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Research Paper

Relation between hepatitis B virus genotypes and gene mutation of basic core promoter in Li nationality ☆

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Abstract

Objective: To investigate the relation between hepatitis B virus(HBV) genotypes and the double mutation of A-to-T nucleotide(nt) 1762 and G-to-A nt 1764 in basic core promoter(BCP T1762/A1764) in patients of the Li nationality. **Methods:**Subjects were 125 HBV DNA positive patients that belong to the Li nationality on Hainan Island. HBV DNA genotype was determined by real time fluorimetry polymerase chain reaction. BCP T1762/A1764 mutation was performed using the direct sequencing method. **Results:**The prevalence rates of genotype B, genotype C, genotype D, genotype C and D mixed infection(genotype C + D) and genotype B and D mixed infection (genotype B + C) were 31.20%, 53.60%, 12.00%, 2.40% and 0.80% respectively. Mutation frequencies in patients infected with HBV genotype C(58.21%) were significantly higher than in those infected with other genotypes ($P<0.01$). The serum viral load of the patients with genotype C(5.74 ± 1.21) was also higher than that of those with genotype B($P<0.01$). **Conclusion:**The major genotypes in the Li nationality were genotype C and genotype B. The infection of genotype D and mixed infection also occurred in the Li nationality. Genotype C HBV has a higher replication rate, and the different degrees of pathogenecity among HBV genotypes may be related to BCP T1762/A1764 mutation frequency.

Keywords: hepatitis B virus; genotype; gene mutation; Li nationality

INTRODUCTION

Hepatitis B virus(HBV) is a major global health problem. Worldwide there are about 2 billion people who are infected with HBV; and more than 350 million people are chronic carriers of the virus[1-2]. Approximately 200 million Chinese are infected with HBV[3]. Chronic HBV infection is the cause of up to 50% of the cases of cirrhosis and 70%~90% of the cases of hepatocellular carcinoma(HCC) in China[4].

HBV infection can cause a broad spectrum of clinical outcomes, ranging from asymptomatic carriage, self-limiting acute hepatitis, fulminant hepatic failure, chronic liver disease, and cirrhosis to hepatocellular carcinoma. Viral gene mutations are important factors that cause different clinical outcomes with HBV infection. Based on a nucleotide sequence divergence > 8% in the complete nucleotide sequence, HBV has been classified into eight genotypes, designated A~H[4-5]. The geographical distribution of HBV genotypes differs. HBV genotype A has a specific geographic distribution, and is found predominantly in Northwest Europe, North America, central and sub-Saharan Africa; genotypes B and C are found in southeast Asia, China, and Japan; genotype D in the Mediterranean, the Middle East, and India; genotype E in Africa; genotype F in America; genotype G in the United States and France, and genotype H in central America[4-6]. HBV genotypes have been shown to play a role in the clinical features and disease outcome, and in the response to antiviral therapy[7].

It is also known that the HBV genomic sequence variations may influence the pathogenicity of HBV[8-10]. The double mutation of A-to-T nucleotide (nt) 1762

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and G-to-A nt 1764 in basic core promoter(BCP T1762/A1764) is one of the common mutations. The mutation might be associated with hepatic injury and HBV replication[8-10]. However, study of the relation between HBV genotypes and BCP T1762/A1764 mutation has not been undertaken in China.

The Li nationality arose from the Baiyue people of China in ancient times. Since the Stone Age, their ancestors have lived in the middle region of Hainan Island, which lies in the Tropics. The Li seldom intermarried with people of other nationalities. To date, no study has been undertaken on HBV genotypes and BCP T1762/A1764 mutations in the Li nationality. However, it is important to know this information for effective health control and treatment. The aim of this study was to investigate HBV genotypes and BCP T1762/A1764 mutations in the Li nationality.

MATERIALS AND METHODS

Clinical data

125 patients with HBV infection belong to the Li nationality on Hainan Island. All patients had attended the Outpatient Department or been in the Inpatient Department of the Affiliated Hospital of Hainan Medical College from February 2005 to September 2008. Clinical data and clinical histories of patients were recorded. All patients were clinically diagnosed with chronic hepatitis B according to the criteria that the Chinese Society of Hepatology and the Chinese Society of Infectious Diseases instituted in 2007[11]. The patients consisted of 66 males and 59 females, age range 17~64 years(mean 31.73±8.52 years). Serum HBV DNA was tested, and only HBV DNA positive patients were used in the study. Patients were also screened for other viral hepatitis, autoimmune hepatitis and acquired immune deficiency syndrome(AIDS). Positive samples were stored in −20℃.

This study protocol was performed to conform to the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Hainan Medical College.

HBV Genotyping

The HBV genotype was determined by real time fluorimetry polymerase chain reaction(PCR) with HBV genotyping Kits(Guangzhou Huayin Medicine Science Limited Company, Guangzhou, China). DNA was extracted from 50 μl serum using DNA extraction Kits (Guangzhou Huayin Medicine Science Limited Company, Guangzhou, China). Real time fluorimetry PCR was carried out using 1U Taq polymerase, 2 μl template and 23 μl reaction liquid of B, C, D type. The real time fluorimetry PCR included an initial activation step that was incubation at 94℃ for 60 s and forty cycles of repeated incubation at 94℃ for 5 s, and 60℃ for 30 s. The used fluorescein was FAM, and fluorescein values were read at 60℃ when the reaction procedure was ended. The sample genotype was determined according to whether or not there was amplification. If the sample was amplified in two or more reaction systems, the genotype was considered to be a mixed infection[12-13].

BCP T1762/A1764 mutation detection

DNA was extracted from 200 μl serum using DNA extraction Kits(Tiangen Biotech Co, Beijing, China). PCR was used to amplify part of the BCP gene nt1643-nt2112(470 bp). The upper primer was nt 1643~1663 5′-CCCAAGGTCTTACATAAGAGG-3′; and the lower primer was nt 2112~2093 5′-GGTGGCCAGATTCATCAACT-3. The PCR reaction was performed in a volume of 50 μl containing 5 μl genomic DNA, 25 μl PCR Master, and 19 μl sterilized double-distilled H_2O. The PCR included an initial activation step that was incubation at 94℃ for 3 min and thirty cycles of repeated incubation at 94℃ for 60 s, 55℃ for 30 s, and 72℃ for 60 s. The final extension was at 72℃ for 10 min. The nucleotide sequence of the PCR product was detected in Chinese Nation Human Genome Center, Shanghai.

Statistical analysis

Data in the text and table were analyzed with the SPSS 12.0 software package. Data are expressed as mean ± SD. Following Levene's test, statistical analysis was performed using Student's *t*-test. Statistical analysis of enumeration data was performed using the Pearson chi-square test. A two-tailed *P*-value < 0.05 was considered statistically significant.

RESULTS

PCR result in HBV BCP gene nt1643-nt2112

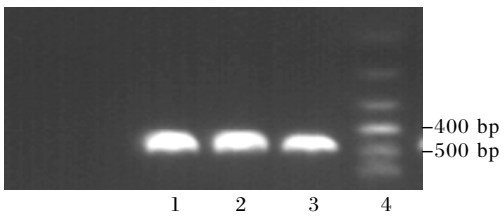
The PCR result in HBV BCP gene nt1643-nt2112 is shown in ***Fig. 1***.

HBV BCP sequencing result

The HBV BCP sequencing result is shown in ***Fig. 2***.

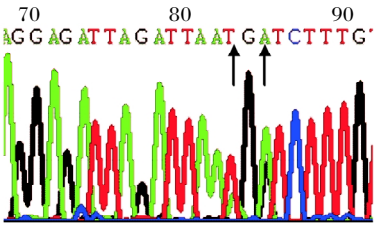
Relation between HBV Genotypes and BCP T1762/A1764 mutation in the Li nationality

The distribution of HBV genotype and BCP T1762/



Lane 1,2,3:the BCP gene fragment; lane 4:markers.

Fig. 1 HBV BCP fragment nt1643-nt2112



The electrophoregram correspond to the BCP T1762/A1764 mutation. Arrows indicate the position of the mutation.

Fig. 2 Sequence analysis of the genomic DNA of the patient revealed BCP T1762/A1764 mutation

A1764 mutation in patients of the Li nationality is shown in ***Table 1***. In 125 patients, we detected genotypes B (31.20%), C(53.60%), D(12.00%), genotype C and D mixed infection(genotype C+D)(2.40%), and genotype B and C mixed infection(genotype B+C,0.80%). Both the mutation frequency and the load of serum HBV-DNA were significantly higher in patients infected with genotype C than in those infected with other genotypes (χ^2=19.57, $P < 0.01$; t = 5.46, $P < 0.01$).

Table 1 **Relation between HBV Genotypes and BCP T1762/A1764 mutation**

HBV genotype	cases(%)	sex(male/female)	age($\bar{x} \pm s$)	BCP mutation(%)	HBV DNA(log, $\bar{x} \pm s$)
B	39(31.20)	21/18	33.18 ± 8.23	7(17.95)	4.71 ± 1.32
C	67(53.60)	35/32	31.36 ± 9.64	39(58.21)	5.74 ± 1.21
D	15(12.00)	7/8	30.60 ± 7.49	3(20.00)	5.36 ± 0.85
C+D	3(2.40)	2/1	28.33 ± 6.72	1(33.33)	5.01 ± 1.38
B+C	1(0.80)	1	27	0	3.96

DISCUSSION

HBV belongs to the family hepadnaviridae, which includes several genera of partially double-stranded DNA of approximately 3.2 kb. Genotypes of HBV are defined by a sequence divergence > 8% over the entire genome. Presently eight genotypes of HBV have been recognized, and designated A to H in the order of discovery[3]. Previous reports described the existence of a strong correlation between genotype and ethnicity[14-15]. In China, there is a significant difference among ethnicities in the distribution of HBV genotypes. The predominant genotype in Tibetans is genotype D[16], and genotype C is the major genotype(79.4%) in the Muslim population in China[17].

In our study, we found genotype C(53.60%) was the major genotype, and genotype B(31.20%) was the next major genotype. Genotype D(12.00%), genotype C+D (2.40%) and genotype B+C(0.80%) were also found in the Li nationality. This is similar to the distribution of HBV genotype in the Han nationality that lives in Nanjing City[18]. The predominance of genotypes C and B suggests that the HBV genome in the Li nationality originated from people of the Han nationality from continental China, or vice versa. The prevalence might be correlated with migration from the Chinese mainland to Hainan Island. However, the distribution of genotypes was different between patients of the Li nationality and other minority ethnic groups[16-17]. Thus ethnicity may be also one of factors that affects the distribution of HBV genotypes in the Li nationality.

Our study showed that the serum viral load of the patient with genotype C was higher than one with genotype B in the Li($P < 0.01$). This was similar to previous reports[19]. Genotype C might also be correlated with high HBV DNA levels[20]. There is no clear explanation for the mechanism behind this observation[21], although it may be related to the higher HBV BCP mutation rate in genotype C than in the other genotypes, and to the capability of genotype C virus to escape from the host's immune response[22].

Several in vitro lines of evidence indicated that BCP T1762/A1764 mutation appeared to enhance viral replication and was associated with advance liver disease. It remains controversial whether BCP double mutation is associated with the pathogenesis of genotype C HBV[23]. To investigate the relation, we detected the gene sequence of BCP in 125 patients. Our study showed that the BCP T1762/A1764 mutation frequency of the patient with genotype C was higher than in patients with other genotypes in the Li nationality group($P < 0.01$). This outcome was similar to previous reports[21]. We presume that the BCP T1762/A1764 mutation may be one of factors that affects HBV pathogenecity.

In conclusion, we found that genotypes C and B are the predominant HBV genotypes in the Li nationality, and genotype C HBV exhibited the higher replication. Differences in pathogenecity among HBV genotypes may be related to BCP T1762/A1764 mutation frequency.

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