LIVER TRANSPLANTATION 14:1420-1427, 2008

ORIGINAL ARTICLE

Impact of the Hepatitis B Virus Genotype on Pre- and Post-Liver Transplantation Outcomes

Paul Gaglio,[1] Sundeep Singh,[2] Bulent Degertekin,[2] Michael Ishitani,[3] Munira Hussain,[2] Robert Perrillo,[4] Anna S. Lok,[2] and the National Institutes of Health Hepatitis B Virus–Orthotopic Liver Transplantation Study Group

[1]Center for Liver Disease and Transplantation, Columbia University College of Physicians and Surgeons, New York, NY; [2]Division of Gastroenterology, University of Michigan Health System, Ann Arbor, MI; [3]Department of Surgery, Mayo Clinic, Rochester, MN; and [4]Ochsner Clinic, New Orleans, LA

Emerging data suggest that the hepatitis B virus (HBV) genotype and the precore and core promoter variants impact the outcome of orthotopic liver transplantation (OLT) for hepatitis B. The aim of this study was to determine if there is a correlation between HBV genotype, precore and core promoter variants, and pre- and post-OLT outcomes. Serum samples from patients participating in the National Institutes of Health HBV-OLT study were tested for HBV genotype and precore and core promoter variants. A total of 123 patients were studied: 43% were Asians, 46% were Caucasians, and 8% were African Americans. HBV genotypes A (35%) and C (35%) were the most prevalent, followed by genotypes D and B. Precore and core promoter variants were detectable in 44% and 90% of patients. Patients with genotype C were more likely to have hepatocellular carcinoma (HCC) at listing ($P < 0.001$). Waitlist mortality was highest among patients with genotype D, while posttransplant mortality was highest among patients with genotype C. Precore or core promoter variants did not correlate with pre- or post-OLT survival. In conclusion, in this US patient population, patients with genotype C were more likely to have HCC at the time of transplant listing and to die after transplant than patients with non-C genotypes. Patients with genotype D had the highest posttransplant survival, but this was offset by higher waitlist mortality. Our study suggests that HBV genotypes but not precore or core promoter variants may have an impact on pre- and post-OLT outcomes of hepatitis B patients. ***Liver Transpl 14:1420-1427, 2008.*** © **2008 AASLD.**

Received October 26, 2007; accepted May 12, 2008.

Eight distinct genotypes of hepatitis B virus (HBV)—A to H—have been identified, each having a characteristic geographic distribution.[1,2] Numerous studies from Asia, where genotypes B and C predominate, have shown that HBV genotype C is associated with more rapid progression to cirrhosis and a higher rate of hepatocellular carcinoma (HCC) in comparison with genotype B.[3-8] Data on the correlation between other HBV genotypes and outcomes of chronic HBV infection are not as well established. One study from Spain found that patients with genotype D were less likely to have sustained biochemical and virological remission after hepatitis B e antigen (HBeAg) seroconversion than those with genotype A infection.[9] An additional observation in this study was that patients with genotype F were more likely to die from liver disease than those with genotype A or D; however, only 19 patients with genotype F were included. Another study of Alaskan natives noted a preponderance of HBV genotype F among patients with HCC.[10]

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease; NIH, National Institutes of Health; OLT, orthotopic liver transplantation.

Paul Gaglio is currently affiliated with the Albert Einstein College of Medicine, New York, NY.
Robert Perrillo is currently affiliated with the Baylor University Medical Center, Dallas, TX.

This study was supported by National Institutes of Health grant U01 DK57577 (to A.S.L.). Innogenetics (Ghent, Belgium) provided reagents for the line probe assays.
Potential conflict of interest: Anna S. Lok receives research support from Innogenetics and serves as a consultant for Innogenetics.

Address reprint requests to Anna S. Lok, M.D., Division of Gastroenterology, University of Michigan Medical Center, 3912 Taubman Center, SPC 5362, Ann Arbor, MI 48109. Telephone: 734-936-7511; FAX: 734-936-7392; E-mail: aslok@med.umich.edu

DOI 10.1002/lt.21563
Published online in Wiley InterScience (www.interscience.wiley.com).

Several recent publications have reported an association between HBV genotype and outcome of orthotopic liver transplantation (OLT). One study from Australia, which included 15 patients with recurrent HBV (8 with genotype D), found that HBV genotype D was associated with more severe recurrence of liver disease.[11] Another study composed of 22 patients from the United States observed that HBV genotype D (n = 5) was associated with the highest risk for HBV recurrence and mortality.[12] A third study analyzing 45 patients, the majority infected with either genotype A or D, found that genotype D was associated with a higher rate of HBV recurrence and graft loss, but the difference was not statistically significant.[13] A fourth study including 119 patients from Hong Kong, all but 2 with genotype B or C infection, reported that 3-year graft survival was similar for patients with genotype B or C. However, patients with genotype B had more frequent hepatitis flares and worse Model for End-Stage Liver Disease (MELD) scores pre-transplant, while those with genotype C had a higher rate of virologic breakthrough due to lamivudine-resistant mutants.[14]

Precore and core promoter HBV variants have also been reported to be associated with a higher incidence of fulminant hepatitis, and core promoter variants have been shown in many Asian studies to be associated with HCC.[15-20] Precore variants have also been reported to be associated with severe recurrent hepatitis post-transplant in a study of 15 patients from Australia, but this finding was not confirmed in another study of 69 patients from Germany.[11,21]

These data suggest that HBV genotypes, as well as precore and core promoter variants, may influence the indications for liver transplant among HBV-infected patients and their outcomes pre- and post-transplant. However, most studies to date have included small numbers of patients, the majority infected with either genotype A or D or genotype B or C. The presence of all 4 major HBV genotypes (A-D) and precore and core promoter variants in the United States provided an opportunity to reassess the impact of HBV genotype and precore and core promoter variants in liver transplant patients with data from the National Institutes of Health (NIH) "Prevention of Recurrent Hepatitis B After Liver Transplantation" study (the NIH HBV-OLT study).[22,23] The aims of the present analysis were to determine if there is a correlation between HBV genotype, precore and core promoter variants, and indications for liver transplantation and if these factors impact pretransplant and posttransplant outcomes.

PATIENTS AND METHODS

Patient Population

The NIH HBV-OLT study is a retrospective-prospective observational study that enrolled hepatitis B surface antigen–positive patients who were 13 years or older from 15 centers in the United States.[24] All patients either were on the liver transplant waiting list or were within 12 months post-transplant. The study was approved by the institutional review board representing each of the participating centers, and written informed consent was obtained from all patients prior to study entry. For patients enrolled at the time of listing, data were collected prospectively. For patients enrolled after placement on the liver transplant list, data after enrollment were collected prospectively, while data prior to enrollment were collected retrospectively up to the time of listing.

All laboratory tests except for HBV DNA and antiviral-resistant mutations were performed with commercially available assays at the participating centers. An additional 10 mL of blood was collected at each visit, centrifuged, divided into aliquots, and stored at −70°C in the participating centers and was batch-shipped to the central laboratory at the University of Michigan, where serum samples were stored at −80°C until testing.

Patients with available serum samples in the central laboratory and detectable HBV DNA levels by polymerase chain reaction assay prior to transplant were included in this analysis. Patients listed for retransplantation and those coinfected with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus were excluded. Of the 196 patients enrolled prior to transplant, HBV genotype, precore, and core promoter data were available in 123 (63%). The remaining patients had undetectable HBV DNA (n = 50) or an unavailable sample (n = 23). Demographic, clinical, and laboratory data were extracted from an electronic database.

HBV Assays

Serum HBV DNA levels were quantified by the Cobas Amplicor HBV Monitor assay (Roche Molecular Systems, Inc., Branchburg, NJ) at the central laboratory. The lower limit of detection of this assay is 200 copies/mL. Samples with values > 100,000 copies/mL were diluted 1:1000-fold to 1:100,000-fold and retested. The HBV genotype and presence of precore (G1896A) and core promoter variants (A1762T/G1764A) were determined with line probe assays (InnoLipa, Innogenetics NV, Ghent, Belgium). The line probe assay can differentiate HBV genotypes A-H and G versus A at nucleotide position 1896 and A versus T and G versus A at nucleotide positions 1762 and 1764 in the precore and basal core promoter regions of the HBV genome.[25]

Statistical Analysis

Categorical data were presented as number and percentage and compared with the chi-square test or Fisher's exact test as appropriate. Continuous variables were expressed as mean and standard deviation unless specified otherwise and compared with the *t* test or Mann-Whitney U test. The serum HBV DNA level was expressed as copies per milliliter and logarithmically transformed. For the analysis of factors associated with OLT indications and pretransplant and posttransplant outcomes, core promoter and precore variants were considered to be present if the bands that detected the

TABLE 1. Characteristics of All 123 Patients and 63 Transplanted Patients at the Time of Listing

	All Patients	Transplanted Patients
Number of patients	123	63
Age, years	51.2 ± 10.8	49.0 ± 11.5
Gender, male	100 (81.3)	47 (74.6)
Ethnicity		
Caucasian	56 (45.5)	29 (46.2)
Asian	53 (43.1)	22 (34.9)
African American	11.0 (8.0)	9 (14.2)
Other	3 (2.4)	3 (4.7)
OLT indication		
Cirrhosis	77 (62.6)	34* (53.9)
HCC	38 (30.8)	23 (36.6)
Acute liver failure	8 (6.6)	6 (9.5)
HBV DNA, Log_{10} copies/mL	5.33 ± 2.09	5.33 ± 1.83
HBV genotype		
A	43 (35.0)	28 (44.4)
B	15 (12.2)	8 (12.7)
C	43 (35.0)	18 (28.6)
D	16 (13.0)	6 (9.5)
Other	6 (4.9)	3 (4.8)
HBeAg-positive	47/97 (48.4)	24/48 (50.0)
Precore, nt 1896		
A ≥ G	44 (35.8)	20 (31.7)
A detectable	54 (43.9)	25 (39.6)
Core promoter, nt 1762/1764		
TA ≥ AG	107 (87.0)	55 (87.3)
TA detectable	111 (90.2)	57 (90.5)

NOTE: Data are expressed as mean ± standard deviation or number (%).
Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; OLT, orthotopic liver transplantation.
*Five had a new diagnosis of HCC while on the waiting list (n = 2) or on explant (n = 3).

mutations had an intensity equal to or stronger than the intensity of the bands that detected the wild-type sequence, that is, if the mutations constituted ≥50% of the virus population.

Outcomes on the transplant waiting list, including deaths, dropouts (deaths or delisting due to disease progression), and transplant-free survival as well as post-OLT survival, were estimated with Kaplan-Meier analysis. Univariate analyses of factors associated with outcomes on the waiting list and post-OLT were performed with Kaplan-Meier analysis with the log rank test. The following variables were included in the analysis: demographics (gender, age, and race/ethnicity), OLT indication, HBeAg status, HBV DNA, HBV genotype, presence of precore and core promoter variants, use of antiviral therapy and virologic breakthrough during antiviral therapy, MELD score at listing (for patients with cirrhosis) and tumor staging within or outside Milan criteria (for patients with HCC), HBV prophylaxis (antiviral therapy only versus hepatitis B immune globulin only versus a combination of antiviral therapy and hepatitis B immune globulin) post-OLT, and HBV and HCC recurrence post-OLT. Continuous variables were dichotomized with the median taken as the cutoff value, except for serum HBV DNA, for which the cutoff used was 5 log_{10} copies/mL. Variables that had a *P* value of <0.1 on univariate analysis were entered into a Cox regression hazards model by forward logistic regression to determine the independent predictors of pre- and post-OLT outcomes. All statistical analyses were performed with SPSS version 14.0.8 statistical software (SPSS, Inc., Chicago, IL).

RESULTS

Characteristics of Patients at Listing

A total of 123 patients were included in this study. Table 1 summarizes the characteristics of these patients at the time of listing. The vast majority of the patients were men (81%), and their mean age was 51.2 years. Asians comprised 43% of the patient population, most (46%) of the remaining patients were Caucasians, and only 8% were African Americans. Cirrhosis with liver failure was the most common indication for transplant listing (62.6%) and was followed by HCC (30.8%) and acute liver failure (6.6%). Approximately half of the patients were HBeAg-positive, and mean serum HBV DNA was 5.3 log_{10} copies/mL. HBV genotypes A (35%) and C (35%) were the most prevalent, followed by genotypes D (13%) and B (12%). Six patients had other genotypes: 2 had genotype E, 2 had genotype F, and 2

TABLE 2. Characteristics of Patients at Listing According to HBV Genotypes

	HBV Genotypes					
	A	B	C	D	Other	*P* Value
Number of patients	43 (35.0)	15 (12.1)	43 (35.0)	16 (13.0)	6 (4.9)	
Age, years	50.9 ± 12.1	49.8 ± 9.1	50.5 ± 10.1	55.9 ± 9.0	49.6 ± 14.6	NS
Gender, male	35 (81.4)	14 (93.3)	36 (83.7)	10 (62.5)	5 (83.3)	NS
Ethnicity						<0.001
Caucasian	30 (69.8)	6 (40)	4 (9.3)	12 (75.0)	4 (66.7)	
Asian	2 (4.7)	9 (60)	38 (88.4)	4 (25.0)	0	
African American	9 (20.9)	0	1 (2.3)	0	1 (16.7)	
Other	2 (4.7)	0	0	0	1 (16.7)	
OLT indication						0.042
Cirrhosis	28 (65.1)	13 (86.6)	21 (48.8)	10 (62.5)	5 (83.3)	
HCC	10 (23.2)	2 (13.3)	21 (48.8)	4 (25.0)	1 (6.7)	
Acute liver failure	5 (11.6)	0	1 (2.3)	2 (12.5)	0	
Labs at listing						
ALT (IU/L)	68 ± 479	64.5 ± 272	48 ± 79.1	47 ± 384	61 ± 25	NS
Albumin (g/dL)	3.1 ± 1.0	3.1 ± 0.5	3.2 ± 0.7	3.1 ± 0.8	3.2 ± 0.4	NS
MELD score (for patients with cirrhosis)	14 ± 8.5	13 ± 8.8	13 ± 5.7	12 ± 8.6	13 ± 4.8	NS
HBV DNA, Log_{10}copies/mL	5.6 ± 2.0	5.4 ± 2.1	5.5 ± 2.2	4.7 ± 2.2	3.6 ± 0.9	NS
HBeAg-positive	25/36 (69.4)	4/15 (26.7)	14/30 (46.7)	2/14 (14.2)	2/2 (100)	0.001
Precore, nt 1896						0.006
A ≥ G	7 (16.3)	8 (53.3)	17 (39.5)	10 (62.5)	2 (33.3)	
A detectable	9 (21.0)	8 (53.3)	22 (51.1)	13 (81.2)	2 (33.3)	
Core promoter, nt 1762/1764						0.01
TA ≥ AG	35 (81.4)	10 (66.7)	43 (100)	14 (87.5)	5 (83.3)	
TA detectable	36 (83.7)	11 (73.4)	43 (100)	15 (93.7)	6 (100)	
Tumor within Milan (for patients with HCC)	6/8 (75.0)	1/2 (50.0)	12/19 (63.2)	2/4 (50.0)	0/1 (0)	NS
On antiviral therapy	28 (65.1)	12 (80)	37 (86)	11 (68.8)	3 (50)	NS
Breakthrough prior to OLT	8/28 (28.5)	4/12 (33.0)	8/37(21.0)	2/11(18.1)	1/3 (33.3)	NS

NOTE: Data are expressed as mean ± standard deviation or number (%).
Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease; OLT, orthotopic liver transplantation.

had mixed genotypes (A+E and A+D). Precore and core promoter variants were detectable in 44% and 90% of patients and constituted at least 50% of the virus population in 36% and 87% of patients, respectively.

Characteristics at Listing According to HBV Genotype

Characteristics of the patients at the time of listing for the 4 major genotypes A to D are listed in Table 2. HBV genotype was significantly correlated with ethnicity ($P < 0.001$); genotype C and to a lesser extent genotype B were predominantly found among Asians, while genotypes A and D were mainly found among Caucasians; and all but 1 African American had genotype A. The mean age of the patients and gender distribution were comparable across genotypes A to D.

HBV genotype was significantly associated with OLT indication ($P = 0.042$). HCC was the indication for listing among 49% of the patients with genotype C, but only 25% of the patients with genotype D, 23% of those with genotype A, and 13% of the patients with genotype B had HCC at listing. By contrast, acute liver failure was the indication for transplant in 13% of patients with genotype D and in 12% of those with genotype A but in only 2% of patients with genotype C and in none of those with genotype B.

A higher proportion of patients with genotypes A (69%) and C (47%) than those with genotypes B (27%) and D (14%) were HBeAg-positive ($P = 0.001$). However, serum HBV DNA levels were similar across these 4 genotypes.

A precore variant was more frequently present in patients with genotypes B and D followed by genotype C and was rarely found among those with genotype A ($P = 0.006$). A core promoter variant was prevalent, regardless of the HBV genotype, being detected in all patients with genotype C and less commonly in patients with genotype B ($P = 0.01$).

MELD scores among patients listed for cirrhosis and tumor staging among patients listed for HCC were similar among the patients with genotypes A to D. The proportion of patients receiving antiviral therapy at listing and the proportion of patients with virologic break-

TABLE 3. Outcomes of Patients on the Transplant Waiting List by Genotype

	Genotype A	Genotype B	Genotype C	Genotype D	Other Genotypes	All Patients
Number of patients at listing	43	15	43	16	6	123
Number of patients who were transplanted (%)	28 (65.1)	8 (53.3)	18 (41.9)	6 (37.5)	3 (50)	63 (51.2)
Cirrhosis	14/28 (50)	6/13 (46.1)	8/21 (38)	4/10 (40)	2/5 (40)	34/77 (44.1)
HCC	9/10 (90)	2/2 (100)	10/21 (47.6)	1/4 (25)	1/1 (100)	23/38 (60.5)
Acute liver failure	5/5 (100)	0	0/1 (0)	1/2 (50)	0	6/8 (75.0)
Number of patients who died (%)	6/43 (13.9)	0/15	2/43 (4.7)	5/16 (31.3)	0/6	13/123 (10.5)
HCC	1/10 (10.0)	0/2	2/21 (9.5)	1/4 (25)	0/1	4/38 (10.5)
Non-HCC	5/33 (15.1)	0/13	0/22	4/12 (33.3)	0/5	9/85 (10.5)
Number of patients who dropped out (%)	6 (13.9)	0	4 (9.33)	6 (37.5)	0	16/123 (13.0)
HCC	1/10 (10.0)	0/2	4/21 (19.0)	1/4 (25)	0/1	6/38 (15.7)
Non-HCC	5/33 (15.1)	0/13	0/22	5/12 (41.6)	0/5	10/85 (11.7)

Abbreviation: HCC, hepatocellular carcinoma.

through prior to transplant were comparable across genotypes A to D.

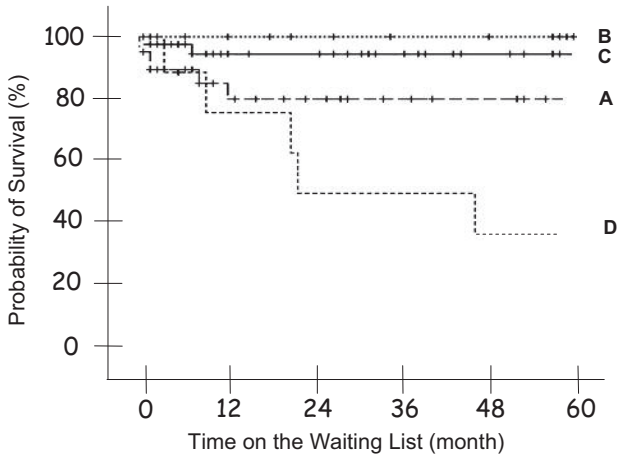
Factors Associated with OLT Indications

Univariate analysis showed that male gender, Asian race, HBV genotype C, and presence of core promoter mutations were associated with HCC. Multivariate analysis identified HBV genotype C as the only independent factor associated with HCC, with an odds ratio of 4.54 (95% confidence interval, 1.99, 10.30; $P < 0.001$). These results were the same whether patients with acute liver failure were included or excluded.

Outcomes While on the Transplant Waiting List

Thirteen (10%) patients died while waiting for OLT; 10 of the patients died because of worsening liver failure (n = 8) or HCC progression (n = 2), while 3 patients died from non–liver-related causes. Patients with genotype D had the highest rate (5 of 16) of waitlist mortality, while none of 15 patients with genotype B died while on the waiting list (Table 3). The 1-, 3-, and 5-year probability of waitlist mortality was 17%, 44%, and 58% for patients with genotype D, 8%, 8%, and 8% for patients with genotype A, 4%, 4%, and 4% for patients with genotype C, and 0%, 0%, and 0% for patients with genotype B ($P = 0.005$; Fig. 1). Cox regression failed to identify any predictor of waitlist mortality.

Three patients were removed from the transplant waiting list because of worsening liver failure (n = 1) or HCC progression (n = 2). The probability of dropout (death or delisting due to disease progression) while on the waiting list was highest among patients with genotype D ($P = 0.005$) compared to patients with genotype non-D, despite similar MELD scores among patients listed for cirrhosis and similar tumor staging among patients listed for HCC.



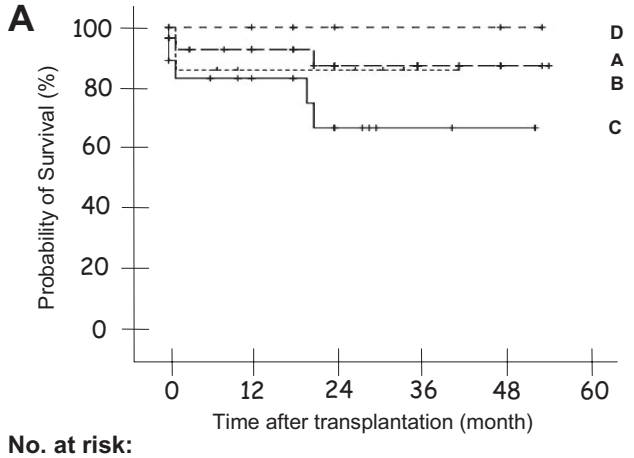
No. at risk:

Genotype A	43	17	12	7	5	2
Genotype B	15	11	9	7	7	4
Genotype C	43	26	24	17	12	8
Genotype D	16	6	4	4	3	3

Figure 1. Probability of survival on the transplant waiting list for patients with hepatitis B virus genotypes A to D ($P = 0.005$).

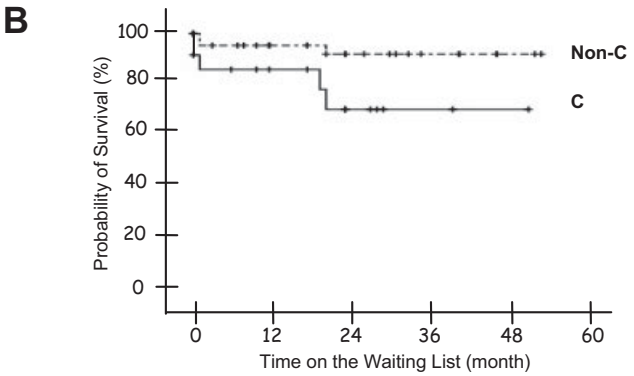
Post-OLT Survival

Sixty-three patients underwent OLT, and 9 died during a median follow-up period of 24 months (range, 0-60). The probability of post-OLT survival at 1, 2, 3, and 4 years was 89%, 83%, 83%, and 83%, respectively. The probability of post-OLT survival was highest among patients with genotype D and lowest among those with genotype C (Fig. 2A), but the difference was not significant in pairwise comparisons and when patients with all 4 major genotypes were compared. Patients with genotype C had a trend toward lower post-OLT survival compared to those with genotype non-C, the probability of post-OLT survival at 1, 2, 3, and 4 years being 83% versus 93%, 67% versus 89%, 67% versus 89%, and



No. at risk:

Genotype A	28	22	15	12	5
Genotype B	8	4	4	1	0
Genotype C	18	12	8	2	1
Genotype D	6	5	3	2	2



No. at risk:

Genotype C	18	12	8	2	1
Genotype Non C	45	33	24	16	7

Figure 2. (A) Probability of survival post-transplant for patients with hepatitis B virus genotypes A to D ($P = 0.867$). (B) Probability of survival post-transplant for patients with genotype C versus patients with genotype non-C ($P = 0.055$).

67% versus 89%, respectively, for patients with genotype C versus patients with genotype non-C ($P = 0.055$; Fig. 2B).

Although genotype C was predominantly found in Asians, post-OLT survival of Asians was not worse than that of non-Asians. Similarly, despite a strong association between genotype C and HCC, post-OLT survival of patients with HCC was not different compared to that of those with cirrhosis.

Univariate analysis showed that HBV genotype (C versus non-C) was the only variable associated with post-OLT survival. None of the variables at listing (including the presence of a precore or core promoter variant) or HBV or HCC recurrence post-OLT correlated with post-OLT survival. Cox regression showed a trend toward an association between HBV genotype C and post-OLT mortality, with a hazard ratio of 3.31 (95% confidence interval, 0.89, 12.34; $P = 0.058$). Five of 18 (27.7%) patients with genotype C died after OLT versus 4 of 45 (8.9%) patients with non-C genotypes (Table 4). The causes of death were perioperative complications (n = 2), primary graft nonfunction (n = 1), sepsis at month 20 (n = 1), and recurrent HCC at month 21 (n = 1) among patients with genotype C and perioperative complications (n = 1), graft versus host disease (n = 1), metastatic HCC at month 1 (n = 1), and other malignancy at month 21 (n = 1) among patients with genotype non-C.

HCC Recurrence

Among the 63 patients who underwent OLT, 23 had HCC at listing, and 5 had HCC diagnosed while on the waiting list (n = 2) or on explant (n = 3). Two of these 28 patients developed HCC recurrence, and both were Asians; 1 had genotype C infection, and 1 had genotype B infection (Table 4). The cumulative probability of HCC recurrence at 1, 2, and 3 years post-OLT was 0%, 13%, and 13%, respectively.

HBV Recurrence

Five (8%) patients had HBV recurrence. The cumulative probability of HBV recurrence at 1, 2, and 3 years post-OLT was 6%, 11%, and 11%, respectively. Of the 5 patients with HBV recurrence, 3 were Caucasians, 1 was Asian, and 1 was African American; 3 had genotype A infection, 1 had genotype B infection, and 1 had genotype C infection (Table 4).

DISCUSSION

In this study, which included 123 hepatitis B patients representing the 4 major HBV genotypes from 15 liver transplant centers in the United States, we observed multiple differences in pre- and post-OLT outcomes and indications for OLT in patients infected with different HBV genotypes. However, the presence or absence of precore or core promoter mutations had no impact on OLT indications or pre- and post-OLT outcomes. The lack of impact of these variants may be related to their presence in a high percentage of patients (precore and core promoter variants were present in 40% and 90% of patients, respectively) and their strong correlation with HBV genotype. Alternatively, these variants may have no influence on outcomes of liver transplantation.

Similarly to studies in Asian countries, we found a strong correlation between genotype C and HCC, as almost half of the genotype C–infected patients had HCC at listing. This study provided an opportunity to compare not only the relationship between HCC and genotype C versus genotype B but also other genotypes not commonly encountered in Asia (A and D). We found that genotype B was less frequently associated with HCC at listing compared to genotypes A and D, the predominant HBV genotypes in Europe. This remained true even when new cases of HCC diagnosed after listing and on explant were included. We acknowledge that the population studied represents a highly selected

TABLE 4. Outcomes of Patients Post-OLT by Genotype

	Genotype A	Genotype B	Genotype C	Genotype D	Other Genotypes	All Patients
Number of patients who were transplanted	28	8	18	6	3	63
Number of patients who died (%)	3/28 (10.7)	1/8 (12.5)	5/18 (27.7)	0/6 (0)	0/3 (0)	9/63 (14.2)
Diagnosis at OLT (%)	0/5	0	0	0/1	0	0/6 (0)
Cirrhosis	2/11 (18.1)	1/5 (20)	3/7 (42.8)	0/4	0/2	6/29 (20.6)
HCC	1/12 (8.3)	0/3	2/11 (18.1)	0/1	0/1	3/28 (10.7)
Acute liver failure	0/5	0	0	0/1	0	0/6 (0)
HBV prophylaxis post-OLT, n (%)						
Antiviral + HBIG	24 (85.7)	6 (75%)	16 (88.9%)	6 (100%)	3 (100%)	55 (87.3%)
Antiviral only	4 (14.3%)	2 (25%)	2 (11.1%)	0	0	8 (12.7%)
Number of patients with HCC recurrence (%)	0/12	1/3 (33.3)	1/11 (9.0)	0/1	0/1	2/28 (7.1)
Number of patients with HBV recurrence (%)	3/28 (10.7)	1/8 (12.5)	1/18 (5.5)	0/6	0/3	5/63 (8.0)

Abbreviations: HBIG, hepatitis B immune globulin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OLT, orthotopic liver transplantation.

group of patients who presented to transplant centers and who qualified for listing for liver transplantation and may not be generalized to other hepatitis B patients. Nevertheless, it is intriguing that genotype B, which is considered a more benign HBV genotype in Asia, may also be associated with a lower risk of HCC than genotypes that are common among Caucasians.

In accordance with previously reported data from the US Acute Liver Failure Study Group, patients listed for acute liver failure were infected predominantly with genotypes A and D.[26] This is likely related to the preponderance of these genotypes among Caucasians and African Americans, in whom HBV infection usually occurs in adulthood. By contrast, genotypes B and C are mainly found among Asians, many of whom were exposed to HBV during infancy or childhood.

Contrary to an earlier study from the United States and another study from Australia, we found that patients with genotype D had the best post-OLT survival.[11,12] None of the 6 genotype D patients died after a median post-OLT survival of 33 months (range, 12-54). We acknowledge that this favorable observation is limited by the small number of genotype D patients transplanted. In this study, patients with genotype C had the highest mortality post-OLT. However, 2 of 5 deaths were related to perioperative complications. Thus, the increased post-OLT mortality observed among patients with genotype C may be a random effect and may not be related to more severe recurrent disease. Whether the higher post-OLT mortality is related to donor factors such as cold ischemic time cannot be determined as donor data were not collected in this study.

Paradoxically, patients with genotype D had the highest waitlist mortality. Patients with genotype C had lower waitlist mortality compared to those with genotype D or A, but this difference was not significant because of the small numbers of patients.

The use of antiviral therapy pre- and post-OLT and the addition of hepatitis B immune globulin post-OLT resulted in a low rate of HBV recurrence of 7.9% 3 years post-OLT. The low rate of HBV and HCC recurrence precluded an analysis of the relationship between HBV genotypes and precore and core promoter variants and recurrent disease post-OLT.

In summary, in this United States–based patient population, which included liver transplant patients with all 4 major HBV genotypes, we observed that patients with genotype C were more likely to have HCC at the time of listing and to die after transplant compared to patients with non-C genotypes. Patients with genotype D had the best post-OLT survival, but this was offset by higher waitlist mortality. Our study suggests that HBV genotypes but not precore or core promoter variants may have an impact on pre- and post-OLT outcomes of hepatitis B patients. These data need to be confirmed in larger studies. However, the ability to enroll patients with diverse HBV genotypes may not be possible as in most countries only 1 or 2 genotypes predominate. Thus, despite the limitations of differences in organ availability and transplant policies, multinational studies will be required to verify our observations.

ACKNOWLEDGMENT

We thank all the investigators and study staff at the participating sites:

California Pacific Medical Center, San Francisco, CA: Natalie Bzowej, M.D., Robert Gish, M.D., and Jamie Zagorski, R.N.

Cedars Sinai Medical Center, Los Angeles, CA: Tram Tran, M.D., and Amy Crumley, R.N.

Columbia University, New York, NY: Paul Gaglio, M.D., and Maria Martin.

Massachusetts General Hospital, Boston, MA: Raymond T. Chung, M.D., Diana Tsui, and Marian Bihrle.

Mayo Clinic, Rochester, MN: Michael Ishitani, M.D., and Heidi Togerson, R.N.

Mount Sinai University Medical Center, New York, NY: Sukru Emre, M.D., Mark Sturdevant, M.D., and Aniceto Javaluyas, M.D.

Ochsner Clinic, New Orleans, LA: Robert Perrillo, M.D., and Cheryl Denham, L.P.N.

Stanford University, Palo Alto, CA: Emmet Keeffe, M.D., and Lucinder Porter, R.N.

University of California, Los Angeles, CA: Steve Han, M.D., Pearl Kim-Hong, and Val Peacock, R.N.

University of Florida, Gainesville, FL: Consuelo Soldevila-Pico, M.D., and Joy Peter, R.N., B.S.N.

University of Miami, Miami, FL: Eugene Schiff, M.D., and Maria Torres.

University of Pennsylvania, Philadelphia, PA: Rajender Reddy, M.D., and Timothy Siropaides.

University of Virginia, Charlottesville, VA: Timothy Pruett, M.D.

Virginia Commonwealth University, Richmond, VA: Velimir A. C. Luketic, M.D., and Stacy McLeod.

University of Michigan, Ann Arbor, MI: Anna Lok, M.D., Bulent Degertekin, M.D., Terese Howell, Donna Harsh, Munira Hussain, Jim Imus, and Morton Brown, Ph.D.

REFERENCES

1. Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology 2004; 40:790-792.
2. Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology 2004;47:289-309.
3. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118:554-559.
4. Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology 2003;37:19-26.
5. Orito E, Ichida T, Sakugawa H, Sata M, Horiike N, Hino K, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology 2001;34:590-594.
6. Yuen MF, Sablon E, Yuan HJ, Wong DK, Hui CK, Wong BC, et al. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. Hepatology 2003;37:562-567.
7. Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, Sung JJ. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut 2004;53:1494-1498.
8. Yu MW, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst 2005;97:265-272.
9. Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. Gastroenterology 2002;123:1848-1856.
10. Livingston SE, Simonetti JP, McMahon BJ, Bulkow LR, Hurlburt KJ, Homan CE, et al. Hepatitis B virus genotypes in Alaska Native people with hepatocellular carcinoma: preponderance of genotype F. J Infect Dis 2007;195:5-11.
11. McMillan JS, Bowden DS, Angus PW, McCaughan GW, Locarnini SA. Mutations in the hepatitis B virus precore/core gene and core promoter in patients with severe recurrent disease following liver transplantation. Hepatology 1996;24:1371-1378.
12. Devarbhavi HC, Cohen AJ, Patel R, Wiesner RH, Dickson RC, Ishitani MB. Preliminary results: outcome of liver transplantation for hepatitis B virus varies by hepatitis B virus genotype. Liver Transpl 2002;8:550-555.
13. Girlanda R, Mohsen AH, Smith H, Sablon E, Yuen MF, O'Grady J, et al. Hepatitis B virus genotype A and D and clinical outcomes of liver transplantation for HBV-related disease. Liver Transpl 2004;10:58-64.
14. Lo CM, Cheung CK, Lau GK, Yuen MF, Liu CL, Chan SC, et al. Significance of hepatitis B virus genotype in liver transplantation for chronic hepatitis B. Am J Transplant 2005;5:1893-1900.
15. Baptista M, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. Hepatology 1999;29:946-953.
16. Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 2003;124:327-334.
17. 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;324:1705-1709.
18. Lindh M, Hannoun C, Dhillon AP, Norkrans G, Horal P. Core promoter mutations and genotypes in relation to viral replication and liver damage in East Asian hepatitis B virus carriers. J Infect Dis 1999;179:775-782.
19. Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med 1991;324:1699-1704.
20. Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, et al. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann Intern Med 1995;122:241-248.
21. Naumann U, Protzer-Knolle U, Berg T, Leder K, Lobeck H, Bechstein WO, et al. A pretransplant infection with precore mutants of hepatitis B virus does not influence the outcome of orthotopic liver transplantation in patients on high dose anti-hepatitis B virus surface antigen immunoprophylaxis. Hepatology 1997;26:478-484.
22. Chu CJ, Keeffe EB, Han SH, Perrillo RP, Min AD, Soldevila-Pico C, et al. Hepatitis B virus genotypes in the United States: results of a nationwide study. Gastroenterology 2003;125:444-451.
23. Chu CJ, Keeffe EB, Han SH, Perrillo RP, Min AD, Soldevila-Pico C, et al. Prevalence of HBV precore/core promoter variants in the United States. Hepatology 2003;38:619-628.
24. Wong SN, Reddy KR, Keeffe EB, Han SH, Gaglio PJ, Perrillo RP, et al. Comparison of clinical outcomes in chronic hepatitis B liver transplant candidates with and without hepatocellular carcinoma. Liver Transpl 2007;13:334-342.
25. Hussain M, Chu CJ, Sablon E, Lok AS. Rapid and sensitive assays for determination of hepatitis B virus (HBV) genotypes and detection of HBV precore and core promoter variants. J Clin Microbiol 2003;41:3699-3705.
26. Wai CT, Fontana RJ, Polson J, Hussain M, Shakil AO, Han SH, et al. Clinical outcome and virological characteristics of hepatitis B-related acute liver failure in the United States. J Viral Hepat 2005;12:192-198.