MAJOR ARTICLE

Genotypic Distribution of Hepatitis B Virus (HBV) Among Acute Cases of HBV Infection, Selected United States Counties, 1999–2005

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Background. Knowledge of the genotypic distribution of hepatitis B virus (HBV) facilitates epidemiologic tracking and surveillance of HBV infection as well as prediction of its disease burden. In the United States, HBV genotyping studies have been conducted for chronic but not acute hepatitis B.

Methods. Serum samples were collected from patients with acute hepatitis B cases reported from the 6 counties that participated in the Sentinel Counties Study of Acute Viral Hepatitis from 1999 through 2005. Polymerase chain reaction followed by nucleotide sequencing of a 435–base pair segment of the HBV *S* gene was performed, and the sequences were phylogenetically analyzed.

Results. Of 614 patients identified with available serum samples, 75% were infected with genotype A HBV and 18% were infected with genotype D HBV. Thirty-two percent of genotype A sequences constituted a single subgenotype A2 cluster. The odds of infection with genotype A (vs with genotype D) were 5 times greater among black individuals than among Hispanic individuals (odds ratio [OR], 5; 95% confidence interval [CI], 2.3–10.7). The odds of infection with genotype A were 49, 8, and 4 times greater among patients from Jefferson County (Alabama), Pinellas County (Florida), and San Francisco (California), respectively, than among those living in Denver County (Colorado). Genotype A was less common among recent injection drug users than it was among non–injection drug users (OR, 0.2; 95% CI, 0.1–0.4).

Conclusions. HBV genotype distribution was significantly associated with ethnicity, place of residence, and risk behavior.

Hepatitis B virus (HBV) has been classified as belonging to at least 8 genotypes (A–H) with an additional provisional genotype (I) recently described from southeast Asia [1]. Understanding of genotype distribution among acute cases is important for epidemiologic characterization of HBV transmission and to control infection [2]. Studies of the distribution of HBV genotypes have largely been conducted among patients with chronic hepatitis B. These studies have associated infection by specific genotypes with outcomes of chronic hepatitis B, such as progression to liver cirrhosis and cancer, and with response to antiviral therapy [3]. Owing to the long time-lapse between acute HBV infection and the diagnosis of chronic hepatitis B and the variable rate of progression from acute to chronic infection, the genotype distributions among individuals with chronic hepatitis cases may be different from those among individuals with acute infection. Furthermore, chronic hepatitis B in the United States is primarily an imported disease, and the majority of people who are affected by it originate from countries where HBV infection is hyperendemic [4]. No study of the genotypic distribution of HBV in acute hepatitis B has been reported from the United States. We report here the extent of spread of HBV genotypes among US cases of acute hepatitis B and

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the demographic characteristics and risk factors associated with infection.

METHODS

The Sentinel Counties Study of Acute Viral Hepatitis was a population-based study that enrolled cases of acute viral hepatitis reported to 6 US county health departments from 1982 through 2006 [5]. The data used for this analysis were collected from cases identified during the period 1999–2005. Participating county and city health departments originated from Jefferson County, Alabama; Pinellas County, Florida; Pierce County, Washington; Multnomah County, Oregon; San Francisco County, California; and Denver County, Colorado. These sites were selected on the basis of the reportedly higher incidence of acute viral hepatitis before 1982. Acute hepatitis B was defined as having a history of sudden onset of signs and symptoms consistent with hepatitis together with serological detection of hepatitis B surface antigen alone or in combination with immunoglobulin M antibody to hepatitis B core antigen. An epidemiological investigation of all reports of acute hepatitis B was conducted by study staff. Risk factors were assigned using mutually exclusive hierarchies of known risk factors for HBV infection that occurred during the 6 months prior to infection [6]. HBV genotyping was performed by nucleotide sequencing of a 435–base pair DNA segment amplified from the HBV *S* gene (from nucleotide position 222 to 656 of HBV genome), as previously described [7]. Phylogenetic analysis was conducted using the neighbor-joining method [8].

Risk-ratio estimates were calculated to determine the association between patient characteristics and HBV genotypes.

RESULTS

Of 1206 patients with acute hepatitis B identified from 1999 through 2005, 614 (51%) had serum samples from which the HBV *S* gene could be amplified and sequenced. Among patients for whom the HBV *S* gene was sequenced, the mean age was 38 years (range, 14–86 years), 439 (71%) were men, 337 (55%) were white, 160 (26%) were black, 76 (12%) were Hispanic, and 15 (2%) were Asian/Pacific Islander. Data for country of birth were available only for patients with cases identified from 2001 through 2005; of 446 such patients, 59 (13%) were foreign born.

Genotype

Phylogenetic trees were constructed on the basis of *S* gene sequences. A total of 462 sequences (75%) were classified as genotype A, from which 456 sequences were determined to belong to subgenotype A2 and 6 to A1. Among genotype A sequences, 3 prominent clusters with 100% similarity at the nucleotide level were identified (Figure 1). The largest cluster, cluster I ($n = 150$; 32%), was identical in sequence to the "prisoners' variant" (HBV^{PV}) sequence that had earlier been characterized in England [9]. Clusters II and III of genotype A consist of 25 (5%) and 43 (9%) sequences, respectively. The remaining 238 (52%) genotype A sequences did not form prominent clusters. A total of 111 sequences, comprising 18% of the total, belonged to genotype D. Of these, 1 cluster, cluster IV ($n = 46$; 41%), segregated separately from the other D sequences (Figure 1). The median genetic difference among the genotype A and genotype D sequences was 0.69% and 1.38%, respectively. The remaining 41 sequences (6%) belonged to genotypes B (0.3%), C (2.9%), E (0.6%), F (1%), G (1.4%), and H (0.5%). In 4 sequences belonging to genotype A ($n = 2$), D ($n = 1$), and G ($n = 1$), nucleotide changes that lead to amino acid changes in the "a" determinant that are known to be associated with vaccine-escape mutations were identified (D144V, D144A, D144E, and T126A). No intergenotype recombination was found. Because of the small numbers in each group belonging to genotypes B, C, E, F, G, and H, cases infected with these genotypes were excluded from additional analyses. Preliminary analyses showed no differences in the demographic characteristics of patients infected with variants belonging to clusters I–IV or to other genotype A and D variants not belonging to these 4 clusters. Therefore, demographic characteristic and risk factor analyses were conducted on the basis of whether patients were infected with either genotype A or genotype D.

Demographic Characteristics

Age and sex of the patients were not significantly different between genotypes A and D (Table 1). However, race/ethnicity and county of residence of the patients were significantly associated with HBV genotype. The odds of being infected with genotype A (vs D) were 5 times greater among black individuals than among Hispanic individuals (odds ratio [OR], 5; 95% confidence interval [CI], 2.3–10.7). Furthermore, the odds of being infected with genotype A were 49 (OR, 49.1; 95% CI, 11.4–212), 8 (OR, 7.9; 95% CI, 4.0–15.4), and 4 (OR, 4.0; 95% CI, 1.8–8.7) times greater among those from Jefferson County (Alabama), Pinellas County (Florida), and San Francisco County (California), respectively, than among those living in Denver County (Colorado). The majority of patients in Jefferson County (98%), Pinellas County (91%), and San Francisco County (83%) were infected with genotype A. Fifty-two percent of the cases from Pierce County and 45% of the cases from Denver were due to infection with genotype D.

Year of Infection and Risk Factors

The distribution of cases by genotype remained relatively constant over the study period (Table 1). For patients with hepatitis B, genotype A was significantly less common among recent

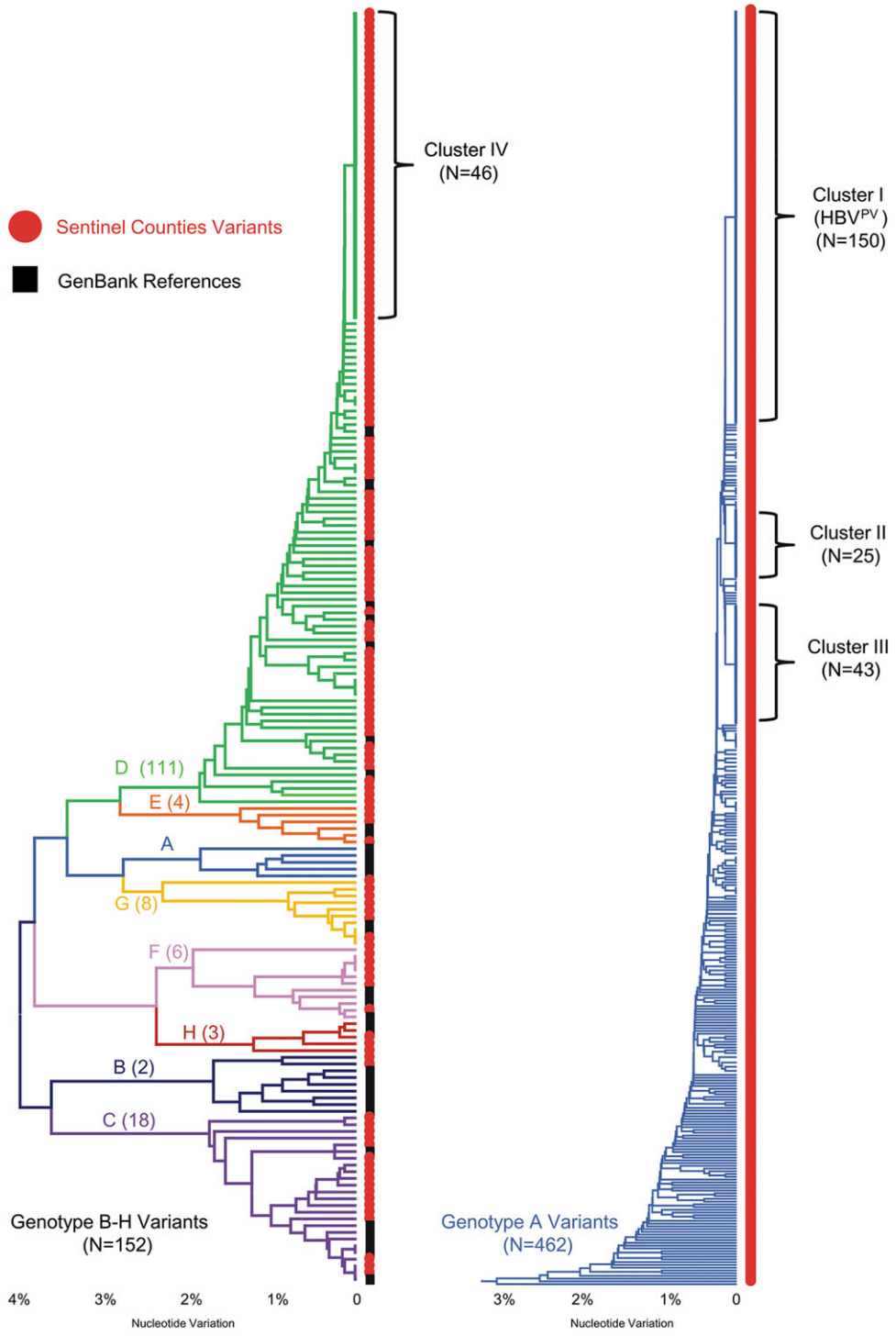

Figure 1. Phylogenetic trees based on a 435–base pair DNA segment amplified from hepatitis B virus *S* gene.

injection drug users than among those who did not use injection drugs (OR, 0.2; 95% CI, 0.1–0.4) (Table 1). The odds of being infected with genotype A also were significantly less among those who had a history of incarceration (OR, 0.6; 95% CI, 0.4–0.9), compared with those without such a history. No significant associations with genotype were found among persons whose risk for infection was male-to-male sex, persons who engaged in heterosexual sex with multiple partners, or those who engaged in either male-to-male sex or heterosexual sex with an HBV-infected partner.

Table 1. Demographic Characteristics and Risk Factors of Patients With Cases of Acute Hepatitis B Virus (HBV) Infection by HBV Genotype, Selected US Counties, 1999–2005

Characteristic	No. of patients				OR (95% CI)
	Genotype A		Genotype D		
Age, years					
14–24	57	78	16	22	0.8 (.4–1.6)
25–44	287	81	68	19	1.0 (.6–1.6)
≥45	118	81	27	19	Referent
Sex					
Male	336	81	77	19	0.9 (.5–1.3)
Female	126	79	34	21	Referent
Race/ethnicity					
White	249	78	69	22	1.6 (.9–2.9)
Black	142	92	13	8	5.0 (2.3–10.7)
Hispanic	46	69	21	31	Referent
County					
Denver	46	55	38	45	Referent
Jefferson	119	98	2	2	49.1 (11.4–212)
Multnomah	79	72	31	28	2.1 (1.2–3.8)
Pinellas	153	91	16	9	7.9 (4.0–15.4)
Pierce	12	48	13	52	0.8 (.3–1.9)
San Francisco	53	83	11	17	4.0 (1.8–8.7)
Year of diagnosis					
1999	55	85	10	15	Referent
2000	70	79	19	21	0.7 (.3–1.6)
2001	69	78	20	22	0.6 (.3–1.5)
2002	57	70	24	30	0.4 (.2–1.0)
2003	79	77	24	23	0.6 (.3–1.4)
2004	64	91	6	9	1.9 (.7–5.7)
2005	68	89	8	11	1.5 (.6–4.2)
Ever jailed					
Yes	195	76	60	24	0.6 (.4–.9)
No	254	85	45	15	Referent
HBV infection risk					
Men who have sex with men					
Yes	125	87	18	13	1.8 (1.0–3.2)
No	203	80	52	20	Referent
Injection drug use[a]					
Yes	42	56	33	44	0.2 (.1–.4)
No	419	85	75	15	Referent
Heterosexual sex with ≥1 person[a]					
Yes	118	84	23	16	1.2 (.7–1.9)
No	320	82	72	18	Referent
Sex with HBV-infected person[a]					
Yes	28	78	8	22	1.1 (.3–3.5)
No	20	77	6	23	Referent
Nonsexual contact with HBV-infected person[a]					
Yes	3	75	1	25	1.2 (.1–12.4)
No	33	72	13	28	Referent

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Totals may vary by subgroup because of missing data.

Country of Birth

Fifty-six percent of the 59 foreign-born patients were infected with HBV genotype A, compared with 78% of those who were US born (data not shown). The D genotype and the non-A, non-D genotypes occurred more frequently among foreign-born individuals (25% and 19%, respectively) than among US-born individuals (17% and 5%, respectively). Although foreign-born persons comprised only 13% of the study population, 34% of the non-A, non-D genotypes occurred among this group.

DISCUSSION

This study is the first to evaluate the distribution of HBV genotypes among acute hepatitis B cases in the United States, and, with 614 cases included, is the largest study of HBV genotypes among acute cases [10, 11]. All 8 known HBV genotypes were evident among our study sample. The most common genotype was genotype A, which occurred in 75% of the samples tested, followed by D, which comprised 18% of the total. Phylogenetic analysis of the *S* gene sequences enabled the assignment of subgenotypes among genotype A but not genotype D sequences. Almost all genotype A sequences belonged to subgenotype A2 (99%), with only 6 sequences classified as subgenotype A1, indicating that, despite the diversity of subtypes worldwide [12, 13], the majority of acute hepatitis B in the United States is caused by a single HBV subgenotype.

The genotypic distribution of HBV among patients with chronic hepatitis B in the United States has previously been reported. A study that tested 527 patients attending liver specialty clinics in 8 states found that genotypes A and D accounted for 35% and 10% of the total number of cases, respectively [4]. Furthermore, that study found a substantially higher representation of genotypes B and C, which accounted for 22% and 31% of cases, respectively, compared with our findings of 0.3% and 2.9% for these genotypes. These differences in genotype distribution are likely explained by the fact that chronic hepatitis B in the United States is largely an imported disease that typically affects persons who originate from regions where HBV infection is hyperendemic, particularly east Asia, where genotypes B and C are dominant [3, 14]. Indeed, the study involving patients with chronic infection who were born in the United States [4] found that 77% and 10% were infected with genotypes A and D, respectively, which are similar to the 75% and 18% that we found.

In our study, the most prominent *S* gene cluster comprised 150 sequences, which constituted 32% of all genotype A and 25% of the total sequences examined. This cluster was found to be identical to that of a variant that is prevalent in Europe [9, 15–17]. It first gained prominence in England, where it was observed to be responsible for outbreaks among inmates of prisons in the north, and so was assigned HBV^{PV} (PV for "prisoners' variant") [9]. HBV^{PV} is, strictly, not a single strain

but a cluster of closely related variants [9, 15]. Although, in earlier investigations, the spread of HBV^{PV} was associated with injection drug use, it was subsequently found to be widely disseminated in Europe among the general population (identified in 23% of sporadic acute hepatitis B cases in England from 1997 through 2001 [15] and in 59% of cases in Ireland from 2004 through 2006) [16] and in outbreaks associated with a variety of transmission risks, including blood transfusion, surgery, and tattooing in England [15]; hemodialysis or renal transplantation in Latvia [17]; and male-to-male sexual activity in Denmark [18]. HBV^{PV} also has emerged in Japan as the dominant variant associated with transmission among men who have sex with men [19]. In our study, the demographic and epidemiologic characteristics of patients infected with HBV^{PV} were not significantly different from those associated with genotype A as a whole (data not shown). Collectively, HBV^{PV} and the other genotype A strains were observed to be more common among black patients and among those from Jefferson County, Pinellas County, and San Francisco County. No significant associations were found between genotype A and specific risk factors, which suggests that this particular genotype spreads along established transmission routes among the US population.

There are some limitations to our study. First, the findings are limited to the geographic location where surveillance was conducted. Second, serum samples were available for HBV genotyping from only approximately one-half of cases, so the findings are not generalizable to acute hepatitis B cases from the Sentinel Counties as a whole. Moreover, such sample size limitations precluded more in-depth analysis (eg, multivariable analysis) of genotype differences among the cases. The findings of this study are limited to symptomatic cases of acute hepatitis B and may not apply to individuals with asymptomatic infection.

Data from this study indicate that the vast majority of patients with acute hepatitis B cases identified from 1999 to 2005 were infected with HBV A2 and that, over this period, the extent of infection with strains from this subgenotype was relatively constant. The extensive infection, particularly by the most dominant cluster of variants, HBV^{PV}, together with the high conservation of the *S* gene, impose limitations to the utility of the *S* gene for reliable molecular epidemiologic investigations of HBV transmissions. Accordingly, the Centers for Disease Control and Prevention conducts whole-genome HBV sequencing for epidemiological investigation of HBV outbreaks. The predominance of HBV genotype A2 also has clinical and public health implications. Acute infection with genotype A, compared with genotypes B, C, and D, has been observed to lead more frequently to chronicity [19]. Furthermore, genotype A also has been determined to favor the emergence of mutations associated with resistance to lamivudine [20]. Another study has found that HBV genotype A is more responsive to interferon therapy, compared with genotype D [21]. Such differences in natural history and responses to antiviral agents highlight the need to maintain vigilance in the conduct of molecular epidemiological surveillance. Molecular epidemiologic surveillance will allow monitoring of the changing prevalence of HBV genotypes in acute and chronic hepatitis B and will help to preempt virological changes that could correspondingly increase the burden of disease borne by people living with HBV infection in the United States. Furthermore, such close surveillance may facilitate identification of highly transmissible or virulent HBV strains.

Notes

Acknowledgments. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Oligner CM, Jutavijittum P, Hubschen JI, et al. Possible new hepatitis B virus genotype, southeast Asia. Emerg Infect Dis **2008**; 14:1777–80.
2. Holmberg SD. Molecular epidemiology of health care-associated transmission of hepatitis B and C viruses. Clin Liver Dis **2010**; 14:37–48.
3. Yuen MF, Lai CL. Hepatitis B virus genotypes: natural history and implications for treatment. Expert Rev Gastroenterol Hepatol **2007**; 1:321–8.
4. Chu CJ, Keeffe E, Han SH, et al. Hepatitis B virus genotypes in the United States: results of a nationwide study. Gastroenterol **2003**; 125:444–51.
5. Nainan OV, Armstrong GL, Han XH, Williams I, Bell BP, Margolis HS. Hepatitis A molecular epidemiology in the United States, 1996–1997: sources of infection and implications of vaccination policy. J Infect Dis **2005**; 191:957–63.
6. Goldstein ST, Alter M, Williams I, et al. Incidence and risk factors for acute hepatitis B in the United States, 1992–1998: implications for vaccination programs. J infect Dis **2002**; 185:713–9.
7. Forbi JC, Vaughan G, Purdy MA, et al. Epidemic history and evolutionary dynamics of hepatitis B virus infection in two remote communities in rural Nigeria. PLoS One **2010**; 5:e11615.
8. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **1987**; 4:406–25.
9. Hallet RL, Ngui SL, Meigh RE, Mutton KJ, Boxall EH, Teo CG. Widespread dissemination in England of a stable and persistent hepatitis B virus isolate. Clin Infect Dis **2004**; 39:945–52.
10. Sugauchi F, Orito E, Ohno T, et al. Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. Hepatol Res **2006**; 36:107–14.
11. Panessa C, Hill WD, Giles E, et al. Genotype D amongst injection drug users with acute hepatitis B virus infection in British Columbia. J Viral Hepat **2009**; 16:64–73.
12. Pourkarim MR, Amini-Bavil-Olyaee S, Lemey P, Maes P, Van Ranst M. Are hepatitis B virus ''subgenotypes'' defined accurately? J Clin Virol **2010**; 47:356–60.
13. Abdou Chekaraou M, Brichler S, Mansour W, et al. A novel hepatitis B virus (HBV) subgenotype D (D8) strain, resulting from recombination between genotypes D and E, is circulating in Niger along with HBV/E strains. J Gen Virol **2010**; 91:1609–20.
14. Toan NL, Song le H, Kremsner PG, et al. Impact of the hepatitis B virus genotype and genotype mixtures on the course of liver disease in Vietnam. Hepatology **2006**; 43:1375–84.
15. Sloan R, Strang AL, Ramsay ME, Teo CG. Genotyping of acute HBV isolates from England, 1991–2001. J Clin Virol **2009**; 44:157–60.

16. Laoi BN, Crowley B. Molecular characterization of hepatitis B virus (HBV) isolates, including identification of a novel recombinant, in patients with acute HBV infection attending an Irish hospital. J Med Virol **2008**; 80:1554–64.
17. Sominskaya I, Mihailova M, Jansons J, et al. Hepatitis B and C virus variants in long-term immunosuppressed renal transplant patients in Latvia. Intervirology **2005**; 48:192–200.
18. Van Houdt R, Bruisten SM, Geskus RB, et al. Ongoing transmission of a single hepatitis B virus strain among men having sex with men in Amsterdam. J Viral Hepat **2010**; 17:108–14.
19. Matsuura K, Tanaka Y, Hige S, et al. Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. J Clin Microbiol **2009**; 47:1476–83.
20. Damerow H, Yuen L, Wiegand J, et al. Mutation pattern of lamivudine resistance in relation to hepatitis B genotypes: hepatitis B genotypes differ in their lamivudine resistance associated mutation pattern. J Med Virol **2010**; 82:1850–8.
21. Hou J, Schilling R, Janssen HLA. Molecular characteristics of hepatitis B virus genotype A confers a higher response to interferon treatment. J Hepatol **2001**; 34(Suppl 1):15.