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Review

Hepatitis B virus subgenotyping: History, effects of recombination, misclassifications, and corrections

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ABSTRACT

Hepatitis B virus (HBV) has evolved into phylogenetically separable genotypes and subgenotypes. Accurately assigning the subgenotype for an HBV strain is of clinical and epidemiological significance. In this paper, we review the recommendations currently employed for HBV subgenotyping, the history of HBV subgenotyping, the effects of recombination on HBV subgenotyping, misclassifications in HBV subgenotyping, and suggestions are made to correct the misclassifications. Finally, proposals are made to guide future HBV subgenotyping.

Contents

1. Introduction

Despite the availability of a safe and well-tolerated vaccine, hepatitis B virus (HBV) still poses a serious threat to global public health, especially in Asia, Africa, and Central and South America, where seroprevalence is highest (Lee, 1997; Simmonds and Midgley, 2005). Estimates suggest that HBV infects more than 350 million people worldwide (Purcell, 1993), and host and viral genetics in combination with environmental factors leads to progression to severe liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC), in a subset of individuals (Cao, 2009).

The 3.2 kb circular genome of HBV comprises two partially overlapping double-stranded DNA strands and is highly heterogeneous. Two principal reasons account for the high level of HBV genetic diversity: firstly, the virally-encoded reverse transcriptase

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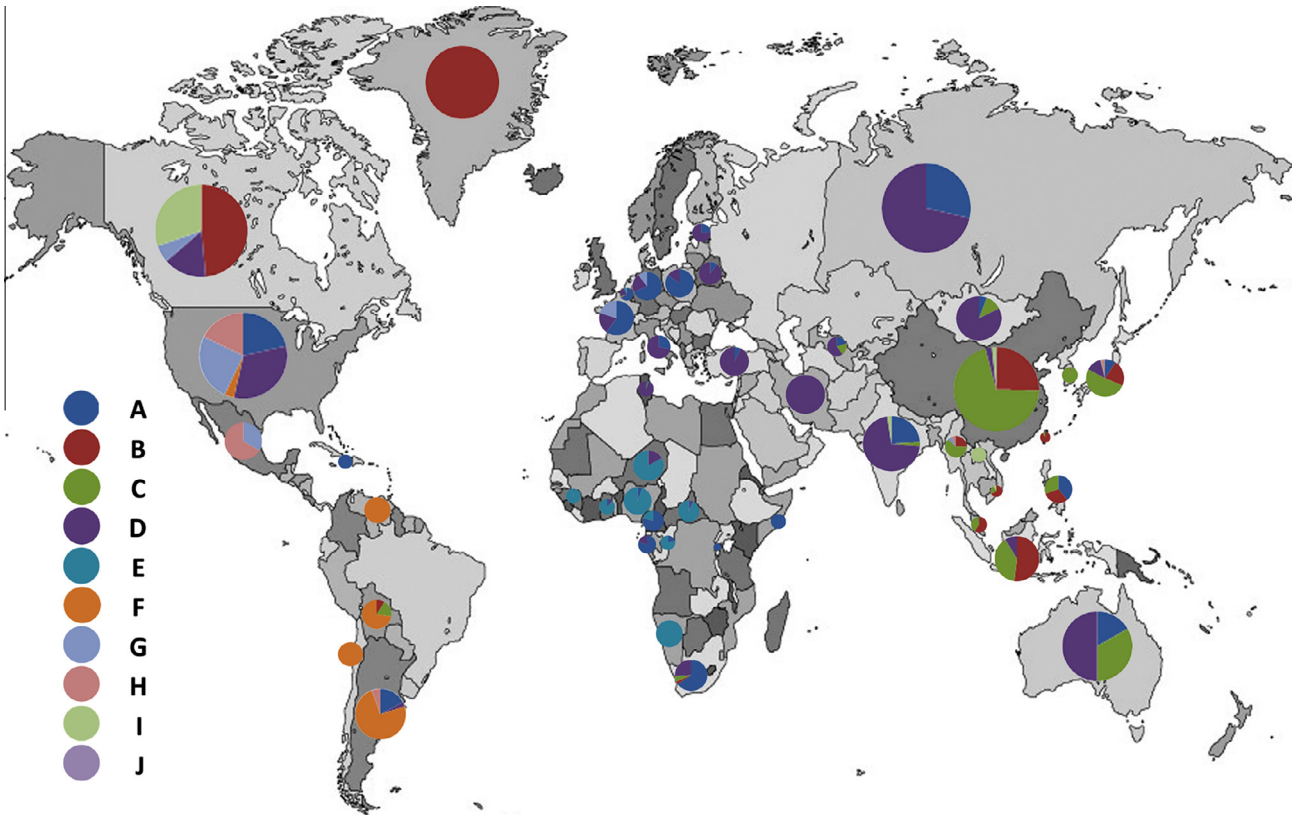

Fig. 1. Geographic distribution of HBV genotypes. The HBV genotyping data was adapted from previous work (Shi et al., 2012a). Only countries/regions with greater than five full-length genome sequences available were included. This dataset included 46 countries/regions and 3179 full-length genome sequences. It should be noted that this geographic distribution of HBV genotypes may not represent actual seroprevalence in a country/region, as there are potential sampling errors due to the bias of including only whole genome analyses. In addition, the size of each circle does not represent the prevalence of HBV infection and burden of disease in the specified geographic region.

(RT) lacks proof-reading ability (Duffy et al., 2008), and secondly, HBV genomes undergo frequent recombination (Shi et al., 2012a; Simmonds and Midgley, 2005; Yang et al., 2006; Ye et al., 2010). HBV has been divided into various genotypes based on the recommendation that different genotypes should diverge by at least 8% over the entire genome (Kramvis et al., 2005; Schaefer, 2007). Some HBV genotypes have been further classified into subgenotypes (Cao, 2009; Schaefer, 2007) and different subgenotypes should show more than 4% nucleotide difference over the genome (Schaefer, 2007).

The accurate designation of HBV genotypes and subgenotypes is significant. Numerous studies have shown that different genotypes and subgenotypes may have different geographic distributions (Fig. 1), disease progression, clinical outcomes, response to antiviral therapy, and prognosis (Akuta and Kumada, 2005; Cao, 2009; Schaefer, 2005; Guettouche and Hnatyszyn, 2005). For example, subgenotype B1 was related to fulminant HBV infections in Japan and subgenotype B2 has been reported to be associated with HCC or HCC recurrence in young patients in East Asia (Ni et al., 2004; Yin et al., 2008). Similarly, patients with subgenotype C2 infections had higher risk of HCC (Chan et al., 2008). In India, subgenotypes D1 and D3 were significantly associated with chronic and occult HBV infections, respectively (Chandra et al., 2009).

In this paper, we review the recommendations used for HBV subgenotyping, the history of HBV subgenotyping, the effects of recombination on HBV subgenotyping, misclassifications in HBV subgenotyping in the literature, and proposals are made to correct the current misclassifications. Finally, we make proposals to guide HBV subgenotyping in the future.

2. Current recommendations for HBV subgenotyping

Three principal recommendations have been proposed and widely adopted to designate novel HBV subgenotypes:

(i) Sequence divergences between the proposed novel subgenotype and all the established subgenotypes should not be less than 4% over the genome (Kramvis et al., 2008; Pourkarim et al., 2010a). Introducing novel HBV subgenotypes based on partial genomes should be avoided.
(ii) From a phylogenetic perspective, the proposed novel subgenotype should be monophyletic. In other words, it should form an independent lineage in the phylogenetic tree (Pourkarim et al., 2010a).
(iii) The proposed novel subgenotype should also be supported with reasonable bootstrap values, usually above 75% (Kramvis et al., 2008; Pourkarim et al, 2010a; Schaefer et al., 2009).

In addition, a few other suggestions have also been proposed, though not widely acknowledged in practice. For example, researchers have proposed that recombinant HBV sequences should not be designated as novel subgenotypes, but reported separately. It has also been suggested that a novel subgenotype should have nucleotide and amino acid polymorphisms specific to itself (Pourkarim et al., 2010a).

3. History of HBV subgenotyping

HBV genotypes A, B, C, D, F and the putative genotype I have been classified into various subgenotypes (Cao, 2009; Olinger

et al., 2008). For the remaining HBV genotypes: E, G and H, no subgenotypes have been described thus far.

In 1997, subgenotype A1 was first described using partial HBV genomic fragments from the *preS2*/*S* region (Bowyer et al., 1997) and were initially named subgenotype A', which was later confirmed by whole genome phylogenetic analysis (Kramvis et al., 2002). In 2004, Sugauchi et al. analyzed 19 HBV whole genome sequences and 20 previously described HBV genotype A whole genomes and found that genotype A was phylogenetically separable into two distinct subgenotypes based on epidemiological distribution: Aa (a: Asia and Africa) and Ae (e: Europe) (Sugauchi et al., 2004). Subsequently, Kurbanov and colleagues classified Aa into subgenotype A1 and renamed Ae as A2 in 2005 (Kurbanov et al., 2005). In addition, Kurbanov and colleagues reported a novel subgenotype Ac (A3), identified in Pygmy and Bantu populations in Cameroon in Western Africa (Kurbanov et al., 2005). Almost at the same time, Hannoun et al. reported a new subgenotype A3 from Gambia, also in Western Africa, and proposed that HBV genotype A is of African origin (Hannoun et al., 2005). In 2006, Olinger et al. described HBV subgenotype A4 in Mali and subgenotype A5 in Nigeria (Olinger et al., 2006) and in 2010, subgenotype A6 was found in African–Belgian patients, with sequence divergence greater than 4% to the five previously described HBV A subgenotypes (Pourkarim et al., 2010b). More recently, subgenotype A7 has been identified from patients from Cameroon, but not from chronically HBV-infected patients from Nigeria, despite the geographical proximity of these nations (Hubschen et al., 2011).

Genotype B was initially divided into two subgenotypes: Bj (j: Japan) and Ba (a: Asia) (Sugauchi et al., 2002). Evidence did not suggest that subgenotype Bj viruses were inter-genotypic recombinants, while those of subgenotype Ba had evidence to support that they were recombinants of HBV genotypes B and C, with their *preC-C* genes derived from genotype C. In 2004, Norder renamed HBV subgenotype Bj as B1 and Ba was renamed as B2 (Norder et al., 2004). In this report, two novel subgenotypes, B3 and B4, were also described (Norder et al., 2004). Subgenotype B3 was composed of four strains from Indonesia, while subgenotype B4 mostly comprised strains from Viet Nam (Dunford et al., 2012) and France. Subgenotype B5 was initially reported in 2006 from the Philippines (Nagasaki et al, 2006). A few months later, Sakamoto and co-workers found HBV strains from the Philippines that differed from subgenotypes B1 to B4, and also designated it as a novel subgenotype, B5 (Sakamoto et al., 2006). Subgenotype B6 was identified in 2007 from indigenous Arctic populations (Sakamoto et al., 2007). As with HBV subgenotype B1, the origin of HBV subgenotype B6 was not apparently the result of a prior recombination event. This observation led Sakamoto et al. to categorize HBV genotype B into two groups: recombinants (B2–B5) and non-recombinants (B1 and B6) (Sakamoto et al., 2007). However, a number of viruses isolated from Yunnan in southwestern China were also subsequently named as subgenotype B6 (Shen et al., 2009). HBV subgenotype B7 viruses were described from the Nusa Tenggara islands in Eastern Indonesia in 2008 (Nurainy et al., 2008). Subsequently, subgenotype B8 was also identified in Indonesia by analyzing a large cohort of patient samples (Mulyanto et al., 2009). More recently, viruses of subgenotype B9 were described from the same geographic region in Indonesia where subgenotype B8 was previously identified (Thedja et al., 2011). This suggested that the distribution of HBV subgenotypes might relate to the ethnic origin of the chronically HBV infected patients (Thedja et al., 2011).

HBV genotype C has the largest number of reported subgenotypes, with at least 16 identified to date. In early 2004, Huy et al. found that genotype C could be classified into at least two subgenotypes: C1 and C2 (Huy et al., 2004). Also in 2004, Norder et al. further divided genotype C into four subgenotypes: C1 from East Asia, C2 mostly from China and Southeast Asia, C3 from Oceania and C4 from Australian Aborigines (Norder et al., 2004; Sugauchi et al., 2001). Subgenotype C5 was subsequently identified from chronic HBV infected patients from the Philippines in 2006 (Sakamoto et al., 2006). Subgenotype C6 was first proposed by analyzing partial HBV genomic fragments (*S* and *preC-C* gene sequences) from Papua, Indonesia (Lusida et al., 2008), which was later confirmed by complete genome sequencing in 2009 (Utsumi et al., 2009). Contemporaneously, a HBV strain isolated from the Philippines was also defined as subgenotype C6 (Cavinta et al., 2009b). After a comparison between these two C6 subgenotypes, the Philippines virus was renamed as subgenotype C7 (Schaefer et al., 2009; Cavinta et al., 2009a). However, some viruses from Nusa Tenggara, Indonesia were also proposed as a novel subgenotype C7 by Mulyanto and colleagues (Mulyanto et al., 2009). To avoid potential confusion in the description of subgenotypes, Mulyanto and colleagues renamed their C7 as C8 in 2010 (Mulyanto et al., 2010). In addition, they also proposed a novel subgenotype C9, which they originally reported as unclassifiable (Mulyanto et al., 2010). Subgenotype C10 was also identified from Indonesia where notably other novel HBV subgenotypes, such as B7, B8 and C7 to C9, have also been described (Mulyanto et al., 2010). This genetic diversity of Indonesian HBV strains was further highlighted in 2011, by two independent research groups who proposed a HBV subgenotype as C11 (Mulyanto et al., 2011; Utsumi et al., 2011). Moreover, Mulyanto et al. reported another novel subgenotype C12 which has the same geographical origin as C11 (Mulyanto et al., 2011). Recently, Mulyanto et al. described further four novel HBV subgenotype C viruses: C13 to C16 also from Papua, Indonesia (Mulyanto et al., 2012). Finally, two more subgenotypes associated with HBV genotype C/D inter-genotypic recombination events, CD1 and CD2, were characterized from Tibet (Cui et al., 2002; Wang et al., 2005, 2007).

HBV genotype D is broadly geographically distributed and is the dominant genotype in the Mediterranean regions (Kramvis and Kew, 2005; Miyakawa and Mizokami, 2003; Schaefer, 2007). Norder and colleagues first classified genotype D into four subgenotypes: D1, D2, D3, and D4, by analyzing 33 full-length HBV genomes in 2004 (Norder et al., 2004). In 2008, Kramvis et al. analyzed 102 HBV genotype D genome sequences and also classified them into four subgenotypes, despite some of the inter-subgenotype divergences being clearly below 4% (Kramvis et al., 2008). Two HBV strains described in Eastern India showed sequence divergences greater than 4% from previously reported subgenotypes D1-D4 and were designated as subgenotype D5 in 2006 (Banerjee et al., 2006). Subgenotype D6 was first proposed based on partial HBV genome sequences in 2008 (Lusida et al., 2008) and was further confirmed using three complete HBV genome sequences in 2009 (Utsumi et al., 2009). Some HBV genotype D strains from Tunisian blood donors were found to be more than 4% divergent over their entire genomes from previously described subgenotypes and were defined as a novel HBV subgenotype, D7, even though evidence of recombination between D7 and genotype E HBV was observed (Meldal et al., 2009). Subgenotype D8 was identified in Niger in 2010 and viruses of this subgenotype were also notably HBV D/E inter-genotypic recombinants (Abdou Chekaraou et al., 2010).

In 2001, genotype F was classified into four clusters with different geographical origins based on the small *S* gene (Mbayed et al., 2001). Later in 2003, HBV genotype F was classified into two clades based on precore mutations (G1896A), frequently observed in anti-HBe-positive carriers of HBsAg with T1858 in the stem region of the RNA encapsidation signal and a non-synonymous mutation (T45L) in the S protein (Norder et al., 2003). In the same year, genotype F was split into four groups using full-length genome sequences for the first time and HBV genotype F I was further subdivided into two subgroups, Ia and Ib (Piñeiro y Leone et al.,

Table 1
Distribution of the recombinant sequences among different HBV subgenotypes.

Genotypes	Subgenotypes	Recombination types	Is the subgenotype entirely or partially composed of recombinant sequences?	Representative references
A	A1	A/C, A/D	Partially	Shi et al. (2012a)
	A2	A/C, A/D	Partially	Owiredu et al. (2001)
	A3	A/E	Partially	Kurbanov et al. (2005)
	A7	A/D	Partially	Shi et al. (2012a)
B	B1	B/C	Partially	Shi et al. (2012a)
	B3	B/C	Entirely	Sakamoto et al. (2007), Shi et al. (2012a)
	B4	B/C	Entirely	Sakamoto et al. (2007), Shi et al. (2012a)
	B5	B/C	Entirely	Sakamoto et al. (2007), Shi et al. (2012a)
	B6 (from China)	B/C	Entirely	Shen et al. (2009), Shi et al. (2012a)
	B7	B/C	Entirely	Nurainy et al. (2008), Shi et al. (2012a)
	B8	B/C	Entirely	Mulyanto et al. (2009), Shi et al. (2012a)
	B9	B/C	Entirely	Shi et al. (2012a), Thedja et al. (2011)
C	C1	A/C, B/C, C/D	Partially	Shi et al. (2012a)
	C2	A/C, B/C	Partially	Shi et al. (2012a)
	C4	C/X	Entirely	Sugauchi et al. (2001)
	C5	A/C, B/C	Entirely	Sakamoto et al. (2006)
	C12	C/G	Partially	Mulyanto et al. (2012)
	C13	B/C	Partially	Mulyanto et al. (2012)
	CD1	C/D	Entirely	Cui et al. (2002), Wang et al. (2007)
	CD2	C/D	Entirely	Wang et al. (2005, 2007)
D	D1	A/D, B/D, C/D	Partially	Shi et al. (2012a)
	D2	C/D	Partially	Shi et al. (2012a)
	D3	A/D, C/D	Partially	Chauhan et al. (2008)
	D7	A/D, D/E	Partially	Meldal et al. (2009), Shi et al. (2012a)
	D8	D/E	Entirely	Abdou Chekaraou et al. (2010)
F	F4	C/F	Partially	Shi et al. (2012a)
I	I1	A/C/G	Entirely	Olinger et al. (2008)
	I2	A/C/G	Entirely	Olinger et al. (2008)

2003). This classification scheme was supported by Devesa and colleagues by analyzing a large number of complete HBV genome sequences (Devesa et al., 2004). Taking the above classifications and the sequences from Bolivia into consideration, Huy et al. proposed that genotype F should be separated into four subgenotypes, F1–F4, with F1 corresponding to F I, F2 to F II, F4 to F IV, previously proposed by Mbayed and co-workers (Mbayed et al., 2001) and F3 to F III as designated by Devesa et al. (Devesa et al., 2004; Huy et al., 2006).

Although the designation of the putative HBV genotype I was challenged due to nucleotide sequence divergence <8% with HBV genotype C (Kurbanov et al., 2008), it has been tentatively classified into two subgenotypes, I1 and I2 (Olinger et al., 2008).

4. Effects of recombination on HBV subgenotyping

Initially it was proposed that recombinant HBV sequences should not be designated as subgenotypes, but reported separately, despite evidence that several subgenotypes were entirely or partially derived from recombination events (Table 1). In particular, all the sequences of subgenotypes B3 to B9, C4, C5, CD1, CD2, D8, I1 and I2 were inter-genotype recombinants (Table 1).

The effects of recombination on HBV subgenotyping have been previously studied by us (Shi et al., 2012b,c). The results showed that inclusion of recombinant HBV sequences can alter (frequently increase) sequence divergence values in a few cases and more significantly can change the topology of the trees. However, for genotypes A, C and D, inter-genotype recombination played a very limited role in HBV subgenotyping when all the sequences available were used to classify HBV subgenotypes. However, it was apparent that if only a few representative sequences are used for HBV subgenotyping, the result may be significantly affected with the inclusion of recombinant sequences.

For genotype B, approximately 93% of the sequences ($N > 800$) were inter-genotype recombinants (Shi et al., 2012a). If recombinants could not be designated as subgenotypes (Pourkarim et al., 2010a), apart from subgenotypes B1 and B6, the remaining subgenotypes should be discarded. This would lead to a major problem how we report such a large number of recombinant sequences not only capturing the major evolutionary characteristics of the sequences, but also the differences between them.

5. Misclassifications in HBV subgenotyping

Although there have been rules for HBV subgenotyping, a number of misclassifications were still reported (Ahn et al., 2009; Schaefer et al., 2009). Below we summarize the misclassifications and present possible explanations.

First, incomplete sampling, particularly with small datasets or partial genome sequences results in misleading phylogenies and as a consequence erroneous designation of novel HBV subgenotypes. This might be the major reason leading to misclassifications in HBV subgenotyping and has been observed in genotypes A, B, C and D (Table 2). For example, there was a reported incongruence in C1 and C2 proposed by Huy et al. and Norder et al. respectively (Ahn et al., 2009). Although Schaefer and colleagues suggested that the designation proposed by Huy et al. should be used (Schaefer et al., 2009), subgenotype C2 proposed by Huy et al. was not monophyletic (Huy et al., 2004).

Second, different sampling sometimes may give rise to different sequence divergence values. The sequence divergences between A7 and A1 to A6 were 5.10 ± 0.37%, 5.34 ± 0.33%, 4.24 ± 0.24%, 3.99 ± 0.25%, 3.81 ± 0.24% and 5.10 ± 0.35% (Hubschen et al., 2011), while those calculated by Pourkarim and colleagues were relatively lower, with 3.90 ± 0.03%, 4.70 ± 0.03%, 3.70 ± 0.02%, 3.50 ± 0.03%, 3.10 ± 0.03% and 4.60 ± 0.03%, respectively. Based on this, Pourkarim and colleagues believed that A7 should not be designated as a novel subgenotype with sequence divergence just below 4% (Pourkarim et al., 2011).

Third, some sequence divergences between existing subgenotypes are less than 4%. For example, sequence divergence between

Table 2
Misclassifications identified in HBV subgenotyping.

Genotype	Misclassifications	Probable reasons	References
A	A3, A4, A5	Sequence divergence below 4%	Andernach et al. (2009), Pourkarim et al. (2010a)
	A3, A4	Not a monophyly	Pourkarim et al. (2010a)
	A7	Sequence divergence below 4%	Pourkarim et al. (2011)
B	B3	Not a monophyly	Shi et al. (2012b)
	B6	Named twice	Sakamoto et al. (2007), Shen et al. (2009)
	B3, B5, B7, B8, B9	Sequence divergence below 4%	Shi et al. (2012b)
C	C1, C2	Named twice	Ahn et al. (2009), Schaefer et al. (2009)
	C2	Not a monophyly	Huy et al. (2004)
	C6	Named twice	Cavinta et al. (2009a,b), Utsumi et al. (2009)
	C11	Named twice	Meldal et al. (2009), Utsumi et al. (2009)
D	D1, D2, D3, D4	Sequence divergence below 4%	Kramvis et al. (2008)
	D3	Not a monophyly	Unpublished data
	D7	Not a monophyly	Unpublished data
	D3, D6	Sequence divergence below 4%	Stanojević et al. (2011)

A3 and A4 was less than 4% (Andernach et al., 2009). Similarly, some of the sequence divergences between subgenotypes D1 to D4 proposed by Kramvis and colleagues were below 4%, although these subgenotypes have been widely accepted (Schaefer et al., 2009). In addition, sequence divergence between D3 and D6 was only 3.0 ± 0.2% (Stanojević et al., 2011).

Fourth, a number of subgenotypes proposed by different research groups were given the same subgenotype name. For example, subgenotype C6 was proposed twice by two research groups (Cavinta et al., 2009a; Utsumi et al., 2009). The same situation was also seen for A3 (Hannoun et al., 2005; Kurbanov et al., 2005), B6 (Sakamoto et al., 2007; Shen et al., 2009), C11 and D6 HBV subgenotypes (Meldal et al., 2009; Utsumi et al., 2009).

It should be noted that all the problems in HBV subgenotyping were found in genotypes A–D and none was found in genotypes F and the putative genotype I. However, this is perhaps attributable to the far smaller number of available sequences for the latter genotypes so further sampling is clearly needed in Latin America and Southeast Asia respectively. In addition, in genotype C, there are several sequences that have not been classified into any known subgenotypes.

6. Proposed corrections and novel classifications

A few suggestions are proposed to correct the misclassifications in HBV subgenotyping and based on these corrections, novel classifications have also been proposed for HBV genotypes A–D (Table 3). For genotypes F and I, old classifications remained unchanged in that no misclassifications were identified in these two genotypes (unpublished results).

Notably, Pourkarim et al. proposed a definition of "quasi-subgenotype" and applied it in HBV subgenotyping (Pourkarim et al., 2010a). A quasi-subgenotype includes previously designated subgenotypes and sequences of these subgenotypes have the same or similar geographic origin. However, current evidence failed to support these subgenotypes either because of sequence divergence less than 4% or non-monophyletic tree topologies. However, when all these subgenotypes are regarded as one single "quasi-subgenotype", both sequence divergences greater than 4% with other known subgenotypes and monophyly are obtained. This definition has been employed successfully to correct the misclassifications in the subgenotyping of HBV genotypes A, B and C (Pourkarim et al., 2010a; Shi et al., 2012b,c).

7. Future directions

Although several corrections have been made, some problems still exist. First, bootstrap values for some HBV subgenotypes are very low. For example, after correction, C2 became a monophyly, however, the bootstrap value to support the quasi-subgenotype C2 was very low (Shi et al., 2012c). Similarly, bootstrap support for subgenotype D1 was only 46% (unpublished results). Second,

Table 3
Proposed corrections and novel classifications of HBV subgenotypes.

Genotypes	Proposed corrections	Novel classifications	References
A	1. A3, A4, A5 and A7 should be designated as a quasi-subgenotype A3 2. A6 should be renamed as A4	A1, A2, quasi-subgenotype A3, and A4 (original A6)	Pourkarim et al. (2010a), Pourkarim et al. (2011)
B	1. B3, B5, B7, B8, B9, and B6 (from China) should be designated as one quasi-subgenotype B3 2. B6 (from Arctic) should be renamed as B5	B1, B2, quasi-subgenotype B3, B4, and B5 (original B6)	Shi et al. (2012b)
C	1. C2 should be classified into two subgenotypes, quasi-subgenotype C2 and tentative C14. Quasi-subgenotype C2 included the old C14 proposed by Mulyanto and colleagues in 2012 2. Two sequences, GQ358157 and GU721029, previously designated as C6 should be re-designated as C12 and C7, respectively 3. C6 from the Philippines has been renamed as C7 4. C11 proposed by Utsumi and colleagues should be renamed as C12	C1, quasi-subgenotype C2, C3 to C10, C11 (proposed by Mulyanto and colleagues), C12 (including C11 proposed by Utsumi and colleagues), C13, tentative C14, C15, C16, CD1 and CD2	Shi et al. (2012c)
D	1. D3 and D6 should be designated as a single subgenotype, newD3 2. D7 and D8 should be designated as a single newD7	D1, D2, new D3 (including original D6), D4, D5, new D7 (original D7 and D8)	Unpublished results

although most recombinant sequences have been classified into certain subgenotypes, a few recombinant sequences cannot be classified into any known subgenotypes (Shi et al., 2012b,c).

Among all the rules proposed by the leading experts in the field, phylogeny should be given the highest priority in HBV subgenotyping. If phylogenetic analysis shows that a subgenotype is not monophyletic, one should be extremely cautious to designate it as a novel subgenotype despite sequence divergence values with other known subgenotypes greater than 4%.

To avoid assigning more subgenotypes which have the same or similar geographical or ethnic origin, and sequence divergence values between them are approximately 4% (depending on different estimate methods and different sequences into analysis), quasi-subgenotype should be used (Pourkarim et al., 2010a).

In addition, most previous analyses have selected a few representative strains of all known HBV genotypes and subgenotypes. This incomplete sampling strategy often showed high bootstrap support for a given subgenotype and with a monophyletic nature. However, when all associated sequences were analyzed together, neither the high bootstrap support nor its monophyletic nature was always replicated. Therefore, we suggest that the designation of a novel subgenotype should be based on a comparison of all available relevant full-length genome sequences in public databases rather than only a few representative strains.

Finally, novel subgenotypes should not be named by individuals, but by the International Committee on Taxonomy of Viruses (ICTV) as proposed previously (Schaefer et al., 2009). Individuals will then be able to present their evidence to support a potential new HBV subgenotype and accordingly, reviewers should be cautious of proposed novel subgenotypes (Schaefer et al., 2009). The ICTV summarizes and verifies all the potential novel subgenotypes and formally names novel HBV subgenotyping classifications annually. Any personally designated ‘‘novel’’ subgenotype without verification by the ICTV should not be acknowledged.

8. Conclusions

Worldwide, HBV infection still poses a serious threat to public health, especially in East Asia, Southeast Asia and Africa. HBV has evolved into various genotypes and subgenotypes, and tracing the evolution and variation of HBV is important for HBV phylogenetic analysis and correctly assigning new HBV genotypes/subgenotypes and also for predicting the virologic response to therapy in chronic hepatitis B infections. However, a number of factors result in misclassifications of several HBV subgenotypes: (1) HBV subgenotypes showing <4% sequence divergence with known subgenotypes, (2) non-monophyletic topologies for certain subgenotypes, (3) subgenotypes named twice, and (4) not including all available HBV whole genome sequences in analyses. In this paper, we review the recommendations currently employed for HBV subgenotyping, the HBV subgenotyping history, the existing misclassifications in HBV subgenotyping and corresponding corrections. We recommend that researchers should comply strictly with previously suggested guidelines and new recommendations proposed in this review for HBV subgenotyping and finally, that HBV subgenotypes should be only named by the ICTV.

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