

## TOPIC HIGHLIGHT

Dieter Glebe, PhD, Series Editor

# Hepatitis B virus taxonomy and hepatitis B virus genotypes

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## Abstract

Hepatitis B virus (HBV) is a member of the hepadnavirus family. Hepadnaviruses can be found in both mammals (orthohepadnaviruses) and birds (avihepadnaviruses). The genetic variability of HBV is very high. There are eight genotypes of HBV and three clades of HBV isolates from apes that appear to be additional genotypes of HBV. Most genotypes are now divided into subgenotypes with distinct virological and epidemiological properties. In addition, recombination among HBV genotypes increases the variability of HBV. This review summarises current knowledge of the epidemiology of genetic variability in hepadnaviruses and, due to rapid progress in the field, updates several recent reviews on HBV genotypes and subgenotypes.

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## INTRODUCTION

Hepatitis B virus (HBV) is the prototype member of a steadily growing family of viruses called hepadnaviruses<sup>[1]</sup>. Hepadnaviruses can be found in both mammals (orthohepadnaviruses) and birds (avihepadnaviruses). HBV, the hepadnavirus infecting humans, is classified into eight genotypes today. HBV genotypes differ by at least 8%<sup>[2]</sup>. Since the first definition of the genotypes A, B, C and D<sup>[2]</sup>, genotypes E<sup>[3]</sup>, F<sup>[4]</sup>, G<sup>[5]</sup> and H<sup>[6]</sup> have been

detected. Due to the genetic diversity of HBV, numerous subgenotypes of HBV have been described<sup>[7]</sup> (Table 1). HBV subgenotypes differ by at least 4%<sup>[8]</sup>.

HBV genotypes and most subgenotypes show a distinct geographic distribution. In Asia, where there is a high prevalence of HBV carriers, strong evidence suggests that HBV genotypes influence the course of disease. Several recent reviews have summarised knowledge on different aspects of HBV genotypes<sup>[7-12]</sup> and on hepadnaviruses that infect species other than homo sapiens<sup>[13-15]</sup>. This review will update recent developments in understanding HBV genotypes and taxonomy.

## TAXONOMY

HBV is a partially double stranded virus that uses reverse transcriptase in its replication cycle. Thus, HBV is similar to many retroviruses found in animals and pararetroviruses in plants<sup>[16,17]</sup>.

After cloning and sequencing the HBV genome<sup>[18]</sup>, several related viruses were discovered in woodchucks (*Marmota monax*)<sup>[19]</sup>, ground squirrels (*Spermophilus beecheyi*)<sup>[20]</sup> and pekin duck (*Anas domestica*)<sup>[21]</sup>. Subsequently, numerous new viruses that are similar to HBV were found in mammals and birds and have been cloned (Tables 1 and 2). All these viruses are classified in the family of hepadnaviridae, including the genus orthohepadnavirus (mammals; Figure 1), and the genus avihepadnavirus (birds; Figure 2). In addition to the avihepadnaviruses listed in Table 2, five new hepadnaviruses were cloned from exotic duck and goose species; i.e., the Chiloe wigeon, mandarin duck, puna teal, Orinoco sheldgoose, and ashy-headed sheldgoose. Sequence comparisons revealed that 4 virus isolates were closely related to existing isolates of duck hepatitis B virus (DHBV), while the mandarin duck virus was closely related to Ross goose hepatitis B virus<sup>[22]</sup>.

In chimpanzees, gorillas, orangutans and gibbons new putative members of hepadnaviridae were discovered and sequenced completely<sup>[14]</sup>. It is now widely accepted that primate hepadnaviruses are indigenous to their hosts. Because hepadnaviruses isolated from apes are grouped as HBV genotypes in phylogenetic analyses, it has been suggested that isolates from apes should be named following the nomenclature used for immune deficiency viruses<sup>[23]</sup> (Table 1), e.g. HBV found in chimpanzees should be called HBVcpz. With only 5% divergence from the chimpanzee HBV isolates, the HBV isolate from gorilla is categorized in the HBV genotype (Figure 3, unpublished

**Table 1 Orthohepadnaviruses and their host**

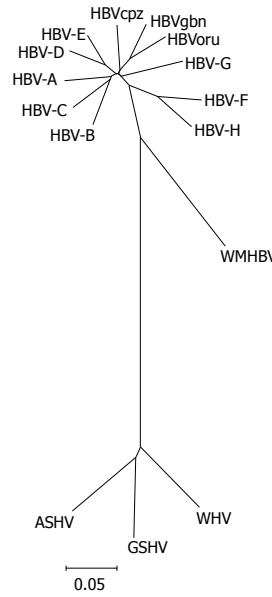
	Host	Ref.
Hepatitis B Virus	Man <i>Homo sapiens sapiens</i>	[75]
Chimpanzee Hepatitis B Virus	Chimpanzee <i>Pan troglodytes</i>	[76]
Gibbon Hepatitis B Virus	White handed gibbon <i>Hylobates lar</i>	[77]
Orangutan Hepatitis B Virus	Orangutan <i>Pongo pygmaeus pygmaeus</i>	[78]
Gorilla Hepatitis B Virus	Gorilla <i>Gorilla gorilla</i>	[79]
Woolly Monkey Hepatitis B Virus	Woolly monkey <i>Lagothrix lagotricha</i>	[80]
Woodchuck Hepatitis Virus	Woodchuck <i>Marmota monax</i>	[19]
Ground Squirrel Hepatitis Virus	Ground Squirrel <i>Spermophilus beecheyi</i>	[20]
Arctic Squirrel Hepatitis Virus	Arctic Squirrel <i>Spermophilus parryi kennicotti</i>	[81]

**Table 2 Avihepadnaviruses and their host**

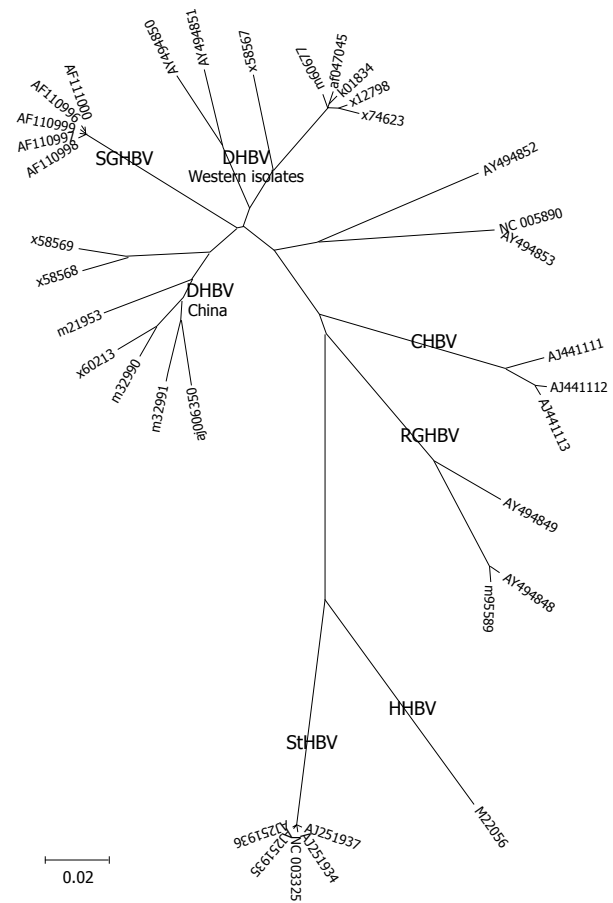
	Host	Ref.
Duck Hepatitis B Virus (DHBV)	Pekin duck <i>Anas domestica</i>	[21]
Grey Teal Hepatitis B Virus (GTHBV)	Grey Teal <i>Anas gibberifrons gracilis</i>	[82]
Heron Hepatitis B Virus (HHBV)	Heron <i>Adrea cinerea</i>	[83]
Maned Duck Hepatitis B Virus (MDHBV)	Maned Duck <i>Chenonetta jubata</i>	[82]
Ross Goose Hepatitis Virus (RGHV)	Ross Goose <i>Anser rossi</i>	[4]
Snow Goose Hepatitis B Virus (SGHBV)	Snow Goose <i>Anser caerulescens</i>	[84]
Stork Hepatitis B Virus (STHBV)	White Stork <i>Ciconia ciconia</i>	[85]
Crane Hepatitis B Virus (CHBV)	Demoiselle cranes <i>Anthropoides virgo</i> Grey crowned cranes <i>Balearica regulorum</i>	[86]

results). Thus, three HBV genotypes from apes can now be differentiated. The chimpanzee and gorilla isolates from Africa are categorized as one genotype, i.e., HBVcpz. The isolates from the South-East-Asian apes, gibbon and orang-utan, are categorized into two genotypes, i.e., HBVgbn and HBVoru, respectively. These genotypes diverge by 8%. Within the gibbon genotype, distinct strains of HBV circulating in geographically separated populations have been described<sup>[24]</sup>.

Avihepadnaviruses are the most distant relatives of HBV with a nucleic acid homology of only 40%. WHV and GSHV as mammalian hepadnaviruses are more closely related to HBV and differ by only 17%. Complete WHV and GSHV genomes from GenBank show a high degree of homology and only one genotype is listed<sup>[25-27]</sup>. However, using degenerate primers, several variant WHV

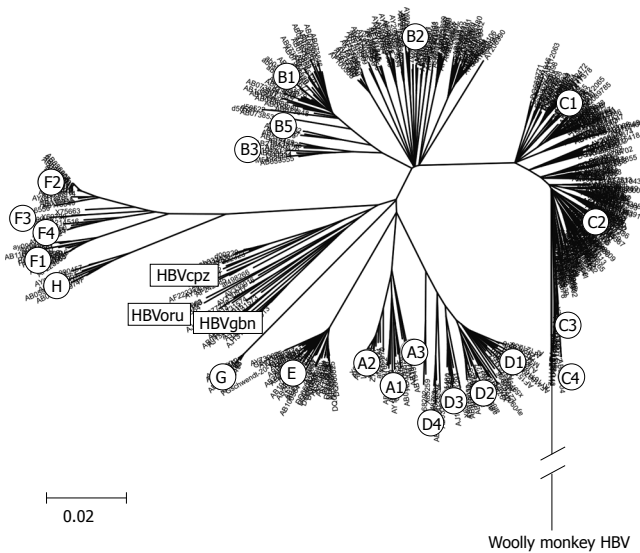


**Figure 1** Phylogenetic tree of orthohepadnaviruses. Complete genomes of HBV genotypes A (X02763), B (D00330), C (M12906), D (V01460), E (X75657), F (X69798), G (AF160501) and H (AY090454); HBVcpz (D00220), HBVoru (NC 002168), and HBVgbn (U46935) were aligned using clustal w with orthohepadnavirus genomes from woolly monkey (AF046996) woodchuck (J02442), ground squirrel (K02715) and the tentative member from arctic squirrel (nc\_001719). The alignment was tested with the neighbour-joining method.



**Figure 2** Phylogenetic tree of the genus avihepadnavirus.

isolates from wild-captured woodchucks were found that showed high divergence with sequencing of small parts of the genome<sup>[28]</sup>. DHBV has two genotypes, in contrast to WHV and GSHV, which have a narrow host range and geographical distribution<sup>[25,26]</sup>, DHBV is found in different avian species with independent isolates in many countries



**Figure 3** Phylogenetic tree of complete HBV genomes. An alignment of 601 complete HBV sequences was performed with Clustal X in the program DNASTar. The alignment was further analysed by boot-strapping using the Neighbourhood-Joining method contained in MEGA version 3.1.<sup>[104]</sup>



**Figure 4** Geographic distribution of HBV genotypes and subgenotypes.

around the world<sup>[29]</sup> (Figure 4).

Human HBV can be grouped into eight genotypes (based on more than 8% difference)<sup>[7,9-12]</sup>. Several attempts have been made to reconstruct the evolution of hepadnaviruses<sup>[30-34]</sup>. Estimating the rate of synonymous substitutions for HBV to be  $4.57 \times 10^{-5}$  per site per year, DHBV has been proposed to have diverged about 30 000 years ago from a common ancestor while GSHV and WHV should have diverged about 10 000 years ago from HBV and the HBV serotypes would be separated by about 3000 years<sup>[31]</sup>. However, as long as we are not able to accurately estimate the mutation rate of HBV over centuries or even millennia, it is not possible to calculate a time point for the separation of HBV genotypes or hepadnaviral species.

### HBV GENOTYPES AND SUBGENOTYPES

HBV genotypes differ by more than 8%<sup>[2,3]</sup>. Phylogenetic analyses using alignments of whole genomes have shown that 8 genotypes, called A, B, C, D, E, F, G and H, of HBV

**Table 3** Fundamental properties of genomes and differences between HBV genotypes

Genotype	Genome length in bp	ORF-differences
A	3221	Insertion of aa 153 and 154 in HBc
B	3215	
C	3215	
D	3182	Deletion of aa 1-11 in preS1
E	3212	Deletion of aa 11 in preS1
F	3215	
G	3248	Insertion of 12 aa in HBc Deletion of aa 11 in preS1
H	3215	

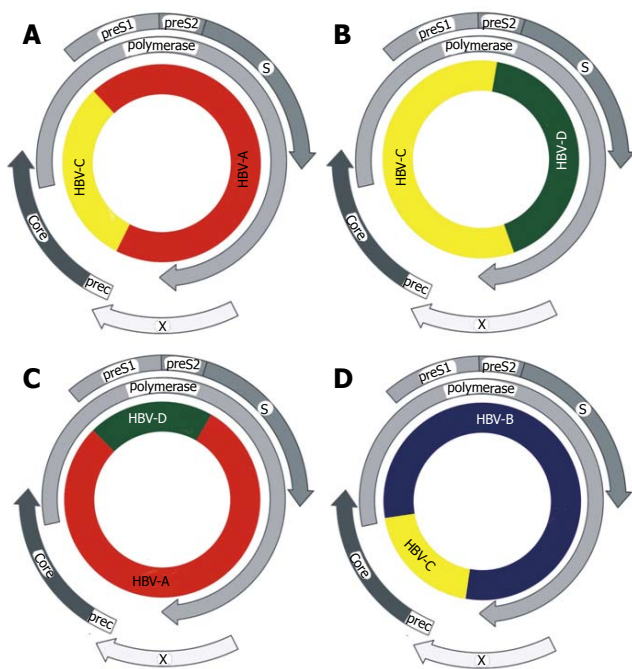
**Table 4** HBV subgenotypes and geographic prevalence

Subgenotype	Synonyms	Geographic origin	Ref.	
A	Aa, A'	Africa, (Asia, South America)	[41,87]	
	A2	Europe		
	A3	Gabon, Cameroon	[88,89]	
	(A4)	Mali	[59]	
	(A5)	Nigeria	[59]	
B	B1	Bj	Japan	[67,90]
	B2	Ba	Asia without Japan	
	B3		Indonesia, Philippines	[7]
	B4		Vietnam	[7]
	B5		Philippines	[91,92]
C	C1	Cs	South East Asia (Vietnam, Myanmar, Thailand, Southern China)	[37-39]
	C2	Ce	Far East (Korea, Japan, Northern China)	
	C3		Micronesia	[7]
	C4		Australia	[93]
	C5		Philippines, Vietnam	[92,94]
D	D1		Mongolia, Belarus, Europe?	
	D2		India?	
	D3		South Africa, East India, Serbia	[40,41]
	D4		Australia	[93]
	D5		East India	[40]
F	F1		South and Central America	[95,96]
	F2		South America	[4]
	F3		Bolivia	[97,98]
	F4		Argentina	[97,98]

can be distinguished<sup>[7,11,12,35]</sup> (Figure 1). In general, HBV isolates found in apes diverge similarly to HBV genotypes in phylogenetic analyses and have been named HBVcpz, -oru, -gor and -gbn for their host's, i.e. chimpanzee, orangutan, gorilla and gibbon, respectively (Table 1)<sup>[23]</sup>. However, as elucidated above, the isolate from gorillas is always categorized into the chimpanzee clade.

A prototypic HBV genome may have a length of 3215 nt, as found in HBV genotypes B, C, F and H. Due to deletions and insertions (Table 3), the other HBV genotypes differ slightly in length of genome (Table 3). Thus HBV genotype G with 3248 nt. is 66 nt longer than genotype D with 3182 bp.

Extensive phylogenetic analyses have shown that HBV genotypes can be further subdivided into subgenotypes (Table 4). HBV subgenotypes differ by at least 4%<sup>[8]</sup>. In



**Figure 5** Schematic genome organisation of recombinants between HBV genotypes. HBV recombinants were described from materials sampled in **A:** Vietnam<sup>[99]</sup>, **B:** Tibet<sup>[68]</sup>, **C:** Africa<sup>[100]</sup> and **D:** Asia<sup>[67]</sup>. The ORF coding for the HBV proteins are shown as arrows, the inner circle represents the HBV genome.

genotypes A, B and C, epidemiological data show that the respective subgenotype pairs A1/A2 (formerly termed Aa/Ae)<sup>[36]</sup>, B1/B2 (formerly Bj/Ba)<sup>[36]</sup> and C1/C2 (formerly Cs/Ce)<sup>[37-39]</sup> differ substantially in many virological and probably some clinical parameters. Subgenotypes also show distinct geographic distribution (Figure 3). However, this is not true for genotype D with subgenotypes D1, D2 and D3 being described as widespread in the world; e.g. D3 was found in Asia (East India)<sup>[40]</sup>, South Africa<sup>[41]</sup> and Europe (Serbia) (Stanojevic *et al*, unpublished results).

Except for genotype E and G, all HBV genotypes can be divided into subgenotypes. The absence of subgenotypes in HBV genotype E has been assumed to be the consequence of a recent genesis for genotype E<sup>[42-45]</sup>. Furthermore, genotype E is not present in Americans of African origin from Venezuela and Brazil<sup>[46,47]</sup>. The case for HBV genotype G appears to be less clear. Genotype G was originally found in the USA, France<sup>[5]</sup> and Germany<sup>[48]</sup>. Later, partial sequencing of HBV genes pointed to a high prevalence of HBV genotype G in Mexico<sup>[49]</sup>. Nevertheless, the geographic origin of HBV genotype G remains unknown<sup>[50]</sup>. To date only a limited number of complete HBV genotype G sequences have been deposited in GenBank that are not classified into subgenotypes.

## DOUBLE INFECTIONS AND RECOMBINANTS

Double infections with two different HBV genotypes have been known since typing was done serologically<sup>[51,52]</sup>. Subsequently, evidence of super infection with HBV isolates of the same or different genotype was described in

**Table 5** Examples for recombination events between of HBV genotypes

Genotype of Backbone	Insert	Recombination Breakpoint		No. in literature	Ref.
		5'	3'		
A	C	1801	2865	3	[99]
A	D	2895	327	3	[100]
		2820	386-586		
		?	670		
B	C	1740-1838	2443-2485	41	[67,73,101,102]
B	C	3120	3171	1	[60]
		3060	3191	1	
		2910	2950	1	
C	B	1731-1838	2437-2479	1	[102]
D	A	129	2339	3	[73,101,102]
		495	780		
		822	1775		
G	C	1860	2460	1	[103]
A	E	882	1060	1	[88]

chronic HBV patients<sup>[53]</sup>. Super infection was accompanied by acute exacerbation of the chronic disease. Additional observations came from patients treated with interferon. Before treatment, HBV genotype A was prevalent. After treatment and relapse, a switch of the genotype to HBV genotype D was described<sup>[54,55]</sup>.

Using different methods for genotyping, several reports described high rates of double infection with two different HBV genotypes in all parts of the world. Using these methods double infections have been found in 4.4%<sup>[56]</sup>, 10.9%<sup>[57]</sup>, 12.5%<sup>[58]</sup>, 14.1% (Kirschberg *et al*, unpublished results), 17.3%<sup>[59]</sup> and 17.5%<sup>[60]</sup> of HBV infected patients. Even triple infections with HBV of genotype A, B and C have been described in 0.9% of HBV infected intravenous drug users<sup>[60]</sup>.

Infection with HBV of genotype G seems to be associated very often with an infection of HBV genotype A<sup>[61]</sup>. This was found in 4 individuals from the USA and in one patient from France<sup>[62]</sup>.

Coinfection with two different HBV genotypes in one patient may lead to an exchange of genetic material between the two strains. However, with current knowledge of HBV replication, the mechanism for this supposed recombination remains enigmatic. No mechanism can be envisioned that would allow an exchange of genetic material between two hepadnaviral genomes at the level of transcription. Nevertheless, numerous authors described changes in the genome of HBV that appear to be the consequences of a recombinatorial event (Figure 5 and Table 5).

Two recent works have comprehensively analysed the prevalence of events in the HBV genome that are reminiscent of recombinations<sup>[63,64]</sup>. About 87% of the putative recombinants were B/C (120) and A/D (29) hybrids. The other recombinants comprised A/B/C, A/C, A/E, A/G, C/D, C/F, C/G, C/U (U for unknown genotype) and B/C/U hybrids. Genotypes A and C showed a higher recombination tendency than did



other genotypes. The results also demonstrated region priority and breakpoint hot spots in the intergenotype recombination. Recombination breakpoints were found to be concentrated mainly in the vicinity of the DR1 region (nt 1640-1900), the preS1/S2 region (nt 3150-100), the 3'-end of the Core gene (nt 2330-2450) and the 3'-end of the Surface gene (nt 650-830)<sup>[63,64]</sup>.

Recombination events between human and chimpanzee<sup>[65]</sup> or gibbon<sup>[63]</sup> HBV sequences have also been described. Discrepant genotyping results from different parts of the genome are indicative of a recombination between genotype A and F<sup>[66]</sup>. Even mosaic genomes with sequences derived from three different genotypes have been described<sup>[59,64]</sup>.

Some recombinants among HBV genotypes have become the dominant subgenotype prevalent in certain geographic regions. Recombination between genotypes B and C has led to the generation of two different strains with distinct geographic distribution<sup>[67]</sup>. Strains of genotype B without recombination are found in Japan (subgenotype B1), whereas strains with recombination between genotype B and C are found throughout Asia (subgenotype B2), sparing Japan<sup>[67]</sup>. Recombinants between HBV genotypes C and D are the leading HBV subgenotype in Tibet<sup>[68-70]</sup>.

It remains open for discussion whether the observed exchanges are the consequence of direct genetic recombination taking place between two HBV strains or if they are the consequence of fast adaptation of HBV to a certain genetic and immunologic environment in different human populations in the world. The high replication capacity of HBV with a release of up to  $10^{13}$  viral particles per day<sup>[71,72]</sup> and the high error rate of the viral polymerase, lead to the production of HBV genomes with all possible single mutations and double mutations of every nucleotide of the HBV genome every day<sup>[72]</sup>. Thus, a fast adaptation of HBV to a new environment is also a possibility.

A hypothetical mosaicism of the HBV genome has already been proposed by Bowyer and Sim<sup>[73]</sup>. This work and later works described most HBV genotypes as a modular genome<sup>[63]</sup> that represents a mixture of small segments coming from many different HBV genotypes. If we expand on this observation, the HBV genome may be made up of a number of allelic modules with different properties; e.g. different binding sites for transcription factors or antigenic epitopes. Thus, a certain combination of these modules would make up an HBV genotype. The findings of Fischer *et al*<sup>[74]</sup> are in support of this speculation. The authors described genotype specific activation or repression of HBV enhancer II, preCore-pregenomic promoter by the transcription factor COUP-TF1.

## CONCLUSION

HBV has been recognised as a prototype member of a family of viruses infecting mammals and birds. Due to its high replication capacity and the high error rate of the viral reverse transcriptase, HBV is able to adapt to the host's environment. This adaptation has led to the emergence

of eight genotypes in humans and three closely related genotypes in apes. The human genotypes have further diverged into at least 24 subgenotypes, with certainly many more to come, and a plethora of recombinants. From the analysis of recombinants there are indications that at least one more genotype remains to be detected.

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