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Multicentre study of hepatitis B virus genotypes in France: correlation with liver fibrosis and hepatitis B e antigen status

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SUMMARY. The clinical significance of hepatitis B virus (HBV) genotypes is still under debate. The aims of this study were to assess the distribution of HBV genotypes in France and to identify the associations between HBV genotypes and patient demographics, severity of liver disease and HBeAg status in patients referred to tertiary care centres. This was a French, multicentre, retrospective study on 262 patients with chronic HBV infection. HBV genotypes were determined using INNO-LiPA. Liver fibrosis damage was evaluated by histological analysis of biopsy samples. Patients were mainly male (74%), of Caucasian (65%), Asian (17%) or African (18%) ethnicity and 36% were HBeAg positive. All A–G genotypes were found, the most frequent being genotypes D (27%) and A (24%), followed by E (13%) and C (12%), and B (7%). Mixed genotypes were detected in 16% of the cases. Genotype A was associated with sexual contact ($P < 0.001$) and genotype D with transfusion ($P < 0.001$) and HBe antibody positivity ($P = 0.03$).The distribution of HBV genotypes differed with regard to the ethnicity, and may reflect migration patterns. Genotypes A and D were the most frequent in France. Genotype A was associated with HBeAg positivity and genotype D with HBe antibody positivity. In our European patients, we find no clear association between a given HBV genotype and liver disease severity.

Keywords: epidemiology, France, genotype, hepatitis B, hepatitis B e antigen, liver fibrosis.

INTRODUCTION

Chronic hepatitis B infection is a major health problem, with approximately 400 million virus carriers worldwide [1]. Seven genotypes (A–G) of the hepatitis B virus (HBV) have been described, with a worldwide and characteristic geographic distribution [2,3]. A further genotype H has been discovered recently in American Indian patients [4]. Currently, four major HBV serologic subtypes (adw, ayw, adr and ayr) and nine minor subtypes have been identified by the antigenic determinants of the hepatitis B s antigen (HBsAg) [5]. The seven HBV genotypes (A–G) have been defined by a divergence higher than 8% in the entire HBV genome [5]. The 11–13% diversity of HBV sequences between HBV genotypes implies a nucleotide substitution rate of around 1.5×10^{-4} per site per year [6].

Serological and genotypic classifications of HBV are well documented. By contrast, the clinical significance of HBV genotypes in terms of clinical outcome [7–9] and therapeutic response to antiviral therapy in patients with chronic HBV infection [10] remains largely unknown. The HBV genotypes B and C are the most prevalent viral strains in Asian people [9]. Genotype C is associated with the development of cirrhosis and hepatocellular carcinoma (HCC) whereas genotype B may be associated with the development of HCC in noncirrhotic young patients [9]. In addition, genotype C is associated with a higher frequency of core promoter mutation and a lower response rate to alpha interferon therapy compared to genotype B [10]. Genotype E is restricted to Africa and genotype F is found in Central and South America, and Alaska [3]. The seventh HBV genotype, named

Abbreviations: HBV, hepatitis B virus; HbsAg, hepatitis B s antigen; HbeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus.

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G, has been discovered in France and has a high (>11.8%) sequence divergence and an increased genome length, with the insertion of 36 bp and two stop codons at positions 2 and 28 of the pre-C region that abolishes the production of the hepatitis B e antigen (HBeAg) [11,12].

Recent European or international studies have shown that genotypes A and D are predominant in Europe [13–15]. In Caucasian patients, a better response to peginterferon alpha-2b and lamivudine was found when patients were infected with the genotype A *vs* genotype D [14]. However, association between HBV genotypes and severity of the liver disease remains debatable, even if one study found more fibrosis in patients with genotype A *vs* genotype D [14].

The aims of the present study were to assess the distribution of HBV genotypes in France and to identify the associations between HBV genotypes and patient demographics, severity of liver disease and HBeAg status, in a retrospective group of patients from five *tertiary care* centres.

PATIENTS AND METHODS

Patients

This was a national (France), multicentre, retrospective, cross-sectional study performed in five centres located in the Paris and Southeast regions, according to the national regulations. The centres were gastroenterology or internal medicine units known for their specific expertise in hepatitis B. Consecutive outpatients having visited the centres during the 10-year period from January 1993 to December 2002 were included in the study provided they were aged 18 years or more and suffered chronic HBV infection documented by the presence of HBsAg in serum for at least 6 months. Selection criterion of patients was histological assessment determination by liver biopsy. However, among the 262 patients, 42 patients have no interpretable biopsy according to the Regev quality criteria (size ≥15 mm, portal tracts ≥5) or detectable viral load. Patients with previous antiviral treatment or vaccination, or having received liver transplant were excluded from the study. None of the patients had decompensated liver disease and none were hepatitis D virus positive.

Demographics, risk factors and general clinical information

Information relating to the patients' demographics, risk factors, virological status and hepatic disorders (clinical, biological and histological) were recorded anonymously and retrospectively from the patients' medical files. The demographic data included sex, date of birth and ethnicity. Risk factors were coinfection with the human immunodeficiency virus (HIV), coinfection with the hepatitis C virus (HCV) and alcohol abuse. The presumed source of HBV infection (drug use, transfusion, sexual, other or unknown) was recorded. Each of the specimens was divided into four aliquots and frozen at −80 °C within 2 h of collection.

Hepatitis B virological characteristics

The HBV status was determined by testing for HBsAg, HBeAg, anti-HBs, anti-HBe and anti-HBc antibodies (Axsym, Abbott Laboratories, Rungis, France). HBV genotype (A–G) determination was performed centrally (Alphabio Laboratory, Marseille, France) by reverse hybridization in a portion of the HBsAg region (nt 328–619) using the INNO-LiPA HBV Genotyping (Innogenetics Inc., Ghent, Belgium) line probe assay [16]. Genotype G was also confirmed by sequence analysis (Truegene, Bayer, Puteaux, France). Anti-HCV and anti-HDV were tested by commercially available assays (HCV EIA II, Anti-Delta; Abbott Laboratories).

Hepatic disorders (histological, biological)

The degree of liver fibrosis was evaluated by histological analysis of biopsy samples and expressed in METAVIR units [17]. Serum alanine aminotransferase (ALT) levels were also recorded. Patients were considered as alcohol consumers when their alcohol consumption was >30 g/day.

Statistical analyses

Data were analysed using the SAS® version 8.02 (SAS Institute Inc., Cary, NC, USA). Only patients with known HBV genotype were considered for the analyses. Missing data were not replaced. Descriptive statistics were provided for all variables. The Kolmogorov–Smirnov test for normality was used with a normality rejection threshold set at $P < 0.05$. Categorical variables were analysed using the chi-square or Fisher's exact test, continuous variables using the Student's *t*-test, with the risk α set at 0.05. Adjusted standardized residuals were estimated to identify significantly contributive cells when the chi-square test was significant. Factors related to liver fibrosis or HBeAg status were investigated using multiple logistic regression models with stepwise analyses. First-term interactions between variables retained in the final models were tested, with the risk α set at 0.10. Because of the low number of patients with genotypes B, C, E, F and G, we did not consider these genotypes in the analysis of the characteristics according to HBV genotypes.

RESULTS

A total of 286 HBsAg-positive patients were enrolled, of whom 170 were from Paris and 116 from Southeast France. Of them, 24 were not considered for further analyses, as among them, six had no HBV genotype determination, four were recorded as both HBeAg positive and HBe antibody positive and 14 were HIV coinfected.

Population characteristics

The main characteristics of the 262 analysed patients are given in Table 1. The population with an average age of 43 ± 15 years, comprised mainly males (195/262, 74%) and Caucasians (170/262, 63%). The proportion of patients with HCV coinfection and alcohol abuse was below 10% (6/262 and 19/262, respectively). The most frequent presumed mode of infection was sexual (42/262, 16%). It was, however, undetermined in 140/262 (53%) of the patients.

Hepatitis B virus phenotype was wild type (HBeAg positive) in 94/262 (36%) of the cases and mutant (HBe antibody positive) in 152/262 (58%) of the cases. As shown in Fig. 1, all A–G genotypes were found in this sample, the most frequent being genotypes D and A [70/262 (27%) and 64/262 (24%), respectively], followed by E and C [34/262 (13%) and 31/262 (12%), respectively], and B [19/262 (7%)]. Mixed genotypes were detected in 41/262 (16%) of the cases. Among the 41 patients with mixed genotypes, 27 (66%) were infected with genotype A, five (12%) with genotype B, five(12%) with genotype C, 27 (66%) with genotype D, seven (17%) with genotype E, nine (22%) with genotype F and 14 (34%) with genotype G. Frequencies of genotypes which occurred together are shown in Table 2.

Table 1 Demographics, risk factors and HBV status

	n (%) of patients
Male sex	195 (74)
Age (year)	
Mean (SD): 43 (15)	
<40	118 (45)
40–49	63 (24)
>49	81 (31)
Ethnicity	
Caucasian	170 (65)
African	47 (18)
Asian	45 (17)
Risk factors	
HCV positive	6 (2)
Alcohol abuse	19 (7)
Presumed source of HBV infection	
Drug use	8 (3)
Transfusion	23 (9)
Sexual	42 (16)
Vertical	36 (14)
Transplantation	13 (5)
Unknown	140 (53)
HBV phenotype	
HBeAg positive	94 (36)
HBe antibody positive	152 (58)
ALT elevated	134 (51)

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SD, standard deviation.

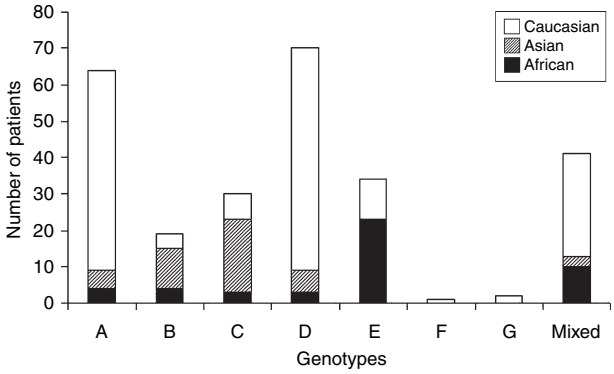

Fig. 1 Distribution of hepatitis B virus genotypes and patient ethnic background by genotype.

Table 2 Frequencies of mixed genotypes

Genotype	*n* (%)	Genotype	*n* (%)
AB	2 (5)	BEG	1 (2)
AD	10 (24)	BF	1 (2)
ADFG	3 (7)	CD	4 (10)
ADG	3 (7)	CDF	1 (2)
AE	2 (5)	DE	2 (5)
AF	2 (5)	DEG	1 (2)
AG	5 (12)	DF	2 (5)
BD	1 (2)	EG	1 (2)

Genotype G was carried by 16 patients. It was pure in two (13%) of them, associated with BE, DE and E in one case each, with ADF and AD in three (19%) cases each and with A in five (31%) cases. The genotypes associated with G were thus A (11/14 mixed genotypes) followed by D (7/14), E and F (3/14 each) and B (1/14). Genotype G was never associated with genotype C in our sample.

Of the 220 patients who underwent liver biopsy, 104 (47%) had no or portal fibrosis (F0 or F1 METAVIR score) and 116 (53%) had fibrosis with septa (F2–F4) including 39 (34%) with cirrhosis (F4). ALT levels were elevated in 66% of the cases.

Characteristics according to HBV genotypes

The population's characteristics by genotype are provided in Table 3 and Fig. 1. Patients carrying genotype D were older than those with mixed genotypes (respective mean age ± SD: 47 ± 17, 42 ± 12 with $P < 0.05$). About half of genotype D patients (33/70, 47%) were aged above 49, whereas only 31% of genotype A patients and 32% of mixed genotype were aged above 49 years.

Table 3 Demographics, HBV status and hepatic disorders according to the main HBV genotypes

	Genotype							
	A	B	C	D	E	F	G	Mixed
Sample size	64 (24)	19 (7)	31 (12)	70 (27)	34 (13)	1	2 (1)	41 (16)
Male sex	50 (78)	13 (68)	23 (74)	52 (74)	27 (79)	1	1	35 (85)
Age (year)								
Mean (SD)	43 (15)	40 (12)	38 (10)	47 (17)	41 (14)	40	37	42 (12)
<40	30 (47)	10 (53)	15 (48)	27 (39)	17 (50)	0	1	18 (44)
40–49	14 (22)	4 (21)	14 (45)	10 (14)	10 (29)	1	1	8 (20)
>49	20 (31)	5 (26)	2 (7)	33 (47)	7 (21)	0	0	13 (32)
Risk factors								
HCV positive	1 (2)	0 (0)	0 (0)	2 (3)	0 (0)	0	0	4 (10)
Alcohol abuse	3 (5)	0 (0)	2 (7)	7 (10)	2 (6)	0	1	4 (10)
Presumed source of HBV infection								
Drug use	3 (5)	0 (0)	0 (0)	2 (3)	0 (0)	0	0	3 (7)
Transfusion	2 (3)	0 (0)	0 (0)	16 (23)	2 (6)	0	1	2 (5)
Sexual	20 (31)	0 (0)	0 (0)	6 (9)	7 (21)	0	1	8 (20)
Vertical	3 (5)	7 (37)	13 (42)	5 (7)	7 (21)	0	0	1 (2)
Transplantation	0 (0)	0 (0)	0 (0)	10 (14)	1 (3)	0	1	1 (2)
Unknown	36 (56)	12 (63)	18 (31)	31 (44)	11 (32)	1	0	22 (54)
HBV phenotype								
HbeAg positive	29 (45)	6 (32)	14 (42)	19 (27)	14 (41)	1	1	10 (24)
HBe antibody positive	30 (47)	11 (58)	17 (55)	46 (66)	20 (59)	0	1	27 (66)
Liver fibrosis (METAVIR)								
F0 or F1	26 (41)	10 (53)	17 (55)	25 (36)	17 (57)	0	0	9 (22)
F2, F3 or F4	26 (41)	8 (42)	10 (32)	38 (54)	13 (38)	1	2	18 (44)
ALT elevated	29 (45)	4 (21)	15 (48)	46 (66)	17 (50)	1	2	21 (51)

ALT, alanine aminotransferase; F0–F1, no or portal fibrosis; F2–F3–F4, fibrosis with septa; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SD, standard deviation.
Values are expressed as *n* (%).

Genotype A was associated with sexual intercourse (31%, $P < 0.001$). Genotype D was associated with transfusion as the presumed source of infection (23%, $P < 0.001$).

The ethnic distribution of genotypes is displayed in Fig. 1. Genotype statistical comparison was performed after pooling the ethnicity data for genotypes B, F, G and mixed genotypes, because of the small sample sizes. The distribution of genotypes differed significantly ($P < 10^{-6}$) according to ethnicity. Genotypes A and D were almost exclusively carried by Caucasian patients. Genotypes B and C were mainly found in Asians, and genotype E in Africans.

Factors relating to HBV phenotype

As shown in Table 4, HBV genotype D was associated with HBe antibody positivity (30% *vs* 20% in HBeAg-positive patients, $P = 0.03$). Genotype A tended to be more frequent in HBeAg-positive patients (31% *vs* 20% in HBe antibody-positive patients, $P = 0.051$). In the group of Caucasian patients, genotype A was associated with HBeAg positivity (59% *vs* 33% in patients with other genotypes, $P = 0.001$).

Factors relating to liver disease severity

The population's characteristics by degree of liver fibrosis are provided in Table 4. The METAVIR fibrosis score was increased when patients were male ($P = 0.02$), older ($P = 0.001$ for mean age, $P = 0.002$ for age classes), Caucasian ($P = 0.064$), when the presumed source of infection was transfusion ($P = 0.004$) or vertical ($P = 0.01$) and when the serum ALT levels were above the upper limit of normal ($P < 0.001$). Although not significant, fibrosis was also more marked in patients carrying mixed genotypes ($P = 0.16$), whereas it was less severe in those with HBV genotype C ($P = 0.06$). Because the presumed source of infection was unknown in 140/262 (53%) of patients, and ALT is a measure of liver disease severity, the probability of having liver fibrosis with septa (F2–F3–F4) was modelled with a forward stepwise logistic regression model that did not include initially the source of infection and ALT. Only one variable was retained in the final model: the age of the patient (OR 1.04 $P = 0.001$). Centre effects and interaction terms were tested but not found significant in each tested model.

Table 4 Factors related to HBV phenotype or liver disease severity

	HBV phenotype			Fibrosis METAVIR score		
	HbeAg positive	Anti-HBe positive	*P*-value	F0–F1	F2–F3–F4	*P*-value
Sample size	94	152		104	116	
Male sex	76 (81)	115 (76)	0.5	79 (74)	98 (85)	0.02
Age (year)						
Mean (SD)	42 (15)	43 (14)	0.4	39 (12)	46 (15)	0.001
<40	46 (49)	64 (42)	0.4	60 (58)	42 (36)	0.002
40–49	19 (21)	42 (28)		22 (21)	31 (27)	
>49	29 (31)	46 (30)		22 (21)	43 (37)	
Ethnicity						
Caucasian	61 (65)	97 (64)	0.2	58 (56)	81 (70)	0.064
African	13 (14)	33 (22)		21 (20)	19 (16)	
Asian	20 (21)	22 (14)		25 (24)	16 (14)	
Risk factors						
HCV positive	2 (2)	5 (3)	0.7	1 (1)	5 (4)	0.2
Alcohol abuse	6 (6)	12 (8)	0.5	8 (8)	8 (7)	0.7
Presumed infection source						
Drug use	2 (2)	5 (3)	0.7	4 (4)	4 (3)	1
Transfusion	8 (9)	13 (9)	0.8	3 (3)	17 (15)	0.004
Sexual	16 (17)	24 (16)	0.3	24 (23)	13 (11)	0.2
Vertical	10 (11)	26 (17)	0.16	26 (25)	10 (10)	0.01
Transfusion	6 (6)	6 (4)	0.34	2 (2)	11 (11)	0.05
Unknown	52 (55)	78 (51)	0.9	45 (43)	63 (54)	0.1
HBV phenotype						
HBeAg positive	–	–	–	39 (38)	40 (35)	0.8
HBe antibody positive	–	–	–	63 (61)	66 (57)	0.7
HBV genotype						
A only	29 (31)	30 (20)	0.051	26 (25)	27 (23)	0.8
B only	6 (6)	11 (7)	0.7	10 (10)	8 (7)	0.4
C only	14 (15)	17 (11)	0.6	17 (16)	10 (9)	0.06
D only	19 (20)	46 (30)	0.03	25 (24)	37 (32)	0.3
E only	14 (15)	20 (13)	0.9	17 (16)	13 (11)	0.2
F only	1 (1)	0 (0)	0.4	0 (0)	1 (1)	1
G only	1 (1)	1 (1)	0.6	0 (0)	2 (2)	0.3
Mixed	10 (11)	27 (18)	0.6	9 (9)	18 (16)	0.2
Liver fibrosis (METAVIR)						
F0 or F1	39 (42)	63 (42)	0.3	–	–	–
F2, F3 or F4	40 (43)	68 (45)		–	–	
ALT elevated	57 (61)	70 (46)	0.1	32 (31)	79 (68)	<0.001

ALT, alanine aminotransferase; F0–F1, no or portal fibrosis; F2–F3–F4, fibrosis with septa; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SD, standard deviation.
Values are expressed as *n* (%).

DISCUSSION

This study is one of the first attempts to investigate the clinical significance of HBV genotypes in France. All HBV genotypes (A–G) were represented in this sample recruited in two demographically major French regions. While the hypothesis of a potential role of some HBV genotypes in the liver fibrosis process was not confirmed by our results, we obtained some evidence to suggest the prevalence of HBe antibodies in patients chronically infected with HBV.

Our results showed a predominance of genotypes A and D in agreement with a previous study [18]. As evidenced by previous studies on the geographic distribution of HBV, genotypes A and D were predominantly found in Caucasians, genotypes B and C in Asians and genotype E in Africans. A high prevalence of mixed HBV genotypes (17%) has

also been found in our sample, which is consistent with the results of another French group [19], but not with US data where no patients had mixed genotypes [20]. In our patients with mixed genotypes, the majority had genotype A, followed by genotypes D and G. A previous virological and epidemiological study reported that genotype G would be selected, accompanied by the recombination with genotype A [12]. As far as we know, our data are the first to find genotype G independently of genotype A. Genotype G was pure in three cases who were recruited in the same centre in Paris and had liver cirrhosis.

Interestingly, 58% of our patients were found positive for HBe antibodies and one patient was recorded as both HBeAg positive and HBe antibody positive. This finding is consistent with the recent results of a nationwide survey performed in France by Cadranel *et al.* [21]. Nevertheless, genotypic analysis performed in a subgroup of patients revealed a mix of wild type and mutant HBV in the majority (80%) of our patients (data not shown). The mutations in the precore stop codon (G1896A) and the basal core promoter (A1762T and G1764A) were more frequently found in HBeAg-negative patients. These findings are in agreement with those of a US nationwide study [20].

The cross-sectional design of our study could not answer the issue of the association of HBV genotypes with HBeAg seroconversion which has been suggested by recent data [8,22]. Our study did not confirm previous results that have suggested a higher prevalence of HBeAg in patients with genotype B compared to genotype C (33% *vs* 45%, $P = 0.51$) [21], but our sample was small. However, we found that genotype A was associated with a higher prevalence of HBeAg. In Caucasian patients, who represented the majority of our patients, genotype A was also significantly associated with a higher prevalence of HBeAg. This result has also been found in a US nationwide study [20].

The issue of the impact of HBV genotype on the severity of liver disease has been studied by several authors, especially in Asia. HBV genotype C has been suggested to be prevalent in patients with cirrhosis and in those with HCC who were older than 50 years compared with age-matched asymptomatic carriers (60% *vs* 23%, $P < 0.001$ and 41% *vs* 15%, $P = 0.005$, respectively) [9]. In our sample, we did not find higher prevalence of liver fibrosis (METAVIR F2, F3 or F4) in patients with genotype C compared to those with genotype B. However, the proportion of patients with genotypes B and C in our sample, 7 and 12% respectively, was too small (19 and 31) to investigate the issue with sufficient statistical power. Nevertheless, we observed a statistical significance between the age of the patients according to ethnicity: Caucasians were older than Africans and Asians with respective ages of 45.3 (±14.6), 38.4 (±12.2) and 37.9 (±9.9) years ($P = 0.005$ and $P = 0.003$). The severity of the liver disease is influenced by the age of the patients. An absence of correlation was found between the severity of liver disease and positive viral load of more than 400 copies/mL (data not shown).

Our data corroborate the results recently reported by Chu *et al.* [19] who did not find any significant relationship with cirrhosis and HBV genotypes. We can hypothesize that, as it has been shown for hepatitis C, HBV genotype could be predictive of the response to antiviral therapy but not related to the severity of liver disease in naive patients.

In conclusion, the lack of an association of HBV genotypes and disease severity from this retrospective study does not exclude the possibility that this may still be the case. Only a population-based study of all HBV patients performed in a prospective manner could definitively evaluate this, because there may be a bias to refer Caucasians with more advanced liver disease to tertiary care centres.

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