Under Construction: Classification of Hepatitis B Virus Genotypes and Subgenotypes

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Hepatitis B virus (HBV) genotypes [1–6] and subgenotypes [7–12] have an impact on the course of disease and treatment decisions. In Germany, HBV genotyping is recommended before starting interferon therapy treatment of HBV infection [13]. Thus, an accurate and universal system of classification is of prime importance.

Several largely overlapping definitions for HBV genotypes have been presented by leading experts in this field in the past [4, 14–17]. However, due to the lack of universally accepted rules, irregularities have accumulated within the last 20 years of HBV genotype research. Some of these are found below.

(1) In a letter to the editor in this issue of *Intervirology* Ahn et al. (pp. 321–322) raise an important point. The assignment of subgenotypes C1 and C2 to clades is contradictory in the literature. As outlined by Ahn et al. there are two influential papers in which C1 following the nomenclature of Norder et al. [4] is C2 according to the classification of Huy et al. [18] and vice versa. However, regarding the figure in the letter, an uninitiated reader might get the impression that Huy et al. [18] also defined subgenotypes C3–C5. Also, only C1–C4 were described by Norder et al. [4]. C5 was described by Sakamoto et al. [19] in 2006. The reason for the discrepancies in nomenclature would have been more obvious if the references in the figure had been Huy et al. [18] and Norder et al. [4], respectively.

(2) Sequencing of only parts of the HBV genomes led to the tentative suggestion of subgenotypes A4 and A5 [20]. In an article in *Emerging Infectious Diseases* sequencing whole genomes of tentative A4 isolates, Andernach et al. [21] now group this subgenotype into the clade of A5.

(3) Based on whole genome sequencing two new subgenotypes have been called C6 in two contemporaneous publications. C6 has been identified in the Philippines [22] and Papua, Indonesia [23]. Isolates from these two publications map into two clades with a divergence of 5.1% and thus make up a new subgenotype C7 from the Philippines [Schaefer S, unpublished].

(4) A similar problem seems to arise with subgenotype D6. There are two concurrent publications that describe a new subgenotype D6 in Tunisia [24] and Papua, Indonesia [23].

(5) Regarding the delimitation of subgenotypes, these have been described as the major divisions within the phylogenetic trees in genotypes by Norder et al. [4]. Later on Kramvis et al. [14] defined subgenotypes as differing by at least 4% and supported this by robust bootstrapping data, although yielding largely overlapping results with the definition by Norder et al. [4]. This competing definition is partly contradictory to the original paper [4], where 4 major clades of genotype D, designated D1–D4, were described with an overall divergence for some of these clearly below 4% [25]. However, these subgenotypes

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are supported by robust bootstrapping values and are now widely accepted.

(6) Recombination in HBV isolates is common [26], e.g. subgenotype B1 is a recombinant between small parts of genotype C and subgenotype B2 [27]. In 2008 a new genotype I was described by Tran et al. [28]. Isolates of this clade seem to be widespread in South East Asia and many additional isolates were described in Vietnam [28] and Laos [29]. However, this genotype had already been described by Hannoun et al. [30] as a recombinant. Because the tentative genotype I is a complex recombination of several human and even gibbon HBV sequences, the designation genotype has been questioned by leading experts from the field [15]. However, the exact parental strains remain to be defined for genotype I as well as genotype G [31]. This is in great contrast to the first intergenotypic HCV 2k/1b recombinant where the parental strains are easily classifiable within already established HCV subtypes [32].

Taken together, there are several points in HBV classification that need mending. To solve the issue we suggest an international consensus with a universal recommendation on how to delimit HBV genotypes and subgenotypes that all experts in the field agree on. In addition, this group of experts will make suggestions to the International Committee on Taxonomy of Viruses (ICTV) containing a list of HBV genotypes and subgenotypes. For each subgenotype a prototype genome should be selected derived from an HBeAg-positive source to avoid phylogenetically irrelevant substitutions (in general the first isolate identified, if not too dissimilar to the consensus of that genotype or subgenotype). After careful analysis, the ICTV should officially acknowledge the rules for HBV classification and HBV subgenotypes.

For the time being we suggest following the widely used definitions, i.e.:

(1) Use the designation for subgenotypes C1 and C2 according to Huy et al. [18], since although they were designated as subgroups in the original paper their designations have been widely used for the corresponding subgenotypes.

(2) Group HBV subgenotype following the clade definitions of Norder et al. [4].

(3) The delimitation of new subgenotypes should be based on comparisons with previously described subgenotypes of that particular genotype by pair-wise comparisons and be supported by strong bootstrapping values.

(4) The tentative subgenotype C6 from the Philippines [22] will be renamed C7.

Apart from these – personal – recommendations, we suggest the following points for consideration by the expert committee for a universal HBV classification scheme.

(1) A new genotype should diverge by less than 8% at pair-wise comparisons of complete genomes [33]. The strain defining a new genotype should not be the result of a recent recombination, where the parental strains may be easily classifiable.

(2) New genotypes/subgenotypes should only be suggested on the background of complete genome sequences of more than one isolate.

(3) If feasible, the ICTV should be contacted during the review process when a new clade with a suggested new designation has been submitted. Only after the ICTV has declared that this new designation is not claimed by a contemporaneous submission in another journal will this designation be used in the accepted and published version.

(4) Reviewers should take care that correct and actual designations are used.

Taken together, Ahn et al. have raised an important point that should be taken care of in a timely manner. Coordinated actions from leading experts from the HBV genotype field and the ICTV are needed.

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