Changes in Serum Levels of HBV DNA and Alanine Aminotransferase Determine Risk for Hepatocellular Carcinoma

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CLINICAL LIVER

BACKGROUND & AIMS: It is not clear whether risk for hepatocellular carcinoma can be accurately determined from long-term changes in serum levels of hepatitis B virus (HBV) DNA or alanine aminotransferase (ALT). **METHODS:** We measured serum levels of HBV DNA and ALT at enrollment and during follow-up analysis of 3160 participants in the REVEAL-HBV study. Development of hepatocellular carcinoma was determined from follow-up examinations and computerized linkage with National Cancer Registry and National Death Certification profiles. Multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox regression models. **RESULTS:** During 38,330 person-years of follow-up, 81 participants developed hepatocellular carcinoma (incidence rate, 211.3/100,000 person-years). The risk for hepatocellular carcinoma was only slightly higher for participants whose follow-up levels of HBV DNA spontaneously decreased to <10,000 copies/mL compared with those with baseline levels of HBV DNA <10,000 copies/mL (control group; HR, 2.25; 95% CI, 0.68–7.37). Compared with the control group, the HRs (95% CI) for long-term levels of HBV DNA that persisted at 10,000 to 100,000 copies/mL, decreased to/persisted at 100,000 to 1,000,000 copies/mL, or decreased to/persisted at 1,000,000 to 10,000,000 copies/mL were 3.12 (1.09–8.89), 8.85 (3.85–20.35), and 16.78 (7.33–38.39), respectively. A gradient in ALT level was significantly associated with hepatocellular carcinoma risk: from all low-normal, to ever high-normal, to transient abnormal, to persistent abnormal ($P_{trend} < .001$). **CONCLUSIONS: Long-term changes in serum levels of HBV DNA and ALT are independent predictors of risk for hepatocellular carcinoma. Regular monitoring of levels of HBV DNA and ALT is important in clinical management of chronic carriers of HBV.**

Keywords: Liver Disease; Long-term Follow-up Study; Chronic Hepatitis B.

Chronic hepatitis B virus (HBV) infection poses a global health challenge, affecting more than 350 million people worldwide[1]; the vast majority of those affected are from the Asia-Pacific region[2] and are mostly infected perinatally or during early childhood.[3] Globally, more than 50 million new cases of HBV infection occur annually.[4] Patients with chronic hepatitis B are at increased risk for developing cirrhosis and hepatocellular carcinoma,[5] resulting in more than half a million deaths annually.

The progression of chronic hepatitis B is a multistage, multifactorial process involving interactions among host, environmental, and viral factors.[6,7] Important HBV biomarkers for prediction of hepatocellular carcinoma risk include hepatitis B e antigen (HBeAg) serostatus,[8] genotype C,[9–11] specific mutants,[10,12,13] and elevated HBV DNA level.[14–16] Cross-sectional, case-control, and cohort studies have shown a significant dose-response association between serum HBV DNA levels at initial evaluation and subsequent risk of hepatocellular carcinoma.[16] Serum HBV DNA levels may vary markedly in the natural progression of chronic hepatitis B; however, most studies were only based on a single HBV DNA measurement at enrollment rather than multiple measurements during long-term follow-up.

Chronic inflammation associated with human immune responses to HBV infection may increase damage and proliferation of hepatocytes. Consequently, persistent injury and regeneration may promote liver carcinogenesis.[17,18] Serum alanine aminotransferase (ALT) level is the most commonly used seromarker of HBV-related liver cell injury.[19] Higher ALT levels imply more active immune response against HBV and more extensive hepatocyte damage.[20] Prospective studies have shown that elevated serum ALT level at enrollment was a risk predictor for HBV-related hepatocellular carcinoma.[10,14] Because serum ALT levels may vary during the progression of chronic hepatitis B,[19] the risk for hepatocellular carcinoma predicted by long-term patterns of ALT levels remains to be elucidated.

Abbreviations used in this paper: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.06.036

This study aimed to identify distinctive groups of long-term changes in serum HBV DNA levels during follow-up and examine risk for hepatocellular carcinoma associated with the patterns of long-term changes in HBV DNA and ALT levels.

Subjects and Methods

Study Cohort Enrollment

The participants in this study included a subcohort of the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HBV (REVEAL-HBV) Study described previously.[14] Of the 3653 participants included in the study cohort, 3584 had no cirrhosis within 6 months of enrollment. Of these, 2020 had serum HBV DNA levels $<10^4$ copies/mL at enrollment (control group). The remaining 1564 participants had serum HBV DNA levels $\geq 10^4$ copies/mL at enrollment, and 187 (12%) of them did not participate in the follow-up examinations. Among 1377 participants with follow-up examinations, 237 (17%) did not have adequate follow-up serum samples for the analysis of long-term changes in HBV DNA levels. The remaining 1140 had at least 2 long-term repeated HBV DNA data 2 or

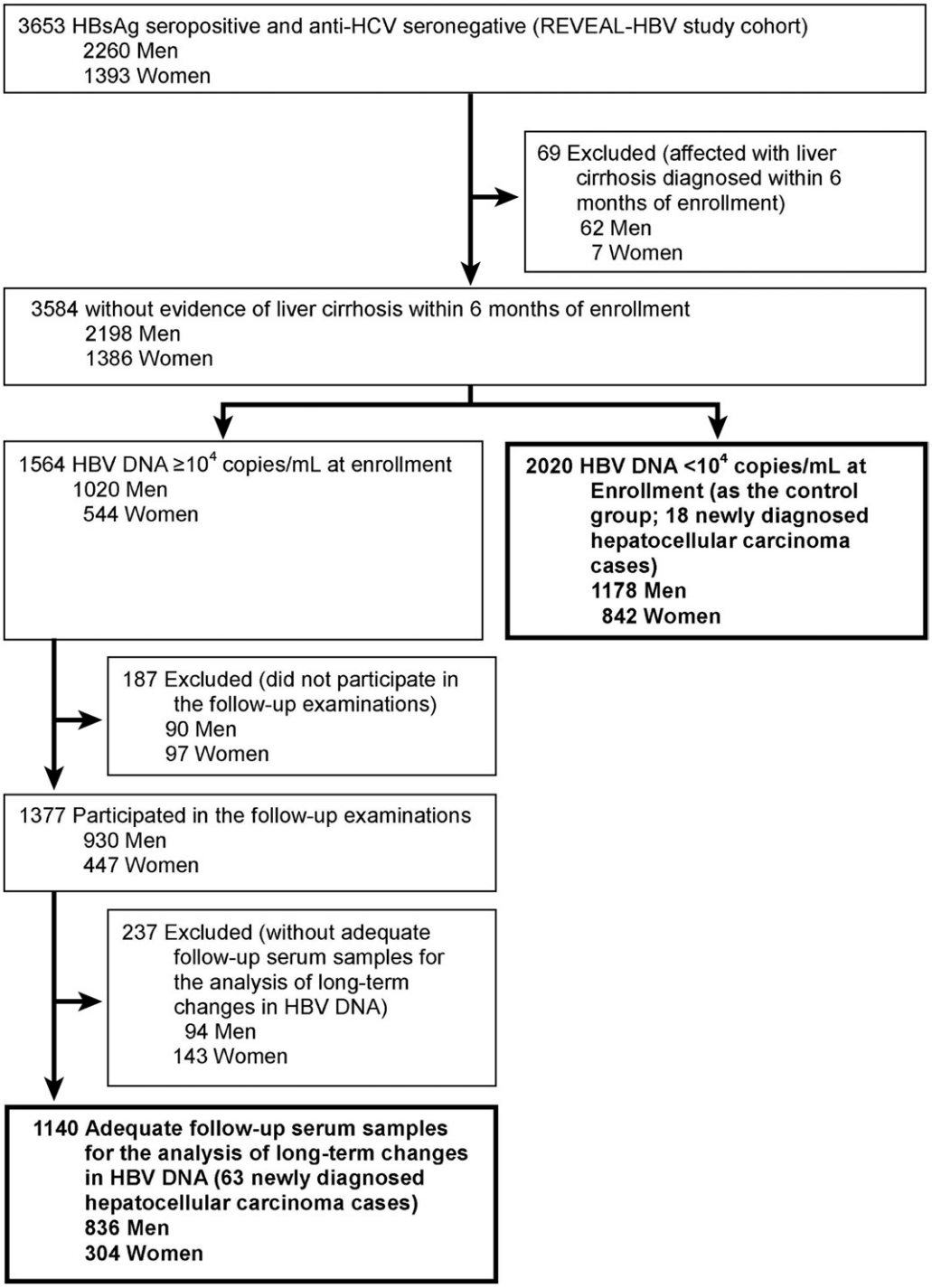


Figure 1. Flow of study participants.

more years apart. These 1140 participants and the 2020 participants in the control group (total, 3160; Figure 1) were included in this study. The average number of repeated HBV DNA measurements per participant available for the group-based trajectory analysis was 4.8 ± 2.1 (mean ± SD). This study was conducted between February 1991 and June 2004 and was approved by the institutional review board of the College of Public Health, National Taiwan University.

Interview and Blood Collection

All participants were personally interviewed by trained public health nurses using a structured questionnaire. Information on sociodemographic characteristics, dietary intake, habits of cigarette smoking and alcohol consumption, personal medical and surgical history, and family history of cancers and other major diseases was collected. A 10-mL blood sample was collected from each participant at enrollment and follow-up examinations (every 6–12 months). The fractionated serum samples were stored at −70°C until tested.

Laboratory Examinations

Serologic testing was performed using commercial kits: hepatitis B surface antigen, HBeAg, and α-fetoprotein by radioimmunoassay (Abbott Laboratories, North Chicago, IL), antibodies against hepatitis C virus by enzyme immunoassay using second-generation test kits (Abbott Laboratories), ALT by serum chemistry autoanalyzer (model 736; Hitachi Co, Tokyo, Japan) using commercial reagents (Biomérieux, Mercy-L'Etoile, France), and serum HBV DNA levels by polymerase chain reaction using the Cobas Amplicor HBV Monitor Test Kit[14,21] and Cobas TaqMan HBV Monitor Test Kit (Roche Diagnostics, Indianapolis, IN). We defined the undetectable lower limit as HBV DNA level <300 copies/mL. According to the manufacturer's instructions, there was an excellent correlation between HBV DNA levels detected by the TaqMan test and the Amplicor test ($R^2 = 0.977$). A real-time polymerase chain reaction–based single-tube assay with fluorescent hybridization probes and LightCycler technology (Roche Diagnostics Applied Science, Mannheim, Germany) was used to determine the genotype of HBV as described by Yeh et al.[22] Of 3160 hepatitis B surface antigen–positive participants, 2297 participants (72.7%) had a detectable viral load and an adequate serum sample for genotyping.

Ascertainment of Newly Developed Hepatocellular Carcinoma

No study participant had hepatocellular carcinoma at enrollment. Newly developed hepatocellular carcinoma was determined by follow-up health examinations or computerized linkage with National Cancer Registry and National Death Certification profiles in Taiwan from January 1, 1991, to June 30, 2004. Overall, 81 incident hepatocellular carcinoma cases were ascertained by the following criteria: histopathologic examination, positive lesion detected by at least 2 imaging techniques (abdominal ultrasonography, angiography, or computed tomography), or an imaging technique with a serum α-fetoprotein level of ≥400 ng/mL.[8,10,14]

Statistical Analysis

The group-based trajectory model[23] was used to identify distinctive groups of long-term changes in serum HBV DNA levels over 11 years of follow-up. Repeated measurements of HBV DNA were $\log_{10}$ transformed and modeled by a censored normal distribution. The group-based trajectory analysis (see Supplementary Materials and Methods) was performed using the PROC TRAJ macro (SAS software version 9.1; SAS Institute Inc, Cary, NC).[24] Model selection was based on the log Bayes factor[24] as a measure of the extent of evidence for improvement in model fit, thereby ensuring model parsimony. Participants were classified into different groups of long-term HBV DNA change based on the highest posterior probabilities of belonging to each group.[23]

The group-based trajectory model identified 9 groups of long-term changes in HBV DNA levels for participants with HBV DNA levels $\geq 10^4$ copies/mL at enrollment. As shown in Figure 2*A*, the predicted long-term changes in HBV DNA levels fit the observed viral loads of the 9 groups very well. There were 137 participants (12.0%) in group A whose viral loads spontaneously decreased from 10^5 copies/mL to undetectable levels from baseline to the fifth year of follow-up, thereafter fluctuating between undetectable levels and $<10^3$ copies/mL after the fifth year of follow-up; 60 (5.3%) in group B whose viral loads spontaneously decreased from 10^5 copies/mL to undetectable levels from baseline to the tenth year of follow-up and then rebounded to low detectable levels ($<10^3$ copies/mL); 54 (4.7%) in group C whose viral loads spontaneously decreased from $>10^7$ to $<10^4$ copies/mL from baseline to the seventh year of follow-up, thereafter fluctuating between 10^3 and 10^4 copies/mL; 309 (27.1%) in group D with persistent viral loads of 10^4 to 10^5 copies/mL; 64 (5.6%) in group E whose viral loads spontaneously decreased from $>10^7$ to $<10^5$ copies/mL; 272 (23.9%) in group F with persistent viral loads of 10^5 to 10^6 copies/mL; 50 (4.4%) in group G whose viral loads spontaneously decreased from $>10^7$ to $<10^7$ copies/mL; 87 (7.6%) in group H with persistent viral loads of 10^6 to 10^7 copies/mL; and 107 (9.4%) in group I with persistent viral loads $>10^7$ copies/mL.

Of these 9 groups, those with similar incidence rates of hepatocellular carcinoma (see Figure 2*B*) were combined into a new group for further analyses. Thus, 5 long-term HBV DNA change groups were obtained. The new group designated as "decrease to $<10^4$ copies/mL" was a combination of the A, B, and C groups; the group of "persistence at 10^4 to 10^5 copies/mL" was the D group; the group of "decrease to/persistence at 10^5 to 10^6 copies/mL" was a combination of the E and F groups; the group of "decrease to/persistence at 10^6 to 10^7 copies/mL" was a combination of the G and H groups; and the group of "persistence at $>10^7$ copies/mL" was the I group. The new group of long-term HBV DNA change was treated as a categorical variable for subsequent analyses.

The person-years of follow-up for each participant were calculated from the date of recruitment to the date of the diagnosis of hepatocellular carcinoma, date at death, or last date of available data from the National Cancer Registry (June 30, 2004), whichever came first. Participants free of hepatocellular carcinoma at their death or at the end of follow-up were censored. Incidence rates of hepatocellular carcinoma were calculated by dividing the number of incident cases of hepatocellular carcinoma by person-years of follow-up. Cumulative incidence of hepatocellular carcinoma by follow-up years was derived using the Nelson–Aalen method. A Cox regression model was used to calculate adjusted hazard ratios with 95% confidence intervals of developing hepatocellular carcinoma for groups of long-term HBV DNA change and long-term patterns of ALT and HBV genotype after adjustment for other risk predictors. Four categories of the long-term patterns of serum ALT levels were defined as follows: "persistent abnormal ALT" for ALT level ≥45 U/L in ≥50% of sequential ALT measurements, "transient ab-

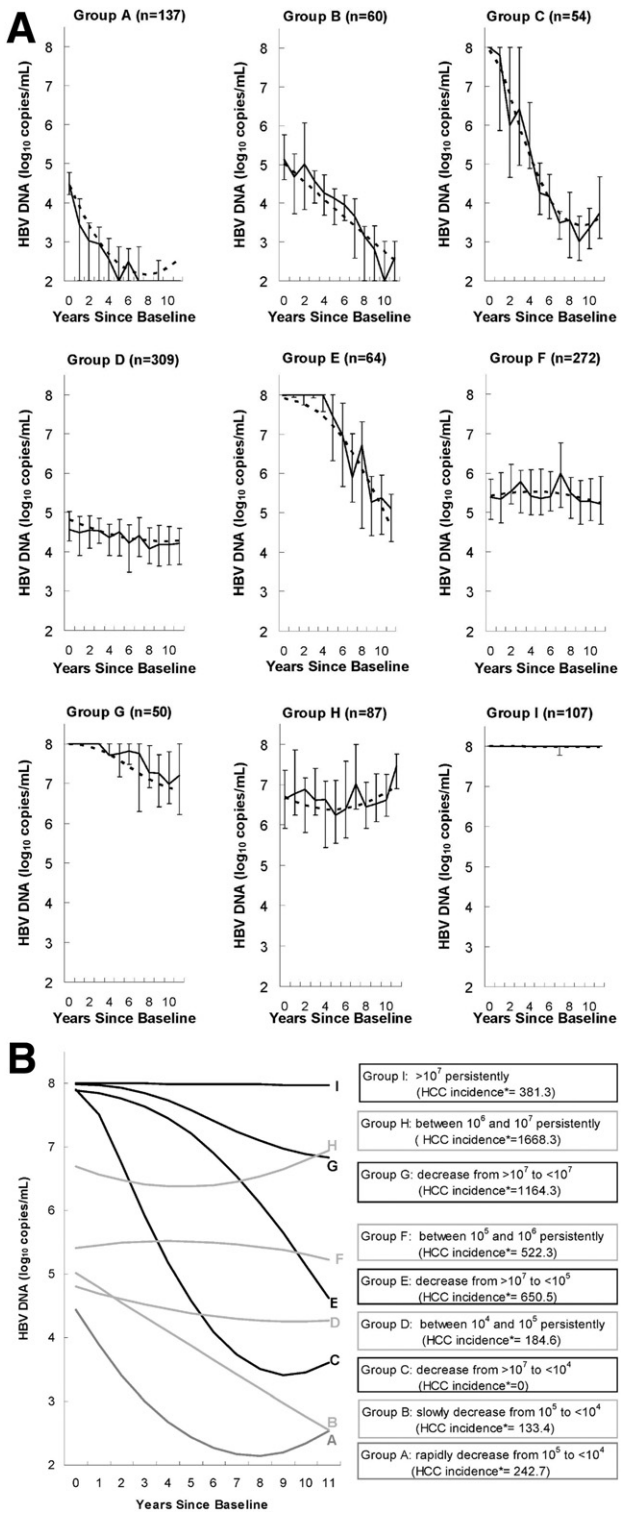


Figure 2. Nine groups of long-term changes in serum HBV DNA levels over the 11-year follow-up. (*A*) The *solid line* represents actual median levels of long-term HBV DNA change over the 11-year follow-up (the lower limit was the 25th percentile of HBV DNA levels and the upper limit was the 75th percentile of HBV DNA levels), and the *dashed line* represents predicted levels of long-term HBV DNA change over the 11-year follow-up. All predicted levels of long-term HBV DNA change were generated using the group-based trajectory model; n indicates total number of participants. (*B*) The plots were generated using the group-based trajectory model. HCC, hepatocellular carcinoma. *The HCC incidence rate was calculated by the following formula: [(Number of Incident Hepatocellular Carcinoma Cases)/(Person-Year of Follow-up)] × 100,000.

normal ALT" for at least 1 ALT level ≥45 U/L but <50% of sequential ALT measurements ≥45 U/L, "ever high-normal ALT" for all sequential ALT measurements <45 U/L and at least 1 ALT level >30 U/L, and "all low-normal ALT" for sequential ALT measurements ≤30 U/L. $P < .05$ was considered statistically significant. SAS software version 9.1 was used for all statistical analyses.

Results

Follow-up Years and Overall Incidence of Hepatocellular Carcinoma

In our study, 81 participants newly developed hepatocellular carcinoma during 38,330 person-years of follow-up (incidence rate, 211.3/100,000 person-years). Among 2020 participants with baseline HBV DNA levels $<10^4$ copies/mL, the mean age was 46 ± 10 (SD) years, the proportion of male subjects was 58%, and 18 new cases of hepatocellular carcinoma were diagnosed (incidence rate, 73.4/100,000 person-years). Among 1140 participants with baseline HBV DNA levels $\geq 10^4$ copies/mL, the mean age was 45 ± 9 (SD) years, the proportion of male subjects was 73%, and 63 new cases of hepatocellular carcinoma were diagnosed (incidence rate, 456.1/100,000 person-years).

Basic Characteristics of 6 Long-term HBV DNA Change Groups

The characteristics of different groups of long-term HBV DNA change are shown in Table 1. Age, sex, habits of cigarette smoking and alcohol consumption, HBeAg seropositivity at enrollment, HBV genotype, elevated serum ALT level at enrollment, and long-term pattern of ALT were significantly associated with groups of long-term HBV DNA change.

Participants in group I with persistent viral loads $>10^7$ copies/mL are unique for several reasons: they were more likely to be younger, had a much higher proportion of female subjects (47%), were almost exclusively HBeAg seropositive (99%), and were mostly genotype C infected (78%). Furthermore, 60% of the participants with persistent viral loads $>10^7$ copies/mL had sequential serum ALT levels <45 U/L.

Cumulative Incidence of Hepatocellular Carcinoma by Long-term HBV DNA and ALT Changes

Figure 3 shows the cumulative incidence of hepatocellular carcinoma by long-term patterns of HBV DNA and ALT. The cumulative incidence of hepatocellular carcinoma at the end of the 13th year of follow-up was 0.9% for participants with HBV DNA levels $<10^4$ copies/mL at enrollment (control group), 2.1% for participants in group A-B-C whose long-term HBV DNA levels decreased to $<10^4$ copies/mL, 2.3% for participants in whom these levels persisted at 10^4 to 10^5 copies/mL (group D), 5.1% for participants in whom these levels decreased to/persisted at 10^5 to 10^6 copies/mL (group E-F), 19.8% for participants in whom these levels decreased to/persisted

Table 1. Characteristics of Different Groups of Long-term HBV DNA Change

	Groups of Long-term HBV DNA change						
	Control group: HBV DNA level $<10^4$ at enrollment (n = 2020)	Group A-B-C: Decrease to $<10^4$ (n = 251)	Group D: Persistence at 10^4 to 10^5 (n = 309)	Group E-F: Decrease to/ persistence at 10^5 to 10^6 (n = 336)	Group G-H: Decrease to/ persistence at 10^6 to 10^7 (n = 137)	Group I: Persistence at $>10^7$ (n = 107)	*P* value
Age (*y*), mean (SD)	46 (10)	45 (10)	46 (9)	45 (9)	44 (9)	43 (10)	.001[a]
Female subjects	842 (42)	65 (26)	92 (30)	71 (21)	26 (19)	50 (47)	<.001[b]
Cigarette smokers[c]	656 (33)	101 (40)	104 (34)	133 (40)	53 (39)	28 (27)	.01[b]
Alcohol drinkers[d]	231 (12)	34 (14)	40 (13)	46 (14)	18 (13)	11 (11)	.75[b]
HBeAg positive at enrollment	22 (1)	65 (26)	18 (6)	88 (26)	101 (74)	106 (99)	<.001[b]
HBV genotype C[e]	412 (33)	76 (35)	68 (23)	81 (25)	80 (62)	79 (78)	<.001[b]
ALT level ≥45 U/L at enrollment	59 (3)	29 (12)	11 (4)	33 (10)	21 (15)	8 (8)	<.001[b]
Long-term pattern of ALT level[f]							
All low-normal[g]	1179 (68)	114 (46)	168 (56)	96 (30)	21 (15)	43 (41)	
Ever high-normal[h]	322 (19)	58 (23)	74 (25)	75 (23)	21 (15)	20 (19)	<.001[b]
Transient abnormal[i]	159 (9)	61 (25)	47 (16)	108 (33)	55 (40)	22 (21)	
Persistent abnormal[j]	62 (4)	14 (6)	11 (4)	45 (14)	40 (29)	19 (18)	

NOTE. All values are expressed as no. (%) unless otherwise noted.
[a]Calculated by analysis of variance.
[b]Calculated by χ^2 test.
[c]Data were not available for 3 participants.
[d]Data were not available for 5 participants.
[e]Data were not available for 863 participants because of baseline HBV DNA level undetectable (n = 519), low viral load (n = 264), and inadequate serum sample for HBV genotyping (n = 80).
[f]Data were not available for 326 participants because of less than 2 measurements of ALT; some percentages do not total 100 due to rounding.
[g]All sequential ALT measurements ≤30 U/L.
[h]All sequential ALT measurements <45 U/L and at least one ALT level >30 U/L.
[i]At least one ALT level ≥45 U/L but <50% of sequential ALT measurements ≥45 U/L.
[j]ALT level ≥45 U/L in ≥50% of sequential ALT measurements.

at 10^6 to 10^7 copies/mL (group G-H), and 7.1% for participants in whom these levels persisted at $>10^7$ copies/mL (group I).

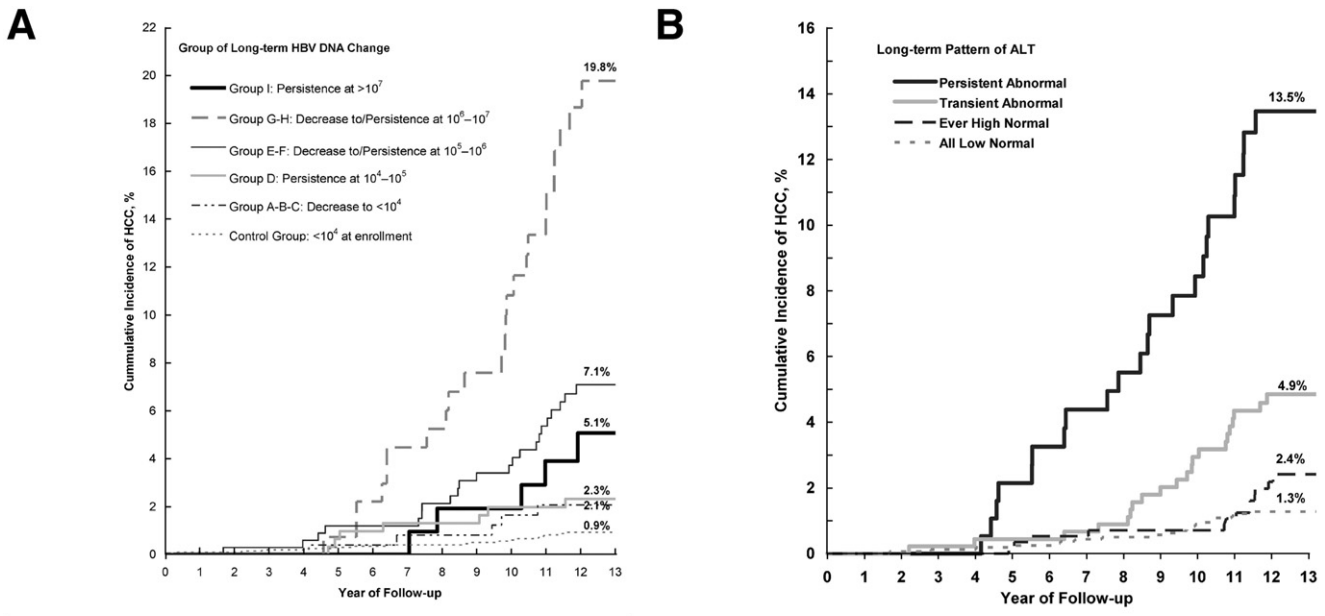
An increasing trend was found in the cumulative incidence of hepatocellular carcinoma with increasing abnormality of long-term serum ALT levels. The cumulative incidences of hepatocellular carcinoma at the end of the 13th year of follow-up were 1.3%, 2.4%, 4.9%, and 13.5% for those with all low-normal ALT, ever high-normal ALT, transient abnormal ALT, and persistent abnormal ALT levels, respectively.

Incidence Rates of Hepatocellular Carcinoma by Long-term HBV DNA and ALT Changes

Table 2 shows the incidence rates of hepatocellular carcinoma for groups of long-term HBV DNA change, long-term pattern of ALT, and HBV genotype. The incidence rates per 100,000 person-years were 73.4 for participants with HBV DNA levels $<10^4$ copies/mL at enrollment (control group), 163.0 for participants whose long-term HBV DNA levels decreased to $<10^4$ copies/mL (group A-B-C), 184.6 for participants in whom these levels persisted at 10^4 to 10^5 copies/mL (group D), 546.8 for participants in whom these levels decreased to/persisted at 10^5 to 10^6 copies/mL (group E-F), 1481.3 for participants in whom these levels decreased to/persisted at 10^6 to 10^7 copies/mL (group G-H), and 381.3 for participants in whom these levels persisted at $>10^7$ copies/mL (group I). The hepatocellular carcinoma incidence rates per 100,000 person-years increased from 101.0 for participants with all low-normal ALT levels to 1033.1 for those with persistent abnormal ALT levels in a dose-response relationship. Participants infected with genotype C had a higher hepatocellular carcinoma incidence rate than those infected with B or mixed genotype (415.3 vs 175.0 per 100,000 person-years).

Multivariate-Adjusted Hazard Ratios of Hepatocellular Carcinoma for Long-term HBV DNA and ALT Changes

Table 3 shows multivariate-adjusted hazard ratios of developing hepatocellular carcinoma for groups of long-term HBV DNA change, long-term ALT pattern, and HBV genotype. Compared with participants with HBV DNA levels $<10^4$ copies/mL at enrollment, the multivariate-adjusted hazard ratios (95% confidence interval) were 2.25 (0.68–7.37), 3.12 (1.09–8.89), 8.85 (3.85–20.35), 16.78 (7.33–38.39), and 3.61 (1.15–11.38) for participants with long-term HBV DNA levels decreased to $<10^4$ copies/mL (group A-B-C), persisted at 10^4 to 10^5 copies/mL (group D), decreased to/persisted at 10^5 to 10^6 copies/mL (group E-F), decreased to/persisted at 10^6 to 10^7 copies/mL (group G-H), and persisted at $>10^7$ copies/mL (group I), respectively, after adjustment for age, sex, habits of



No. at Risk (Group of Long-term HBV DNA Change, copies/mL)														
Persistence at >10^7	107	107	107	107	107	107	107	107	105	105	105	103	102	102
Decrease to/Persistence at 10^6–10^7	137	137	137	137	137	136	134	131	130	127	123	120	114	113
Decrease to/Persistence at 10^5–10^6	336	336	335	335	334	332	332	332	329	325	324	319	314	314
Persistence at 10^4–10^5	309	309	309	309	309	307	306	305	305	305	303	303	302	302
Decrease to <10^4	251	251	251	251	251	250	250	249	249	249	247	246	246	246
<10^4 at enrollment	2020	2018	2018	2016	2016	2014	2013	2012	2011	2010	2008	2004	2002	2002

No. at Risk[a] (Long-term Pattern of ALT, U/L)														
Persistent Abnormal[b]	191	191	191	191	191	187	185	183	181	178	176	172	168	168
Transient Abnormal[c]	452	452	452	451	450	450	450	449	448	443	439	433	431	431
Ever High Normal[d]	570	570	570	570	570	569	567	567	566	566	566	564	558	557
All Low Normal[e]	1621	1621	1620	1619	1619	1617	1617	1614	1613	1612	1607	1603	1601	1601

Figure 3. Cumulative incidence of hepatocellular carcinoma by (*A*) group of long-term HBV DNA change and (*B*) long-term pattern of ALT. Both *P* values for log-rank tests were <.001.[a]Data were not available for 326 participants because of <2 measurements of ALT level. [b]ALT level ≥45 U/L in ≥50% of sequential ALT measurements. [c]At least one ALT level ≥45 U/L but <50% of sequential ALT measurements ≥45 U/L. [d]All sequential ALT measurements <45 U/L and at least one ALT level >30 U/L. [e]All sequential ALT measurements ≤30 U/L.

cigarette smoking and alcohol consumption, long-term ALT pattern, and HBV genotype. The long-term pattern of serum ALT levels was independently associated with the development of hepatocellular carcinoma. Compared with participants with all low-normal ALT levels, the multivariate-adjusted hazard ratio (95% CI) was 1.63 (0.73–3.61) for ever high-normal ALT levels, 3.08 (1.41–6.71) for transient abnormal ALT levels, and 5.75 (2.71–12.23) for persistent abnormal ALT levels. The increasing trend was statistically significant ($P_{trend} < .001$). Genotype C was associated with a higher risk of hepatocellular carcinoma than was B or mixed genotype, showing a multivariate-adjusted hazard ratio (95% confidence interval) of 2.05 (1.20–3.51).

Discussion

In this analysis, 9 groups of long-term HBV DNA change were identified to illustrate spontaneous changes in HBV viral loads. Regardless of baseline HBV DNA levels, participants with spontaneous viral load reduction to $<10^4$ copies/mL during follow-up had a similar risk of hepatocellular carcinoma to those with a baseline HBV DNA level $<10^4$ copies/mL. We found that the group of long-term HBV DNA change was a strong independent risk predictor of hepatocellular carcinoma after taking age, sex, long-term ALT pattern, HBV genotype, and habits of cigarette smoking and alcohol consumption into consideration. In other words, participants with similar HBV DNA levels at enrollment had different risks of hepatocellular carcinoma depending on their HBV DNA levels during follow-up. For participants with the same HBV DNA levels at enrollment, greater decreases in serum HBV DNA levels during follow-up were associated with lower risk of hepatocellular carcinoma. This finding is consistent with those of clinical trials, which have shown improvements in liver histologic findings and reduction in the incidence of advanced liver disease with effective antiviral therapy.[25–27] It suggests the importance of regular monitoring of serum HBV DNA levels and therapy to lower HBV viral load in patients with chronic hepatitis B.

The risk of hepatocellular carcinoma was primarily determined by HBV DNA levels at enrollment for the participants who had persistent HBV DNA levels during follow-up. In other words, the higher the persistent HBV viral load, the higher the risk of subsequent hepatocellular carcinoma except in the cases of persistent viral loads $>10^7$ copies/mL. Participants in group I with persistent viral loads $>10^7$ copies/mL had a higher proportion of young, female, HBeAg-seropositive (99%), ALT normal participants than those in other long-term HBV DNA change groups. They had a moderate risk of developing hepatocellular carcinoma even after taking age, sex, long-term ALT pattern, HBV genotype, and habits of cigarette smoking and alcohol consumption into consideration. HBV-infected persons in the immune tolerance phase are

Table 2. Incidence Rate of Hepatocellular Carcinoma by Group of Long-term HBV DNA Change, Long-term Pattern of ALT, and HBV Genotype

Variable	No. (%) of participants (N = 3160)	Person-years of follow-up	No. of hepatocellular carcinoma cases	Incidence rate (per 100,000 person-years) (95% CI)	Crude hazard ratio (95% CI)	*P* value
Group of long-term HBV DNA change						
Control group: HBV DNA level $<10^4$ at enrollment	2020 (64)	24,515.4	18	73.4 (46.3–116.5)	1.00 (referent)	
Group A-B-C: Decrease to $<10^4$	251 (8)	3067.6	5	163.0 (67.8–391.6)	2.23 (0.83–6.00)	.11
Group D: Persistence at 10^4 to 10^5	309 (10)	3791.9	7	184.6 (88.0–387.2)	2.51 (1.05–6.01)	.04
Group E-F: Decrease to/persistence at 10^5 to 10^6	336 (11)	4023.1	22	546.8 (360.1–830.5)	7.52 (4.03–14.01)	<.001
Group G-H: Decrease to/persistence at 10^6 to 10^7	137 (4)	1620.2	24	1481.3 (992.9–2210.0)	20.63 (11.20–38.02)	<.001
Group I: Persistence at $>10^7$	107 (3)	1311.3	5	381.3 (158.7–916.1)	5.23 (1.94–14.08)	.001
Long-term pattern of ALT[a]						
All low-normal	1621 (57)	19,810.4	20	101.0 (65.1–156.5)	1.00 (referent)	
Ever high-normal	570 (20)	7054.1	13	184.3 (107.0–317.4)	1.81 (0.90–3.64)	.10
Transient abnormal	452 (16)	5557.7	21	377.9 (246.4–579.5)	3.75 (2.03–6.91)	<.001
Persistent abnormal	191 (7)	2226.3	23	1033.1 (686.5–1554.7)	10.57 (5.81–19.25)	<.001
HBV genotype[b]						
B or mixed genotype	1501 (65)	18,287.1	32	175.0 (123.7–247.4)	1.00 (referent)	
C	796 (35)	9630.5	40	415.3 (304.7–566.2)	2.40 (1.51–3.82)	<.001

CI, confidence interval.

[a]Data were not available for 326 participants because of less than 2 measurements on ALT.

[b]Data were not available for 863 participants because of baseline HBV DNA level undetectable (n = 519), low viral load (n = 264), and inadequate serum sample for HBV genotyping (n = 80).

characterized by HBeAg seropositivity and high viral load but normal serum ALT levels with minimal liver disease progression.[19,28–30] A large proportion of participants with persistent viral loads $>10^7$ copies/mL were remaining in their immune tolerance phase during the long-term follow-up period. Their moderate risk of hepatocellular carcinoma may be attributable to their persistent immune tolerance phase resulting from certain unique interactions between viral and host characteristics. The mechanism and risk of hepatocarcinogenesis may be different between patients with chronic hepatitis B in the immune tolerance phase and those in the immune clearance phase. Further study is needed to elucidate and differentiate these mechanisms.

A recent case-cohort study of male adult chronic HBV carriers has reported only 3 groups of long-term HBV DNA change.[31] The limited number of long-term change groups identified in the previous study may be due to the small number of participants with serum HBV DNA levels $>10^4$ copies/mL at enrollment. Owing to a large number of participants with serum HBV DNA levels $>10^4$ copies/mL at enrollment, 9 distinctive groups of long-term HBV DNA change were identified in our study. They included 4 groups of long-term HBV DNA change with baseline levels $>10^7$ copies/mL and another 5 groups with baseline levels $<10^7$ copies/mL. We were therefore able to examine the impact of long-term sequential changes in HBV viral load on subsequent hepatocellular carcinoma risk in a more refined manner.

In this study, a significant biologic gradient was associated with hepatocellular carcinoma risk: long-term patterns of ALT from all low-normal, ever high-normal, and transient abnormal to persistent abnormal levels. The risk of hepatocellular carcinoma started to increase at ever high-normal ALT levels. The threshold of high-normal ALT level associated with increased risk of hepatocellular carcinoma in this study was set as 30 U/L partly because it has been reported that the risk of liver-related mortality is increased even in patients with high normal ALT levels.[32] These data suggest that lowering the upper limit of normal for ALT might be important in the medical management of patients with chronic hepatitis B. Consistent with previous findings, the frequency of abnormal ALT levels (≥45 U/L) during follow-up was important to evaluate the risk of hepatocellular carcinoma.[15] Serum ALT levels usually reflect the degree of HBV-related liver cell injuries and may vary during the course of chronic HBV infection.[19,33,34] It is important to monitor serum ALT levels regularly to predict the risk of hepatocellular carcinoma.

In consideration of the cost, only the last follow-up HBV DNA samples were tested for participants with baseline HBV DNA levels $<10^4$ copies/mL; only 82 (0.9%) of them had HBV DNA levels $\geq 10^5$ copies/mL at the last

Table 3. Multivariate-Adjusted Hazard Ratio of Developing Hepatocellular Carcinoma for Group of Long-term HBV DNA Change, Long-Term Pattern of ALT, and HBV Genotype

Variable	Model I[a]		Model II[b]		Model III[b]	
	Adjusted hazard ratio (95% CI)	*P* value	Adjusted hazard ratio (95% CI)	*P* value	Adjusted hazard ratio (95% CI)	*P* value
Group of long-term HBV DNA change						
Control group: HBV DNA level $<10^4$ at enrollment	1.00 (referent)		1.00 (referent)		1.00 (referent)	
Group A-B-C: Decrease to $<10^4$	2.12 (0.78–5.73)	.14	1.92 (0.70–5.28)	.21	2.25 (0.68–7.37)	.18
Group D: Persistence at 10^4 to 10^5	2.54 (1.06–6.10)	.04	2.36 (0.92–6.06)	.07	3.12 (1.09–8.89)	.03
Group E-F: Decrease to/persistence at 10^5 to 10^6	8.38 (4.44–15.81)	<.001	6.55 (3.28–13.06)	<.001	8.85 (3.85–20.35)	<.001
Group G-H: Decrease to/persistence at 10^6 to 10^7	24.36 (13.02–45.61)	<.001	15.86 (7.94–31.68)	<.001	16.78 (7.33–38.39)	<.001
Group I: Persistence at $>10^7$	7.19 (2.66–19.46)	<.001	3.90 (1.37–11.04)	.01	3.61 (1.15–11.38)	.03
Long-term pattern of ALT[c]						
All low-normal	Not included		1.00 (referent)	<.001[d]	1.00 (referent)	<.001[d]
Ever high-normal			2.14 (1.03–4.47)	.04	1.63 (0.73–3.61)	.23
Transient abnormal			4.17 (2.00–8.70)	<.001	3.08 (1.41–6.71)	.005
Persistent abnormal			8.00 (3.98–16.05)	<.001	5.75 (2.71–12.23)	<.001
HBV genotype[e]						
B or mixed genotype	Not included		Not included		1.00 (referent)	
C					2.05 (1.20–3.51)	.009

CI, confidence interval.
[a]Adjusted for age, sex, cigarette smoking, alcohol drinking, and serum levels of ALT at enrollment.
[b]Adjusted for age, sex, cigarette smoking, alcohol drinking, total number of repeated measurements of ALT, and the other factors listed in the table.
[c]Data were not available for 326 participants because of less than 2 measurements on ALT.
[d]For trend test.
[e]Data were not available for 863 participants because of baseline HBV DNA level undetectable (n = 519), low viral load (n = 264), and inadequate serum sample for HBV genotyping (n = 80).

follow-up examination, and none were afflicted with hepatocellular carcinoma. Therefore, participants with baseline HBV DNA levels $<10^4$ copies/mL served as the control group. Because participants in this study were aged 30 years or older with genotype B/C HBV infection in early childhood, the findings of this study may not be directly applicable to chronic HBV carriers younger than 30 years of age or infected in adulthood by other HBV genotypes.

There were some short-term fluctuations of follow-up HBV DNA levels during the observation period in each group of long-term HBV DNA change. However, for the overall 11-year follow-up, we did not found any group with an obvious increasing trend of long-term HBV DNA levels in this study. The rebound in serum HBV DNA levels is frequently observed for those who stopped antiviral treatments, but only 16 of 1140 participants (1.4%) had rebound in this cohort study on the natural history of chronic hepatitis B.

In conclusion, a dynamic change in serum levels of HBV DNA over time is a strong risk predictor of hepatocellular carcinoma, independent of long-term pattern of ALT and HBV genotype. Spontaneous decrease of HBV DNA levels to $<10^4$ copies/mL is associated with a significantly lowered risk of hepatocellular carcinoma. Regular monitoring of serum levels of HBV DNA and ALT is recommended for the clinical management of patients with chronic hepatitis B.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2011.06.036.

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Received December 8, 2010. Accepted June 3, 2011.

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Acknowledgments

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Conflicts of interest

The authors disclose the following: Dr Iloeje is an employee of and holds stock in Bristol-Myers Squibb Co. Dr Su was an employee of and held stock in Bristol-Myers Squibb Co previously. Professor Chien-Jen Chen was supported by research grants from Bristol-Myers Squibb to conduct the laboratory tests of HBV DNA for this study. Drs Chen, Lee, Yang, Chang, Jen, Hsiao, Wang, You, and Lu disclose no conflicts.

Funding

Supported by research grants from the Department of Health, Executive Yuan (Taipei, Taiwan); Academia Sinica (Taipei, Taiwan); National Health Research Institute (Taipei, Taiwan) (research grants awarded to C.-J.C.); Bristol-Myers Squibb (Wallingford, CT) to conduct the laboratory tests for this study; and National Taiwan University Hospital (Taipei, Taiwan) to conduct the laboratory test on HBV genotype for this study. The funding organizations of this study had no role in the design or conduct of the study, nor did they have any role in the collection, management, analysis, and interpretation of the data as well as the preparation, review, or approval of the manuscript. All data handling and statistical analyses were performed by staff at the National Taiwan University and Academia Sinica. At no time did the funding organizations have access to the data.

Supplementary Materials and Methods

Group-Based Trajectory Model

To identify distinctive groups of long-term changes in serum HBV DNA levels over 11 years of follow-up, the group-based trajectory model[1] was used. The analysis assumed that the heterogeneous study population contained participants with multiple long-term patterns of serum HBV DNA levels. The technique was used to cluster participants following similar long-term patterns of serum HBV DNA levels in the same group, although long-term patterns of serum HBV DNA levels were never exactly the same among participants. In other words, the analysis was designed to group participants with similar patterns of long-term HBV DNA change together and to discriminate different groups of participants with dissimilar patterns of long-term HBV DNA change over time.

Four key outputs of the group-based trajectory model[1] included (1) the optimal number of groups of long-term HBV DNA change, (2) the shape of each long-term HBV DNA change group, (3) the group assignment for each participant (based on the highest posterior probabilities of belonging to each group), and (4) the proportion of study population in each group.

In this study, repeated measurements of serum HBV DNA levels were $\log_{10}$ transformed and modeled by a censored normal distribution.[1] The group-based trajectory analysis was performed with the PROC TRAJ macro in SAS version 9.1.[2] Suppose $Y_i = \{y_{i1}, y_{i2}, y_{i3} \ldots y_{iT}\}$ represented the long-term repeated measurements of serum HBV DNA levels ($\log_{10}$ copies/mL) on a participant i over T periods and $P(Y_i)$ denoted the probability of Y_i. The group-based trajectory model assumes that the study population was composed of a mixture of J underlying groups of long-term HBV DNA change such that

$$P(Y_i) = \sum_j \pi_j P^j(Y_i),$$

where $P^j(Y_i)$ was the probability of Y_i given membership in group j and π_j was the probability of group j. The basic model also assumed that conditional upon membership in group j, the random variables, y_{it}, $t = 1,2 \ldots T$, were independent. Thus, $P^j(Y_i) = \prod^T P^j(y_{it})$. The censored normal distribution was used to define $P^j(y_{it})$, and the linkage between time (years of follow-up) and long-term HBV DNA levels was established via a latent variable, y_{it}^{*j}, that represented the predicted levels of HBV DNA of subject i at time t given membership in group j. The PROC TRAJ macro in SAS allowed estimation of up to a fourth-order polynomial relationship between time (years of follow-up) and long-term HBV DNA levels. It was assumed that

$$y_{it}^{*j} = \beta_0^j + \beta_1^j X_{it} + \beta_2^j X_{it}^2 + \beta_3^j X_{it}^3 + \beta_4^j X_{it}^4 + \varepsilon_{it},$$

where ε_{it} was a disturbance assumed to be normally distributed with a zero mean and a constant variance σ^2. Furthermore, the model's coefficients (β_0^j, β_1^j, β_2^j, β_3^j, β_4^j) determined the shape for a specific group (j) of long-term HBV DNA change.

Model Selection

The group-based trajectory approach involved a 2-stage model selection process.[3] First, we chose the optimal number of long-term HBV DNA change groups based on testing the comparative fit of a series of models with different numbers of groups using an SAS macro named PROC TRAJ.[2] In the second stage, the focus turned to determining the preferred order of the polynomial specifying the shape of each long-term HBV DNA change group given the first-stage decision on the number of groups.

The Bayesian information criterion (BIC) value was obtained for each model tested and was a fit index[3] to compare competing models that included different numbers of long-term HBV DNA change groups or long-term HBV DNA change groups of various shapes. However, it was difficult to judge which model was clearly better without a concrete standard for calibrating the magnitude of change in BIC. The log Bayes factor[2] provided useful statistics for calibrating the substantive importance of a difference in the BIC scores of 2 nested models. The log Bayes factor approximation was defined by the formula.

$$2\log_e(B_{10}) \approx 2(\triangle \mathrm{BIC}),$$

where $\triangle BIC$ was the BIC value of the alternative (more complex) model less the BIC value of the null (simpler) model.[2] The log Bayes factor was interpreted as a measure of the extent of evidence for improvement of model fit, thereby ensuring model parsimony. The log Bayes factor values ranging from 0 to 2 were interpreted as weak evidence for the more complex model, values ranging from 2 to 6 were interpreted as moderate evidence, values ranging from 6 to 10 were interpreted as strong evidence, and values greater than 10 were interpreted as very strong evidence.

In the first stage (to identify the optimal number of groups of long-term HBV DNA change) of this study, a series of models that contained from one to 10 groups and included both a linear and a quadratic term for each group was systematically examined and compared. BIC rose steadily (that is, became less negative) as the number of groups increased from 1 (BIC= −10,715.74) to 9 (BIC= −8493.08) groups and thereafter began a steady decline (BIC = −8507.16 for 10 groups). The estimate of the log Bayes factor for the comparison of the 9- versus 8-group model equaled 42.96 (far exceeding 10). Thus, according to the log Bayes factor, this evidence was very strong in favor of the 9-group model.

In the second stage of this study, the focus turned to determining the preferred order of the polynomial specifying the shape of each long-term HBV DNA change group given the first-stage decision on the number of groups. The fit of each competing model was then compared using the estimate of the log Bayes factor. Finally, a quadratic 9 groups of long-term HBV DNA change was identified as the best-fit model.

Group Assignment

Participants were classified into different groups of long-term HBV DNA change based on the highest posterior probabilities of belonging to each group.[1] The group of long-term HBV DNA change for each participant was treated as a categorical variable for subsequent analyses.

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