レベルB 表1… PEG-IFN・リバビリン併用療法の薬剤投与量

ペガシス®・コペガス®		
ペガシス®	コペガス®	
180μg/週 (体重に関係なし)	体重≦60kg	600mg/日
	60kg<体重≦80kg	800mg/日
	80kg<体重	1,000mg/日
ペグイントロン®・レベトール®		
ペグイントロン®	レベトール®	
1.5μg/kg/週 35~45kg：60μg、46~60kg：80μg、 61~75kg：100μg、76~90kg：120μg、 91~120kg：150μg	体重≦60kg	600mg/日
	60kg<体重≦80kg	800mg/日
	80kg<体重	1,000mg/日

豆知識

高ウイルス量とは、HCV-RNA量がリアルタイムPCR法で5.0 logIU/mL以上、アンプリコア®(Amplicor®)法で100 KIU/mL以上、プローブ法で1Meq/mL以上、コア抗原で300 fmol/L以上を意味します。リアルタイムPCR法はTaqMan®法とAccuGene®法の二つがあります。

3. 投与量 レベルB

薬剤投与量は、ペガシス®は体重にかかわらず180μg/週で、ペグイントロン®は体重別に投与量が決められています。また、リバビリンはコペガス®、レベトール®とも、体重別に投与量が決められています(表1)。

2 PEG-IFN・リバビリン併用療法の効果判定

抗ウイルス療法の第一目標はHCVを排除することです。ウイルス学的治療効果はHCV-RNAの有無によって評価します。

1. 効果判定の時期 レベルB

ウイルス学的効果は、治療開始後4週時点のHCV-RNAの陰性化(rapid virological response；RVR)、治療開始12週時点の陰性化(complete early virological response；c-EVR)、治療終了時点の陰性化(end-of-treatment response；ETR)が定義されています。

2. 最終治療効果判定 レベルA

最終的な効果は、治療終了24週後のHCV-RNAの陰性化(end-of-follow response)により評価し、HCV-RNA陰性であれば、ウイルス学的著効(sustained virological response；SVR)と判定します。HCV-RNA陽性の場合は、抗ウイルス療法中のHCV-RNA陰性化の有無により、再燃(relapse)と無効(non-response；NR)に区別されます(図1)。

3 PEG-IFN・リバビリン併用療法中の検査スケジュール

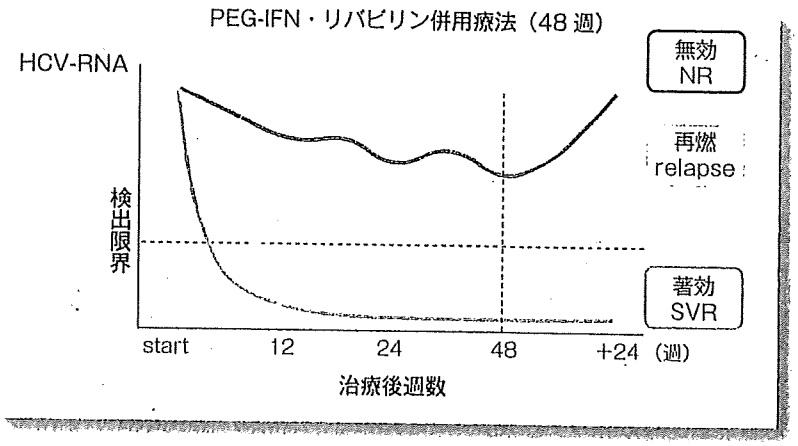
1. HCV-RNA量測定 レベルB

PEG-IFN・リバビリン併用療法では、後述するように、HCV-RNA減少量や、陰性化時期が治療効果の予測に重要です。よって、治療開始から4週ごとにHCV-RNA量の測定が必要です。

2. 血液検査 レベルB

PEG-IFNの副作用は、IFN単独投与よりも臨床症状はやや軽いですが、逆に血球減少は強くみられます。特に、ペガシス®では、治療後期においても急激な血小板減少がみられることがあります。また、リバビリン投与により、特に治療早期

レベルA 図1… 抗ウイルス療法における効果判定



レベルA 表2… PEG-IFN・リバビリン併用療法における薬剤減量基準

ペガシス®／コペガス®併用療法

検査項目	数値	ペガシス®	コペガス®
好中球	＜750/μL	90μgへ減量	変更なし
ヘモグロビン	＜10g/dL	変更なし	1,000→600mg 800→600mg 600→400mg
好中球	＜500/μL	中止	中止
血小板	＜50,000/μL		
ヘモグロビン	＜8.5g/dL		

ペグイントロン®／レベトール®併用療法

検査項目	数値	ペグイントロン®	レベトール®
白血球	＜1,500/μL	半量へ減量	変更なし
好中球	＜750/μL		
血小板	＜80,000/μL		
ヘモグロビン	＜10g/dL	変更なし	1,000→600mg 800→600mg 600→400mg
白血球	＜1,000/μL	中止	中止
好中球	＜500/μL		
血小板	＜50,000/μL		
ヘモグロビン	＜8.5g/dL		

に溶血性貧血による貧血の進行がみられます。

このため、ペグイントロン®・レベトール®併用療法では治療開始後8週まで、ペガシス®・コペガス®併用療法では投与終了まで、投与当日に血液検査（血算）を行う必要があります。血球減少に対しては、薬剤減量基準が決められています（表2）。

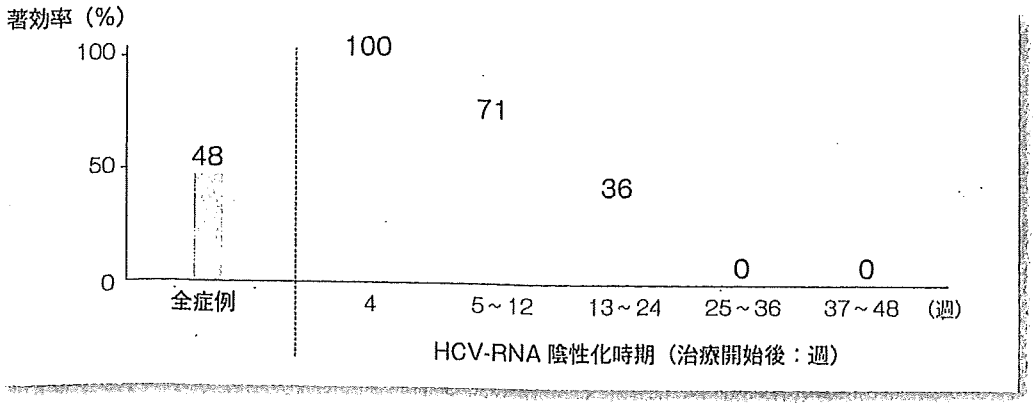
4 PEG-IFN・リバビリン併用療法の実際

1. 1型・高ウイルス量症例の治療成績

レベルB

本邦における1型・高ウイルス量症例に対するPEG-IFN・リバビリン併用48週投与の国内臨床

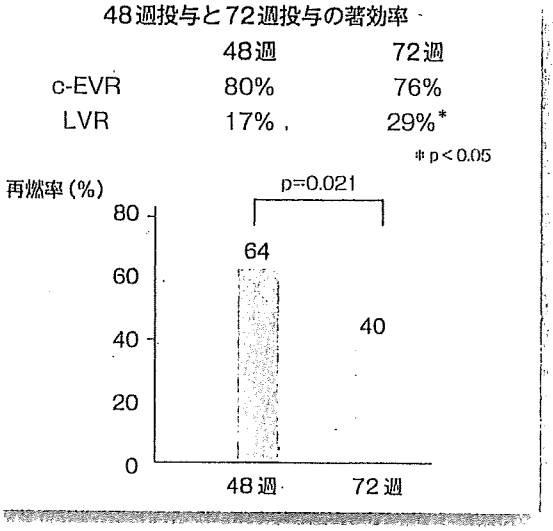
エビデンスB 図2… 国内臨床試験でのPEG-IFNα-2b・リバビリン48週併用療法の治療成績（HCV-RNA陰性化時期別）



エビデンスB 表3… PEG-IFN・リバビリン併用療法における治療効果に寄与する因子（1型・高ウイルス量症例）

因子	要因	難治要因
ウイルス因子	HCVコア蛋白変異	70番・91番mutant
	ISDR変異	Wild（変異数0～1個）
宿主因子	年齢	高齢（65歳以上）
	性別	女性
	肝線維化	肝線維化進展

エビデンスB 図3… 海外大規模試験による長期投与治療成績



文献1改変

試験では、著効率が48%、副作用中止率が18%でした。HCV-RNA陰性化時期別の著効率は、治療開始後4週の時点でHCV-RNAが陰性化した症例で100%、5～12週に陰性化した症例では71%、13～24週に陰性化した症例では36%でした（図2）。

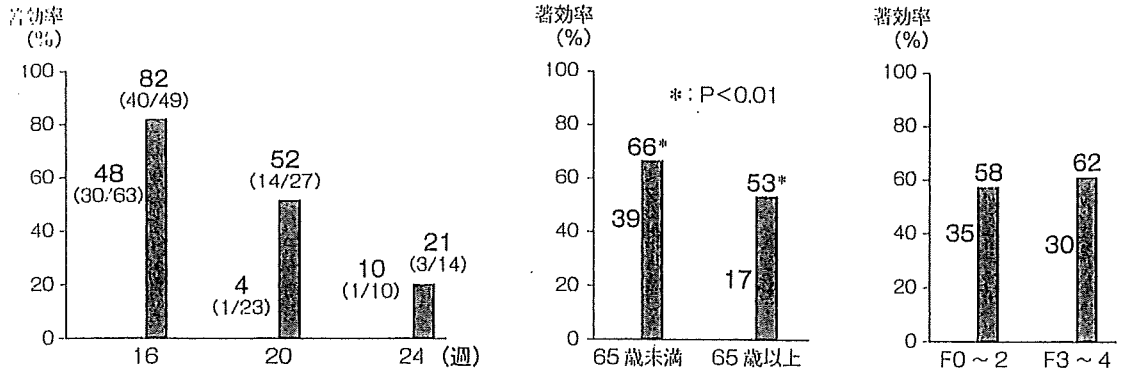
2. 治療効果に寄与する因子

1型・高ウイルス量症例に対するPEG-IFN・リバビリン併用48週投与では、若齢者、男性、肝線維化の進展度が軽度である症例で治療効果（著効）が期待できます。一方、高齢女性や、ISDR変異の少ない症例（0～1個）、HCVコア蛋白の70番アミノ酸が変異型である症例はPEG-IFN・リバビリン併用療法に抵抗性で、ウイルス排除率が低率です（表3）。

3. 1型・高ウイルス量症例に対する治療への反応性からみた治療戦略 エビデンスB

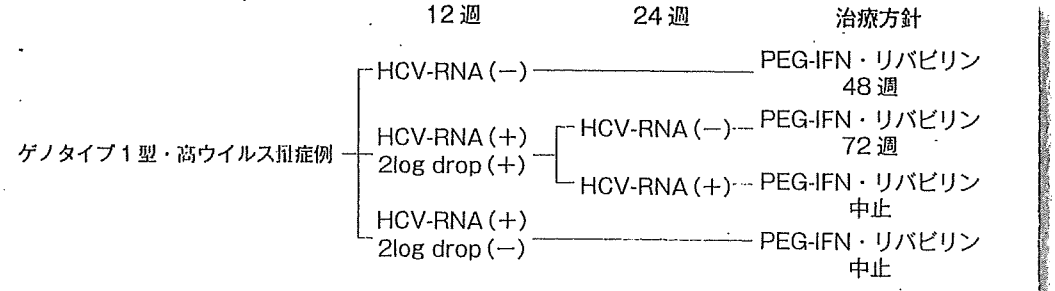
PEG-IFN・リバビリン併用48週投与では、治療開始後12週時点におけるHCV-RNA減少率が治療効果を予測する重要な因子です。治療開始後12週時点までの、治療開始前の100分の1以下へのHCV-RNA減少をpartial early virological response（p-EVR）と定義しますが、p-EVRが得られない症例では48週治療において著効が得られる可能性はほとんどなく、この時点で治療を終

図4… LVR症例における長期投与治療成績（OLF；Osaka Liver Forum）



48週、■72週

図5… 抗ウイルス療法への反応性に基づいた治療方針（1型・高ウイルス量症例）



了することが推奨されています（12週ルール）。

治療開始後24週の時点でHCV-RNA陰性化が認められない症例でも、48週治療において著効が得られる可能性はほとんどないため、この時点で治療を終了することが推奨されています（24週ルール）。

治療開始後13～24週にHCV-RNAが陰性化した症例をlate virological response（LVR）と定義しますが、LVR例では48週治療において、治療終了後のHCV-RNA再燃が高率に起こります。こうしたLVR症例には、PEG-IFN・リバビリン併用72週投与によって、48週投与と比べて、再燃率が低下することがわかっています[1]（図3）。LVR症例にPEG-IFN・リバビリン併用72週投与を行った場合、LVR症例のなかでも、HCV-RNA陰性化時期の早い症例では著効が得られやすく、また、48週投与では治療効果が不十分である高齢者や、肝線維化の進展した症例においても、著効率が向上します（図4）。

1型・高ウイルス量症例に対しては、個々の症例における抗ウイルス療法に対する反応性を考慮した治療方針の決定（response-guided therapy）が重要です（図5）。

4. 1型・高ウイルス量以外の治療成績

本邦における、1型・高ウイルス量以外の症例に対するPEG-IFN・リバビリン併用24週投与の国内臨床試験では、著効率が87%、副作用中止率が21%でした。

1型・高ウイルス量以外の症例に対するPEG-

IFN・リバビリン併用24週投与では、年齢や性別に関係なく、良好な治療効果(著効)が期待できます。

以上、本章で述べたPEG-IFN・リバビリン併用療法の治療成績は、アンプリコア®法によって判定されたものです。TaqMan®法により感度が上がることにより、治療効果(SVR)に対するpositive predictionやnegative predictionに相違がみられるため、注意が必要です。

5 PEG-IFN・リバビリン併用療法のup to date レベルC

1. 治療期間

治療早期にHCV-RNA陰性化が得られた症例での著効率が高率であることから、抗ウイルス療法に対する反応性の良い症例に対する短期投与が試られました。

ゲノタイプ1型ではRVR例に24週あるいは48週投与を行った結果、HCV-RNA量400KIU/mL未満では24週投与で83%、48週投与で84%の著効率と差を認めませんでしたが、HCV-RNA量が400KIU/mL以上では24週投与で73%、48週投与で87%と24週投与で著効率が低率でした[2]。ゲノタイプ2型に対しては、RVR例に16週あるいは24週投与を行った結果、16週投与で62%、24週投与で85%と有意に16週投与の著効率が低率でした[3]。

以上の結果からは、RVRが得られても可能なかぎり、1型・高ウイルス量では48週、1型・高ウイルス量以外では24週の標準投与を行うべきだと考えられます。

2. 薬剤投与量

従来から薬剤投与量について、「予定投与量の80%以上のPEG-IFN、リバビリンを、予定投与期間の80%以上の期間投与した場合に、著効率が高率である」という報告がなされていました。すべて80%なので、80／80／80(トリプル・エイティ)則といわれています[4]。

その後、高用量の薬剤投与について検討され、高用量のPEG-IFN投与ではHCV-RNA減少率が大きく[5]、高用量のリバビリン投与ではHCV-RNA陰性化症例の治療後再燃率が低率[6]であることが報告されました。

3. 副作用対策

PEG-IFN・リバビリン併用療法におけるリバビリンの副作用を軽減するため、欧米ではエリスロポエチン(Epo)併用などが試みられています(日本では保険適用外)。リバビリンの副作用である溶血性貧血に対して、IFN・リバビリン併用療法中に貧血が進行(ヘモグロビン＜12g/dL)した症例にEpo(40,000U/L)を8週間投与すると、Hbは2.2g/dL回復し、リバビリン内服量の維持やQOLの改善がみられています[7]。

文献

1) Berg, T. et al. Extended treatment duration for hepatitis C virus type 1 : comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. Gastroenterology. 130, 2006, 1086-97.
2) Mangia, A. et al. Individualized treatment duration for hepatitis C genotype 1 patients : a randomized controlled trial. Hepatology. 47, 2008, 43-50.
3) Shiffman, ML. et al. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N. Engl. J. Med. 357, 2007, 124-34.
4) McHutchison, JG. et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology. 123, 2002, 1061-9.
5) Fried, MW. et al. Improved outcomes in hepatitis C patients with difficult to treat characteristics : randomized study of higher doses of peginterferon α-2a and ribavirin. Hepatology. 48, 2008, 1033-43.
6) Shiffman, ML. et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. Hepatology. 46, 2007, 371-9.
7) Afdhal, NH. et al. Epoetin alfa maintains ribavirin dose in HCV-infected patients : a prospective, double-blind, randomized controlled study. Gastroenterology. 126, 2004, 1302-11.

Hepatology Research 2009; 39: 648–656 doi: 10.1111/j.1872-034X.2009.00519.x

Original Article

Case–control study for the identification of virological factors associated with fulminant hepatitis B

Atsunori Kusakabe,[1,2] Yasuhito Tanaka,[1] Satoshi Mochida,[3] Nobuaki Nakayama,[3] Kazuaki Inoue,[4] Michio Sata,[5] Norio Isoda,[6] Jong-Hon Kang,[7] Yasukiyo Sumino,[8] Hiroshi Yatsuhashi,[9] Yasuhiro Takikawa,[10] Shuichi Kaneko,[11] Gotaro Yamada,[12] Yoshiyasu Karino,[13] Eiji Tanaka,[14] Junji Kato,[15] Isao Sakaida,[16] Namiki Izumi,[17] Fuminaka Sugauchi,[2] Shunsuke Nojiri,[2] Takashi Joh,[2] Yuzo Miyakawa[18] and Masashi Mizokami[1,19]

[1]Departments of Clinical Molecular Informative Medicine and [2]Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, [3]Division of Gastroenterology and Hepatology, Internal Medicine, Saitama Medical University, Saitama, [4]Showa University Fujigaoka Hospital, Yokohama, [5]Kurume University School of Medicine, Kurume, [6]Jichi Medical University, Tochigi, [7]Center for Gastroenterology, Teinekeijinkai Hospital, Sapporo, [8]Toho University Omori Medical Center, Tokyo, [9]National Hospital Organization Nagasaki Medical Center, Nagasaki, [10]Iwate Medical University, Morioka, [11]Department of Signal Transduction, Cancer Research Institute, Kanazawa University, Kanazawa, [12]Kawasaki Hospital, Okayama, [13]Sapporo-Kosei General Hospital, Sapporo, [14]Shinsyu University Graduate School of Medicine, Matsumoto, [15]Sapporo Medical University Hospital, Sapporo, [16]Yamaguchi University Hospital, Ube, [17]Musashino Red Cross Hospital, Musashino, [18]Miyakawa Memorial Research Foundation, Tokyo, and [19]Research Center for Hepatitis and Immunology, International Medical Center of Japan, Kohnodai Hospital, Ichikawa, Japan

Background: Host and viral factors can promote the development of fulminant hepatitis B (FHB), but there have been no case–control studies for figuring out virological parameters that can distinguish FHB.

Methods: In a case–control study, virological factors associated with the development of FHB were sought in 50 patients with FH developed by transient hepatitis B virus (HBV) infection (FH-T) and 50 with acute self-limited hepatitis B (AHB) who were matched for sex and age. In addition, 12 patients with FH developed by acute exacerbation (AE) of asymptomatic HBV carrier (ASC) (FH-C) were also compared with 12 patients without FH by AE of chronic hepatitis B (AE-C).

Results: Higher HBV DNA levels, subgenotype B1/Bj, A1762T/G1764A, G1896A, G1899A and A2339G mutation were significantly more frequent ($P < 0.05$), while hepatitis B e-antigen was less frequent in the FH-T patients than AHB. In multivariate analysis, G1896A mutation (odds ratio [OR], 13.53; 95% confidence interval [CI], 2.75–66.64), serum HBV DNA more than 5.23 log copies/mL (OR, 5.14; 95% CI, 1.10–24.15) and total bilirubin more than 10.35 mg/mL (OR, 7.81; 95% CI, 1.77–34.51) were independently associated with a fulminant outcome by transient HBV infection. On the other hand, in comparison with the patients between FH-C and AE-C groups, there was no significant difference of virological factors associated with the development of FHB.

Conclusion: A number of virological factors have been defined that may distinguish FH-T from AHB in a case–control study. The pathogenic mechanism of FHB between transient HBV infection and AE of ASC would be different.

Key words: acute exacerbation of asymptomatic hepatitis B virus carrier, fulminant hepatitis, genotypes, transient hepatitis B virus infection

INTRODUCTION

IN JAPAN, 634 patients with fulminant hepatitis (FH) were registered from 1998–2003. Of them, 41.8% were infected with hepatitis B virus (HBV) that is the most frequent cause of FH there.[1] HBV is classified into eight genotypes (A–H) based on a sequence divergence of more than 8% in the entire genome of approximately

Correspondence: Dr Yasuhito Tanaka, Department of Clinical Molecular Informative Medicine, Nagoya, City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya 467-8601, Japan. Email: ytanaka@med.nagoya-cu.ac.jp
Received 22 August 2008; revision 26 January 2009; accepted 24 February 2009.

3200 nucleotides.[2–5] They have distinct geographical distributions and are associated with the severity of liver disease.[6,7] Furthermore, subgenotypes have been reported for HBV/A, B and C, and they are named A1/Aa (Asian/African type) and A2/Ae (European type),[8] B1/Bj (Japanese type) and B2/Ba (Asian type),[9] and C1/Cs (Southeast Asian type) and C2/Ce (East Asian type).[10,11] HBV genotypes/subgenotypes and mutations in the pre-core region and the core promoter can influence the viral replication and expression of hepatitis B e-antigen (HBeAg).[6,12]

Acute HBV infection in adulthood resolves in the most cases by far, but can induce FH or go on to become chronic in some. It has been reported that host and viral factors may influence the development of fulminant hepatitis B (FHB), but the pathogenesis of FHB remains unclear. As for virological factors associated with FHB, mutations in the core promoter (A1762T/G1764A)[13] and the pre-core region (G1896A)[14–16] have been reported in association with the development of FHB in Asia and the Middle East. Additional mutations, including T1753V, T1754V and A2339G in the core gene are implicated, also.[17,18] In regard of HBV genotypes, subgenotype B1/Bj is highly associated with the development of FHB in Japan.[15] In contrast, an association of HBV genotypes with the fulminant outcome has not been reproduced in patients from the USA and Europe.[19–22] Such a discrepancy would be attributed, at least in part, to distinct geographical distributions of HBV genotypes/subgenotypes over the world.

The original definition by Trey *et al.*[23] about fulminant hepatic failure is widely used all over the world. On the other hand, in Japan, the diagnosis of FH was contingent on a slight modification of Trey's original definition by the Inuyama Symposium (Aichi, Japan in 1981). Furthermore, the Intractable Liver Diseases Study Group of Japan modified the criteria for the etiology of FH and late-onset hepatic failure in 2002. According to the criteria of the Intractable Liver Diseases Study Group of Japan, there are two clinical entities of FHB that are induced, respectively, by transient HBV infection and acute exacerbation (AE) of an asymptomatic HBV carrier (ASC).[1]

Recently, FH developing in ASC who undergo AE is increasing in Japan.[1] In patients with hematological malignancy, in particular, rituximab and/or glucocorticoid, can reactivate HBV for the development of FHB.[24] The outcome is poor for FHB precipitating in ASC who undergo acute exacerbation,[1] but it has been difficult to identify it by clinical examinations.

As there have been no case–control studies for figuring out virological parameters that can distinguish FHB, a case–control study was conducted on the patients with FH by transient HBV infection and acute self-limited hepatitis B (AHB) in this study, for the identification of virological factors that influence a fulminant outcome. In addition, the patients with FH by AE of ASC, which is assumed as a different clinical condition from transient HBV infection, were also compared with the patients without FH by AE of chronic hepatitis B (CHB) in a case–control study.

METHODS

Patients

DURING 9 YEARS from 1998 to 2006, in twenty-six hospitals all over Japan, sera were obtained from the 50 FH patients by transient HBV infection (the FH-T group) and the 50 patients with AHB (the AHB group) who were controlled for age and sex. As the elder patients with FHB were enrolled in this study (mean age, 42.8 years), the mean age of AHB patients became relatively high (42.9 years, Table 1). Furthermore, the 12 FH patients developed by AE of ASC (the FH-C group) were also compared with the 12 patients without FH by AE of CHB who were matched by age and sex (the AE-C group).

All the serum samples tested for this study were collected at hospitalization. All 124 patients had hepatitis B surface antigen (HBsAg) in serum. Infection with hepatitis A virus and hepatitis C virus, as well as alcoholic hepatitis, were excluded in them.

The diagnosis of acute hepatitis B was based on sudden manifestation of clinical symptoms of hepatitis and detection of high-titered immunoglobulin (Ig)M anti-hepatitis B core (HBC). Patients with initial high-titered anti-HBC (>90% inhibition by a 1:200 diluted serum) were excluded. The diagnosis of FH was contingent on a slight modification by Inuyama Symposium (Aichi, Japan in 1981) of the original definition by Trey *et al.*:[23] (i) coma of grade II or higher; and (ii) a prothorombin time less than 40% developing within 8 weeks after the onset of hepatitis. To exclude AE of ASC in FH-T and AHB groups, we confirmed the negativity of HBsAg before onset of FHB or AHB and no family histories of hepatitis were found among all the patients. Furthermore, serum HBsAg in all patients with FH-T or AHB became naturally seronegative within 24 weeks. AE of ASC or CHB was defined as the elevation of alanine aminotransferase (ALT >300 IU/L) or total bilirubin (T.bil >3.0 mg/dL).[25] All 24 patients with AE of ASC or CHB could be confirmed positive for serum HBsAg before the onset of acute liver injury.

Table 1 Baseline characteristics between fulminant hepatitis B patients by transient infection (FH-T) and acute self-limited hepatitis B (AHB) patients

Features	FH-T ($n = 50$)	AHB ($n = 50$)	Differences *P*-value
Age (years)	42.8 ± 16.1	42.9 ± 14.6	Matched
Men	25 (50%)	25 (50%)	Matched
ALT (IU/L)	3788 ± 2856	2170 ± 1350	<0.001
AST (IU/L)	3131 ± 3673	1676 ± 1851	<0.05
Total bilirubin (mg/dL)	14.8 ± 8.6	9.5 ± 9.8	<0.01
Prothrombin time (%)	16.9 ± 11.2	72.8 ± 26.0	<0.001
HBeAg positive	15 (30%)	28 (56%)	<0.01
Core protein (log U/mL)	3.21 ± 1.28	3.01 ± 1.00	NS
HBcrAg (log U/mL)	5.30 ± 1.32	5.95 ± 1.13	<0.01
HBV DNA (log copies/mL)	5.97 ± 1.87	4.98 ± 1.17	<0.005
Deceased	19 (38%)	0 (0%)	<0.001

AHB, acute self-limited hepatitis B; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FH-T, fulminant hepatitis B by transient HBV infection; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NS, not significant.

Serological markers of HBV infection

Hepatitis B surface antigen, HBeAg and the corresponding antibody (anti-HBe) were determined by enzyme immunoassay (EIA) (AxSYM; Abbott Japan, Tokyo, Japan) or chemiluminescence enzyme immunoassay (CLEIA) (Fujirebio, Tokyo, Japan). Anti-HBC of IgM and IgG classes were determined by radioimmunoassay (Abbott Japan). Core protein constituting the viral nucleocapsid and HBV core-related antigen (HBcrAg), both of which correlate with HBV DNA in serum, were measured by CLEIA as described elsewhere.[26,27]

Quantification of serum HBV DNA

Hepatitis B virus DNA sequences spanning the S gene were amplified by real-time detection polymerase chain reaction (RTD-PCR) in accordance with the previously described protocol[28] with a slight modification;[8] it has a detection limit of 100 copies/mL.

Sequencing and molecular evolutionary analysis of HBV

Nucleic acids were extracted from serum samples (100 μL) using the QIAamp DNA extraction kit (Qiagen, Hilden, Germany) and subjected to PCR for amplifying genomic areas bearing enhancer II/core promoter/pre-core/core regions [nt 1628–2364], as described previously.[29] The target of PCR covered several mutations which were associated with FHB. Amplicons were sequenced directly with use of the ABI Prism Big Dye ver. 3.0 kit in the AMI 3100 DNA automated sequencer (Applied Biosystems, Foster City, CA, USA). All sequences were analyzed in both forward and backward directions.

Hepatitis B virus genotypes were determined by molecular evolutionary analysis. Reference HBV sequences were retrieved from the DDBJ/EMBL/GenBank database and aligned by CLUSTAL X, then genetic distances were estimated with the 6-parameter method in the Hepatitis Virus Database (http://s2as02.genes.nig.ac.jp/).[30] Based on obtained distances, phylogenetic trees were constructed by the neighbor-joining (NJ) method with the mid-point rooting option. To confirm the reliability of the phylogenetic trees, bootstrap resampling tests were performed 1000 times.

Statistical analysis

Statistical differences were evaluated by the Mann–Whitney *U*-test, Fisher's exact probability test and χ^2-test, where appropriate. Differences were considered to be statistically significant at $P < 0.05$. Multivariate analyses with logistic regression were utilized to sort out independent risk factors for FHB. STATA Software ver. 8.0 was employed for all analyses.

RESULTS

Baseline characteristics of the patients with FHB by transient HBV infection and AHB

TABLE 1 COMPARES baseline clinical characteristics of the 50 FH-T patients and the 50 AHB who

were matched for age and sex. The peak ALT, AST and T.bil levels were significantly higher (3788 ± 2856 vs 2170 ± 1350 IU/L, $P < 0.001$; 3131 ± 3673 vs 1676 ± 1851 IU/L, $P < 0.05$; and 14.8 ± 8.6 vs 9.5 ± 9.8 mg/dL, $P < 0.01$, respectively), while HBeAg was less frequent (30% vs 56%, $P < 0.01$) in the FH-T patients than AHB. The level of HBcrAg was significantly lower (5.30 ± 1.32 vs 5.95 ± 1.13 log U/mL, $P < 0.01$), while HBV DNA loads were higher (5.97 ± 1.87 vs 4.98 ± 1.17 log copies/mL, $P < 0.005$), in the FH-T patients than AHB. The level of core protein in sera tended to be higher in the FH-T patients than AHB (3.21 ± 1.28 vs 3.01 ± 1.00 log U/mL). Death occurred more often in the FH-T patients than AHB (38% vs 0%, $P < 0.001$).

HBV Genotypes and enhancer II/core promoter/pre-core/core Mutations in Patients with FHB by transient HBV infection and AHB

Figure 1(a) compares the distribution of HBV genotypes/subgenotypes between the FH-T and the AHB patients. The subgenotype C2/Ce was most prevalent in both patients with FH-T and AHB (66% and 62%, respectively), whereas B1/Bj was more frequent in the FH-T patients than AHB (22% vs 6%, $P < 0.05$). Likewise, mutations in enhancer II/core promoter/pre-core/core regions are compared between the FH-T and AHB patients in Figure 1(b). A1762T/G1764A, G1896A, G1899A and A2339G mutation were more frequent in the FH-T patients than AHB (48% vs 16%, $P < 0.001$; 62% vs 6%, $P < 0.001$; 24% vs 4%, $P < 0.001$; and 8% vs 0%, $P < 0.05$, respectively).

Figure 2(a) compares various mutations between the 11 FH-T patients and the three AHB patients who were infected with B1/Bj. Only G1896A was significantly more frequent (73% vs 0%, $P < 0.05$), while the lack of any mutations was less common (0% vs 33%, $P < 0.05$) in the FH-T patients than AHB. In comparison with the 33 FH-T patients and the 31 AHB patients who were infected with C2/Ce (Fig. 2b), A1762T/G1764A (70% vs 19%, $P < 0.001$), G1896A (61% vs 6%, $P < 0.001$) and the combination of all three mutations (A1762T/G1764A and G1896A) (45% vs 6%, $P < 0.001$) were significantly more frequent, while the lack of any mutations was less common (9% vs 70%, $P < 0.001$) in the FH-T patients than AHB. Interestingly, all the AHB patients with both G1896A and A1762T/G1764A mutations suffered acute severe hepatitis B that was defined by prothrombin time less than 40% but without coma of grade II or higher.

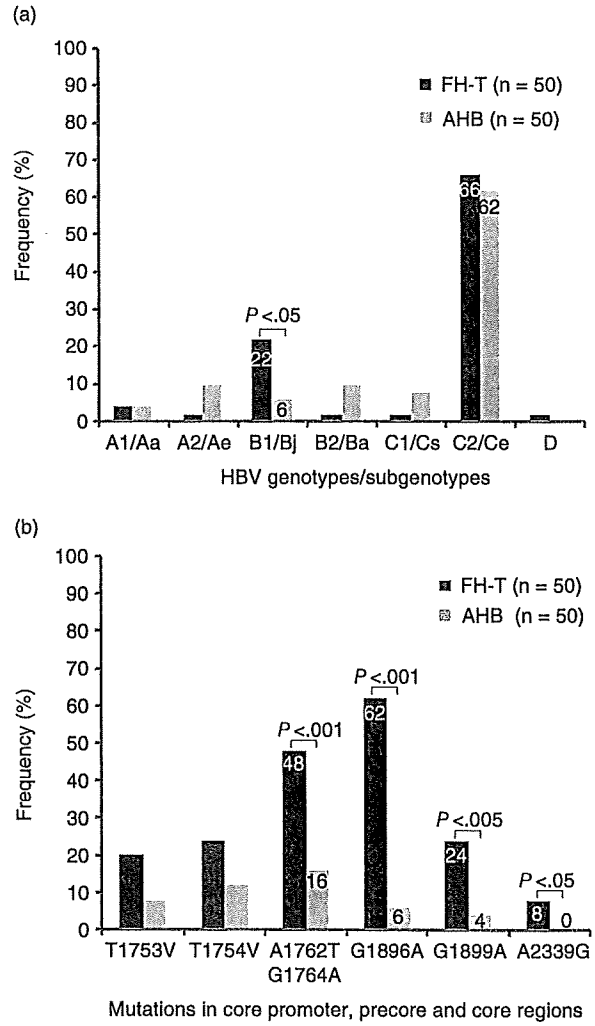


Figure 1 Genotypes/subgenotypes (a) and mutations in core promoter, pre-core and core regions (b) between the 50 transient hepatitis B virus infection (FH-T) and the 50 acute self-limited hepatitis B (AHB) patients.

Factors independently associated with the development of FHB by transient HBV infection

The following independent factors, promoting the development of FHB, were evaluated by multivariate analysis: ALT, AST, T.bil, HBeAg, HBV DNA, core protein, HBcrAg, genotypes/subgenotypes (B1/Bj or not) and mutations (T1753V, T1754V, A1762T/G1764A, G1896A, G1899A and A2339G). T.bil more than 10.35 mg/dL (OR, 7.81 [95% CI, 1.77–34.51], $P = 0.0067$), G1896A mutation (OR, 13.53 [95% CI,

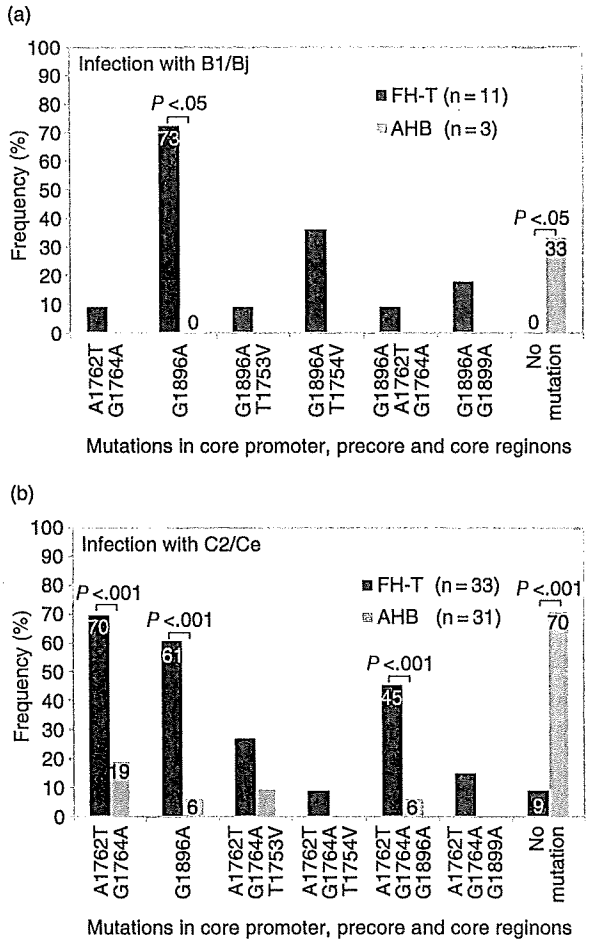


Figure 2 Frequencies of core promoter, pre-core and core mutations compared between the transient hepatitis B virus infection (FH-T) and the acute self-limited hepatitis B (AHB) patients who were infected with HBV of subgenotype B1/Bj (a) or C2/Ce (b).

2.75–66.64], $P = 0.0014$) and serum HBV DNA more than 5.23 log copies/mL (OR, 5.14 [95% CI, 1.10–24.15], $P = 0.0379$) were independent risk factors for the development of FHB by transient HBV infection (Table 2). Other mutations (T1753V, T1754V, A1762T/G1764A, G1899A and A2339G) were not significantly associated with the development of FHB by transient HBV infection, however.

Baseline clinical characteristics for distinguishing between the patients with FHB by AE of ASC (FH-C) and those without FHB by AE of CHB (AE-C)

Table 3 compares baseline clinical characteristics between the 12 FH-C patients and the 12 AE-C patients who were matched for age and sex. The levels of T.bil were significantly higher in the FH-C patients (15.0 ± 7.3 vs 7.3 ± 8.8 mg/dL, $P < 0.05$), but the peak ALT and AST levels tended to be slightly higher in the FH-C patients than AE-C (887 ± 681 vs. 641 ± 620 IU/L and 701 ± 451 vs 601 ± 753 IU/L, respectively). There were also no significant differences in levels of sera HBV DNA, core protein and HBcrAg between these two groups (7.44 ± 1.51 vs 6.60 ± 1.10 log copies/mL, 5.04 ± 1.45 vs 5.07 ± 1.07 log U/mL, and 6.35 ± 1.70 vs 6.29 ± 1.95 log U/mL, respectively).

HBV genotypes and enhancer II/core promoter/pre-core/core mutations between the patients with FH-C and those with AE-C

There were no significant differences in the frequencies of any HBV genotypes between the 12 FH-C patients and the 12 AE-C patients (Fig. 3a). In addition, there were also no significant differences in the frequencies

Table 2 Multivariate analysis for factors independently associated with fulminant hepatitis by transient HBV infection

Factors	Odds ratio	95% confidence interval	*P*-value
Total bilirubin (mg/dL)†			
<10.35	1		
≥10.35	7.81	1.77–34.51	0.0067
G1896A mutation			
Absent	1		
Present	13.53	2.75–66.64	0.0014
HBV DNA (log copies/mL)†			
<5.23	1		
≥5.23	5.14	1.10–24.15	0.0379

†Median values. HBV, hepatitis B virus.

Table 3 Baseline characteristics between patients with FH by AE of ASC (FH-C) and those without FH by AE of CHB (AE-C)

Features	FH-C ($n = 12$)	AE-C ($n = 12$)	Differences *P*-value
Age (years)	51.7 ± 14.7	49.9 ± 5.6	Matched
Male	10 (83%)	9 (75%)	Matched
ALT (IU/L)	887 ± 681	641 ± 620	NS
AST (IU/L)	701 ± 451	601 ± 753	NS
Total bilirubin (mg/dL)	15.0 ± 7.3	7.3 ± 8.8	<0.05
Prothrombin time (%)	25.8 ± 6.6	48.4 ± 21.5	<0.005
HBeAg positive	4 (33%)	3 (25%)	NS
Core protein (log U/mL)	5.04 ± 1.45	5.07 ± 1.07	NS
HBcrAg (log U/mL)	6.35 ± 1.70	6.29 ± 1.95	NS
HBV DNA (log copies/mL)	7.44 ± 1.51	6.60 ± 1.10	NS

AE, acute exacerbation; ALT, alanine aminotransferase; ASC, asymptomatic HBV carrier; AST, aspartate aminotransferase; CHB, chronic hepatitis B; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NS, not significant.

of any specific mutations between these two groups (Fig. 3b).

DISCUSSION

THE MAGNITUDE OF liver injuries depends on the replication level of HBV and cytotoxic immune responses of the host raised against viral epitopes in general.[31] Various viral factors have been proposed that promote the development of FHB, represented by pre-core (G1896A) and core promoter (A1762T/G1764A) mutations.[13–16] Impact of virological factors on the development of FHB has remained controversial, however, especially because these mutations are rarely detected in the patients from the USA and France.[19–21] It has been argued that the development of FHB is not promoted by these mutations and is dependent on host factors including the human leukocyte antigen (HLA) environment.[22]

The expression of HBeAg is terminated by G1896A mutation in the pre-core region at the translation level,[32] and downregulated by the A1762T/G1764A double mutation at the transcription level.[33,34] Lamberts *et al.* are the first to implicate a negative influence of HBeAg on the replication of HBV.[35] Should HBeAg suppress the replication of HBV, presumably by inhibiting the encapsidation of pre-genome,[35] the lack or decrease of HBeAg would enhance the reproduction of HBV. Furthermore, HBeAg acts as a tollerogen to T cells recognizing epitopes on core protein, thereby, obviating immune injury of hepatocytes.[36,37] In the absence or decrease of HBeAg, therefore, hosts would mount vigor cytotoxic T-cell responses to core epitopes excessively presented on hepatocytes, and develop severe liver injuries culminating in FHB.[38]

There is a possibility that influence of viral factors such as HBV mutants with a HBeAg-negative phenotype, on the induction of FHB, may have been confounded by host factors and created disagreement. Therefore, the sheer influence of virological factors on FHB would need to be evaluated in case–control studies, as has been attempted to sort out the influence of HBV genotypes on development of cirrhosis and hepatocellular carcinoma.[8] These backgrounds have instigated us to identify virological factors accelerating the severity of liver disease in the 50 FHB patients by transient HBV infection and the 50 AHB patients who were of the same ethnicity and matched for age as well as sex.

In this case controlled study, A1762T/G1764A, G1896A, G1899A and A2339G mutation were significantly more frequent in the patients with FH-T than AHB, providing further corroboration of previous studies;[13–16] these mutations could enhance viral replication. Interestingly, our recent study using an *in vitro* replication model, showed that A2339G mutation in the core region enhanced viral replication and the effect of A2339G mutation may be associated with inhibition of the cleavage of the core protein by a furin-like protease, resulting in the high expression of the complete core protein.[18] Such enhanced HBV would induce significant immune response, resulting in development of FHB.

In multivariate analysis, higher levels of serum HBV DNA and G1896A mutation were independent virological risk factors for the development of FHB by transient

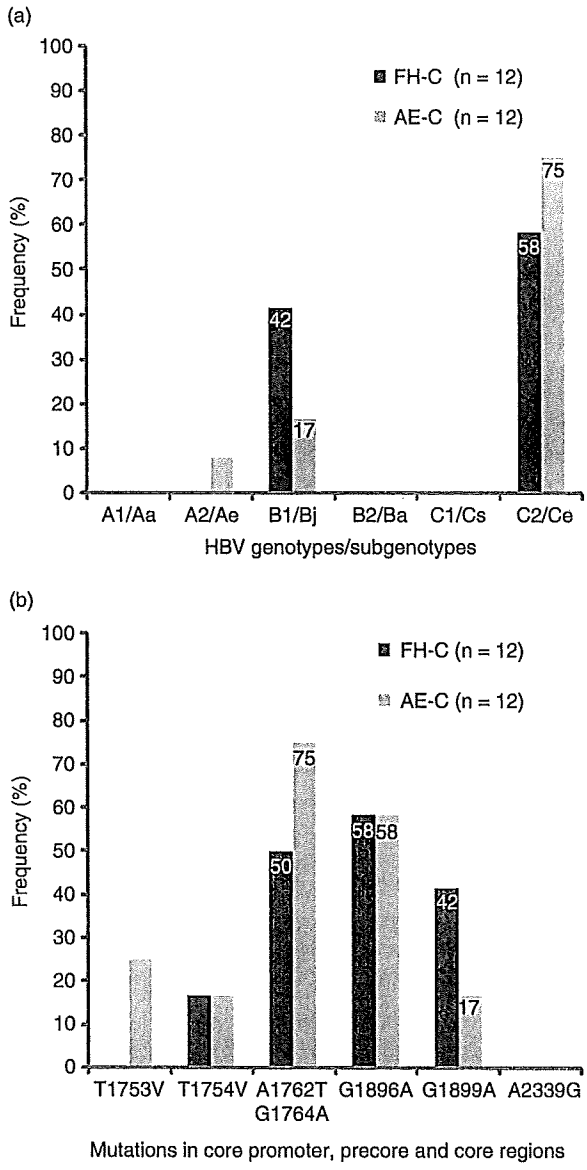


Figure 3 Genotypes/subgenotypes (a) and mutations in core promoter, pre-core and core regions (b) between the 12 transient hepatitis B virus infection (FH-T) and the 12 acute self-limited hepatitis B (AHB) patients.

HBV infection (Table 2). In particular, G1896A mutation was the most important factor associated with the development of FHB. Host responses, represented by T.bil, contributed to the development of FHB as well.

As for HBV genotypes, B1/Bj alone was significantly more frequent in the FH-T patients in univariate analysis. In the patients infected with B1/Bj, G1896A was more frequent in those with FH-T than AHB. In *in vitro* replication analysis, Ozasa *et al.*[15] observed extremely high expressions of intra- and extracellular HBV DNA in culture transfected with an HBV clone of B1/Bj genotype having the G1896A mutation; a high replication would be induced by this pre-core mutation for the induction of FHB. Our clinical results stand in support of this *in vitro* analysis. Taken altogether, chances for developing severe acute or FH would be high in the patients with acute hepatitis who are infected with HBV/B1 having the pre-core mutation. By contrast, in patients infected with C2/Ce, G1896A or A1762T/G1764A, or both was much more frequent in the FH-T patients than AHB. Of note, the co-occurrence of G1896A and A1762T/G1764A mutations was invariably accompanied by either FHB or acute severe hepatitis B in this study. Hence, these pre-core and core-promoter mutations might have addictive or synergetic effects for exacerbating hepatitis, when they emerge in the patients infected with C2/Ce. Such high-risk patients deserve special care and surveillance for signs and symptoms of fulminant or severe acute hepatitis B.

In the present study, serum levels of HBV DNA were significantly higher in the patients with FH-T than AHB. High serum levels of HBV DNA have been reported in patients with FHB;[39] they are followed by rapid decrease as the sequel of virus elimination operated by vigorous immune responses. Because of rapid and extensive elimination of HBV by the host immune system, HBV DNA in serum, in general, has decreased to low levels in patients with FHB at the presentation.[40] HBV DNA levels may be subject to the time that has elapsed from the onset of hepatitis to its measurement.[39] Also, serum levels of core protein (the product of the C gene) closely correlate with serum HBV DNA levels in patients with hepatitis B,[27] and they were compared between the FH-T patients and AHB. The core protein was determined by the newly developed CLEIA method; it is much easier and less expensive than the determination of HBV DNA. The level of core protein has turned out to be marginally higher in the FH-T patients than AHB (Table 1), and therefore might not contribute to an early diagnosis of FHB by transient infection.

Fulminant hepatitis B by AE of ASC is assumed as a different clinical condition from FHB by transient HBV infection. In this study, as there was no case–control study on virological factors associated with FHB for the patients with AE of ASC, we also attempted to identify virological factors associated with the development of FHB in the 12 FH-C and the 12 AE-C patients who were

matched for age as well as sex. Disappointingly, no differences of virological factors such as HBV genotypes and pre-core mutations, which were strongly associated with the development of FHB by transient infection, were found between the FH-C and AE-C patients (Fig. 3a,b). Furthermore, there were also no significant differences about HBeAg-positive rate and the levels of serum HBV DNA or core protein (Table 3), suggesting that several host factors may play a more important role in the development of FHB in ASC instead of virological factors. In this case–control study, however, there seems to be some problems: a small number of patients, different duration of HBV infection, different clinical stage (ASC or CHB) at the onset of AE, and HBV quasispecies complexity. Further investigations are needed to identify factors associated with FHB precipitating in asymptomatic HBV carriers.

In conclusion, virological factors associated with enhancement of viral replication seemed to be important for the development of FHB in the patients by transient HBV infection. But no virological factors were identified for differentiation of the FH-C patients from the AE-C patients. Hence, the pathogenic mechanism of FHB between transient HBV infection and AE of ASC would be different.

ACKNOWLEDGMENTS

WE WOULD LIKE to thank Dr S. Baba, Showa University Hospital, Dr Y. Koga, Kurume University School of Medicine and the other doctors for collecting serum samples in this study. We would also thank Dr N. Maki, Advanced Life Science Institute (Saitama, Japan) for measuring core protein in serum. This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan, and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology.

REFERENCES

1 Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G. Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol Res* 2008; 38: 646–57.
2 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 (Pt 5): 1201–8.
3 Okamoto H, Tsuda F, Sakugawa H *et al.* Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* 1988; 69: 2575–83.
4 Stuyver L, De Gendt S, Van Geyt C *et al.* A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* 2000; 81 (Pt 1): 67–74.
5 Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* 2002; 83 (Pt 8): 2059–73.
6 Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes. *Intervirology* 2003; 46: 329–38.
7 Chu CJ, Lok AS. Clinical significance of hepatitis B virus genotypes. *Hepatology* 2002; 35: 1274–6.
8 Tanaka Y, Hasegawa I, Kato T *et al.* A case-control study for differences among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. *Hepatology* 2004; 40: 747–55.
9 Sugauchi F, Orito E, Ichida T *et al.* Hepatitis B virus of genotype B with or without recombination with genotype C over the pre-core region plus the core gene. *J Virol* 2002; 76: 5985–92.
10 Huy TT, Ushijima H, Quang VX *et al.* Genotype C of hepatitis B virus can be classified into at least two subgroups. *J Gen Virol* 2004; 85 (Pt 2): 283–92.
11 Tanaka Y, Orito E, Yuen MF *et al.* Two subtypes (subgenotypes) of hepatitis B virus genotype C: a novel subtyping assay based on restriction fragment length polymorphism. *Hepatol Res* 2005; 33: 216–24.
12 Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus–large-scale analysis using a new genotyping method. *J Infect Dis* 1997; 175: 1285–93.
13 Sato S, Suzuki K, Akahane Y *et al.* Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. *Ann Intern Med* 1995; 122: 241–8.
14 Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the pre-core region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. *N Engl J Med* 1991; 324: 1699–704.
15 Ozasa A, Tanaka Y, Orito E *et al.* Influence of genotypes and pre-core mutations on fulminant or chronic outcome of acute hepatitis B virus infection. *Hepatology* 2006; 44: 326–34.
16 Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath E. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 1991; 324: 1705–9.
17 Imamura T, Yokosuka O, Kurihara T *et al.* Distribution of hepatitis B viral genotypes and mutations in the core promoter and pre-core regions in acute forms of liver disease in patients from Chiba, Japan. *Gut* 2003; 52: 1630–7.
18 Sugiyama M, Tanaka Y, Kurbanov F, Nakayama N, Mochida S, Mizokami M. Influences on hepatitis B virus replication by a naturally occurring mutation in the core gene. *Virology* 2007; 365: 285–91.

19 Laskus T, Persing DH, Nowicki MJ, Mosley JW, Rakela J. Nucleotide sequence analysis of the pre-core region in patients with fulminant hepatitis B in the United States. *Gastroenterology* 1993; 105: 1173–8.
20 Liang TJ, Hasegawa K, Munoz SJ *et al.* Hepatitis B virus pre-core mutation and fulminant hepatitis in the United States. A polymerase chain reaction-based assay for the detection of specific mutation. *J Clin Invest* 1994; 93: 550–5.
21 Feray C, Gigou M, Samuel D, Bernuau J, Bismuth H, Brechot C. Low prevalence of pre-core mutations in hepatitis B virus DNA in fulminant hepatitis type B in France. *J Hepatol* 1993; 18: 119–22.
22 Karayiannis P, Alexopoulou A, Hadziyannis S *et al.* Fulminant hepatitis associated with hepatitis B virus e antigen-negative infection: importance of host factors. *Hepatology* 1995; 22: 1628–34.
23 Trey C, Lipworth L, Chalmers TC *et al.* Fulminant hepatic failure. Presumable contribution to halothane. *N Engl J Med* 1968; 279: 798–801.
24 Ng HJ, Lim LC. Fulminant hepatitis B virus reactivation with concomitant listeriosis after fludarabine and rituximab therapy: case report. *Ann Hematol* 2001; 80: 549–52.
25 Fujiwara K, Mochida S, Matsui A. [Prospective study for the efficiency of lamivudine for the patients with acute exacerbation of HBV carrier.] *Annual Report of Intractable Liver Disease Study Group of Japan, the Ministry of Health, Welfare and Labor* 2004. (In Japanese.)
26 Kimura T, Rokuhara A, Sakamoto Y *et al.* Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J Clin Microbiol* 2002; 40: 439–45.
27 Kimura T, Rokuhara A, Matsumoto A *et al.* New enzyme immunoassay for detection of hepatitis B virus core antigen (HBcAg) and relation between levels of HBcAg and HBV DNA. *J Clin Microbiol* 2003; 41: 1901–6.
28 Abe A, Inoue K, Tanaka T *et al.* Quantitation of hepatitis B virus genomic DNA by real-time detection PCR. *J Clin Microbiol* 1999; 37: 2899–903.
29 Sugauchi F, Mizokami M, Orito E *et al.* A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. *J Gen Virol* 2001; 82 (Pt 4): 883–92.
30 Shin IT, Tanaka Y, Tateno Y, Mizokami M. Development and public release of a comprehensive hepatitis virus database. *Hepatol Res* 2008; 38: 234–43.
31 Chisari FV, Ferrari C Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; 13: 29–60.
32 Carman WF, Jacyna MR, Hadziyannis S *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; 2 (8663): 588–91.
33 Buckwold VE, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on pre-core gene expression and viral replication. *J Virol* 1996; 70: 5845–51.
34 Okamoto H, Tsuda F, Akahane Y *et al.* Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. *J Virol* 1994; 68: 8102–10.
35 Lamberts C, Nassal M, Velhagen I, Zentgraf H, Schroder CH. Precore-mediated inhibition of hepatitis B virus progeny DNA synthesis. *J Virol* 1993; 67: 3756–62.
36 Chen MT, Billaud JN, Sallberg M *et al.* A function of the hepatitis B virus pre-core protein is to regulate the immune response to the core antigen. *Proc Natl Acad Sci USA* 2004; 101: 14913–8.
37 Chen M, Sallberg M, Hughes J *et al.* Immune tolerance split between hepatitis B virus pre-core and core proteins. *J Virol* 2005; 79: 3016–27.
38 Bocharov G, Ludewig B, Bertoletti A *et al.* Underwhelming the immune response: effect of slow virus growth on CD8+-T-lymphocyte responses. *J Virol* 2004; 78: 2247–54.
39 Sainokami S, Abe K, Sato A *et al.* Initial load of hepatitis B virus (HBV), its changing profile, and pre-core/core promoter mutations correlate with the severity and outcome of acute HBV infection. *J Gastroenterol* 2007; 42: 241–9.
40 Tassopoulos NC, Papaevangelou GJ, Roumeliotou-Karayannis A, Ticehurst JR, Feinstone SM, Purcell RH. Search for hepatitis B virus DNA in sera from patients with acute type B or non-A, non-B hepatitis. *J Hepatol* 1986; 2: 410–8.

CORRESPONDENCE

Pretreatment Prediction of Virological Response to Peginterferon Plus Ribavirin Therapy in Patients with Chronic Hepatitis C, Using Viral and Host Factors: Some Concerns

To the Editor:

We read with great interest the article published in a recent issue of HEPATOLOGY.[1] Shirakawa et al. enrolled 120 patients with chronic hepatitis C (CHC) infected with genotype 1 hepatitis C virus (HCV-1) and high baseline viral loads (HVL), defined by HCV RNA levels $\geq 10^5$ international units (IU)/mL measured by quantitative Cobas Amplicor assays (Roche Diagnostics Co. Ltd, Tokyo, Japan), who underwent combination therapy scheduled for 48 (85%) or 72 (15%) weeks. The authors concluded that the sequence of interferon sensitivity determining region of the HCV, the T-helper type 1 and type 2 (Th1/Th2) ratio, body weight, and neutrophil count can be useful for accurately predicting actual sustained virologic response (SVR) rate before combination therapy.

The definition of HVL ($\geq 10^5$ IU/mL) by the authors is not in accordance with the Asian Pacific Association for the Study of the Liver consensus statements ($\geq 4 \times 10^5$ IU/mL)[2] or other studies ($\geq 8 \times 10^5$ IU/mL).[3,4] Whether this definition influences the results deserves further study. The rates of rapid virologic response (RVR, defined as HCV RNA negative at week 4) and SVR were 45% and 21.7%, respectively, in their study. We examined the 200 Taiwanese patients with HCV-1 CHC in our randomized trial published recently[5] and found that 150 (75%) of these patients had serum HCV RNA $\geq 10^5$ IU/mL (HVL). The RVR and SVR rates of 150 patients were 64% and 34.7%, respectively, after receiving combination therapy with peginterferon alpha-2a plus oral ribavirin. A significantly higher SVR rate in patients treated for 48 weeks than 24 weeks (78.5%, 62 of 79 versus 35.4%, 34 of 71, $P < 0.001$) was observed. Recently, Ide et al. reported a 36% (20 of 56) SVR rate in Japanese patients with CHC and HVL treated with peginterferon alpha-2b/ribavirin for 48 weeks.[6] Accordingly, we have noticed the SVR rate and RVR rate in Taiwanese patients with HCV-1 with HVL treated for 24 or 48 weeks were higher than Japanese patients treated for 48 or 72 weeks. The important role of the weight-based ribavirin exposure during the first 4 weeks of combination therapy on the achievement of an RVR has been highlighted previously.[7] A cut-off point of 13 mg/kg/day of the first 4 weeks of weight-based ribavirin exposure have been reported associated with the achievement of an RVR.[8] Patients in the study by Shirakawa et al. would have an exposure of less than 13.1 mg/kg/day ribavirin exposure by 4 weeks (starting dose: for body weight 60 kg or less; 600 mg/day, 61 kg to 80 kg; 800 mg/day, 81 kg or more; 1000 mg/day) which is lower than 17.4 mg/kg/day (starting dose: for body weight 75 kg or less; 1000 mg/day, 75 kg or more; 1200 mg/day) in Taiwanese patients infected with HCV-1. We agree that the valuable finding of pretreatment predictors for SVR rate by Shirakawa et al. should be applauded. The relative low SVR and RVR rates, even with longer duration of treatment, compared to Taiwanese patients infected with HCV-1 with HVL raise a concern of relative suboptimal ribavirin exposure, and the actual role of these pretreatment predictors have to be confirmed by further studies.

CHIA-YEN DAI[1-3]
WAN-LONG CHUANG[1,2]
JEE-FU HUANG[2,4]
MING-YEN HSIEH[2]
MING-LUNG YU[1,2,4]
[1]*Faculty of Internal Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[2]*Hepatobiliary Division, Department of Internal Medicine, and*
[3]*Department of Occupational Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[4]*Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan*

References

1. Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, et al. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. HEPATOLOGY 2008;48:1753-1760.
2. Asian Pacific Association for the Study of the Liver (APASL) Hepatitis C Working Party, McCaughan GW, Omata M, Amarapurkar D, Bowden S, Chow WC, Chutaputti A, et al. Asian Pacific Association for the Study of the Liver consensus statements on the diagnosis, management and treatment of hepatitis C virus infection. J Gastroenterol Hepatol 2007;22:615-633.
3. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.
4. Hadziyannis SJ, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon alfa-2a (40 kilodaltons) and ribovirin combination therapy in chronic hepatitis C: randomized study of the effect of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-355.
5. Yu ML, Dai CY, Huang JF, Chiu CF, Yang YH, Hou NJ, et al. Rapid virological response and treatment duration for chronic hepatitis c genotype 1 patients: a randomized trial. HEPATOLOGY 2008;47:1884-1893.
6. Ide T, Hino T, Ogata K, Miyajima I, Kuwahara R, Kuhara K, et al. A Randomized study of extended treatment with peginterferon alpha-2b plus ribavirin based on time to HCV RNA negative-status in patients with genotype 1b chronic hepatitis C. Am J Gastroenterol 2009;104:70-75.
7. Yu ML, Dai CY, Huang JF, Chuang WL. Rapid virological response to peginterferon and ribavirin for hepatitis C genotype 1: the role of weight-based ribavirin exposure. HEPATOLOGY 2008;48:1019-1020.
8. Rodriguez-Torres M, Sulkowski M, Chung RT, Hamzeh FM, Jensen D. Association of pretreatment and on-treatment factors with rapid virological response in HCV genotype 1-infected patients with peginterferon alfa- 2a/ribavirin [Abstract]. HEPATOLOGY 2007;46(Suppl):817A.

Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/hep.22826
Potential conflict of interest: Nothing to report.

Reply:

First, we have to point out a mistake that Dai et al. made in their letter: the rapid virological response (RVR) and sustained virological response (SVR) rates are described inversely with respect to our data and maybe with respect to their data.

Dai et al. reported that the SVR rate of 64% in their study is apparently higher than the rate of 45% in our study. We think that this difference can be attributed to the difference in the mean ages of the patients enrolled in the two studies. The mean age in their study seems to have been around 50 years[1] and was over 10 years lower than that in our study (62 years old). Younger patients tended to show higher SVR rates in our study as well, and the SVR rate in patients who were younger than 55 years old was 61%, which is comparable to the rate reported by Dai et al.

It is possible that a higher administration dose of ribavirin during the first 4 weeks is associated with a higher RVR rate. However, we cannot comment on this because our study was not designed for that purpose.

We adopted the definition of high viral load generally used in Japan (10^5 IU/mL). Since Dai et al. suggested that a difference in the definition of high viral load influenced the results, we recalculated factors associated with SVR in 103 patients whose viral load was higher than 4×10^5 IU/mL and also in 89 patients whose viral load was higher than 8×10^5 IU/mL. The same four prediction factors as those provided by the original analysis of 120 patients whose viral load was higher than 10^5 IU/mL were again selected.

JOURNAL OF CLINICAL MICROBIOLOGY, May 2009, p. 1476–1483 Vol. 47, No. 5
0095-1137/09/$08.00+0 doi:10.1128/JCM.02081-08

Distribution of Hepatitis B Virus Genotypes among Patients with Chronic Infection in Japan Shifting toward an Increase of Genotype A▽

Kentaro Matsuura,[1,2] Yasuhito Tanaka,[1]* Shuhei Hige,[3] Gotaro Yamada,[4] Yoshikazu Murawaki,[5] Masafumi Komatsu,[6] Tomoyuki Kuramitsu,[7] Sumio Kawata,[8] Eiji Tanaka,[9] Namiki Izumi,[10] Chiaki Okuse,[11] Shinichi Kakumu,[12] Takeshi Okanoue,[13] Keisuke Hino,[14] Yoichi Hiasa,[15] Michio Sata,[16] Tatsuji Maeshiro,[17] Fuminaka Sugauchi,[2] Shunsuke Nojiri,[2] Takashi Joh,[2] Yuzo Miyakawa,[18] and Masashi Mizokami[1,19]

Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan[1]; Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan[2]; Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan[3]; Department of Basic Laboratory Sciences, Kawasaki Medical School, Kawasaki Hospital, Okayama, Japan[4]; Division of Medicine and Clinical Science, Faculty of Medicine, Tottori University, Tottori, Japan[5]; Department of Gastroenterology, Akita City Hospital, Akita, Japan[6]; Kuramitsu Clinic, Akita, Japan[7]; Department of Gastroenterology, Yamagata University School of Medicine, Yamagata, Japan[8]; Department of Medicine, Shinshu University School of Medicine, Matsumoto, Japan[9]; Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan[10]; Department of Internal Medicine, Division of Gastroenterology and Hepatology, St. Marianna University School of Medicine, Kawasaki, Japan[11]; Department of Gastroenterology, Aichi Medical University School of Medicine, Aichi, Japan[12]; Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan[13]; Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan[14]; Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Ehime, Japan[15]; Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan[16]; First Department of Internal Medicine, University Hospital, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan[17]; Miyakawa Memorial Research Foundation, Tokyo, Japan[18]; and Research Center for Hepatitis and Immunology, Kohnodai Hospital International Medical Center of Japan, Ichikawa, Japan[19]

Received 29 October 2008/Returned for modification 17 December 2008/Accepted 2 March 2009

Acute hepatitis B virus (HBV) infection has been increasing through promiscuous sexual contacts, and HBV genotype A (HBV/A) is frequent in patients with acute hepatitis B (AHB) in Japan. To compare the geographic distribution of HBV genotypes in patients with chronic hepatitis B (CHB) in Japan between 2005 and 2006 and between 2000 and 2001, with special attention to changes in the proportion of HBV/A, a cohort study was performed to survey changes in genotypes of CHB patients at 16 hospitals throughout Japan. Furthermore, we investigated the clinical characteristics of each genotype and examined the genomic characteristics of HBV/A isolates by molecular evolutionary analyses. Of the 1,271 patients, 3.5%, 14.1%, and 82.3% were infected with HBV/A, -B, and -C, respectively. In comparison with our previous survey during 2000 and 2001, HBV/A was twice as frequent (3.5% versus 1.7%; $P = 0.02$). The mean age was lower in the patients with HBV/A than in those with HBV/B or -C. Based on phylogenetic analyses of 11 full-length genomes and 29 pre-S2/S region sequences from patients, HBV/A isolates were imported from Europe and the United States, as well as the Philippines and India. They clustered with HBV/A from AHB patients and have spread throughout Japan. HBV/A has been increasing in CHB patients in Japan as a consequence of AHB spreading in the younger generation through promiscuous sexual contacts, aided by a tendency of HBV/A to induce chronic hepatitis. The spread of HBV/A infection in Japan should be prevented by universal vaccination programs.

Hepatitis B virus (HBV), a member of the *Hepadnaviridae*, is a circular, partially double-stranded DNA virus and is one of the major causes of chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).

The HBV genome is composed of approximately 3,200 nucleotides. HBV is classified into eight genotypes, designated A to H, based on an intergroup divergence of 8% or more in the complete nucleotide sequence (3, 23, 26, 37). They have distinct geographical distributions and are associated with differences in clinical and virological characteristics, such as severity of liver disease and response to antiviral therapies (7, 8, 12, 13, 22, 28). Furthermore, subgenotypes have been reported for HBV/A, -B, and -C and named A1 to -3 (17, 38), B1 to -6 (31, 32, 40), and C1 to -6 (20, 31, 45). Equally, other genotypes are classified into subgenotypes. There have been increasing lines of evidence to indicate influences of HBV subgenotypes on the outcome of liver disease and the response to antiviral therapies (1, 39, 44).

In 2001, we reported the geographic distribution of HBV genotypes in Japan (27). Of the 720 Japanese patients with chronic HBV infection (CHB), 12 (1.7%) harbored HBV/A, 88 (12.2%) HBV/B, 610 (84.7%) HBV/C, 3 (0.4%) HBV/D, and 7 (1.0%) mixed genotypes. HBV/C was detected in over 94%

* Corresponding author. Mailing address: Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya 467-8601, Japan. Phone: 81-52-853-8292. Fax: 81-52-842-0021. E-mail: ytanaka@med.nagoya-cu.ac.jp.

▽ Published ahead of print on 18 March 2009.

of patients on the Japanese mainland, while HBV/B was found in 64% of those in Okinawa, the southernmost islands, and 44% of those in the Tohoku area in the northern part of the mainland.

Recently, acute HBV infection (AHB) has been increasing in Japan, predominantly through promiscuous sexual contacts. In addition, it was reported that HBV/A was more frequent in patients with acute hepatitis than in those with chronic hepatitis (29, 41, 49). Recent studies suggest that the chances for progression to chronic disease may differ among patients acutely infected with HBV of distinct genotypes (21, 25); patients infected with HBV/A run an increased risk of becoming HBV carriers. Hence, it is of utmost concern whether chronic HBV/A infection is increasing in Japan.

In the present study, we compared the geographic distribution of HBV genotypes in Japan during 2005 and 2006 with 2000 and 2001, with special attention to changes in the proportion of HBV/A. Furthermore, we investigated the clinical characteristics of each genotype and examined the genomic characteristics of HBV/A isolates by molecular evolutionary analyses.

MATERIALS AND METHODS

Patients. From September 2005 to October 2006, sera were collected from 1,370 consecutive patients with CHB at 16 representative hospitals that were liver centers in their respective regions throughout Japan for the purpose of investigating the geographic distribution of HBV genotypes in Japan. All of the patients were diagnosed after they had been followed for at least 12 months. Patients diagnosed with AHB were excluded from the study; they had a sudden onset of clinical symptoms of hepatitis, along with high-titer antibody to HBV core antigen of the immunoglobulin M class in serum. Their sera were tested for alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), and hepatitis B e antigen (HBeAg), as well as antibody to HBeAg (anti-HBe) (Dinabot, Tokyo, Japan). Four clinical diagnoses were established for them. The inactive carrier state was defined by the presence of HBV surface antigen (HBsAg) with normal ALT levels over 1 year (examined at least four times at 3-month intervals) and without evidence of portal hypertension. Chronic hepatitis was defined by elevated ALT levels (>1.5 times the upper limit of normal [35 IU/liter]) persisting over 6 months (with at least three bimonthly tests). Cirrhosis was diagnosed principally by ultrasonography (coarse liver architecture, nodular liver surface, blunt liver edges, and hypersplenism), platelet counts of <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 study protocol conformed to the 1975 declaration of Helsinki and was approved by the ethics committees of the respective institutions. Every patient or his/her next of kin gave informed consent to the purpose of the study.

Genotypes and subgenotypes of HBV. The six HBV genotypes (A to F) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV Genotype EIA; Institutes of Immunology Co., Ltd., Tokyo, Japan). The method depends on the combination of epitopes on pre-S2 region products detected by monoclonal antibodies that were specific for each of them (46, 47). Subgenotypes of HBV/A, designated A1 and A2, were determined by direct sequencing of the pre-S2/S gene, followed by a phylogenetic analysis.

Quantification of HBV DNA and sequencing. HBV DNA levels in sera were quantitated with a commercial kit (Amplicor HBV Monitor; Roche Diagnostics, Basel, Switzerland) with a detection range from 2.6 to 7.6 log copies/ml. Nucleic acids were extracted from 100 μl of serum using the Qiaamp DNA Blood Minikit (Qiagen GmbH, Hilden, Germany). Eleven complete HBV/A genomes and 29 pre-S2/S region sequences were amplified by PCR with appropriate primer sets, as described previously (40). The amplified HBV DNA fragments were directly sequenced using the ABI Prism Big Dye kit version 3.0 (Applied Biosystems, Foster City, CA) in an ABI 3100 automated DNA sequencer (Applied Biosystems). All sequences were analyzed in both forward and reverse directions. Complete and partial HBV genome sequences were aligned using GENETYX version 11.0 (Software Development Co., Ltd., Tokyo, Japan).

TABLE 1. Characteristics of 1,271 CHB patients

Parameter	Value
Characteristic	
Male gender [no. (%)]	766 (60.3)
Age (yr; mean ± SD)	51.4 ± 14.0
Diagnosis	
Inactive carrier state [no. (%)]	206 (16.2)
Chronic hepatitis [no. (%)]	786 (61.8)
Cirrhosis [no. (%)]	175 (13.8)
HCC [no. (%)]	104 (8.2)
Antiviral treatment [no. (%)]	577 (45.4)
Blood tests	
Platelets (10^4/mm^3)	21.4 ± 30.2
ALT (IU/liter)	59.8 ± 103.0
ALP (IU/liter)	270.4 ± 136.0
γ−GTP (IU/liter)	47.4 ± 66.1
HBV markers	
HBeAg [no. (%)]	399 (31.4)
HBV DNA (median [range] [log copies/ml])	4.2 (<2.6 to >7.6)

Molecular evolutionary analysis of HBV. Reference sequences were retrieved from the DDBJ/EMBL/GenBank databases with their accession numbers for identification. To investigate the relationship between HBV isolates from patients with chronic and acute hepatitis B in Japan, HBV/A isolates (AH1 to -10) were randomly retrieved from them and sequenced in our previous study (29). Nucleotide sequences of HBV DNA were aligned by the program CLUSTAL X, and genetic distance was estimated by the six-parameter method (10) in the Hepatitis Virus Database (36). Based on these values, phylogenetic trees were constructed by the neighbor-joining method (30) with the midpoint rooting option. To confirm the reliability of the phylogenetic trees, bootstrap resampling tests were performed 1,000 times.

Statistical analysis. Categorical variables were compared between groups by the χ^2 test or Fisher's exact test and noncategorical variables by the Mann-Whitney U test. A P value of less than 0.05 was considered significant.

Nucleotide sequence accession numbers. The DDBJ/EMBL/GenBank accession numbers of the complete genome sequences of HBV isolates JPN_CH1 to -11 are AB453979 to AB453989.

RESULTS

Distribution of HBV genotypes among patients with CHB. Of the 1,370 serum samples, the genotype could not be determined for 99 (7.2%) by EIA due to low HBsAg levels, leaving 1,271 for analysis in this study (Table 1). Of these, 206 (16.2%) were inactive carriers, 786 (61.8%) had chronic hepatitis, 175 (13.8%) cirrhosis, and 104 (8.2%) HCC. They had a mean age of 51.4 ± 14.0 years and included 766 (60.3%) men. They had a median HBV DNA level of 4.2 log copies/ml, and 399 (31.4%) of them were positive for HBeAg. Antiviral treatment had been given to 577 (45.4%) of them with interferon, lamivudine, adefovir pivoxil, or entecavir.

The genotypes were HBV/A in 44 (3.5%), HBV/B in 179 (14.1%), HBV/C in 1,046 (82.2%), and HBV/D in 2 (0.2%) (Table 2). In comparison with our previous report on the distribution of genotypes in Japan in 2001 (27), HBV/A was more frequent in this study (3.5% versus 1.7%; $P = 0.02$). Of the 16 hospitals in this study, 10 overlapped with those in our previous report from 2001. In these 10 hospitals, HBV/A was more frequent in the present than in the previous survey (3.6% versus 1.7%; $P = 0.04$).

The distribution of HBV genotypes in Japan differed by

TABLE 2. Distribution of HBV Genotypes

Genotype	No. (%)	
	2005–2006 (n = 1,271)	2000–2001[a] (n = 720)
A	44 (3.5[b])	12 (1.7)
B	179 (14.1)	88 (12.2)
C	1,046 (82.3)	610 (84.7)
D	2 (0.2)	3 (0.4)
Mixed	0 (0.0)	7 (1.0)

[a] From Orito et al. (27).
[b] $P = 0.02$.

geographic location (Fig. 1). HBV/C was the most prevalent in the majority of areas. In the Tohoku area, the northern part of the Japanese mainland (Honshu), HBV/B was more prevalent than in the other areas of the Japanese mainland. In Okinawa, the southernmost islands of Japan, HBV/B was predominant. Of note, HBV/A was more frequent in the Kanto area (9.5%), the metropolitan area, and Okinawa (9.1%) than in the other areas.

Clinical differences among HBV/A, -B, and -C. Clinical backgrounds were compared among the patients infected with HBV/A, -B, and -C (Table 3). HBeAg was significantly less prevalent in the patients infected with HBV/B than in those infected with HBV/A or -C ($P < 0.01$ for each). When the positivity of HBeAg was stratified by age, HBeAg was markedly less common in patients infected with HBV/B than in those infected with HBV/A or -C who were older than 40 years of age (7/157 [4.5%] versus 4/19 [21.1%] [$P < 0.05$] or 215/755 [28.5%] [$P < 0.01$]) (Fig. 2). There were no significant differences in HBV DNA levels among patients infected with the three genotypes. As antiviral treatments might have influenced the severity of liver disease, clinical states were compared among patients infected with HBV/A, -B, and -C who did and did not receive it; antiviral treatments did not affect the above-mentioned trends represented in Table 3 in age, diagnosis, and HBeAg, as well as ALT and HBV DNA levels (data not shown).

Additionally, we compared the distributions of age and liver diseases in patients infected with HBV/A, -B, and -C. In patients infected with HBV/C, the prevalence of cirrhosis and HCC increased in those older than 50 years of age compared to younger patients (Fig. 3), whereas in the patients infected with HBV/B, cirrhosis and HCC were rare in elderly patients. The proportion of patients younger than 40 years of age was higher in those infected with HBV/A than in those infected with HBV/B or -C (25/44 [56.8%] versus 22/179 [12.3%] or 288/1,046 [27.5%]; $P < 0.01$ for each), while cirrhosis and HCC were also found in those older than 50 years of age infected with HBV/A.

Coinfection with human immunodeficiency virus type 1 (HIV-1) was found in 6 of the 44 (13.6%) patients infected with HBV/A compared to only 3 of the 1,046 (0.3%) patients infected with HBV/C ($P < 0.0001$); it occurred in none of the 179 patients infected with HBV/B.

Phylogenetic analyses. Among the 44 HBV/A isolates, the complete genome was sequenced successfully in 11 (JPN_CH1 to -11). Seven of them were classified as HBV/A2 and four as HBV/A1. A phylogenetic tree was constructed based on the complete genome sequences of these 11 isolates, along with those from two patients with AHB and those from 40 HBV/A isolates retrieved from the database (Fig. 4). Of the seven HBV/A2 isolates, the four from patients with CHB in this study formed a cluster with the Japanese isolates retrieved from the database and two from patients with AHB. Of the other three isolates, JPN_CH5 clustered with French and U.S. isolates, JPN_CH6 with German isolates, and JPN_CH7 with

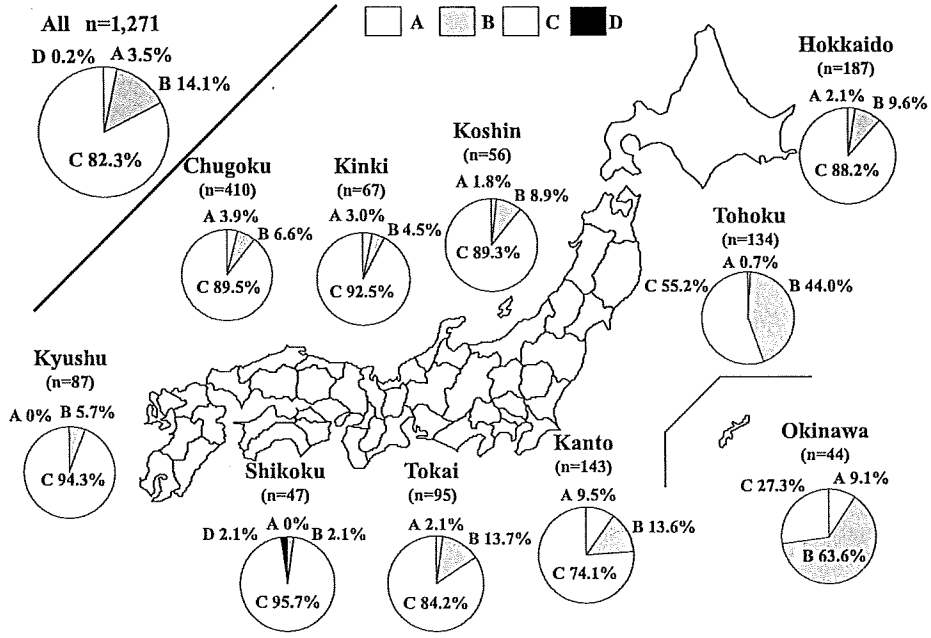


FIG. 1. Geographic distribution of HBV genotypes in patients with chronic HBV infection in Japan during 2005 and 2006.

TABLE 3. Clinical characteristics of individuals chronically infected with HBV of different genotypes

Parameter	Value for genotype:		
	A (n = 44)	B (n = 179)	C (n = 1,046)
Male gender [no. (%)]	32 (72.7)	112 (62.6)	621 (59.4)
Age (yr [mean ± SD])	41.3 ± 14.9[a]	55.8 ± 13.7[b]	48.8 ± 13.3
Diagnosis			
Inactive carrier state [no. (%)]	13 (29.5)[c]	63 (35.2)[b]	129 (12.3)
Chronic hepatitis [no. (%)]	26 (59)	103 (57.5)	656 (62.7)
Cirrhosis [no. (%)]	3 (6.8)	10 (5.6)[b]	162 (15.5)
HCC [no. (%)]	2 (4.5)	3 (1.7)[b]	99 (9.5)
Anti viral treatment [no. (%)]	13 (29.5)[d]	48 (26.8)[b]	516 (49.3)
Blood tests			
Platelet (10^4/mm^3)	23.3 ± 21.9	25.9 ± 35.9[e]	20.6 ± 29.5
ALT (IU/liter)	56.2 ± 83.8	42.2 ± 104.2[e]	63.0 ± 103.3
ALP (U/liter)	247.1 ± 123.0	255.5 ± 97.9	273.9 ± 141.9
γ-GTP (U/liter)	39.6 ± 34.6	49.3 ± 63.4	47.5 ± 67.6
HBV markers			
HBeAg [positive rate(%)]	15 (34.0)[f]	17 (9.5)[b]	367 (35.1)
HBV DNA (median [range]) (log copies/ml)	4.2 (<2.6–>7.6)	4.1 (<2.6–>7.6)	4.2 (<2.6–>7.6)

[a] $P < 0.01$, A versus B or C.
[b] $P < 0.01$, B versus C.
[c] $P < 0.01$, A versus C.
[d] $P < 0.05$, A versus C.
[e] $P < 0.05$, B versusC.
[f] $P < 0.01$, A versus B.

Spanish and Italian isolates. All four HBV/A1 isolates in this study formed a cluster with Philippine and Indian isolates.

In addition, the pre-S2/S region sequences of a total of 29 isolates were determined, including the 11 isolates whose complete genomes were sequenced. Of these, 21 (72%) were classified as HBV/A2 and the remaining 8 as HBV/A1. A phylogenetic tree was constructed based on the pre-S2/S region sequences from the 29 isolates, along with those from 10 patients with AHB infected with HBV/A and 47 HBV/A isolates retrieved from the database (Fig. 5). The 21 HBV/A2 isolates in the present study formed a cluster with Japanese, American, and European isolates retrieved from the database and those from patients with acute hepatitis. In addition, some of them were highly homologous with each other. Likewise, HBV/A1 isolates from eight patients with chronic hepatitis in this study were highly homologous with those from two patients with acute hepatitis and isolates from the Philippines and India. Based on the phylogenetic analyses, HBV/A isolates were imported from Europe and the United States, as well as the Philippines and India, and had infiltrated throughout Japan.

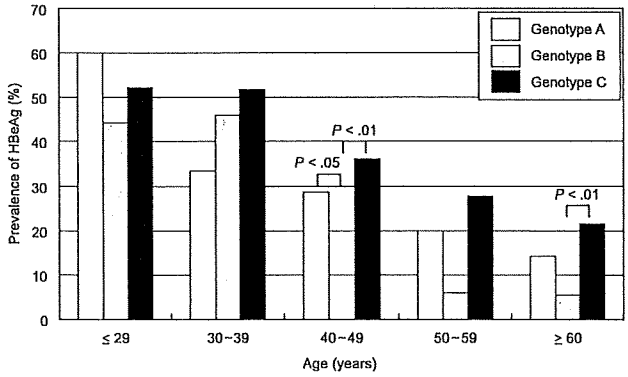


FIG. 2. Prevalence of HBeAg among patients infected with HBV of different genotypes stratified by the age.

DISCUSSION

Perinatal transmission from carrier mothers to their babies has been the principal route for establishing persistent HBV infection in Asian countries (19). In Japan, passive and active immunoprophylaxis with HBV immune globulin and vaccine has been mandated for babies born to HBeAg-positive carrier mothers since 1986; this was extended to HBeAg-negative carrier mothers in 1995. As a result, HBsAg has become rare in Japanese born after 1986; it was detected in only 0.2% of first-time blood donors younger than 19 years of age in 2000 (24). However, AHB has been increasing in Japan, predominantly through promiscuous sexual contacts.

In Japan, HBV/A is detected rarely among patients with CHB but is frequent in those with acute hepatitis (14, 25, 29, 41, 43). Yotsuyanagi et al. reported the distribution of genotypes in 145 Japanese patients with AHB and found HBV/A in 27 (19%), HBV/B in 8 (5%), and HBV/C in 109 (75%) (49). HBV/A is more frequent in metropolitan areas than other areas. The majority of patients with HBV/A infection in metropolitan areas have had extramarital sexual contacts with multiple irregular partners, through which they could have contracted infection. In support of this view, among men who have sex with men (MSM) who are coinfected with HBV and HIV-1 in Tokyo, most were infected with HBV/A (15, 35).

In Japan, AHB in adulthood becomes chronic in only ~1%

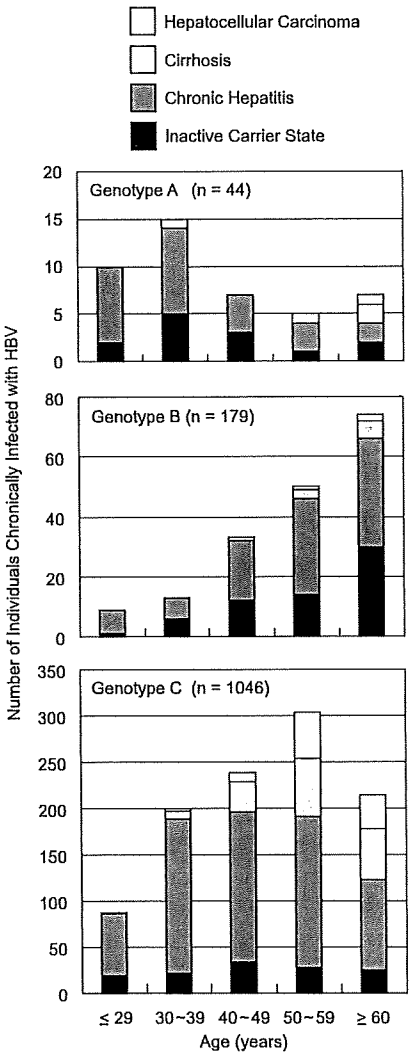


FIG. 3. Distribution of HCC, cirrhosis, chronic hepatitis, and inactive carrier state among the 1,271 patients infected with HBV of different genotypes stratified by the age.

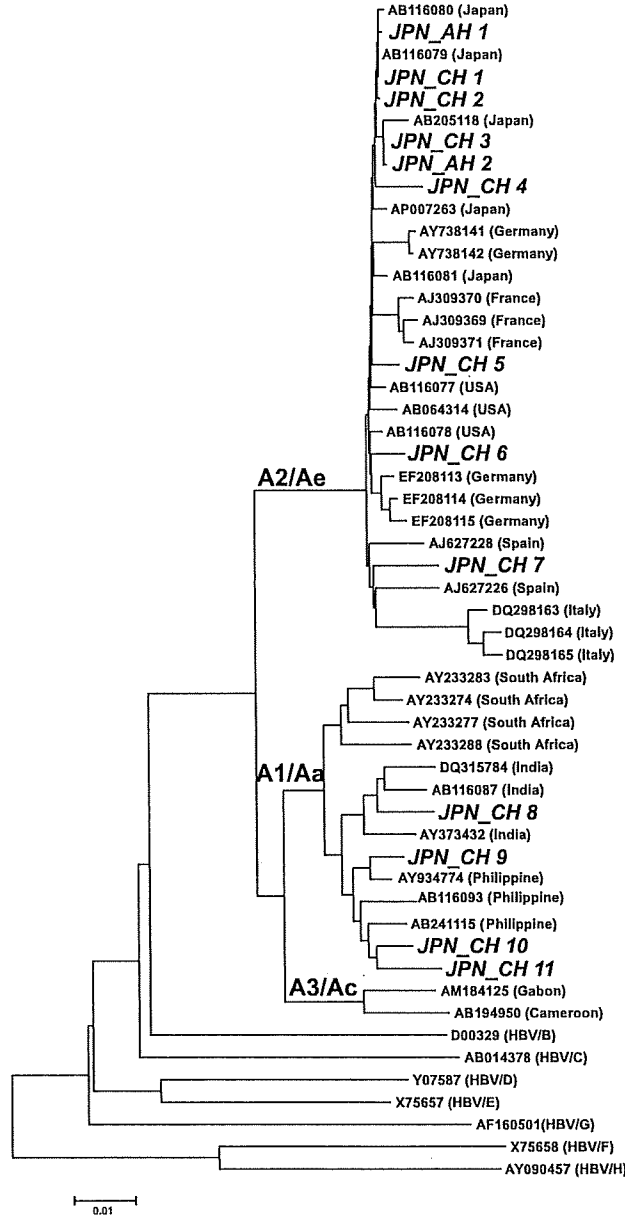


FIG. 4. Phylogenetic tree constructed based on the complete genome sequences of HBV/A isolates. Those from 11 patients with chronic infection in this study are shown in boldface italic (JPN_CH1 to -11), along with two isolates (JPN_AH1 and -2) from patients with acute hepatitis in Japan reported in our previous study (17). Representative isolates were retrieved from the DDBJ/EMBL/GenBank databases, including 21 HBV/Ae, 10 HBV/Aa, and 2 HBV/Ac isolates, along with 7 HBV isolates representative of the other seven genotypes. Isolates from the databases are identified by accession numbers, followed by the country of origin. The bar at the bottom spans 0.01 nucleotide substitutions per site.

of cases. This is much less than the progression to chronic disease (close to 10%) in Europe and the United States, where HBV/A prevails (34). Recent studies have suggested that the chances for persistence may differ among patients acutely infected with HBV of distinct genotypes (21, 25). In particular, acute infection with HBV/A may bring about an increased risk of progression to chronic disease. Therefore, an increase of acute infection with HBV/A would result in a surge of HBV/A among patients with CHB in Japan. In actuality, in comparison with our previous results during 2000 and 2001 (27), HBV/A was twice as frequent in this study (3.5% versus 1.7%; $P = 0.02$). HBV/A has been increasing in patients with CHB in the Kanto area, where HBV/A in patients with acute hepatitis is more frequent than in the other areas. In the islands of Okinawa, also, HBV/A was found to be prevalent in this study. Of the four patients infected with HBV/A there, two were coinfected with HIV-1. They were both MSM, and they were suspected to have been infected with HIV through sexual contacts on the Japanese mainland. It has been reported that HIV infection increases the probability that AHBs will become chronic (2, 11, 33, 48). Because they share routes of transmission and the risk for HIV-1 and HBV infections, approximately