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EDITORIAL

Clinical relevance and public health significance of hepatitis B virus genomic variations

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Abstract

Ten hepatitis B virus (HBV) genotypes (A-J) and 34 HBV subgenotypes have been identified so far. HBV genotypes and subgenotypes have distinct geographical distributions, and have been shown to differ with regard to clinical outcome, prognosis, and response to interferon treatment. Infection with subgenotype A2 is frequently associated with high viral load, resulting in acute infection *via* horizontal transmission. Genotypes A and B are more sensitive to interferon treatment than genotypes D and C, respectively. Genotype B is more frequent in acute hepatitis than genotype C, whereas genotype C (C2) is more frequently associated with an increased risk of hepatocellular carcinoma (HCC), mostly cirrhotic, as compared with genotype B (B2). Genotype mixture is associated with high viral load and worse outcome of HBV infection. HBV mutations in the S genes, especially amino acids substitution at position 145 (G145R), are associated with immune escape, whereas mutations in the PreS or S genes which impair HBsAg secretion could present a risk to blood safety. HBV variants harboring mutations in the viral polymerase gene that confer resistance to nucleoside analogs may be selected during antiviral therapy. Different genotypes have distinct mutation patterns in the PreS and EnhⅡ/BCP/Precore regions. PreS deletions, C1653T, T1753V, and A1762T/G1764A are associated with an increased risk of HCC. HCC-associated HBV mutants may not transmit *via* mother-to-child transmission, and are likely generated during HBV-induced pathogenesis. Examination of HBV mutations alone or in combination and host genetic susceptibility will be helpful in classifying the HBV-infected subjects who will develop HCC and need active antiviral treatments.

Key words: Hepatitis B virus; Genotype; Subgenotype; Mutation; Clinical; Public health; Evolution

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INTRODUCTION

Hepatitis B virus (HBV) belongs to the hepadnaviridae, a family of enveloped viruses with an incomplete double-stranded DNA genome of 3.2 kb. The double-stranded DNA genome of HBV contains four overlapping open reading frames that encode the surface protein, the core protein, a polymerase, and a multifunctional nonstructural protein called X. The PreS region, which consists of PreS1 (nucleotides 2848-3204) and PreS2 (nucleotides 3205-154) domains, overlaps a region encoding the polymerase gene. The enhancer Ⅱ (EnhⅡ; nucleotides 1636-1744) and basic core promoter (BCP; nucleotides 1751-1769) regions overlap with the X gene (nucleotides 1374-1835).

Infection with HBV is a major public health problem. Approximately 45% of the world's population lives in regions where HBV infection is endemic. Approximately 2 billion people have been exposed to HBV, and more than 300 million are chronically infected with HBV[1]. In Asia and most of Africa, chronic HBV infection is common and usually acquired perinatally or in childhood[2]. Chronic HBV infection is one of the most important determinants of the occurrence of liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Most HCC

cases (> 80%) occur in either Eastern Asia or in sub-Saharan Africa where HBV is endemic[3]. HBV infection contributes to more than 50% of HCC cases worldwide and 70%-80% of HCC cases in highly HBV endemic regions. The relative risks of HCC among people infected with HBV ranges from 5 to 49 in case-control studies and from 7 to 98 in cohort studies[4]. The incidence of HCC (per 100 000 person/year) among people with chronic HBV infection ranges from 400 to 800 in males and from 120 to 180 in females[4]. Standard HBV vaccination dramatically decreases HCC prevalence among vaccinees aged 6-19 years[5]. HBV genomic variations, including genotypes, subgenotypes, and HBV mutations in the PreS region and the Enh Ⅱ /BCP/Precore region are associated with the development of LC and HCC in different HBV replication and hepatitis B e antigen (HBeAg) status.

HBV GENOTYPES/SUBGENOTYPES AND THEIR CLINICAL RELEVANCE

Distribution of HBV genotypes and subgenotypes

Eight genotypes (genotypes A-H) have been identified by a sequence divergence greater than 8% in the entire HBV genome or a sequence divergence greater than 4% in the S region[6]. HBV isolated in Vietnam and Laos has been suggested to form a ninth genotype I [7,8]. The designation has been questioned due to complex recombination. Recently, a HBV strain isolated from a Japanese patient has been provisionally designated HBV genotype J. HBV genotype J is closer to gibbon/orangutan genotypes than to human genotypes in the P and large S genes and closest to Australian aboriginal strains and orangutan-derived strains in the S gene, whereas it is closer to human than ape genotypes in the C gene[9]. Genotypes have further been separated into subgenotypes if the divergence in whole nucleotide sequence is between 4% and 8%[10]. Currently, subgenotypes 1-5 of genotype A, subgenotypes 1-8 of genotype B, subgenotypes 1-8 of genotype C, and subgenotypes 1-7 of genotype D have been identified[11-37]. HBV genotypes and subgenotypes have distinct geographical distributions (Table 1), and often present demographic characteristics. HBV genotype A1, A3, A4, and A5 are endemic in Africa, especially in West Africa, whereas genotype A2 is endemic in Europe[11-15]. Genotypes B and C are predominant in Asian and Pacific islanders[6,10,16-22,28]. Of HBV genotypes B and C, subgenotypes B2 and C2 are endemic in most parts of Asia. Subgenotype B1 is endemic in Japan[16]. Subgenotype C4 is encountered in Aborigines from Australia, and frequently termed as the Australian aboriginal strain[9,19]. Subgenotypes B3-B8, C1, C3, and C5-C8 have been isolated in South Asia, especially in Indonesia and the Philippines[17-22,28]. Genotype D is endemic in the entire Old World including Africa, Northern and South Eastern Asia, the Mediterranean area, and most European countries[13,23-26,32-34,37]. Subgenotype D1 is predominant in Moslem ethnicity. Subgenotype D2 is endemic in Russia and the Baltic region[26,32]. Subgenotypes D2, D3, and D5 have been found in India[32]. Subgenotypes D4 and D6 are endemic in Oceania and Indonesia, respectively[19,33]. A new subgenotype D7 has been found in Tunisia[34]. HBV genotype E is endemic in Western and Central Africa[13,27,35]. HBV genotypes F, G, and H are endemic in America[29-31,36]. More subgenotypes have been found in South Asia, Oceania, and Africa than other areas in the world, probably indicating evolutionary history of HBV. The distribution of HBV genotypes often provides clues about human migration. HBV genotype B is more frequent than genotype C in Taiwan[38,39]. Although genotype C is more frequent than genotype B in Mainland China[40], HBV genotype B accounts for more than 80% in Zhangzhou (our unpublished data), a city at the coastal line of Fujian Province of Mainland China where early Taiwan residents had migrated from. Subgenotype C1 is endemic in Southern Guangdong Province of Mainland China[41], this subgenotype is also endemic in Hong Kong[42]. Hong Kong residents are mostly from Southern Guangdong Province of Mainland China. HBV genotype E is endemic in Africa. As genotype E is essentially absent from the Americas despite the Afro-American slave trade until at least the beginning of the 19th century, genotype E strains may have been introduced into the general African population only within the past 200 years[14,35]. The genetic diversity of HBV and the geographical distribution of its subgenotypes provide a tool to reconstruct the evolutionary history of HBV and may help to complement genetic data in the understanding of the evolution and past migrations of man[19].

Genotype mixture and its clinical relevance

In an HBV endemic area, infection with more than one HBV genotype often results in genotype recombination and genotype mixture (co-infection or super-infection with multiple HBV genotype in an infected person). With the use of multiplex PCR, a genotype mixture has been frequently identified in the HBV-infected subjects[43,44]. HBV genotype mixture has been associated with high viral load in patients with chronic hepatitis B (CHB) as compared with patients with a single genotype, also associated with increased *in vitro* HBV replication[45]. In our recent study, the prevalence of genotype mixture in asymptomatic hepatitis B surface antigen (HBsAg) carriers (ASC), patients with HCC, and patients with CHB is 5.4%, 10.6%, and 13.7%, respectively. Genotype mixture (mostly mixed genotype B with genotype C) is associated with higher viral load and more severe course of the disease than HBV genotype C alone[40]. These results indicate that co-infection or superinfection with multiple genotypes is associated with worse prognosis of HBV infection.

Clinical relevance of HBV genotypes/subgenotypes

HBV genotypes and subgenotypes have been shown to differ with regard to clinical outcome, prognosis, and response to antiviral treatment. Infection with HBV genotype A is associated with high viral load which facilitates viral transmission. High replication rates of genotype A

Table 1 Geographic distribution and important clinical relevance of HBV genotypes and subgenotypes

Genotype	Subgenotype	Geographic distribution	Important clinical relevance	Ref.
A	A1	Africa	ND	[13-15]
	A2	Europe	Acute infection, chronicificaton, more sensitive to interferon treatment than genotype D	[11,12,47,48]
	A3	West Africa	ND	[13-15]
	A4	West Africa	ND	[15]
	A5	West Africa	ND	[14,15]
B	B1	Japan	Fulminant hepatitis	[16]
	B2	Most of Asia, except Korea	Acute hepatitis, HCC in mostly those younger than 50 years	[6,10]
	B3	Indonesia	ND	[17,18]
	B4	Indonesia, Vietnam	ND	[18,19]
	B5	Indonesia, Philippines	ND	[17,18,22]
	B6	Indonesia	ND	[28]
	B7	Indonesia	ND	[17,18]
	B8	Indonesia	ND	[17]
C	C1	South Asia, Southern China	HCC and LC	[18]
	C2	Northeast Asia, China	HCC and LC mostly in those older than 50 years	[6,10]
	C3	Indonesia, Oceania	ND	[18,19]
	C4	Australia	ND	[19]
	C5	Indonesia, Philippines	ND	[17,22]
	C6	Indonesia, Philippines	ND	[17,21]
	C7	Indonesia	ND	[17]
	C8	Philippines	ND	[20,21]
	C9	Tibet, China	ND	Unpublished
D	D1 (mainly D)	Middle East, the Mediterranean area	Chronic liver disease, HCC	[13,23-25, 37,81]
	D2	Russia, the Baltic region, India	No apparent clinical relevance	[26,32]
	D3	Indonesia, India	Occult HBV infection	[17,32]
	D4	Oceania	ND	[19]
	D5	India	No apparent clinical relevance	[32]
	D6	Indonesia	ND	[33]
	D7	Tunisia	ND	[34]
E	ND	Western and Central Africa	ND	[13,27,35]
F	F Ⅰa	Central America	HCC	[29,36,62]
		Chile, Alaska		
	F Ⅰb	Argentina, Japan, Venezuela, USA		
	F Ⅱ	Brazil, Venezuela, Nicaragua		
	F Ⅲ	Venezuela, Panama, Columbia		
	F Ⅳ	Argentina, Bolivia, France		
G	ND	Mexico, Canada	ND	[30,31]
H	ND	Mexico	ND	[30]
I	ND	Vietnam, Laos	ND	[7,8]
J	ND	Japan (might be from Borneo)	HCC	[9]

HBV: Hepatitis B virus; ND: Not determined; HCC: Hepatocellular carcinoma.

in adults lead to an increased risk of horizontal transmission of HBV by sexual activity because a high concentration of HBV DNA in serum is associated with high concentrations in semen and other body fluids of HBV carriers[46]. Genotype A also tends to cause chronic infection following an acute course. This has been demonstrated in Japan where genotype A introduced from Europe has started to increase sharply in patients with acute infection since 1991, and gradually in those with chronic infection[47]. As compared with HBV genotype D endemic in Europe, genotype A is more sensitive to interferon α treatment[48]. Spontaneous HBeAg seroclearance was significantly higher in genotype A carriers than in carriers of genotypes A, B, D, and F. After losing HBeAg, those with genotypes C and F were more likely to revert to the HBeAg-positive state[49]. Infection with subgenotype B2 is associated with HCC or HCC recurrence in young, mostly noncirrhotic, patients in Mainland China and Taiwan[39,40,50], whereas infection with subgenotype B1 is frequently associated with fulminant hepatitis B in Japan[16]. Infection with HBV genotype C is associated with increased risks of LC and HCC at an older age as compared with infection with the HBV genotype B[38,51,52]. Although HBV subgenotypes C1 and C2 are associated with the risk of HCC, only HBV subgenotype C2 is independently associated with an increased risk of HCC[53]. Genotype B has recently been shown by us to be more likely to cause acute hepatitis B, while the serum viral load of ASCs with genotype B is significantly higher than that of ASCs infected with genotype C[54]. As compared with genotype C, HBV genotype B has been shown to be associated with earlier HBeAg seroconversion, and associated with better response to interferon therapy in HBeAg-positive chronic hepatitis[55,56]. Early HBeAg seroconversion typically confers a favorable outcome[57]. In HBeAg-negative patients, detectable HBV DNA and HBV genotype C are associated with more severe liver damage[58]. Thus, infection with HBV genotype C is as-

sociated with worse clinical outcome as compared with genotype B. Data from India have shown that HBV subgenotype D1 is significantly associated with chronic liver disease, whereas HBV subgenotype D3 is significantly associated with occult HBV infection. No apparent clinical relevance was observed in those infected with HBV subgenotypes D2 and D5[32]. There are very limited data on the association of genotype E with its clinical relevance. Population-based prospective cohort studies have found that HBV genotypes C and F are associated with the highest risk for HCC or LC[59]. HBV genotypes E, F, and H appear to be sensitive to IFN-α treatment[60]. The recombination of two genotypes is frequent in the area where the two genotypes are endemic[6,15,61], probably due to the selection of viral growth advantage. Lower rates of response to IFN-α treatment in patients with HBV genotype G might be related to the frequent occurrence of double infection[60].

CLINICAL AND PUBLIC HEALTH SIGNIFICANCE OF HBV MUTATIONS

Association of HBV PreS and S region mutations with immune escape, occult infection, and the development of HCC

HBV genomic variations in the PreS and S regions which are selected during the infection course are of clinical and public health importance. The HBV envelope is composed of 3 forms of HBsAg, the so-called large (L, coded for by the PreS1/S2/S gene), middle (M, the PreS2/S gene), and small (S, the S gene) proteins. The small or major peptide is 226 amino acids in length, and the M and L proteins are assembled by amino-terminal extension of 55 amino acids at the PreS2 domain and of 108-119 amino acids of the PreS1 domain. HBsAg is the main target for viral neutralization, either by natural or vaccine-induced anti-HBs. A central major hydrophilic region (MHR, approximately residues 103-173) exposed at the surface of viral particles. The MHR itself is structured into five regions, including three central loops held together by disulphide bonds. The immunodominant "a" determinant (residues 124-147), against which most neutralizing antibodies are directed and which is the major target of HBsAg detection tests, is formed by loops 2 and 3[62]. HBV with mutations in the portion of the S gene coding the "a" determinant of hepatitis surface antigen, including a glycine to arginine substitution at position 145 (G145R) and other S gene mutations in the region of amino acids 120-147, can potentially evade neutralizing anti-HBs antibody and infect vaccinated people. G145R is by far the most common immune escape mutant, whereas the most important immune escape mutants with substitutions outside of the "a" determinant is P120S/T. Mutations in the S genes within "a" determinant (but not G145R) are partially responsible for occult HBV infection, which are characterized by the presence of HBV DNA in serum in the absence of detectable HBsAg, and could present a risk to blood safety[63]. Mutations in PreS are also associated with occult HBV infection[64], probably due to the inactivation of the overlapping PreS2/S promoter which causes impaired HBsAg secretion.

The 5' flanking region of the S gene coding the PreS1 and PreS2 domains is overlapped by the region of the *P* gene coding the spacer domain of the viral polymerase. Genotype D and non-human primate isolates inherently have a 33 nucleotide deletion at or near the beginning of the PreS1 open reading frame[10,62]. The PreS1 protein contains the hepatocyte binding site (amino acids 21-47) and is known to be essential for virion assembly and for the transporting of virions out of the hepatocyte[65]. The PreS1 and PreS2 regions play an essential role in the interaction with immune responses because they contain several epitopes for T or B cells[66]. There is little evidence supporting the idea that PreS mutants are transmissible, therefore, the PreS mutation might generate during the pathological process following the infection. The PreS mutations emerge in chronic infections, often in patients treated with interferon, and seem to represent desperate attempts to escape from host immune surveillance[62]. Many of the mutations affecting the PreS domains of the envelope proteins are deletions. More recently, PreS deletions are frequently associated with an increased risk of HCC, especially in those infected with HBV genotype C[66-69]. Our recent meta-analysis showed that the frequencies of the PreS deletion mutation consecutively increased during the progression of chronic HBV infection from ASC states to LC or HCC ($P_{trend} < 0.001$), while the frequencies of mutations at the promoter sites of PreS1 and PreS2 were significantly higher in the patients with HCC than in the patients without HCC ($P < 0.001$, $P = 0.032$, respectively)[70]. It is suggested that PreS deletion and nucleotide substitution mutations at the promoter sites of PreS1 and PreS2 may serve as useful biomarkers for predicting the clinical outcomes of HBV-infected patients, especially for predicting HCC.

Polymerase mutations associated with drug resistance

HBV variants harboring mutations in the viral polymerase gene that confer resistance to antiviral drugs may be gradually selected during long-term antiviral therapy with nucleoside analogs. The main polymerase gene mutations conferring resistance to nucleoside analogs have been well characterized. Lamivudine resistance mutants harbor a M204V or I substitution in the YMDD motif of the C domain of the polymerase/reverse transcriptase. Adefovir resistance mutants harbor a N236T and/or A181V amino acid substitution in the D and B domains of viral polymerase, respectively[71,72]. Entecavir resistance mutations occurred on a background of lamivudine resistance, as these patients received entecavir for lamivudine failure, with a combination of substitutions I169T and M250V, or T184G and S202I. These additional mutations clearly conferred an increased level of entecavir resistance compared to the initial lamivudine resistant strain[62]. Resistance to telbivudine has been associated with a M204I mutation in the viral polymerase[73]. Table 2 summarizes drug-resistance-associated amino acid substitutions in the 5 domains of HBV polymerase. On the other hand, the reappearance of wild-type virus

Table 2 HBV mutations associated with drug resistance

Nucleoside analogs	Mutations in the polymerase domains (amino acids)				
	A	B	C (YMDD)	D	E
Lamivudine/ Emtricitabine	-	V173L L180M	M204I/V	-	-
Adefovir	-	A181V	I233V	N236T	-
Entecavir	-	I169T T184G	S202I	M250V	-
Telbivudine	-	-	M204I	-	-
Famciclovir	-	L180M	-	-	-

-: Not reported.

as the major viral population after cessation of drug treatment is probably due to persistence of non-mutated cccDNA molecules in hepatocytes even after long-term drug treatment[62,74]. Naïve patients infected with adefovir resistance mutants have been reported[71]. In this case, drug resistant mutants transmitted to a naïve subject may be stable since there will be no competition with wild-type virus.

The association of viral mutations in the Enh Ⅱ/BCP/Precore region with hepatocarcinogenesis

The core promoter, positively and negatively regulated by Enhancer Ⅱ and to some extent by Enhancer Ⅰ, controls the transcription of precore mRNA and pregenomic RNA that can be the mRNA for both core protein and the viral polymerase and is the template for viral replication. HBeAg expression indicates active viral replication. There are two classes of mutants that affect HBeAg expression, BCP mutants and precore mutants. Although viral loads are generally several logs lower in HBeAg-negative patients than in HBeAg-positive patients and children born to HBeAg-positive mothers have a much higher risk of contracting chronic HBV infection than children born to HBeAg-negative mothers[62], some combined mutations in the Enh Ⅱ/BCP/Precore region like 1766/1768, 1762/1764/1766, 1753/1762/1764, and 1753/1762/1764/1766 mutations have been associated with high HBV DNA production in the *in vitro* transfection studies[75,76]. HBV core promoter mutations other than those at 1762/1764 appear to upregulate viral DNA replication and, at the same time, greatly reduce HBeAg production. Although expression of HBeAg has been associated with an increased risk of HCC in a prospective study[77], high viral load in HBeAg-negative patients is often associated with worse outcome of chronic HBV infection, especially in those with HBV carrying mutations at the PreS and Enh Ⅱ/BCP/Precore regions[66,78-82].

Several mutations at the Enh Ⅱ/BCP/Precore region have been recently associated with an increased risk of HCC. These mutations include C1653T, T1753V, T1766/A1768, and A1762T/G1764A[81-92]. Our recent meta-analysis using published data up to August 31, 2008 has shown that C1653T, T1753V, and A1762T/G1764A are each associated with an increased risk of HCC, whereas precore mutations G1896A and C1858T are not associated with the risk of HCC, regardless of HBeAg status and HBV genotype[70]. A1762T/G1764A has been shown to be a valuable biomarker for identifying a subset of male HBsAg carriers who are at extremely high risk of HCC in a prospective study[92]. In a community-based prospective study, A1762T/G1764A and genotype C have been associated with an increased risk of HCC, whereas G1896A in the precore region has been associated with decreased risk of HCC[84]. G1896A has been associated with fulminant hepatitis in Japan[93]. Since the Enh Ⅱ/BCP/Precore region overlaps with X gene in the HBV genome, mutations in the Enh Ⅱ/BCP/Precore region should be included in evaluating the role of HBV X protein on the development of HCC. That is to say, the mutated X protein might be more carcinogenic than the wild-type X protein in HBV-induced hepatocarcinogenesis.

C1653T, T1753V, and A1762T/G1764A are increasingly more prevalent as chronic HBV infection progresses from the asymptomatic HBsAg carrier state to liver cirrhosis or HCC, indicating that these mutations accumulate before the diagnosis of HCC[70]. This finding suggests that these HBV mutations may serve as useful biomarkers for predicting clinical outcomes of the patients with CHB, especially with regard to predicting whether they will develop HCC. Like the PreS mutants, HCC-associated HBV mutants in the Enh Ⅱ/BCP/Precore region, e.g. A1762T/G1764A mutants, may not transmit *via* mother-to-child vertical transmission because the children whose mothers carrying HBV mutants were mostly found to be infected with wild-type form of the same viruses[94,95]. These HBV mutations are likely generated during HBV-induced pathogenesis. A1762T/G1764A is frequently detected approximately 10 years before the diagnosis of HCC[70]. It is therefore necessary to set up likely checkpoints in the life time for the examination of the HCC-associated HBV mutations in HBV-infected subjects. Recent epidemiological studies demonstrated that male sex, old age, high HBV DNA (> 10 000 copies/mL), viral mutations in the PreS and the Enh Ⅱ/BCP/Precore regions, HBV genotypes (C and F), cirrhosis, and family history were associated with an increased risk of HCC[50-52,65,79-92,96,97]. Further study should focus on systemic evaluation of these risk factors for the prediction of HCC.

Combined HBV mutations in the PreS and the Enh Ⅱ/BCP/Precore regions are becoming important in evaluating HCC risk of HBV-infected subjects[66,69,98]. In a meta-analysis, we have demonstrated that the frequencies of A1762T/G1764A+C1653T (8.6%), A1762T/G1764A+T1753V (14.6%), A1762T/G1764A+PreS mutation (2.2%), and A1762T/G1764A+C1653T+T1753V (3.2%) are low in ASCs, whereas the frequencies of A1762T/G1764A-based combined mutations are statistically significantly higher in patients with HCC than in patients without HCC[70]. For the prediction of HCC in HBV-infected subjects, A1762T/G1764A alone has a sensitivity and specificity of 70.6% (95% CI = 68.7% to 72.5%) and 60.6% (95% CI = 68.7% to 62.0%), respectively, whereas C1653T+T1753V and A1762T/G1764A+C1653T+T1753V has high specificity [92.6% (95% CI = 89.2% to 96.0%) and 93.9% (95%

CI = 90.5% to 97.2%), respectively] but low sensitivity [20.6% (95% CI = 14.9% to 26.3%) and 24.3% (95% CI = 17.5% to 31.1%), respectively][70]. These mutations, alone or in combination, might be reasonably arranged as predictive markers for the prediction of HCC.

INTERACTIONS BETWEEN HBV AND HOST SUSCEPTIBLE GENES

HBV genetic variations are necessary but insufficient for HBV-induced hepatocarcinogenesis. HBV genotype-associated mutations might be selected by the host immune system and in turn promote host hepatocarcinogenesis. It is possible that the mutated X protein could transactivate host oncogenes responsible for the development of HCC or that transactivators encoded by some oncogenes select the specific HBV mutations during HBV-induced hepatocarcinogenesis. There are many important trans-activating nuclear factors binding sites located in the PreS and the Enh Ⅱ/BCP/Precore regions[66,69,70]. Mutations in the PreS and the Enh Ⅱ/BCP/Precore regions might alter the binding ability of some potential trans-activating factors and therefore alter viral replication and/or change expression profiling of some related host genes. The genetic predisposition of some host genes like *MDM2* and *p53* gene polymorphisms, cytokine and TGF-β1 gene polymorphisms, and DNA repair gene polymorphisms have been associated with HBV-induced hepatocarcinogenesis[99-102]. Our recent study demonstrated that nuclear factor κB1 gene promoter *NFKB1*-94ATTG2 allelic carriage, IκBα gene promoter *NFKBIA*-826T and *NFKBIA*-881AG allelic carriage, and HBV genotype C are independently associated with an increased risk of HCC, while the estimated haplotype frequency of *NFKBIA* promoter -881G-826T-519C is significantly higher in the patients with HCC than in the HBV-infected subjects without HCC[103]. Even so, it is largely unknown so far how HBV variations interact with host genetic susceptibility. Understanding the interactions between HBV genetic variations and host genetic susceptibility is undoubtedly helpful in classifying the HBV-infected subjects who will develop HCC in future and need active anti-viral treatments and extensive surveillance of HCC.

CONCLUSION

Ten HBV genotypes (A-J) and 34 subgenotypes have been identified so far. HBV genotypes and subgenotypes have distinct geographical distributions, and have been shown to differ with regard to clinical outcome, prognosis, and response to interferon treatment. Infection with subgenotype A2 is frequently associated with high viral load, resulting in acute infection *via* horizontal transmission. Genotypes A and B are more sensitive to interferon treatment than genotypes D and C, respectively. Genotype B is more common in acute hepatitis than genotype C, whereas genotype C (C2) is more frequently associated with an increased risk of HCC, mostly cirrhotic, as compared with genotype B (B2). Genotypes C and F are frequently associated with the development of HCC. Viral load of the patients with genotype mixture is usually higher than that of those infected with unique genotype. HBV mutations in the S genes, especially amino acid substitutions at position 145 (G145R), are associated with immune escape, whereas the mutations in the PreS or S genes which cause impaired HBsAg secretion could present a risk to blood safety. HBV variants harboring mutations in the viral polymerase gene that confer resistance to antiviral drugs may be selected during antiviral therapy with nucleoside analogs. Genetic diversity of HBV is partly due to virus/host interactions and partly due to parallel evolution in geographically distinct areas. Different genotypes have a distinct pattern of mutations in the PreS and Enh Ⅱ/BCP/Precore regions. PreS deletions, C1653T, T1753V, and A1762T/G1764A are associated with an increased risk of HCC. HCC-associated HBV mutants may not transmit *via* mother-to-child vertical transmission, and are likely generated during HBV-induced pathogenesis. Frequent examination of HBV mutation alone or in combination as well as genetic susceptibility will be helpful in classifying the HBV-infected subjects who will develop HCC and need active anti-viral treatments.

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