

Hepatitis B genotypes: Relation to clinical outcome in patients with chronic hepatitis B in Saudi Arabia

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Abstract

AIM: To identify the most common hepatitis B virus (HBV) genotype in Saudi Arabia, and correlate the prevailing genotypes with the clinical outcome of patients.

METHODS: Patients were consecutively recruited from the hepatology clinics of two tertiary care referral centers. Patients were categorized into 4 different groups: group 1, patients with hepatitis B and normal liver enzymes; group 2, patients with hepatitis B and abnormal liver enzymes but without cirrhosis; group 3, patients with hepatitis B and liver cirrhosis; group 4, patients with hepatitis B and hepatocellular carcinoma. All patients had a positive hepatitis B surface antigen (HBsAg). Genotyping of HBV was performed by nested PCR-mediated amplification of the target sequence and hybridization with sequence-specific oligonucleotides.

RESULTS: Seventy patients were enrolled in this study. They were predominantly male (72.9%) in their mid-forty's (mean age 47 years). Forty-nine (70%) patients were hepatitis B envelope antigen (HBeAg) negative. The majority of patients (64%) acquired HBV through unknown risk factors. Hepatitis B genotyping revealed that 57 patients (81.4%) were genotype D, 1 patient (1.4%) had genotype A, 1 patient (1.4%) had genotype C, and 4 patients (5.7%) had genotype E, while 7 patients (10%) had mixed genotype (4 patients ADG, 1 patient DE, 1 patient DF, and 1 patient ADFG). Based on univariate analysis of genotype D patients, significant predictors of advanced liver disease were age, gender, aspartate transaminase, alanine transaminase, albumin,

bilirubin, and alkaline phosphatase (all $P < 0.001$). In multivariate analysis decreased hemoglobin ($r = -0.05$; 95% CI: -0.08 to -0.03; $P = 0.001$) and albumin levels ($r = -0.004$; 95% CI: -0.007 to -0.001; $P = 0.002$) were highly significant predictors of advanced liver disease. In patients with HBV genotype D, HBeAg negativity was found to increase across advancing stages of liver disease ($P = 0.024$).

CONCLUSION: This study highlights that the vast majority of Saudi patients with chronic hepatitis B have genotype D. No correlation could be observed between the different genotypes and epidemiological or clinical factors. The relationship between genotype D and HBeAg status in terms of disease severity needs to be further elucidated in larger longitudinal studies.

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Key words: Hepatitis B virus; Genotype D; Cirrhosis; Hepatocellular carcinoma; Saudi Arabia

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INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem with over 350 million chronic carriers of the virus with the risk of developing chronic hepatitis, cirrhosis or hepatocellular carcinoma (HCC). HBV is a circular, partially double-stranded DNA virus of approximately 3200 nucleotides. This highly compact genome contains four open reading frames encoding the envelope (PreS1, PreS2, S), core (core, precore), polymerase, and X proteins^[1]. HBV genotypes represent naturally occurring strains of HBV that have evolved over the years and reflect the geographical distribution of HBV throughout the world. Up to now, eight different HBV genotypes have been identified and shown to cluster in different areas of the world^[2,3]. They display an 8% inter-group divergence in the complete nucleotide sequence of HBV and differences

in the nucleotide homology of the surface gene, which result in different hepatitis B surface antigen (HBsAg) serotypes^[4-6].

Genotype A is mainly found in Northwestern Europe, North America and Africa^[7], whereas genotypes B and C have been described in South-Eastern Asian populations^[8,9]. Genotype E and F are seen in East Africa and the New World, respectively. Genotype D is most often found in southern Europe, parts of Central Asia, India, Africa and the Middle East. Genotype G is a recently determined genotype in France, America, and Germany while genotype H has been reported in patients from Central America^[10,11].

Recently, a number of publications have examined the impact of HBV genotype on disease pathogenesis and the clinical outcomes in patients with chronic hepatitis B. Most of these natural history studies, in view of the bimodal distribution of HBV genotypes in Asia and Western countries, have compared either genotypes B and C^[11,12], or genotypes A and D^[13]. The clinical impact of HBV genotypes when studied in Indian patients with a mixed population of both genotypes A and D, has shown that genotype D has a higher likelihood of developing advanced cirrhosis compared to genotype A^[13].

Chronic hepatitis B is an important medical problem in Saudi Arabia although, with the implementation of HBV vaccination of children, the prevalence has dramatically reduced from 6.7% in 1989 to 0.3% in 1997^[14,15]. However, little is known about the prevalence and distribution of HBV genotypes in Saudi Arabia. Furthermore, the association between the distinct genotypes and the severity of liver disease in the country remains unreported. The epidemiological studies of HBV genotypes arising from the Middle-Eastern region suggest that genotype D is perhaps the most common^[16-20]. Accordingly, the objectives of our study were to identify the most common HBV genotype in Saudi Arabia, and to elucidate the relationship between the prevailing genotypes with the clinical outcome of patients.

MATERIALS AND METHODS

Enrollment of study cohort

Patients were recruited prospectively from two tertiary-care hepatology clinics at King Khalid University Hospital and the Riyadh Military Hospital in Riyadh, Saudi Arabia. These medical centers serve as referral centers for population groups resident in different geographical regions of the country. The Medical Ethics Committee in both centers approved the study protocol and all patients signed an informed medical consent indicating agreement to participate in this study. Results of all abnormal tests were provided to patients or their immediate relatives, as was deemed appropriate.

Between May 2004 to December 2005, 70 consecutive adult HBsAg positive patients were recruited at the two centers. Patients were interviewed in person by participating investigators (AAA, FMS, NA, KS) at recruitment by using a structured questionnaire. Information on socio-demographic characteristics, alcohol consumption, personal medical and surgical history, time

of disease diagnosis, area of birth and upbringing, and family history of liver disease or cancers was collected. Patients who were HBsAg positive for a period exceeding 6 mo and who had not received any antiviral therapy for HBV in the preceding 6 mo were included in the study.

Serological evaluation

HBV markers [HBsAg and antibody to HBsAg (HBsAb)] were measured using standard commercial assays. In addition, further serological testing for hepatitis B envelope antigen (HBeAg) and anti-HBe antibody was also performed using the same commercial kits. HBV DNA was determined by a sensitive PCR based assay (COBAS Amplicor; Roche Diagnostics) with a lower limit of detection of approximately 200 copies/mL.

Virologic testing

Genotypic testing was performed in only those with a detectable HBV DNA (qualitative) in serum. HBV genotyping was determined from serum samples by performing nested PCR-mediated amplification of the target sequence and hybridization with sequence-specific oligonucleotides at Bioscientia Laboratory in Germany.

Study design

The patients were recruited into four groups: group 1, patients with hepatitis B and normal liver enzymes; group 2, patients with hepatitis B and abnormal liver enzymes and no laboratory or radiological features of cirrhosis; group 3, patients with liver cirrhosis secondary to hepatitis B; and group 4, patients with hepatitis B and HCC. General exclusion criteria included: (1) anti-HCV antibody positive; (2) identifiable other causes of chronic liver disease defined as (high serum iron and ferritin, abnormal serum ceruloplasmin, history of significant alcohol consumption, antinuclear antibody > 1:320, antismooth muscle antibody > 1:320, antimitochondrial antibody > 1:40); (3) history of hepatotoxic medications in the preceding three months of presentation; (4) history of antiviral therapy in the last 6 mo.

Group specific inclusion criteria for group 1 (HBV with normal liver enzymes) were: (1) persistently normal alanine transaminase (ALT) and aspartate transaminase (AST) (normal ALT and AST on at least two occasions separated by at least 3 mo), (2) normal serum bilirubin, albumin and International Normalized Ratio (INR), (3) normal complete blood count (CBC), (4) normal abdominal ultrasound (US) without features of liver cirrhosis or portal hypertension. Inclusion criteria for group 2 (HBV with abnormal liver enzymes) included: (1) persistently elevated ALT and AST (more than two times the upper limit of normal on at least two occasions separated by at least 3 mo), (2) normal CBC, (3) normal US without features of cirrhosis or portal hypertension. Patients were excluded from groups A and B if there was any abnormality in the CBC or any signs of cirrhosis or portal hypertension on abdominal US. Inclusion criteria for group 3 (HBV with liver cirrhosis) included: Any four of the following features of cirrhosis (1) platelet count < $100 \times 10^9/L$, (2) evidence of esophageal varices on endoscopy, (3) ultrasonographic features consistent with cirrhosis, (4)

albumin level less than 30 g/L, (5) INR more than 1.4 and (6) bilirubin level more than 30 $\mu\text{mol/L}$. Patients were also included in this group if there was histological evidence of liver cirrhosis regardless of the above criteria. Inclusion criteria in group 4 (HBV with HCC) included: Evidence of HCC defined as two of the followings: (1) α -fetoprotein > 400 ng/L, (2) liver mass detected by triphasic computed tomography (CT) or magnetic resonance imaging (MRI) of the abdomen, (3) fine needle aspiration (FNA) or liver biopsy showing HCC.

Ascertainment of cirrhosis and hepatocellular carcinoma

All participants had screening abdominal ultrasonography (US) at the time of recruitment into the study. The US was performed and interpreted by trained radiographers according to a standardized protocol, and the records reviewed by the investigators. Cirrhosis was diagnosed ultrasonographically based on the appearance of the liver surface, liver parenchymal texture, portal vein size, splenic size, presence of ascites and varicose veins in the portal and perisplenic area.

All patients with an α -fetoprotein > 400 ng/L (or a persistently rising α -fetoprotein) or with high clinical suspicion of HCC underwent a CT and/or MRI of the liver. The diagnosis of HCC was based upon the two published European guidelines towards the diagnosis and management of HCC^[21,22]. Enhancement of a liver lesion during the arterial phase and contrast washout during the portal phase, in patients with background cirrhosis secondary to HBV was considered diagnostic of HCC. FNA or liver biopsy was obtained only where considerable doubt existed towards HCC diagnosis.

Statistical analysis

Descriptive statistics are summarized as mean \pm SD. For continuous variables, ANOVA was used for comparison of the four groups. Fisher's exact or chi-square test was used for categorical variables. Univariate analysis was performed to identify important baseline characteristics associated with genotype D. Variables examined included age, gender, White blood cells, hemoglobin, platelet count, HBeAg status, albumin, ALT, AST and bilirubin. A multivariate logistic regression model was developed using forward model to assess the effect of baseline variables on disease advancement. All tests were two sided with a 5% level of significance. All analyses were performed using STATA 9.1.

RESULTS

Seventy patients were enrolled in the period from May 2004 to Dec 2005. Mean age of the patients was 47 years, and 51 patients (72.9%) were male while 19 (27.1%) were female. All geographical regions of the Kingdom were represented, with 51.5% from the central region where the vast majority of the Saudi population reside and also where the study was conducted; 21.4% from the southern region, 8.5% from the eastern region, 13% from the western region and 5.6% from the northern region, vastly representing the population distribution across the country. There were 17 patients included in group 1, 22 patients in

Table 1 Biochemical, hematological and virological parameters of all enrolled patients distributed across the four groups

Parameter (mean value)	Group 1 (n = 17)	Group 2 (n = 22)	Group 3 (n = 19)	Group 4 (n = 12)	P
ALT (U/L)	41.88	177.7	52	96	0.001
AST (U/L)	22	80	55.3	150	0.001
ALP (U/L)	100	111	116.4	238.8	0.001
INR	1.3	1.1	1.4	1.2	0.003
Bilirubin ($\mu\text{mol/L}$)	10	13.8	40.4	30	0.001
Albumin (g/L)	40	37.6	28.9	25.4	0.005
WBC ($10^9/\text{L}$)	7.1	6.1	4.9	7.2	0.160
Hemoglobin (g/L)	125	129.6	118.4	62	0.740
Platelets ($10^9/\text{L}$)	253	202	121.3	213	0.230
HBV DNA level					
< 200 copies/mL	5	2	3	1	NS
200- 10^5 copies/mL	10	8	8	8	NS
> 10^5 copies/mL	2	12	8	3	NS

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase; WBC: White blood cells.

group 2, 19 patients in group 3, and 12 patients in group 4. The majority (45 of 70 patients, 64%) had acquired HBV through unknown risk factors, while 11 patients (15.7%) reported blood transfusion, 7 (10%) reported a prior history of surgery or dental procedures and 4 (5.7%) reported a family history of HBV infection.

Most patients (52 of 70 patients, 74%) did not express HBeAg including 65% (11 of 17 patients) in group 1, 55% (12 of 22 patients) in group 2, 95% (18 of 19 patients) in group 3, and 92% (11 of 12 patients) in group 4. Amongst the patients who were HBeAg positive, only two patients (11.1%) had cirrhosis or HCC. The vast majority of HBeAg positive patients were either carriers with normal ALT (6 of 18 patients, 33.3%), or raised ALT (10 of 18 patients, 55.6%). One patient expressed HBeAg as well as anti-HBe. Two patients were negative for both markers. In group 1, 15 patients (88.2%) had HBV DNA < 10^5 copies/mL and were also HBeAg negative. Two patients (11.8%) had HBV DNA > 10^5 copies/mL and were also HBeAg positive. In group 2, 9 patients (41%) had an HBV DNA < 10^5 copies/mL and were all HBeAg negative. In group 3, 11 patients (58%) had an HBV DNA of < 10^5 copies/mL and all were also HBeAg negative. In group 4, 9 patients (75%) had an HBV DNA of < 10^5 copies/mL and all were also HBeAg negative. Although the proportion of patients with a high viral load (> 10^5 copies/mL) was greater in groups 2 and 3, this did not reach statistical significance (Table 1).

Table 2 shows the genotyping results according to the clinical status of the studied patients. The majority of the patients were genotype D (57 of 70 patients; 81.4%), with 58.8% (10 of 17 patients) in group 1, 95.5% (21 of 22 patients) in group 2, 84.2% (16 of 19 patients) in group 3, and 83.3% (10 of 12 patients) in group 4. One patient (1.4%) had genotype A, and one (1.4%) had genotype C. Four patients (5.7%) were genotype E, and 7 patients (10%) were mixed genotypes (4 patients ADG, 1 patient DE, 1 patient DF, 1 patient ADFG). There were no significant differences between groups in terms of

Table 2 HBV genotype distribution across different stages of liver disease and in relation to HBeAg status in 70 patients

Study cohort (n)	HBV genotype % (n)				
	D	E	A	C	Mixed
Group 1 (17)	58.8 (10)	-	5.9 (1)	-	35.3 (6)
Group 2 (22)	95.5 (21)	-	-	4.5 (1)	-
Group 3 (19)	84.2 (16)	15.8 (3)	-	-	-
Group 4 (12)	83.3 (10)	8.3 (1)	-	-	8.3 (1)
HBeAg + (18)	88.9 (16)	-	5.6 (1)	5.6 (1)	-
HBeAg - (52)	78.8 (41)	7.7 (4)	-	-	13.5 (7)

genotypes. In addition, we found no difference between different genotypes in terms of patient age, gender, area of upbringing, liver enzyme level, or serum albumin level. HBeAg negative genotype D patients comprised 50%, 57%, 93.8% and 75% across groups 1, 2, 3, and 4 respectively ($P = 0.024$) (Table 3).

The baseline characteristics according to disease severity (groups 1 to 4) are listed in Table 1. Patients in group 4 (HCC) were more likely to have a higher level of alkaline phosphatase (ALP) than those in groups 1, 2 and 3 respectively, (238 *vs* 100, $P = 0.004$; 95% CI 45-230), (238 *vs* 111, $P = 0.003$; 95% CI 45-208), (238 *vs* 116, $P = 0.036$; 95% CI 6.8-201), and also a higher level of AST than those in groups 1, 2 and 3 respectively (150 *vs* 22, $P = 0.001$; 95% CI 52-203), (150 *vs* 80, $P = 0.06$; 95% CI 3-145), (150 *vs* 60, $P = 0.02$; 95% CI 13-65). Furthermore, group 4 (HCC) patients were more likely to have a significantly lower level of albumin. In addition, 83.3% (10 of 12) of the patients in group 4 were genotype D compared to 81.4% (57 of 70) of the recruited patients who expressed the same genotype ($P = 0.30$). However, there was no significant difference in WBC, hemoglobin, and platelet count between the four groups.

Table 3 depicts the patients with genotype D according to disease severity. Patients in group 4 were older (mean age 61.5 years), tended to have higher levels of ALT, AST, and bilirubin, and lower levels of albumin. These differences were significant between the groups (all $P = 0.001$). However, there was no significant difference between the four groups with respect to hemoglobin ($P = 0.70$), platelet count ($P = 0.11$) and WBC ($P = 0.10$). In view of the small sample size in each group we did not perform a head-to-head comparison between the groups.

In univariate analysis of genotype D patients, age, gender, AST, ALT, albumin, bilirubin, and ALP were significant predictors of advanced liver disease (all $P < 0.001$). However, in multivariate analysis decreased hemoglobin ($P = 0.001$) and albumin levels ($P = 0.002$) were highly significant predictors of advanced liver disease (Table 4).

DISCUSSION

Chronic hepatitis B is an important medical problem in Saudi Arabia. Al-Faleh *et al* in the late 1980's showed that up to 7% of Saudi children were positive for HBsAg^[14]. After the introduction of universal vaccination of all Saudi children in 1989, the incidence of hepatitis B

Table 3 Hepatitis B virus genotype D patients' biochemical, hematological and virological parameters distributed across the four groups (mean \pm SD)

Parameter	Group 1 (n = 10)	Group 2 (n = 21)	Group 3 (n = 16)	Group 4 (n = 10)	P
Age (yr)	45 \pm 18	35 \pm 9.9	52.1 \pm 7.7	61 \pm 12	0.015
ALT (U/L)	40.3 \pm 8	176.9 \pm 100.7	54.1 \pm 30.4	99.3 \pm 79.9	0.001
AST (U/L)	21.1 \pm 6.5	78.6 \pm 56.8	63.8 \pm 48.5	158.3 \pm 167.6	0.001
ALP (U/L)	98.6 \pm 39.9	112.6 \pm 41	143.8 \pm 6.2	239.9 \pm 201	0.001
Bilirubin (μ mol/L)	10.6 \pm 4.9	14.1 \pm 10.2	48.6 \pm 60.4	27.8 \pm 23.2	0.001
Albumin (g/L)	41.5 \pm 3.2	37.8 \pm 4.6	27.8 \pm 7.9	24.7 \pm 8.4	0.007
WBC (10^9 /L)	7.5 \pm 2.2	6.3 \pm 1.7	6.12 \pm 3.1	6.2 \pm 1.9	0.100
Hemoglobin (g/L)	127.7 \pm 42.5	129.2 \pm 57.5	65.5 \pm 55.9	62.5 \pm 54.5	0.704
Platelets (10^9 /L)	250 \pm 84.3	207.2 \pm 56	130.8 \pm 56	217.6 \pm 97.4	0.113
HBeAg -/+	5/5	12/9	15/1	9/1	0.024

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase; WBC: White blood cells.

Table 4 Predisposing factors for advanced hepatitis B virus genotype D liver disease

	Variable	Coefficient	95% CI	P
Univariate analysis	Age	-0.01	-0.02 to 0.0005	< 0.001
	Gender	0.5	0.03 to 1.1	< 0.001
	AST	0.2	0.09 to 0.3	< 0.001
	ALT	0.04	0.01 to 0.03	< 0.001
	ALK	0.01	-0.001 to 0.02	< 0.001
	Albumin	-0.2	-0.3 to -0.9	< 0.001
Multivariate analysis	Bilirubin	0.08	0.0009 to 0.1	< 0.001
	Hemoglobin	-0.05	-0.08 to -0.03	0.001
	Albumin	-0.004	-0.007 to -0.001	0.002

CI: Confidence interval; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase.

infection has declined to as low as 0.3%^[15]. In spite of this dramatic decline, the burden of decompensated liver disease secondary to hepatitis B is expected to increase significantly in the next 40 years as the previously infected children start aging.

Hepatitis B genotyping has received immense attention recently and its clinical implications are being investigated extensively throughout the world. This study is the first to show that genotype D is the most prevalent genotype in Saudi Arabia. In this prospective study all regions of the country are represented as well as the entire spectrum of chronic HBV infection ranging from the carrier state to HCC. Our study concurs with reports from other parts of the Middle East like Egypt, Yemen, Turkey, Iran, and Tunisia^[16-20], all showing that genotype D is the most common genotype in this region.

HBV genotypes may contribute in part to the wide variation in prevalence rates of HBV infection in different parts of the world through difference in rates of replication and ability to evade immune clearance. However, studies comparing the replication capacity and immune response of the various HBV genotypes have not been performed. Nevertheless, many studies have shown a strong relationship between HBV genotypes and mutations in the precore and core promoter regions that abolish or

diminish the production of HBeAg^[23-26]. In our population which is predominantly HBeAg negative (presumably indicating pre-core or core promoter mutation), the majority of patients had genotype D thereby supporting the above observation.

Hepatitis B genotypes have been correlated with various epidemiological, virological, and clinical variables. It has been recently reported from China that patients with genotype B have a lower prevalence of HBeAg than those with genotype C^[12]. Several other studies reported a correlation between HBV genotype and HBeAg clearance. Similarly in our study, we found a lower prevalence of HBeAg among our patients with genotype D (28%), suggesting that HBeAg clearance occurred at higher rates among patients with genotype D.

Correlation between the clinical outcomes of patients with HBV and their genotypes has also been reported. One study found that HBsAg carriers with genotype B had lower histological activity scores^[23]. Three other studies involving a total of 939 Chinese patients with chronic HBV infection found that genotype C was more prevalent in patients with cirrhosis^[27-29]. In our study, while 58.8% in group 1 (HBV with normal ALT) were genotype D, the number of patients with the same genotype across advancing stages of liver disease, in groups 2, 3, and 4 comprised 95.5%, 84.2%, 83.3% respectively ($P = 0.4$). These findings suggest that genotype D does not correlate with advancing liver disease. However, this could be related to the small sample size of the present study, and probably due to the predominance of genotype D in all clinical forms. Further analysis in large-scale longitudinal studies is required to better delineate this relationship.

In our patients with HBV genotype D, HBeAg negativity was found to increase significantly across more advancing stages of liver disease ($P = 0.024$). Previous studies have revealed that HBeAg positive genotype C^[6] or genotype A^[30] patients were more likely to have active liver disease. Progress of liver disease in relation to HBeAg status has not been reported as yet in the various studies dealing with genotype D^[13,19,20]. Although this observation is significant, the association between HBeAg status and genotype D, in terms of severity of liver disease needs to be studied further before any additional conclusions can be derived.

Since predictors of advanced HBV liver disease were described before as a whole or in relation to individual genotypes other than in genotype D^[31], our study describes for the first time these predictors in genotype D. Hemoglobin and albumin levels are independent predictors of advanced liver disease. Furthermore, group 4 patients (HCC) with genotype D showed significantly higher biochemical parameters (AST, ALT, and bilirubin) and lower albumin levels compared to early stage liver disease.

The relation between HBV genotype and HCC is inconclusive. One study found that genotype B was associated with hepatocellular carcinoma at an earlier age^[28], but this finding was not confirmed by other studies^[11,27,32]. Another study in Indian patients reported that genotype D was commonly found to be associated with HCC in patients < 40 years of age^[13]. However, in

our study, none of our HCC patients with genotype D (10 patients) were < 40 years of age (mean age 61 ± 12).

In Saudi Arabia HCC is the second most common cancer in men^[33], and in a country where hepatitis B is endemic this could imply a correlation between the most prevalent genotype (D) and HCC. In our patients, 83.3% of the patients in group 4 (HCC) were genotype D compared to 81.4% of the overall number of recruited patients who expressed the same genotype. This figure did not reach statistical significance ($P = 0.30$). Moreover, since HBV is thought to be directly carcinogenic because of the integration of HBV DNA into the cellular DNA of the host^[34], it may also explain the observed lack of correlation between genotype D and the development of HCC.

There were no significant differences between groups in terms of genotypes. This is likely secondary to the fact that the vast majority of patients had genotype D making comparisons with the other relatively rarer genotypes difficult. Along with the limitations imposed by the small sample size across the different groups, the study was also restricted by the absence of histology in groups 1 and 2, thereby possibly misallocating some of these patients into either more or less active groups.

In conclusion, genotype D is the most common genotype in Saudi Arabia. Because of the fact that the vast majority of the patients have genotype D, no correlation could be observed between different genotypes and epidemiological or clinical factors. A large-scale study is required to obtain further information on the role of genotype D and its impact on the progress of liver disease.

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