Author's response to reviews

Title: Subgenotype reclassification of genotype B hepatitis B virus

Authors:

Weifeng Shi (wf.shi@qiat.ac.cn)
Chaodong Zhu (zhucd@ioz.ac.cn)
Wei Zheng (zhengwei_001@126.com)
Michael J Carr (michael.carr@ucd.ie)
Desmond G Higgins (des.higgins@ucd.ie)
Zhong Zhang (zh.tsmc@yahoo.com.cn)

Version: 2 Date: 19 August 2012

Author's response to reviews: see over
Dear Editor:

Thank you and the two reviewers very much for reviewing our manuscript. Your comments were very helpful for us to improve our manuscript. Now we have revised our manuscript according to your comments point by point. We think the concerns made by the reviewers have been appropriately addressed.

Your sincerely,

Weifeng Shi

Response to the reviewers:

Reviewer: Mahmoudreza Pourkarim

1- Introduced strains as genotypes "I" are result of recombination between several strains from different genotypes. They are just considered as recombinant strains and accepting them as real genotype has not been confirmed by expert scientists in the field of classification of HBV. I would like to suggest the authors to avoid a controversial article by leaving out this genotype from text and just referring to confirmed genotypes (A-H).

Response: This is a good point. Yes, genotype I has not been widely accepted and we have changed the text to avoid a controversial article.

2- To resolve the problem of indefinite strains in subgenotyping of genotype A, quasi-subgenotype A3 was proposed. Also, authors of present manuscript proposed the same term for indefinite subgenotypes of genotype B. Apparently, authors did not enough focused on the novel term and used quasi-genotype B3 which is not correct. If authors are proposing a novel term for subgenotyping, it should be quasi-subgenotype B3 and quasi-genotype B3 is definitely incorrect.

Response: Definitely, quasi-subgenotype B3 rather than quasi-genotype B3 should be used. It is a typo. We are sorry for this and have modified it in the main text.

3- Authors should correct the Figure 2. In front of a compressed branch instead of "B3,B5,B6,B7,B8,B9==> new B3, quasi-subgenotype B3 should be introduced.
Response: We have made this change in the new Figure 2.

Reviewer: Samad Amini-Bavil-Olyaee

1- Tables 1 and 2 should be mixed together (as Table 1) and also all mean nucleotides distance should be presented as “percentage ± SD” that it makes all variables constant within the manuscript. Therefore, SD from upper part of each table should be removed and is brought up in front of each variable.

Response: We have mixed tables 1 and 2 and SD values in the upper right corner have been removed.

2- The authors should add a new table (Table 3) to present the comparison of mean nucleotide divergence (percentage ± SD) of B1, B2, QSB3, B4 and B5 (previous B6).

Response: We have added the table that the reviewer asked. However, in fact, the contents of the new table have already been listed in the main text.

3- Please edit “quasi-genotype B3” to “quasi-subgenotype B3” in the entire manuscript.

Response: The other reviewer also mentioned this. It is a typo. We feel sorry for this and we have revised it.

4- Table 3 should be renamed to Table 2 and all data should be presented as mean percentage ± SD, and all SD should be removed.

Response: We have done this.

5- Please define a cut-off value for the bootstrap numbers in the trees. I would recommend 45% as cut-off and vanish all values below 45% in trees.

Response: We have removed most of the bootstrap values below 45% in Figure 1 to make the tree look clearer. However, some values below 45% were still kept as they were close to the root and were important and helpful in defining the potential subgenotypes.

6- If the authors really want to follow Pourkarim at al (Ref 25) roles, then they should remove all recombinant strains from all analysis. I would suggest that. Of course the authors mentioned that all B2 are recombinant, I am wondering that all 655 isolates are recombinant or not. If few of them are recombinant, so they can be excluded from analyses.

Response: We would like to follow the method Pourkarim at al used. However, there was a huge difference between genotype B and other genotypes in that most (803 out of 860) genotype B
isolates were potential recombinants and all subgenotype B2 sequences (n=655) were recombinants [Ref 16]. While for other genotypes, only a small number of sequences were identified to be recombinants [Ref 16]. It has also been reported that genotype B could be classified into two types, non-recombinant (B1 and B6) and recombinant (B2 to B5) [Ref 3]. Therefore, recombinants cannot be excluded from the analysis of genotype B HBV.

7- Please check that whether isolates EU660226 and GQ377595 and Bx isolates are recombinant or not (using BootScan analysis); if so, please remove them from trees and analysis.

Response: We have previously proved that EU660226 and GQ377595 and Bx were B/C recombinants using BootScan and other methods [Ref 16]. However, sequences in cluster 3 (quasi-subgenotype B3) were all B/C recombinants (Figure 1). This makes it difficult to give a reasonable excuse to remove them from analysis (why not remove other B/C recombinants?). Therefore, we think it is better not to exclude them from analysis.

8- Please add scale bar for trees.

Response: This is a good question. To be honest, in the old figures 1 and 2, there was a scale bar, respectively. However, as you can see from the figures, Figure 1 was a schematic phylogenetic tree, but not the real tree. We did this because the real tree was too big to visualize in a single page. Similarly, Figure 2 was a cartoon, not a real phylogenetic tree either. Therefore, we removed the scale bar from the figures. However, we have added a scale bar in the upper left corner of additional file 1. Additional file 1 was the real phylogenetic tree we constructed.

9- Please add this references to the manuscript. Pourkarim et al. HBV subgenotype misclassification expands quasi-subgenotype A3, CMI;17(6):947-9.

Response: We have added this reference.