[Responses to Reviewers' comments]

Manuscript Number: 24719

Title: Genomic change in hepatitis B virus associated with development of hepatocellular carcinoma

Thank you for your valuable comments and suggestions. All the authors have discussed your comments in great depth. We have revised your comments and made some changes of our manuscript according to your suggestions. Here we are resubmitting our final version of manuscript reflect your kind recommendations.

Let us address your comments and suggestions as followings:

#1. Reviewer's code 01560464:

1) The whole X, S, basal core promoter (BCP), and precore regions of HBV from patient's serum or liver tissue samples were sequenced using the direct sequencing method. The results showed that the presence of T1753V mutation in HBV X-gene significantly increased the risk of HCC development in patients chronically infected with genotype C HBV. It is important value to reveal the role of T1753V mutation in HBV X-gene in the development of HCC. 2) The design, method and results of the retrospective

cohort study are reasonable and credible. I suggest that the article can be published in the form of retrospective study in World J Gastroenterology.

Answer)

We do much appreciate your valuable comments.

#2. Reviewer's code 02444743:

This was retrospective study of gene changes of 240 CHB patients infected with genotype C HBV and HCC in Korea, the study found that T1753V mutation in HBV X-gene significantly increases the risk of HCC. Even if the current study had longer follow-up period (1-237 months), the patients sample and follow-up duration is also short for patients from CHB to HCC. In addition, some comment should be concerned. 1. This study included 240 patients with CHB, but "N = 234" in Table 1, why? 2. If n=240, the number of patents with C1653T mutation in the X region is 25, the rate of mutation is 25/240= 10%; not 12%, T1753V is 14% not 15%; and so on. 3. The "mutation in the X region, T1753V, was seen in 15% (n = 33)"in text, but n=32 in Figure 3, why? Wild type is 175, what is wild type? 4. The results of "Effects of combined mutations in HBV genome on HCC development" and Figure 4 legend should be revised. For example, "Patients with both BCP A1762T/G1764A mutations also had a significantly higher occurrence rate of HCC (P < 0.05) (Figure 4B)", it is confused.

Response to reviewer's specific comments:

1. This study included 240 patients with CHB, but "N = 234" in Table 1, why?

Answer) Sorry for the typo. We correct the number to "N = 240" in Table 1.

Reaction's as following;

(Table 1: page 26)

Table 1. Baseline characteristics of patients with chronic hepatitis B

Variables	*N* = 240
Age, years[a]	48 (27-86)

2. If n=240, the number of patents with C1653T mutation in the X region is 25, the rate of mutation is 25/240= 10%; not 12%, T1753V is 14% not 15%; and so on.

Answer) We made another mistakes in our calculation. We correct the proportion of mutations including C1653T, T1753V, and G1896A.

Reaction's as following;

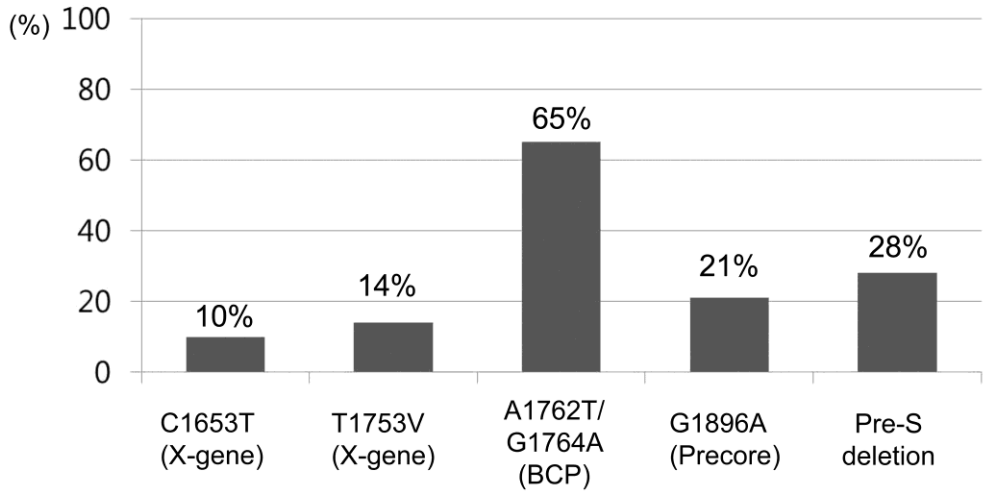
(Abstract: page 5, 3rd paragraph)

Out of 240 CHB patients, 25 (10%) had C1653T and 33 (14%) had T1753V mutation in X region; 157 (65%) had A1762T/G1764A mutations in BCP region, 50 (21%) had G1896A mutation in precore region and 67 (28%) had pre-S deletions.

(RESULTS: page 10, 2nd paragraph)

The C1653T mutation in the X region was found in 10% of the study cases (n = 25). Another mutation in the X region, T1753V, was seen in 14% (n = 33). The BCP double mutation, A1762T/G1764A was detected in 65% (n = 157). The G1896A mutation in the PC region was seen in 21% (n = 50) and the pre-S deletion was found in 28% (n =67).

(Figure 1: page 20)



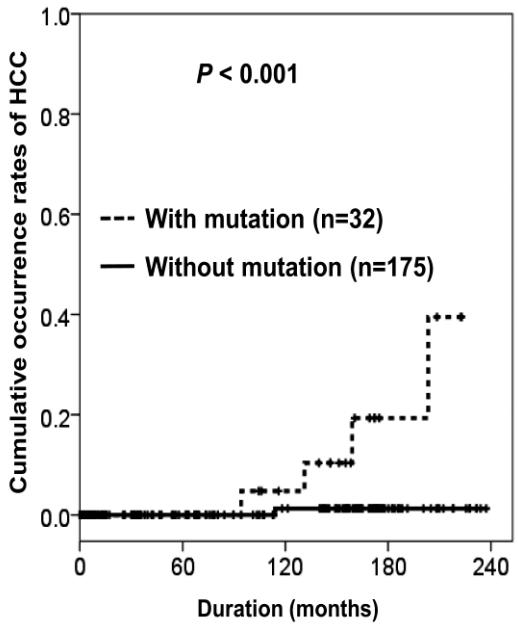
3. The "mutation in the X region, T1753V, was seen in 15% (n = 33)"in text, but n=32 in Figure 3, why? Wild type is 175, what is wild type?

Answer) As you had commented, the number of the patients with T1753V mutation is 33 among the entire subjects. However, we could analyze the cumulative occurrence rates of HCC in relation to the presence of T1753V mutation only in 207 patients out of 240 patients; 32 in patients with T1753V and 175 in patients without T1753V respectively because of the missing value

from some of the patients. In Figure 3, wild type means that the patient without T1753V mutation. We think this word may be inappropriate, so we have changed terms "without mutation" instead of "wild type" in Figure 3 to avoid any confusion.

Reaction's as following;

(Figure 3: page 22)



4. The results of "Effects of combined mutations in HBV genome on HCC development" and Figure 4 legend should be revised. For example, "Patients with both BCP A1762T/G1764A mutations also had a significantly higher occurrence rate of HCC (P < 0.05) (Figure 4B)", it is confused.

Answer) Sorry for typo again and also not explained clearly in some text. In the

results of "Effects of combined mutations in HBV genome on HCC development", we intended to reveal the effects of the combination of the BCP double mutation and other mutations such as T1753V, G1896A, and pre-S deletion. As you have pointed out, we correct the sentence in the results and we revised Figure 4 legend.

Reaction's as following;

(RESULTS; Page 11, 1st paragraph)

Patients with both BCP A1762T/G1764A mutations and the G1896A mutation also had a significantly higher occurrence rate of HCC ($P < 0.05$) (Figure 4B)

(Figure 4 legend; Page 25)

Figure 4. Cumulative occurrence rates of HCC in relation to the combination of the BCP A1762T/G1764A double mutation and with the T1753V mutation in the X region (A), the BCP A1762T/G1764A double mutation and the G1896A mutation in the PC region (B), the BCP A1762T/G1764A double mutation and the pre-S Deletion (C), and the BCP A1762T/G1764A double mutation and the C1653T mutation in the X region (D)

Thank you very much for reviewing our manuscript.

We are looking forward to having your positive feedback.

Sincerely,

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