity[36].

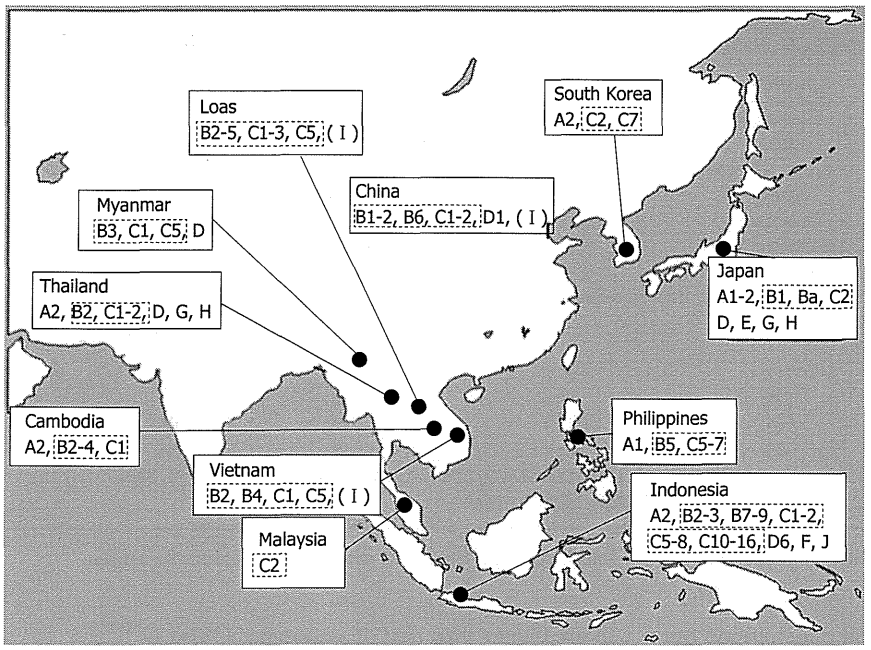

Figure 1 Genotype/subgenotype distribution in East and Southeast Asia. Subgenotypes of genotypes B and C commonly found in Asia are encircled with dotted lines.

Genotype A is highly prevalent in Sub-Saharan Africa (A1 or Aa: a for Africa), Northern Europe (A2 or Ae; e stands for Europe) and Western Africa (A3). Genotypes B and C are the major HBV genotypes circulating in East and Southeast Asia[37] (Figure 1) and co-infection has led to a frequent occurrence of recombination between these two genotypes[38,39]. Subgenotype B1 (or Bj; j for Japan) is found almost exclusively in Japan and B2 (or Ba; a for Asia) is found in the rest of Asia[40,41], but mainly in China and Vietnam. B1 is not a recombinant while B2 is considered to be B/C recombinants with the precore and core genes from genotype C. B3 is mostly found in Indonesia[42] while B4 is in Vietnam[5]. B5 was initially reported in 2006 from the Philippines[43]. B6 was identified in 2007 from the Arctic[39]. B7 to B9 were isolated in eastern Indonesia during the years 2007 to 2011[44-46]. C1 (or Cs: s for Southeast Asia) is the dominant strain in Southeast Asia and southern China, while C2 (or Ce: e for East Asia) is found mainly in East Asia (South Korea and Japan) and the northern part of China, C3 in Oceania[47] and C4 in the Aborigines from Australia[48]. C5 was initially reported in 2006 from the Philippines with B5[44]. C6 was identified from a Papuan population in Indonesia[13,49] and the Philippines[50] in 2008. Surprisingly, ten novel subgenotypes (C7 to C16) were isolated in Indonesia during 2009 to 2012[45,51-54]. Subgenotypes D1 to D4 of genotype D are widely distributed globally[5], D5 in India[55] and D6 in Papua, Indonesia[13]. Genotype E is found mainly in sub-Saharan Africa. Genotypes F and H are found mainly in South and Central America, respectively. Genotype G has been found in Europe, United States and Japan. Genotype I was originally identified in Laos[56], Vietnam and Southern China. However, this classification is still controversial as the sequence divergence hovers at but is slightly less than 8%, with a close relationship to genotype C[35]. Genotype J was found in a Japanese soldier who was thought to have been infected in the forests in Kalimantan, Indonesia, during World War II[57]. Thus, novel HBV genotypes and novel subgenotypes have been found in Southeast Asia, especially in Laos, Vietnam, the Philippines and Indonesia, all consisting of many islands and ethnic groups. In addition to genotypes B and C which are common in Asia, an increasing rate of infection with rare HBV genotypes, such as genotypes A, D, E, G and H, has been recognized throughout Asia. Globalization may yield HBV strains of possible novel genotypes containing novel nucleotide sequences in the precore/core region[58]. The distribution of genotypes/subgenotypes varies even in different regions of a country, as observed in Indonesia, which may partly be related to the ethnic origin of the infected patients.

HBV GENOTYPES AND DISEASE PROGRESSION

Chronic HBV infections usually progress to liver cirrhosis and HCC. Several studies revealed that the presence of hepatitis B e antigen (HBeAg) and high levels of HBV DNA were independent risk factors for the development of liver cirrhosis and HCC[59-62]. HBV genotypes are also related to the clinical characteristics[63]. In northeast Asian countries, where genotypes B and C are prevalent, the dominant mode of transmission is vertical (mother-to-child). A large number of studies have shown that genotype B is associated with HBeAg seroconversion at an earlier age, more sustained remission after HBeAg seroconversion, less active hepatic necroinflammation, a slower rate of progression to cirrhosis, and a lower rate of HCC development compared to genotype C[59,64-67]. On the other hand, genotypes D and A are prevalent in the southwest Asian countries, such as India and Pakistan[68]. The transmission route among Pakistanis, includ-

Table 2 Summary of nucleos(t)ide analogues

	Lamivudine	Adefovir	Entecavir	Telbivudine	Tenofovir	Ref.
Analogue type	Nucleoside	Nucleotide	Nucleoside	Nucleoside	Nucleotide	
Introduction (yr)	1999	2002	2005	2006	2008	
Product name (company)	Zefix (GSK)	Hepsera (Gilead)	Baraclude (BMS)	Sebivo (Novartis)	Viread (Gilead)	
Dose	100 mg	10 mg	0.5 mg	600 mg	300 mg	
	Once daily	Once daily	Once daily	Once daily	Once daily	
Advantage	Low cost	Effective for HIV coinfection		Possible for pregnancy	Effective for HIV coinfection	[89]
Disadvantage	High rate of drug resistance	Renal dysfunction Fanconi anemia	Not recommend for pregnancy	Renal dysfunction	Renal dysfunction Fanconi anemia	
Undetectable HBV-DNA						
HBeAg positive	36%	21%	67%	60%	76%	[90]
HBeAg negative	89%	72%	90%	88%	93%	
HBeAg seroconversion	22%	12%	21%	23%	21%	[91]
Drug-resistance	24%	0%	0.2%	4%	0%	[92]
Drug-resistant mutation	V173l, L180M, A181T, M204V/I	A181V/T, N236T	I169T, L180M, T184A/F/L/S, S202G/I, M204V, M250V	M204V/I	A181V/T, N236T	

GSK: Glaxo Smith Kline; BMS: Bristol-Myers Squibb; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen.

ing Afghan refugees, is not only vertical transmission but also through unsterilized materials and intravenous drug use[69,70]. Reports concerning the risk factors of advanced liver diseases are still limited in those countries.

Mutations in the viral genome, including the X region, are also important factors in association with disease progression. A study from Taiwan revealed that the precore G1896A wild-type and the BCP A1762T/G1764A mutation were strongly associated with HCC development among genotype C[71]. A study from north India also showed that the BCP A1762T/G1764A mutation was associated with progressive liver diseases among genotype D[72]. In Japan and South Korea, the T1653C mutation was reported as a predictive factor for the development of advanced liver diseases in HBV genotype C2 infection[73,74]. Whereas the C/A1753T and C1858T mutations were associated with advanced liver diseases in genotype C1 infection in Vietnam, C1638T and T1753V were independent risk factors for advanced liver diseases in genotype B3 infection in Indonesia[42,75]. In addition, several studies from Taiwan and Japan showed that the pre-S mutation also contributed to the progressive liver disease and HCC[76,77]. The progression from acute hepatitis to chronic infection occurs more frequently in genotype A (23%) compared with genotypes B (11%) and C (7%)[78]. This might change genotype distribution in the future. In Japan, indeed, the prevalence of genotype A in chronic hepatitis B increased from 1.7% to 3.5% during the period between 2000 and 2006[79].

HBV GENOTYPE AND ANTIVIRAL THERAPY

The purpose of antiviral therapy for chronic hepatitis B is the sustained suppression of HBV replication, biochemical remission, HBeAg seroconversion and ultimately HBsAg seroconversion. The annual rate of spontaneous HBsAg seroclearance is approximately 0.4%-2.3%, and the HBsAg seroclearance rates of genotypes A and B are higher than that of genotypes C and D[80,81].

Interferon (IFN) and nucleos(t)ide analogues (NA) are commonly used for the treatment of chronic hepatitis B. Antiviral regimens for chronic hepatitis B are decided based on the age, HBV-DNA viral load, alanine aminotransferase (ALT) levels and the degree of fibrosis. In general, younger patients with high ALT levels are recommended to be treated with IFN therapy and older and/or clinically advanced patients with NA. Due to the economic growth, the treatment of chronic hepatitis B has become universal in most developed and developing Asian countries. However, most of the clinical studies about antiviral therapy were reported from developed countries, with few studies being reported from developing countries. IFN has antiviral, antiproliferative and immunomodulatory effects. The response to IFN treatment is poorer in Asian patients compared with Caucasian patients, which may be due partly to the difference in the genotype distribution[82]. It was shown that patients infected with HBV genotypes A and B showed better response than those with genotypes C and D[83-87]. A meta-analysis also revealed that IFN therapy was more effective in patients infected with genotype A than in those with genotype D, and also more effective in genotype B than in genotype C infection[88].

Currently, lamivudine, adefovir, entecavir, telbivudine and tenofovir have been approved for the treatment of chronic hepatitis B (Table 2). Lamivudine (Zeffix®) was first introduced in 1999 and the clinical efficacy was shown by a long-term follow-up study[93,94]. However, drug-resistant mutations, especially multidrug-resistant mutations, are the major concern with patients receiving long-term NA treatment. It was reported that the drug resistance against lamivudine monotherapy reached 70% after 4 years of treatment[95,96]. Entecavir (Baraclude®) is widely used and a first-line drug in many Asian countries, including China, South Korea, Thailand, Hong Kong and Japan. Entecavir is still expensive but the occurrence of drug resistance is very low for naïve patients.