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REVIEW

Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance

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Abstract

The Hepatitis B Virus (HBV) has a worldwide distribution and is endemic in many populations. It is constantly evolving and 10 genotypic strains have been identified with varying prevalences in different geographic regions. Numerous stable mutations in the core gene and in the surface gene of the HBV have also been identified in untreated HBV populations. The genotypes and viral variants have been associated with certain clinical features of HBV related liver disease and Hepatocellular carcinoma. For example Genotype C is associated with later hepatitis B e antigen (HBeAg) seroconversion, and more advanced liver disease. Genotype A is associated with a greater risk of progression to chronicity in adult acquired HBV infections. Genotype D is particularly associated with the precore mutation and HBeAg negative chronic hepatitis B (CHB). The genotypes prevalent in parts of West Africa, Central and South America, E, F and H respectively, are less well studied. Viral variants especially the Basal Core Promotor mutation is associated with increased risk of fibrosis and cancer of the liver. Although not currently part of routine clinical care, evaluation of genotype and viral variants may provide useful adjunctive information in predicting risk about liver related morbidity in patients with CHB.

Key words: Chronic hepatitis B; Genotype; Pre-core; Basal core promotor; Mutations

Core tip: Chronic hepatitis B (CHB) is a major global cause of liver related morbidity and mortality. Genotypes of the Hepatitis B virus have distinct geographical distributions and are known to influence a number of clinical features of disease and response to treatment. Certain well recognised viral mutations are also known to influence clinical risk of cirrhosis and hepatocellular carcinoma but in addition may have implications for vaccination programs and screening of blood for donation. This review examines the current state of knowledge about genotype and viral variants of CHB and their utility in the management of this disease.

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INTRODUCTION

Chronic hepatitis B (CHB) is a global health problem and a leading cause of cirrhosis and hepatocellular carcinoma (HCC) worldwide. The Hepatitis B virus (HBV) is a hepatotropic virus of the family hepadnaviridae. It comprises a central icosahedral core protein (HBcAg) which contains the viral DNA and HBV viral polymerase. This core (also called the nucleocapsid) is surrounded by a lipid membrane studded with viral proteins which are the small, medium and large HBV surface proteins. The entire virion is 42 nmol/L and was originally referred to as the Dane particle following its discovery by an English pathologist DS Dane[1]. In the past, HBV was divided into serotypes which were subgroups based on the antigenic determinants of the hepatitis B surface antigen (HBsAg) and 4 subtypes were known, adr, adw, ayr and ayw[2].

GENOTYPES IN CHB

In 1988 it was first suggested by Okamoto *et al*[3] that HBV could be divided into 4 genotypes based on a divergence of $\geqslant$ 8% in the complete genomic sequence and genotypes A,B,C and D were identified. The relationship between serotypes and genotypes is not clearly known and the same serotype may be classified into different genotypes[3]. Genotyping may be performed by a number of different techniques including restriction fragment length polymorphism, line probe assay, the enzyme-linked immunosorbent assay or genotype specific polymerase chain reaction[4]. Direct sequencing can also be used and for commercial purposes, genotype can usually be determined through a partial sequence especially of the S gene since it is usually more conserved than other parts of the HBV genome. Following the initial description of genotypes A-D, Norder *et al*[5] also proposed genotypes E and F which differed by more than 4% in the *S* gene from the other genotype groups and this has become an alternative criterion for classification of distinct genotypes. Genotype G is the least common of the genotypes and was reported in 2000 from samples of French and American patients[6] but its geographic origin is still unknown[7]. The precore and core regions of genotype G are aberrant with a 36-nucleotide insertion within the core gene making it the longest of the HBV genotypes[8]. Stop codons in the precore region are also present and some have suggested it is not able to produce hepatitis B e antigen (HBeAg)[8] although others report high HBeAg levels in HIV/HBV coinfected patients with genotype G[9]. However, this may be due to coinfection with genotype A[10]. It is thought that genotype G requires the presence of another genotype, most commonly genotype A2 to enhance its viral replication[11]. Although monoinfection has been reported, this was transient[12]. Genotype H has been shown to be prevalent in central America and the Amazon region and is closely related to genotype F HBV. It is thought to perhaps have split off from genotype F within the "New World"[13]. It is particularly common in Mexico[14].

Genotype I described in Vietnam[15] may not meet the criteria for a novel genotype since the diversity in its complete genome sequence is only 7% from that of its closest neighbour, genotype C[16]. Genotype J is a novel variant described in a Japanese patient who had previously travelled to Borneo. It is thought to be phylogenetically positioned between human and primate HBV variants being close to strains which had been previously found in orang-utans and gibbons[17].

This genetic variability in the HBV may come about through natural mutation or by recombination. Natural mutation rates are high in HBV. The HBV is error prone since its reverse transcriptase lacks proof reading ability and it is estimated that the rate of nucleotide substitutions per site per year is approximately $1.4\text{-}3.2 \times 10^{-5}$[18] which is 10 times higher than other DNA viruses and 100 times higher than the human genome[19]. Recombination, in which DNA exchange or cross over of parts of a gene sequence occurs between two different viruses, is thought to be another potential mechanism for the development of divergent strains of HBV[20] and has been described between numerous different genotypes[21,22]. Recent evidence suggests that the core gene may be a preferred site for recombination to take place as noted in West African patients with A/E recombinant strains of HBV[23].

SUBGENOTYPES

Subgenotypes are also described if there is a divergence of > 4% (but less than 7.5%) of the nucleotide sequence in the complete genomic sequence[19]. There have been numerous (up to 40) subgenotypes reported amongst genotypes A-D and F. Geographic distribution varies for subgenotypes and certain clinical outcomes have also been attributed to some of them however confounding factors are difficult to control for in many of these studies. Divergence of < 4% between subgenotypes are referred to as "clades"[24]. There has been concern raised by experts in the field about the accuracy of classification of some of the newly reported subgenotypes[25]. Pourkarim *et al*[25] suggest that applying phylogenetic analysis over a full length genome sequence rather than partial sequence only is critical to avoid misclassification. They also suggest that recombinant strains should not necessarily be introduced as independent subgenotypes and propose the term "recombino-subgenotype" to identify HBV strains that show strong evidence of recombination in their nucleotide divergence.

GEOGRAPHICAL DISTRIBUTION OF GENOTYPES

The geographical distribution of the different geno-

Table 1 Geographic distribution of genotypes by continent

	Region	Genotype distribution
Africa	Subsaharan Africa (Egypt, Algeria, Libya)	Genotype D
	West Africa (Guinea Bissau, Ghana, Cameroon)	Genotype E and also A
	Central Africa	Genotype E and also A
	East Africa (Malawi, Tanzania)	Genotype A
	South Africa	Genotype A
Europe	Mediterranean Basin (Greece, Italy, Spain)	Genotype D in majority. Gentype A also seen in Spain
	Western Europe	Mixtures of A-D from various migrant groups
	Eastern Europe	A (Czech republic, Poland) and D (Russia, Croatia, Romania)
Americas	North America	Mixtures of A-D from various migrant populations
		Genotype F and B in Alaskan natives
	Central America	Genotype H (Mexico)
		Genotype F (Costa Rica)
	South America	Genotype F predominant and Genotypes A and D in Brazil/Argentina
Asia	Western Asia (Iran, Yemen, Saudi Arabia, Turkey)	Genotype D
	Central Asia (Uzbekistan, Tajikistan, Afghanistan, Pakistan)	Genotype D
	South Asia (India and Pakistan)	Genotype D in India but also Gentoype A

types is quite varied and for most of the older known genotypes and many regions, is well documented. Many parts of the world have dominant genotypes although frequently there are at least 2 prevalent genotypes.

Patients may also be coinfected with more than one genotype since in many parts of the world 2 or more genotypes are commonly found eg B and C in Asia[26]. Genotype G also, as mentioned, appears to require the presence of genotype A[10] or H for chronic infection[27]. The distribution of HBV genotypes in different continents are detailed in Table 1.

AFRICA

In East Africa (including Malawi and Tanzania) genotype A is found in the vast majority (> 90%) of patients[28,29]. In South Africa also, the dominant genotype is A[30]. In other parts of Africa, for example West Africa, Genotype E is prevalent[31,32] and this appears to stretch into parts of central Africa also[33]. There has been some work suggesting that HBV genotype E introduction and expansion in West Africa has been a relatively recent phenomenon[34,35] . In sub-Saharan (or Northern) Africa including Egypt, Algeria and Libya) which forms part of the Mediterranean Basin, genotype D predominates in up to 80% of patients[36,37].

EUROPE

In the European countries of the Mediterranean basin, in particular Greece[38], Italy[39], and Spain, the predominant genotype is Genotype D[40]. Genotype D is also found in about 50% of cases in Eastern Europe, with genotype A in approximately 30%[26]. Although some countries have a higher proportion of genotype A, *e.g.,* 86% in Poland and 67% in Czech republic, Genotype D is found in the majority of Russian (93%), Romanian (67%) and Croatian (80%) patients[41,42]. The proportions of genotypes A and D are similar at about 30%-40% in the remainder of Europe (the EU and northern Europe) with smaller contributions from other genotypes including B and C, most likely due to migration[43].

ASIA

In Western Asia, *e.g.,* Turkey[44] and the Middle East including Iran[45], the prevalent genotype is D. Central Asian countries of Uzbekistan and Tajikistan also have a preponderance of genotype D infection of up to 88%[46,47]. Southern Asian countries similarly show predominantly genotype D infection, *e.g.,* 95% of cases in Afghanistan[48] and in a majority of Indian patients[49-51] and 65% of Pakistani patients[52]. However genotype A is also seen in India[53] and a recent study from Eastern India highlights a shift in the prevalences of genotypes with an increase in Genotypes A and C along with a decrease in that of genotype D in East India[54].

Moving further east into South East Asia and China, genotypes B and C start to predominate. The relative prevalences of genotypes in many countries of south east Asia and regions of China are set out in Table 2[55-79] . In brief however, genotype C is seen in the majority of patients of Cambodian, Thai, Laotian and Myanmar ethnicity. Genotype B is predominant in Vietnamese cohorts and some parts of Indonesia and Malaysia. In China, Genotype C is prevalent in most areas although in other parts, Genotype B is seen frequently. Japan has a predominance of genotype C (82% in a study of 1271 patients)[80]. This study also reported an increase in the prevalence of genotype A from 1.7% to 3.5% from the period 2001-2006 which is thought to be due to persistence of sexually acquired acute HBV in adulthood. In Korea genotype C2 predominates[81].

AMERICAS

Among Indigenous populations living in the Arctic, and northern Canada and Greenland genotype B

Table 2 Prevalence of different hepatitis B virus genotypes in Southeast Asian countries and China

	No. in study	Genotype distribution	Notes	Ref.
Laos	386	42.2% B 55.4% C 2.4% not typable ? I	Cohort of patients from Vientiane city and central provinces. 19 patients did not group into genotype A-H ? genotype I	[55]
Cambodia	12	67% C , 33% B (subtype 4)		[56]
	22	72% C 28% B		[57]
Vietnam	76	51% B, 48.7% C	Chronic cohort	[58]
	40	75% B 18% C 2.5% B + C, 5% not determined	Based in Hanoi	[59]
Indonesia	54	76% B 24% C		[60]
	54	100% B	Surabaya	[61]
	27	85% C 7.4% B 7.4% D	Papua	[62]
Malaysia	86	60% B 34% C 2% D	Genotype B 80% in ethnic Chinese Genotypes B and C equal prevalence in Ethnic Malays Gentoype D in Indian patients	[63]
	51	56.9% B 31.4% C 7.8% B + C 2% each D and E		[64]
Thailand	224	86.6% C (Subgeno C1) 11.2% B 0.44% each of A and D 3 suspected recombinations	Myanmar ethnicity 97.5% genotype C Laos ethnicity 71% C 26% B, Cambodia 84% C, 12% B	[65]
	216	89.3% C 7.4% B, 1.9% B + C, 0.5% A	Northern Thailand adult voluntary blood donors	[66]
	53	90.6% C 7.5% B 1.9% B + C	Children in Chiang Mai	[67]
	332	73.2% C 20.8% B 3.3% A 2.7% unclassified	Cohort included CHB and HCC patients and found that genotype B was not associated with HCC in younger patients	[68]
Philippines	100	51% A 22% B 27% C		[69]
	50	28% A 12% B 26% C 6% Mixed A + C/A + B + C 28% Non typable		[70]
China	101	36% B 64% C	Hong Kong 42% B Shnaghai 39% B Beijing 20% B	[71]
	121	33% B 63.6% C 1.7% B/C 1.7% D	From Beijing China	[72]
	126	38.1% B 54.8% C 0.8% D, 3.2% unknown 1.6% B/C, 1.6% A/C	Yunnan China	[73]
	142	9.2% B 88% C 2.8% D	Northern China (Harbin University China)	[74]
	142	4.2% A 14.1% B 78.9% C 1.4% D	Southern China (Nanning)	[75]
	786	63.23% B 34.99% C 0.89% A and D each	Southern China (Guizhou)	[76]
	220	1.4% A 17.2% B 81.4% C	Shanghai China	[77]
China (Hong Kong)	776	1.5% A 32.5% B 62.6% C 3.4% Mixed	Hong Kong	[78]
Tibet	26	96% C/D recombinant 4% C	Sequences based on surface Ag gene showed that 25 clustered with genotype D and 1 clustered with genotype C. However based on core gene all clustered with genotype C	[79]

(subgenotype B6) has been found to be prevalent[82]. Genotype F has also been shown to be predominant in Alaskan native Inuit populations. Genotype F is also found in South and central America and is thought to be the most prevalent genotype in most of these countries[83] although in Brazil, and Argentina genotypes (plural) A and D are also seen[84]. In Central America, genotype H HBV is prevalent, being found in approximately 75% of patients in a small study in Mexico[85].

In the United States, CHB is found primarily in migrant populations where the mix of different genotypes reflects the various immigrant groups. A large study of 694 patients in the United States identified a strong correlation between ethnicity and genotype and found that in patients of Asian background, genotypes B and C were most common and in those of white or African American background who usually acquired hepatitis B in adulthood through sexual transmission, genotype A was most common[86].

AUSTRALIA AND THE PACIFIC

In Australia, Bell *et al*[87] showed in 2005 that in the cohort at St Vincent's Hospital, Melbourne, 8% had

Table 3 Clinical associations with hepatitis B virus genotypes

	A	B	C	D	E	F	G	H
Progression to chronicity	+++	++	+++	++				
Histological inflammation	++	++	+++	+++		+/-		
Histological fibrosis	+	+	++	++		+/-		
Association with advanced liver disease	+	++	+++	++		+/-		+
Association with HCC	+ (subgeno A1)	+	++	++		++ (subgeno F2)		+
Early HBeAg seroconversion	++	+++	+	+++		+++		++
Sustained remission after HBeAg Seroconversion	+++	+++	++	++		++		
HBsAg clearance	+++	++	+	++				+++
Response to IFN Tx	+++	++	+	+/-	+	+++	+	++
Association with PreCore mutations	-	++	+	+++	++	+++ (F1 but not F2)		
Association with BCP mutation	++		++		++			++

+/-: Possible association; - : No association; +: Slight association; ++: Moderate association; +++: Strong association. HBeAg: Hepatitis B e antigen; BCP; Basal core promotor; HCC: Hepatocellular carcinoma; IFN: Interferon.

genotype A, 29% B, 41% C and 22% D reflecting the multicultural nature of Australian Society and the patterns of migration from the Mediterranean region and more recently South East Asia. There are few studies from the Pacific Island nations however one from the Solomon Islands where Hepatitis B is hyperendemic (prevalence of 21%), found a predominance of genotypes C and D which appeared ethnicity specific[88].

GENOTYPE AND CLINICAL OUTCOMES

There are a number of studies documenting the effect of genotype on various clinical outcomes. Many of these provide comparisons of 2 prevalent genotypes in a region, *e.g.,* A *vs* D, or B *vs* C. Table 3 sets out some of what is known about the different genotypes and clinical associations. There is a paucity of information about genotype E and its associations with clinical outcome and more work is needed to further elucidate its impact on HBV related liver disease.

GENOTYPE AND RISK OF CHRONICITY

Genotype A appears to have the highest risk of progression to chronicity following acute adult acquired Hepatitis B and resolution of acute hepatitis B is often prolonged in genotype A[89]. In a cohort of Asian patients, genotype C2 was independently associated with progression to chronicity, compared to genotype B[90]. Acute infection with genotype D appears to be more commonly associated with acute liver failure than other genotypes[91].

GENOTYPE AND HBEAG/HBSAG CLEARANCE

Many Asian studies have documented the more prolonged HBeAg positive phase and delayed HBeAg seroclearance of genotype C in comparison to Genotype B[92,93] including Chu *et al*[94] who showed that HBeAg seroconversion occurs about 10 years earlier in genotype B compared to genotype C. A study of 1158 Alaskan natives which also looked at timing of seroconversion found that HBeAg seroconversion in genotype C (Subgenotype C2) patients lagged behind that of other genotypes by approximately 3 decades. Age at HBeAg seroconversion of the 75th percentile of patients was 32 years in genotype A2, 27.5 years in B6, 27.3 years in D, 24.5 in F1 but 58.1 years in C2[95]. Genotype C patients are more prone to repeated episodes of acute exacerbation with failure of HBeAg seroconversion[96] and HBeAg sero-reversion after HBeAg loss[95]. Rates of spontaneous HBsAg clearance are higher in genotype B compared to C[97]. Sustained remission following HBeAg seroconversion has been reported to be more commonly seen in genotype A than D as was HBsAg clearance[98] .

GENOTYPE AND HBV DNA LEVELS

Genotype C has been reported to have a significantly higher viral load than genotype B[99]. Viral load in genotype D has also been shown to be significantly higher than in Genotype A[100]. Genotype E is reported as being more likely to be associated with HBeAg positive disease and higher HBV DNA levels than Genotype D thus perinatal infection of infants from infected mothers is likely be an important factor in transmission for African people infected with genotype E[101].

GENOTYPE AND LIVER DISEASE

Genotype C patients are more prone to the complications of advanced fibrosis and cirrhosis[102-104] than Genotype B patients. Some small studies have shown that histological inflammation is more significant in genotype C than genotype B patients[71,105] . Genotype F, in studies of Arctic, South American and Spanish populations, also appears to be associated with worse liver disease[98,106,107]. Genotype A appears to have a

more favourable prognosis than genotype D with one small Indian study of 52 patients (46% Genotype A and 48% genotype D) showing more severe histological disease in genotype D[108] and others also attributing more severe liver disease to genotype D compared to A[109]. Genotype D is associated with HBeAg negative CHB and reports from Mediterranean countries of high rates of cirrhosis associated with HBeAg negative disease are now thought to possibly be attributable to genotype D[110]. Genotype H infection in Mexican patients is often adult acquired and thus is frequently associated with low viral loads and low risk of chronic liver disease and HCC[111]. Occult HBV infection is reported to be commonly seen in genotype H patients however this may be partly due to the suboptimal sensitivity of HBsAg assays used[112]. Furthermore the contribution of HBV genotype H to liver disease in Mexican populations is difficult to establish as alcohol, HCV coinfection and obesity are common cofactors[14].

GENOTYPE AND HCC

Genotype C has been shown to carry an increased risk for the development of HCC in the REVEAL study cohort, with an adjusted hazard ratio of 2.35[113]. In addition there is data to suggest that HCC in genotype C is associated with a higher tumour recurrence rate[114]. Genotype B on the other hand may be more likely to be associated with HCC in non cirrhotic patients and has been reported to have higher rates of solitary tumour and more satellite nodules than genotype C[115]. Genotype B has been reported to be more prevalent in patients with HCC developing at a younger age compared to age matched inactive carriers (80% *vs* 52% in those < 50 years and 90% in those < 35 years)[116]. Genotype A in Africans has also been shown to be associated with HCC and at a much younger age than in other groups. Subgenotype A1 which is the most prevalent type in sub-Saharan Africa appears to be the main factor associated with this increased risk[117]. However the contribution of aflatoxin, human immunodeficiency virus (HIV) coinfection and dietary iron overload are also factors to be considered [118]. Genotype F has also been shown to be a risk factor for HCC especially in young Alaskan natives in a case control study[119] while the rates of HCC in genotype H affected populations in Mexico are low[111].

GENOTYPE AND TREATMENT FOR HBV

Response rates to treatment with Peg IFN differ by genotype. In HBeAg positive patients treated with 52 wk of Peg IFN α-2β, HBeAg loss varied with genotype, being 47% in genotype A, 44% in Genotype B, 28% in genotype C and 25% in D[120]. A small study of Peg IFN treatment in Genotype E patients also showed poor responsiveness[121]. Limited data currently available on genotype F patients response to IFN suggests similar response to genotype A[107]. Genotype G in a small number of patients' treated with standard IFN showed poor responsiveness[122]. Based on pooled data of the 2 largest global trials of Peg IFN in HBeAg positive patients Buster *et al*[123] recommend Peg IFN be used in all genotype A patients, and in genotype B and C patients with a high alanine aminotransferase (ALT) and a low HBV DNA. In HBeAg negative CHB also, genotypes B and C have been shown to have higher response rates to Peg IFN treatment compared to genotype D[124].

Quantitative HBsAg (qHBsAg) levels are increasingly being used as predictors of response in Peg IFN therapy for CHB. In HBeAg positive disease, for patients with genotype A and D, absence of any decline in qHBsAg at week 12 has a negative predictive value (NPV) of 97-100% for poor response and in genotypes B and C, week 12 qHBsAg levels of >20000 IU/mL has a high NPV[125]. In HBeAg negative patients treated with Peg IFN, a stopping rule in genotype D based on no decline in HBsAg and < 2 $\log_{10}$ drop in HBV DNA at week 12 of therapy has also become part of recent guidelines[126] based on a very high negative predictive value for sustained response[127]. In HBeAg negative patients treated with Peg IFN, the on treatment kinetics of HBsAg has been shown to vary between genotypes. Long term virological response to Peg IFN treatment has been shown to be predicted by end of treatment qHBsAg with varying threshold levels of qHBsAg identified for different genotypes[128]. HBV genotype does not appear to influence response rates to nucleoside analogue therapy, however the patterns of drug resistant mutations that develop have been reported to be different in different genotypes[129].

VARIANT VIRUSES

HBV replicates at a high rate through the reverse transcription of an RNA intermediate and has a high spontaneous rate of error resulting in numerous mutations arising in the HBV genome[130]. Thus HBV exists as a quasispecies, ie a heterogeneous viral population composed of closely related but non identical genomes[131]. The predominant strain selected out is determined by factors such as host immune response, viral replication fitness and exogenous pressures such as antiviral therapy[132]. The most frequently occurring natural HBV variants are the precore and the basal core promotor (BCP) mutations. They result in a reduction or abolition of HBeAg production. During the early course of perinatally acquired CHB , *i.e.,* the immunotolerant phase, these mutations in the core or precore region are uncommonly seen, but they emerge during the immune clearance phase as a result of immune selective pressures[133].

HBEAG VARIANTS

Precore mutations

A point mutation at nucleotide 1896 in the HBV with

substitution of G for A results in a stop codon at this point thus preventing production of HBeAg although without affecting replication and HBcAg production[134]. The formation of this "precore" mutation is essentially precluded in certain genotypes in particular A and H and it occurs most frequently in genotype D and to a lesser extent in Genotypes B, C and E[24]. The reason for this relates to the fact that nucleotide 1896 is important in maintaining a stem loop structure (epsilon) which is necessary for encapsidation of the pregenomic RNA into the nucleocapsid. Nucleotide 1896 is opposite nucleotide 1858 in the stem loop structure and thus genotypes with a T at nucleotide position 1858 (*e.g.,* genotype D) are more likely to predispose to the development of the G1896A mutation since T-A pairing is more stable than T-G pairing[135]. Likewise genotypes with C at position 1858 (genotype A) are much less prone to development of the precore mutant virus since C-A pairing is weaker than than C-G pairing[136]. Subgenotype F2 similarly codes for C at position 1858 but F1 does not , so precore mutation may occur in some but not other genotype F CHB patients[137].

It has been suggested that stabilisation of the epsilon encapsidation signal may increase the replicative fitness of HBV which may be one reason for it being selected out[133]. Although HBV DNA levels are lower in HBeAg negative disease, this impairment in virion productivity is not thought to be related to Precore and BCP mutants but instead be the result of an independent process[138]. The HBeAg and its relationship to the host immune response is complex. It has been shown to be able to tolerize T cells[139] and cause immunomodulation of Toll-like receptor mediated signalling pathways to evade immune responses and thus is thought to contribute to viral persistence[140]. However its presence in the cytosol also acts as a target for the inflammatory response[141]. Thus it has been suggested that HBeAg may act as a tolerogen or an immunogen in different circumstances and that loss of HBeAg may be a favourable biological characteristic that renders the HBV less vulnerable to immune attack[142]. Numerous other mutations have been described in the precore region including a point mutation at G1899A (commonly seen in association with G1896A)[143]. It has been shown that viral mutation rates increase in the immune clearance phase when compared to the immune tolerant phase[144] and in fact there is significant viral sequence diversity present in the months/years leading up to HBeAg seroconversion[145]. Furthermore, the high rates of nucleotide substitution continue post seroconversion, with ongoing immune selection pressure being applied in the immune control phase although at lower viral loads[146].

Clinically, the G1896A precore mutation is a major cause of HBeAg negative chronic hepatitis and it appears to be associated with reports of fulminant hepatitis, especially in acute adult acquired HBV[147] although not in fulminant cases of CHB[148]. It was shown to be associated with a slightly lower risk of HCC than wildtype virus in a subanalysis of the REVEAL study cohort[113]. However, also recently published is a meta-analysis of 85 case control studies of 16745 patients with 5781 cases of HCC which reports that the precore mutations G1896A, G1899A as well as deletions in Pre-S region were associated with an increased risk of HCC[149].

BCP region mutations

Mutations in the core promoter region occur most commonly at nucleotides 1762 (adenine (A) to thymine (T)) and 1764 [guanine (G) to adenine (A)] and are usually found together[150, 151]. Once again this is thought to possibly confer some compensatory advantage to the virus and Tacke *et al*[152] showed that BCP mutations increased viral replication levels to above those of wildtype virus, including in strains with Lamivudine resistant mutations also present. Other mutations in the basal core promotor region have also been described, including at positions 1653, 1753-1757, 1766, 1768 and these are usually seen in addition to the A1762T and G1764A variants. Overall the association with HBeAg negativity is less strong in patients with BCP mutations than in those with precore mutations The double BCP reduces the production of HBeAg by approximately 70%[153] and this may be even further reduced in HBV variants with the additional mutations in position 1753 and 1766[153]. The BCP mutation has been found to occur more frequently in genotype C than B[78,154] and one possible reason for this may be because genotype C is more likely to have a C at nucleotide position 1858, which largely precludes formation of the precore mutation[155]. The prevalence of the BCP mutation does appear to vary between genotypes being reported at 41%, 27%, 60% and 42% in genotypes A, B, C and D[86], thus the lower prevalence in genotype A compared to C suggests that other factors apart from the nucleotide at position 1858 are also important. The BCP mutations have been implicated quite strongly in more advanced liver disease and in the development of HCC. Yuen reported an association with higher ALT levels in patients with BCP mutations compared to those with wildtype[78]. The authors also subsequently looked at 66 patients with liver biopsies, 71% of whom had the BCP mutations and found these patients had more severe necroinflammation than those without[156]. Lin *et al*[157] also showed that the BCP mutation was associated with the development of cirrhosis and HCC although this was restricted to males. Increasing prevalence of the BCP double mutation was reported by Kao *et al*[154] in patients with more significant liver disease, being 3% in inactive carriers and 64% in patients with HCC. Orito *et al*[158] also described a significant association between BCP and more advanced liver disease (OR = 4.1, 95%CI: 1.6-10.2). Recently a study by Tseng *et al*[159] of 251 spontaneous seroconverters (Genotypes B and C) showed that a higher proportion of BCP mutation (> 45%) was associated with an OR of

2.81 for the risk of cirrhosis. However, whether the BCP mutant HBV is causative or simply a reflection of more significant immune pressure and thus immune mediated inflammation and fibrosis is not known. The association of the BCP mutation with HCC has been documented by a number of groups who reported the BCP mutation was significantly associated with HCC in both genotypes B and C[154,160], in cirrhotic and non cirrhotic HCC[161,162] and by Baptista who found a high prevalence of the mutations in black African patients with HCC[163].

More recently the REVEAL study group also reported an increased risk of HCC in patients with the BCP A1762T/G1764A double mutant compared to wildtype (HR = 1.73, 95%CI: 1.13-2.67). Genotype C also had a higher hazard ratio compared to genotype B (HR = 1.76, 95%CI: 1.19-2.61) and the highest risk was amongst those with the BCP mutations, Genotype C and wildtype virus at the 1896 precore variant site (adjusted HR = 2.99, 95%CI: 1.57-5.7)[113]. There is no strong evidence that the presence or absence of the precore or BCP mutations affects the response to Interferon or nucleos(t)ide analogue treatment. However some studies have shown that the proportion of pre-core and BCP mutant virus present prior to treatment correlates with the chance of HBeAg seroconversion with an approximately 2% increase in HBeAg seroconversion rates per 1% increase of PC and BCP mutant percentages[164].

HBSAG VARIANTS

Vaccine escape mutants

Variations in the HBsAg protein can result in viral infection developing in a vaccinated subject. Anti-HBs is directed towards a highly conserved region of the surface protein (amino acids 99-160) which includes the major "a" determinant of this protein. HBsAg mutations resulting in amino acid substitutions in the region 137-147 of the surface protein can change the conformational epitope in the "a" determinant so that it is not recognized by the neutralizing anti-HBs antibodies. In particular the G145R vaccine escape mutant is known to be stable and replication competent[165,166]. The infectivity of HBsAg mutants is currently thought to be low, however another problem is their lack of detectability by serological tests[167] as was reported in a recent case of HBV with a 4 amino acid repeat insertion at position 115 in the surface protein[168]. The development of vaccine escape mutants has been thought in some parts to be related to the emergence of anti-viral drug resistant mutants because of the overlap of the polymerase gene (where nucleot(s)ide analogue associated resistant mutations occur) with the surface antigen domains recognized by anti-HBs[169]. There is evidence however that emergence of the vaccine escape mutants predates mass vaccination programs[170]. It is thought possible that immune pressure exerted on HBV by anti-HBV due to expanding vaccination programs will result in an increasing problem of vaccine escape mutants[167]. The sG145R vaccine escape mutant has also been detected in 2 of 65 patients in an Australian indigenous cohort, despite wild type polymerase gene sequences in all[171]. Graft infection with HBV following liver transplant in patients who received hepatitis B Immune globulin therapy post transplant has also been shown to be due to the development of *S* gene mutant HBV[172].

S escape mutants

Other mutations in the S protein, eg due to missense mutations in the *S* gene have also been described and are a particular concern for screening of blood donors since they result in false negatives for HBsAg serological testing[173]. The prevalence of 8 mutations associated with HBsAg diagnostic failure, including P120T, T126S, Q128H, G130N, S143L D144A and G145R was found to be approximately 1% in a study of 11,221 HBV sequences encompassing genotypes A-H[174].

A study of 4.4 million Dutch blood donations identified 23 HBsAg negative but HBV DNA positive persons and also reported the presence of multiple escape mutations in the S gene especially in Genotype D patients with occult HBV[175]. Other reasons for HBsAg negativity in the setting of HBV DNA positivity in this study were early acute HBV infection (prior to development of HBsAg), occult HBV infection, genotype G HBV with decreased HBsAg production and suppressed infection after vaccination.

Pre-S mutations

Mutations in the pre-S region, including Pre-S2 deletions, pre-S1-S2 deletions and Pre-S2 start codon mutations have been described[176,177]. Pre-S mutants have been shown to be associated with decreased synthesis and secretion of HBsAg[178]. It has been also been shown that Pre-S2 deletions are associated with more advanced liver disease[179-181] and possibly with the development of HCC especially in younger patients[162,182].

CONCLUSION

HBV genotype and viral variants have clear implications for many clinical aspects of CHB and should become more routinely utilised to help predict likely clinic course, *e.g.,* longer duration of HBeAg phase and higher risk of progression to cirrhosis in genotype C and increased HCC risk in genotypes C and F. It could be argued that formal testing of genotype should be done rather than it being assumed on the basis of ethnicity especially as populations worldwide become increasingly cosmopolitan and subtle shifts in HBV genotype prevalences are already reported (*e.g.,* in Africa and India). Easy to use nomograms derived from the Taiwanese REVEAL study's cohort for predicting HCC risk show further refinement in risk

stratification with the addition of genotype to other parameters[183]. Similarly nomograms that incorporate genotype into predictability of sustained response to Peg IFN therapy can assist with management decisions in CHB patients[123]. Thus practical tools already exist for use in clinics which are based on existing knowledge about the impact of genotype in CHB. Further research into the genotypes E, F and H are required. International agreement amongst experts on issues related to genetic and phylogenetic classification of genotypes and subgenotypes are also important for the future in this evolving area.

With regard to viral variants, knowledge of variants harboured by patients should also be increasingly incorporated into clinical decision making. Revill and Locarnini recently argued that given the evidence that the BCP mutation is an important viral biomarker of the risk of cirrhosis in genotype B and C patients, detection and quantification of BCP mutants should be performed and used as triggers for treatment in Asian CHB patients[184]. Further work on the significance of these mutations in other genotypes was also recommended.

Further elucidation of the clinical significance of other viral variants is warranted. Importantly from a population health perspective, ongoing monitoring of prevalences of vaccine escape and *S* escape mutants is necessary and further research into vaccines that remain efficacious against mutant forms of HBV will be needed.

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