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Original Article

Molecular epidemiological study of hepatitis B virus in Thailand based on the analysis of *pre-S* and *S* genes

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Aims: This study was undertaken to determine the prevalence and characteristics of hepatitis B virus (HBV) genotypes, antigen subtypes, "a" determinant variants and *pre-S* gene mutations circulating on a large scale in Thailand.

Methods: The sequences of the *Pre-S1*, *Pre-S2* and *S* regions were determined in serum samples of 147 HBsAg and HBV DNA-positive subjects who had been enrolled from the nationwide seroepidemiological survey conducted on 6213 individuals in 2004.

Results: The results showed that genotypes C, B and A accounted for 87.1%, 11.6% and 1.3%, respectively. The distribution of the HBV antigen subtypes was: adr (84.4%), adw (14.2%) and ayw (1.4%). Regarding the "a" determinant, 2/43 (4.65%) and 2/104 (1.92%) samples of vaccinated and non-vaccinated subjects, respectively, displayed mutations, all of which were Thr126Asn. Sequencing analysis showed the *pre-S* mutations in 14 (9.5%) samples, with *pre-S2* deletion as the most common mutant (4.1%) followed by *pre-S2* start codon mutation (2.9%), both *pre-S2* deletion and start codon mutation (2.0%), and *pre-S1* deletion (0.7%). The *pre-S* mutations were associated with older age and higher mean serum HBsAg level.

Conclusion: This study demonstrated that HBV genotype/subtype C/adr and B/adw were the predominant strains circulating in Thailand. The "a" determinant variants seemed to be uncommon, and might not be attributed to vaccine-induced mutation.

Key words: genotype, hepatitis B virus, *pre-S* mutation, subtype, Thailand

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is associated with a diverse clinical spectrum of liver injury ranging from asymptomatic carriers to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC).[1] HBV, a member of the *hepadnaviridae*, is a relaxed circular double-stranded DNA virus of approximately 3200 base pairs (bp) in length, with four overlapping open reading frames encoding the polymerase (P), core (C), envelope (pre-S1/pre-S2/S) and X proteins.[1] The virus shows remarkable genetic variability and is currently classified into eight genotypes, designated A to H, and four major serotypes, ayw, ayr, adw and adr, based on entire genome and S gene sequence analysis.[2,3] HBV genotype distribution appears to show varying geographic patterns.[4] For instance, genotypes A and D are predominant in Western countries and India, whereas genotypes B and C prevail in South-East Asia, China and Japan. Genotype E is restricted to Africa and genotype F is found in Central and South America. In Thailand, a highly endemic area of HBV infection, genotypes C and B are predominant in patients with chronic liver disease and in the general population, accounting for approximately 70–90% and 10–30%, respectively.[5–9]

Since 1992, HBV vaccination has been integrated into the expanded program on immunization (EPI) in Thailand. Based on our recent study, the subsequent coverage rates with the complete three-dose course of HBV

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vaccine were 82.3% and 97.3% in 1999 and 2004, respectively.[10,11] Hepatitis B surface antigen (HBsAg), used in current vaccines, contains an "a" determinant located between amino acids (aa) 121 and 149, which is believed to be the major target of polyclonal antibody against the antigen (antibody to hepatitis B surface antigen, anti-HBs). The emergence of "a" determinant variants has been observed in some vaccinated individuals and may remain undetected by current diagnostic assays.[12] Although the *S* gene mutants' potential significance for failure of immunization has been studied in some endemic countries,[13] it has so far remained unclear whether the "a" determinant variants play a role in vaccine failure in Thailand. In this study, using the serum samples from our recent large-scale survey,[10] we investigated the HBV genotypes and antigen subtypes circulating in four geographic areas of Thailand. We also determined the frequency of "a" determinant variants in these subjects, specifically those related to vaccine escape. Finally, we examined the prevalence and characteristics of the *pre-S* gene mutations predominant in these populations.

METHODS

Study population

FROM MAY TO October 2004, we conducted a nationwide seroepidemiological survey on 6213 healthy subjects from four provinces including Chiangrai, Udon Thani, Chonburi and Nakhon Si Thammarat, chosen as geographic representations of populations in the north, north-east, center and south of the country, respectively. The details of the study have been reported elsewhere.[10] In every province, approximately 1500 individual serum samples were collected and stored at −70°C until analysis. All serum samples were examined for HBsAg by using commercially available automated ELISA assays (AxSYM; Abbott Laboratory, North Chicago, IL, USA). Of these, 246 (4%) serum samples were seropositive for HBsAg. In the present study, 201 serum samples were available for hepatitis B e antigen (HBeAg) assay (Enzygnost HBe monoclonal; Dade Behring, Marburg, Germany) and molecular characterization of HBV. Table 1 shows the age distribution of the subjects regarding previous HBV vaccination. The project was approved by the Ministry of Public Health and the ethical committee of the Faculty of Medicine, Chulalongkorn University. The subjects or the parents of all participating children also consented to the study.

Table 1 Age distribution of the subjects regarding previous HBV vaccination

Age	No	Vaccine	Vaccine complete
(A)			
0–10	1932	1655	1617
10–20	1208	741	588
20–30	803	368	214
30–40	792	281	123
40–50	763	224	87
>50	715	159	31
Total	6213	3428	2660
(B)			
0–10	10	9	9
10–20	35	19	15
20–30	44	30	22
30–40	49	23	9
40–50	37	17	6
>50	26	8	1
Total	201	106	62

(A) 6213 healthy subjects; (B) 201 HBsAg-positive subjects.

HBV-DNA preparation, amplification and direct sequencing

Total DNA was extracted from 100 μL stored serum (−70°C) by using proteinase K/SDS in Tris buffer, followed by phenol/chloroform extraction and ethanol precipitation. DNA pellets were dissolved in 30 μL sterile water and subjected directly to polymerase chain reaction (PCR)-based amplification. PCR was performed by using primers to amplify the *Pre-S1*, *Pre-S2* and *S* genes. The primers consisted of a forward primer *Pre-S1* F (nt 2,817–2838: 5′-TCACCATATTCTTGGG AACAAGA-3′) and reverse primer R4 (nt 689–668: 5′-ATGGCACTAGTAACCTGAGCC-3′). The PCR conditions comprised initial predenaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1.30 min. Amplicons were analyzed by electrophoresis on 2% agarose gel stained with ethidium bromide and observation under UV light. For automated DNA sequencing, the PCR products were purified from the gel using the Gel Extraction Kit (Perfectprep Gel Cleanup; Eppendorf, Hamburg, Germany) according to the manufacturer's specifications. The sequencing reaction was performed using the Gene Amp PCR System 9600 (Perkin-Elmer, Boston, MA, USA). The sequencing products were subjected to a Perkin Elmer 310 Sequencer (Perkin-Elmer). The results were analyzed and HBV genotypes were determined by BLAST analysis. HBV-DNA sequences were also subjected to

Table 2 The prevalence of HBV genotypes in different geographic regions of Thailand

	Genotype (%)			Total
	A	B	C	
Chiangrai (northern)	1 (1.61)	10 (16.13)	51 (82.26)	62
Nakhon Si Thammarat (southern)	1 (5.00)	–	19 (95.00)	20
Udon Thani (north-east)	–	1 (2.17)	45 (97.83)	46
Chonburi (central)	–	6 (31.58)	13 (68.42)	19
Total	2 (1.36)	17 (11.56)	128 (87.08)	147

phylogenetic analysis. The distribution of the HBV subtypes was deduced from amino acid sequences at positions 122 (Lys, Arg for d, y determinants) and 160 (Lys, Arg for w, r determinants).[3]

Phylogenetic analysis

Nucleotide sequences were multiply aligned by using the program CLUSTAL_X (version 1.83). Alignments were then fed into phylogenetic trees that were constructed for each subalignment by using the neighbor joining methods implemented by the MEGA program. The statistical validity of the neighbor joining methods was assessed by bootstrap re-sampling with 1000 replicates. The reference strain from GenBank accession numbers AB11946, AB112063 for genotype C subgenotype Cs; AB014360, D50520, X75665, AB042282 and AY641558 for genotype C subgenotype Ce; D23677, D23678 and D23679 for genotype B; AB116089 and AB116083 for genotype A subgenotype Aa; AJ012207, AY128092 and X70185 for genotype A.

Statistical analysis

Data were expressed as mean ± SD, and percentages as appropriate. Comparisons between groups were analyzed by the χ^2 or Fisher's exact test for categorical variables and by the Mann–Whitney *U*-test or Student's *t*-test for quantitative variables. *P*-values below 0.05 were considered significant. All statistical analyses were performed using the SPSS software for Windows 10.0 (SPSS, Chicago, IL).

RESULTS

Distribution of HBV genotypes

OF THE 201 HBsAg-positive subjects, HBeAg was detected in 56 serum samples (27.9%). One hundred and forty-seven (73.1%) of the 201 HBsAg-positive subjects were positive for HBV-DNA in the sera, detected by PCR. The mean age of the subjects was 33.14 ± 14.03 years and 49.7% were male. Of those positive for HBV-DNA, 128 (87.1%) cases were determined as genotype C and were determined as subgenotype Cs, 17 (11.6%) cases belonged to genotype B, and two (1.3%) cases to genotype A, with all of these being determined as subgenotype Ae. The distribution of the HBV antigen subtypes among these subjects was: adr (84.4%), adw (14.2%) and ayw (1.4%). In this study, all cases with genotype A belonged to subtype adw. For genotype B, 15 of them belonged to subtype adw and two to subtype ayw. For genotype C, 124 and four of them belonged to subtypes adr and adw, respectively. HBV genotype and subtype prevalence according to geographic distribution is shown in Tables 2 and 3, respectively. Although genotype C was the most common

Table 3 The prevalence of HBV subtypes in different geographic regions of Thailand

	Subtype (%)			Total
	adr	adw	ayw	
Chiangrai (northern)	49 (79.03)	12 (19.35)	1 (1.61)	62
Nakhon Si Thammara (southern)	19 (95.00)	1 (5.00)	–	20
Udon Thani (north-east)	45 (97.83)	–	1 (2.17)	46
Chonburi (central)	11 (57.89)	8 (42.11)	–	19
Total	124 (84.35)	21 (14.23)	2 (1.36)	147

Table 4 The clinical and virological data of the subjects with the "a" determinant mutations

	Age	Sex	Genotype	Subtype	Vaccine	HBsAg (S/N)
NK652	33	M	C	adr	–	324.54
NK052	58	F	C	adr	–	374.06
NK110	13	F	C	adr	+	389.18
UD767	8	M	C	adr	+	268.64

genotype in each geographic area, the prevalence of genotype B was significantly higher in the central part of Thailand compared to other regions ($P = 0.007$). Similarly, the prevalence of subtype adw was significantly higher in the central part of Thailand than in other regions ($P = 0.001$).

The obtained sequences were submitted to GenBank under accession numbers DQ361314–DQ361535. The sequences were also aligned with those of the isolates of known genotype and subjected to phylogenetic analysis (Fig. 1).

Prevalence and characterization of the "a" determinant mutations

Subsequent sequencing revealed the prevalence and variation of the "a" determinant mutations among the populations studied. Four out of 147 samples were found to have mutations, all of which were Thr126Asn. Of these, 2/43 (4.65%) and 2/104 (1.92%) originated from vaccinated and non-vaccinated subjects, respectively. There were no statistically significant differences between the vaccinated and non-vaccinated groups ($P = 0.355$). The clinical and virological data of these four subjects are shown in Table 4.

Prevalence and characterization of *pre-S* mutations

Based on direct sequencing, *pre-S* mutations were detected in 14 of 147 cases (9.5%). Among these, 13 cases (92.9%) belonged to genotype C. As for the prevalence of *pre-S* mutations according to site, *pre-S2* deletion was the most common (4.1%), followed by *pre-S2* start codon mutation (2.9%), both *pre-S2* deletion and start codon mutation (2.0%), and *pre-S1* deletion (0.7%). The mean age of patients with *pre-S* mutations ($n = 14$) was significantly higher than that of patients without the mutants ($n = 133$) (41.2 ± 11.4 years vs. 32.3 ± 15.0 years, $P = 0.033$). In addition, the mean HBsAg level in patients with *pre-S* mutations was significantly higher than in those without the mutants ($378–8 \pm 64.4$ vs. 305.7 ± 111.0, $P = 0.017$). The alignment of amino acid sequences of the entire *pre-S1*/*pre-S2* region of the 14 samples is shown in Figure 2.

DISCUSSION

MOLECULAR EPIDEMIOLOGICAL STUDIES provide valuable information on understanding the prevalence and characteristics of HBV genotypes and mutations from different areas of the world. Genotypes of HBV are generally subtype-specific, although some subtypes are heterogeneous. In general, subtype adw is usually found in genotypes A and B, while adr occurs in genotype C.[14] In this nationwide study, we confirmed the predominance of categories C/adr and B/adw among the HBV strains in Thailand, which accounted for more than 95% of cases. These findings are not surprising; they reflect the typical genotypes and subtypes circulating in Thailand and South-East Asia. Besides the epidemiological data, there is now increasing information suggesting that HBV genotypes may play an important role in causing different disease profiles in chronic HBV infection. It has been shown that HBV genotype C is more commonly associated with severe liver diseases and the development of cirrhosis compared to genotype B.[15] Genotype C is also associated with a lower rate of hepatitis B e antigen (HBeAg) seroconversion and a lower response rate to alpha interferon therapy compared to genotype B.[16]

From the recent large-scale survey, we found that among subjects who had had complete vaccination, the HBsAg carrier rate was 21 of 2151 (0.98%).[10] The potential causes for these failures are unclear, but might include improper administration of the HBV vaccine, false-positive HBsAg results, intrauterine infection, low viral replication with or without integration of viral DNA into hepatocyte genomes and S gene mutation. The emergence of S gene variants, with mutations mainly occurring within the "a" determinant, has been observed in some vaccinees in several regions of the world.[12,17,18] Naturally occurring escape mutants have also been reported in chronic carriers after long-term

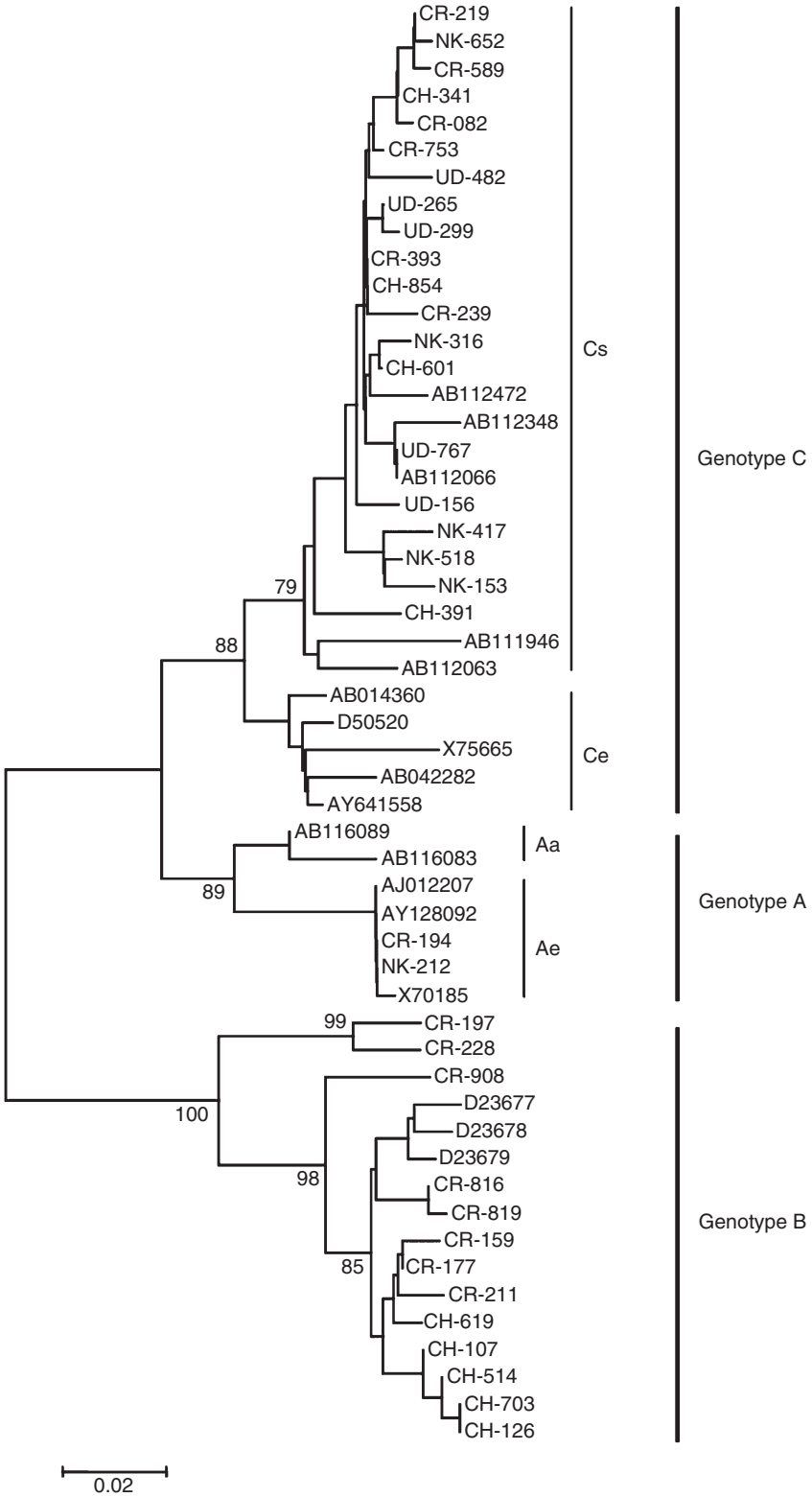

Figure 1 Phylogenetic analysis of the genotype of hepatitis B virus in different geographic regions of Thailand: Chiangrai (CR), Chonburi (CH), Nakhon Si Thammara (NK) and Udon Thani (UD).

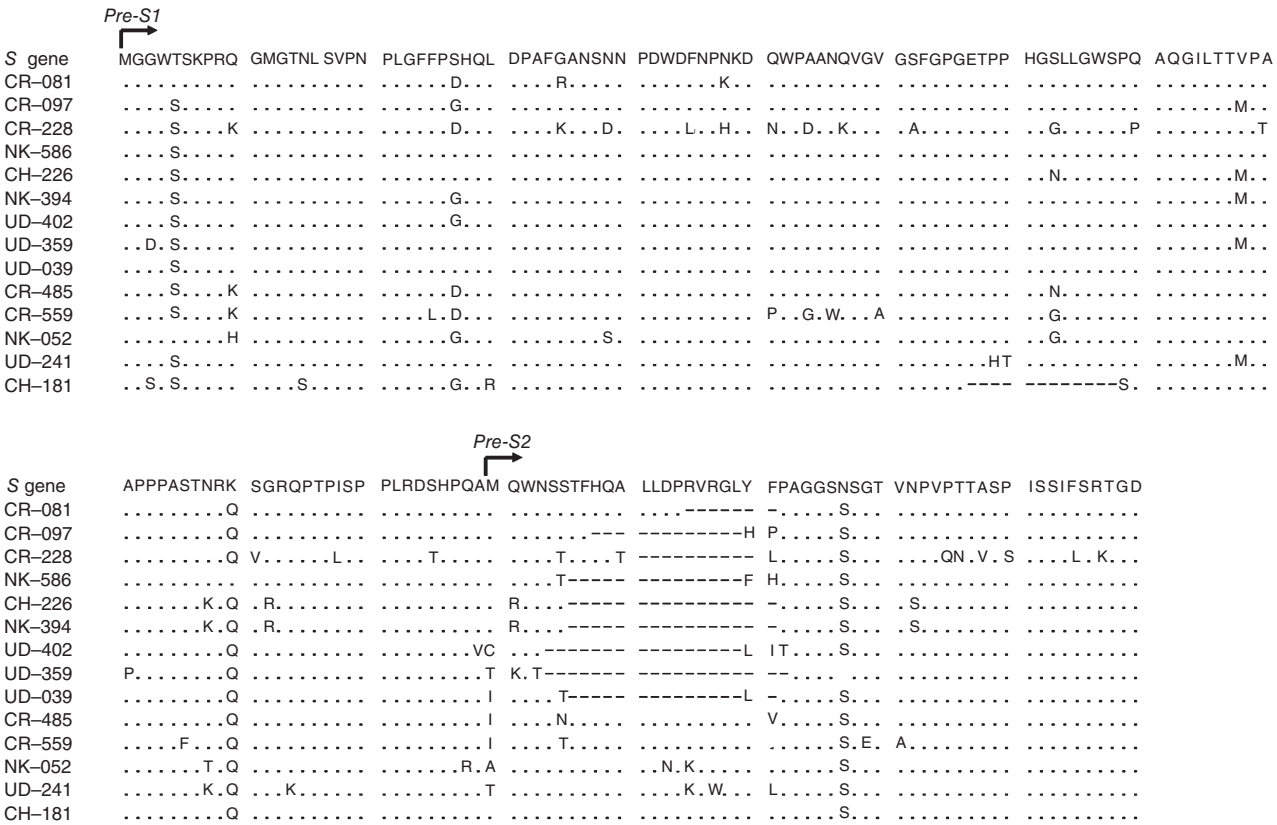

Figure 2 The amino acid sequences alignment of the entire *pre-S1*/*pre-S2* of 14 *pre-S* mutations samples. Chiangrai (CR), Chonburi (CH), Nakhon Si Thammara (NK) and Udon Thani (UD).

follow-up. A study in Taiwan showed an increase in the prevalence of "a" determinant mutants in children from 7.8% before to 23.1% 15 years after the introduction of universal vaccination against HBV.[19] The prevalence of the HBsAg mutants was also significantly higher among those fully vaccinated than among those not vaccinated. This finding suggests that vaccination might have increased a selection pressure on the emergence of surface mutants in relation to wild-type HBV. In this study, our data showed that only two vaccinated subjects and two non-vaccinated subjects had the same mutant-bearing virus affecting amino acid position 126. As a result, it seems that "a" determinant HBV mutants may be uncommon among chronic carriers from Thailand, and the prevalence of the variants might not be associated with vaccination. However, it should be emphasized that all cases included in the study were HBsAg positive and, consequently, those patients with mutations rendering the S protein undetectable with the antibodies tested were excluded. Moreover, since a viral HBV population infecting a host is usually distributed as a quasispecies,[20] variants are expected to coexist with wild-type strains in most carriers. As such mutations were detected by direct sequencing of the PCR products without cloning, quantitative analysis for the relative amount of mutant or wild-type virus in mixed infection was not feasible in this report. Thus, the true proportion of Thai patients carrying "a" determinant variants could be higher than that observed in this study.

Interestingly, the characteristics of the "a" determinant mutations detected in the present study were that they occurred only at position 126. Indeed, the most common mutation causing vaccine escape involves the mutation at position 145, which is located in the second loop of the "a" determinant.[12] Subsequent studies conducted on vaccinated and non-vaccinated individuals have also demonstrated other variants affecting amino acid positions 120, 123, 124, 126, 129, 131, 141 and 144.[17,18] These changes in the "a" determinant may have emerged through host immunoselective pressure, as previously mentioned. Alternatively, it is also possible that the mutant is infectious and has been transmitted by

another individual. It should also be noted that the vast majority of studies on vaccine- or hyperimmune globulin-induced escape mutations have investigated subtype adw or ayw of HBV, in which position 126 is different from adr (threonine in subtypes adw and ayw [genotypes A, B and D] and isoleucine in subtype adr [genotype C]). Thus, differences in genotype may be another reason for the preferred mutations in the first loop, especially at position 126 in the present study investigating only subtype adr/genotype C.

The *pre-S1* and *pre-S2* regions are highly immunogenic and potentially under selective pressure by the immune system because they contain both B- and T-cell epitopes.[21] The prevalence of *pre-S* mutations is variable and considerably different among different geographic areas. For example, Huy *et al*. reported that the prevalence of HBV *pre-S* mutants ranged from 0% to 36% in an analysis of HBV-DNA-positive serum samples from individuals residing in 12 countries, including Thailand.[22] In that report, the prevalence of *pre-S* mutations among Thai patients amounted to 10.5%, which was consistent with the results of our study (9.5%). Regarding the site of mutations, our report showed that *pre-S2* deletion was the most common mutation type, followed by *pre-S2* start codon mutation, both *pre-S2* deletion and start codon mutation, and *pre-S1* deletion. These results are also in agreement with those of recent reports from Japan and Korea, according to which deletion in *pre-S2* regions and *pre-S2* start codon mutations were among the most commonly prevailing.[22,23] Interestingly, our data showed a higher prevalence of *pre-S* mutations in patients infected with genotype C than those with genotype B. Taking into consideration that these mutations were predominantly found in genotype C, it is possible that this genotype may be more prone to develop such mutations. Moreover, the mean age of patients with *pre-S* mutations was significantly higher than that of those without the mutants. This observation also confirmed previous data suggesting that the prevalence of *pre-S* mutations tends to increase in direct relation to the patient's age.[22–24] The mutations found in this study, along with similar observations in previous reports, could help to elucidate the evolutionary pattern of mutations in the clinical course of persistent HBV infection.

In conclusion, our study demonstrated that HBV genotype/subtype C/adr and B/adw were the predominant strains circulating in Thailand. Furthermore, the "a" determinant variants seemed to be uncommon in HBV carriers, and might not be attributed to vaccine-induced mutation.

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REFERENCES

1 Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. *N Engl J Med* 2004; **350:** 1118–29.
2 Kramvis A, Kew M, Francois G. Hepatitis B virus genotypes. *Vaccine* 2005; **23:** 2409–23.
3 Norder H, Hammas B, Lofdahl S, Courouce AM, Magnius LO. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. *J Gen Virol* 1992; **73:** 1201–8.
4 Allain JP. Epidemiology of Hepatitis B virus and genotype. *J Clin Virol* 2006; **36** (Suppl 1)**:** S12–17.
5 Jutavijittum P, Jiviriyawat Y, Yousukh A, Kunachiwa W, Toriyama K. Genotypes of hepatitis B virus among voluntary blood donors in northern Thailand. *Hepatol Res* 2006; **35:** 263–6.
6 Payungporn S, Tangkijvanich P, Jantaradsamee P, Theamboonlers A, Poovorawan Y. Simultaneous quantitation and genotyping of hepatitis B virus by real-time PCR and melting curve analysis. *J Virol Methods* 2004; **120:** 131–40.
7 Sugauchi F, Chutaputti A, Orito E *et al.* Hepatitis B virus genotypes and clinical manifestation among hepatitis B carriers in Thailand. *J Gastroenterol Hepatol* 2002; **17:** 671–6.
8 Tangkijvanich P, Mahachai V, Komolmit P, Fongsarun J, Theamboonlers A, Poovorawan Y. Hepatitis B virus genotypes and hepatocellular carcinoma in Thailand. *World J Gastroenterol* 2005; **11:** 2238–43.
9 Theamboonlers A, Jantaradsamee P, Kaew-In N, Tangkijvanich P, Hirsch P, Poovorawan Y. The predominant genotypes of hepatitis B virus in Thailand. *Ann Trop Med Parasitol* 1999; **93:** 737–43.
10 Chongsrisawat V, Yoocharoen P, Theamboonlers A *et al.* Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Trop Med Int Health* 2006; **11:** 1496–502.

11 Poovorawan Y, Theamboonlers A, Vimolket T *et al.* Impact of hepatitis B immunisation as part of the EPI. *Vaccine* 2000; **19:** 943–9.
12 Carman WF, Zanetti AR, Karayiannis P *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336:** 325–9.
13 Purcell RH. Hepatitis B virus mutants and efficacy of vaccination. *Lancet* 2000; **356:** 769–70.
14 Norder H, Courouce AM, Coursaget P *et al.* Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47:** 289–309.
15 Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* 2000; **118:** 554–9.
16 Sumi H, Yokosuka O, Seki N *et al.* Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; **37:** 19–26.
17 Coleman PF. Detecting hepatitis B surface antigen mutants. *Emerg Infect Dis* 2006; **12:** 198–203.
18 Cooreman MP, Leroux-Roels G, Paulij WP. Vaccine- and hepatitis B immune globulin-induced escape mutations of hepatitis B virus surface antigen. *J Biomed Sci* 2001; **8:** 237–47.
19 Hsu HY, Chang MH, Ni YH, Chen HL. Survey of hepatitis B surface variant infection in children 15 years after a nationwide vaccination programme in Taiwan. *Gut* 2004; **53:** 1499–503.
20 Domingo E. Genetic variation and quasi-species. *Curr Opin Genet Dev* 1992; **2:** 61–3.
21 Mimms L. Hepatitis B virus escape mutants: "pushing the envelope" of chronic hepatitis B virus infection. *Hepatology* 1995; **21:** 884–7.
22 Huy TT, Ushijima H, Win KM *et al.* High prevalence of hepatitis B virus pre-s mutant in countries where it is endemic and its relationship with genotype and chronicity. *J Clin Microbiol* 2003; **41:** 5449–55.
23 Choi MS, Kim DY, Lee DH *et al.* Clinical significance of pre-S mutations in patients with genotype C hepatitis B virus infection. *J Viral Hepat* 2007; **14:** 161–8.
24 Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006; **130:** 1153–68.