Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: LNA RT-PCR and melting curve analysis were used to identify two polymorphisms in codon 269 of HBV Pol, which may be of value for the deeper understanding of epidemiology and disease progression of CHB. However, the manuscript does not seem to be the final manuscript, with multiple annotations, figure s1 has only text descriptions but no figure.

→ As reviewer 1 suggested, we deleted the text descriptions of figure S1, as it was misstated (p. 30). In addition, we performed further language polishing, so that our revised manuscript will meet the publication requirement (Grade A).

Reviewer #2:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: No comments.

→As reviewer 2 suggested, we performed further language polishing, so that our revised manuscript will meet the publication requirement (Grade A).

Reviewer #3:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: On the whole, the manuscript is well organized, concise and coherent, and the use of language and grammar is accurate and appropriate. The title of this manuscript is simple and easy to understand, the abstract is brief and clear, the key words reflect the key points, the introduction fully describes the background, status quo and significance of the research, the method introduces the research methods in detail, the result clearly lists the actual application, and the discussion and summary are relatively accurate. It is well known that HBV genotype C2 infection has obvious clinical or virological characteristics, including higher risk of HCC, lower response rate of interferon or prolonged HBeAg positive period. However, the question of which factor can explain several obvious clinical and virological features of C2 genotype infection has not been resolved. Therefore, the authors propose the hypothesis that this may be related to the existence of two HBV Pol RT polymorphisms, rt269L and rt269I. In order to confirm this hypothesis, the authors developed a new simple and sensitive LNA-RT-PCR assay using melting curve analysis for the identification between two polymorphisms within codon 269 of HBV Pol, rt269L and rt269I (three genotypes,

rt269L1, rt269L2 and rt269I). Different from the traditional direct sequencing, it has the advantages of simple operation, low possibility of cross contamination, and can improve the hybridization affinity of complementary sequences, showing strong mismatch discrimination. In this manuscript, the method was applied to 94 clinical samples, and 87 samples could be identified, 86 of which were consistent with the results of traditional direct sequencing, indicating that LNA RT-PCR analysis could isolate two polymorphisms of the rt269 codon of HBV Pol from clinical samples, with high sensitivity (92.6%, 87/94 samples) and specificity (98.9%, 86/87 samples). Moreover,this assay can find out almost exact ratio between two types within specimens from mixed cases (23/24 cases), suggesting its feasibility in analysis of quasi-species distribution in mixed samples. There are some unique insights in this manuscript. Using this new method, it is found that the frequency of "L1" type is the highest, which indicates that "L1" type is the main cause of HBV infection in South Korea. Type I may be a variant of L1, rather than an independent polymorphism. Studies based on the direct sequencing protocol also showed that "L1" type was more related to higher HBV replication, HBsAg levels and HBeAg positive serum status than "I" type. **There are also something need to be improved, such as the description of clinical significance is too simple, and no detailed inclusion and exclusion criteria are listed.** Of the 94 samples, 7 could not be identified, and 1 was different from the direct sequencing, which needs further analysis**. If "I" type may be a variant of "L1" type, whether "L" and "I" mixed type can be considered as an intermediate process of variation, and how variation is generated, these need to be further discussed.** The subjects included in this manuscript are all patients at the initial stage of drug use, and are samples from one medical institution**. Whether the results obtained are representative is also a question to be discussed.** There may be some mistakes in the sentence that our LNA based RT-PCR assays showed that WT, 'L1' type (n=68, 78.2 %) is found in our cohort with the highest frequency, followed by 'I' type (n=12, 13.8%) and 'L2' type (n=3, 3.4%) (Table 3) in the discussion.We know from the manuscript that in the positive detected 87 samples, 63 and 24 samples were identified either singly or in a mixed manner, respectively. Of the 63 samples identified singly, the prevalence of 'L1' type, 'I' type and 'L2' was 82.5% (n=52), 12.7% (n=8) and 4.8% (n=3), respectively. Of the 24 mixed form samples, the prevalence of samples with the almost same ratio of L1 and I (co-dominant cases) was 29.2% (n=7). The prevalence of L1 (L1+I or L1+L2) and I dominant (L1+I) cases was 54.2% (n=13) and 16.7% (n=4), respectively. So the number of L1 cases should be 65 (52+13).

1) **There are also something need to be improved, such as the description of clinical significance is too simple, and no detailed inclusion and exclusion criteria are listed.**

1-1) As reviewer 3 suggested, we supplemented the clinical significance of our LNA based RT-PCR assay in discussion section (p. 12)

"Since our LNA-based RT-PCR assays can identify L1 of higher infectivity and other variants (L2 or I type) related to disease progression from large serum

samples without time-consuming or labor intensive sequencing procedures, it could help in the management or treatment of chronic patients in genotype C2 endemic nations, including China, Japan and South Korea."

1-2) As reviewer 3 suggested, detailed inclusion and exclusion criteria are described in the material and method section - patient part, as described below (p. 5-6):

"For this study, serum samples were used from 94 patients who visited Seoul National University Hospital (2005-2007) and met the inclusion criteria of hepatitis B surface antigen (HBsAg) positivity and HBV DNA positivity (for more than 6 months) and were LMV, ADV, ETV, LdT, TNF, and peg-IFN treatment-naïve. All patients had negative tests for hepatitis C virus, human immunodeficiency virus and markers for coexisting autoimmune liver disease, and alcohol or drug addiction."

This is also well stated in the content of the approved IRB.

Selection criteria (in IRB content): Treatment-naive hepatitis B patient samples are used for the experiment. All patients were diagnosed with chronic hepatitis B and confirmed that no treatment such as nucleos(t)ide analogs (NAs) or interferon was initiated. These selection criteria include HBVs antigen (HBsAg) positive results for more than 6 months and detection of HBV-DNA virus. In this study, patient serum human-derived products passed the above existing IRB review (NO. 1012-131-346) will be used for experiments, and donors agreed to use human-derived materials for secondary research purposes, and will confirm and use them within the specified storage period.
Exclusion criteria: Exclusion from hepatitis C infection or co-infection of acquired immune syndrome (HIV), autoimmune liver disease, alcohol or drug addiction.

2) **If "I" type may be a variant of "L1" type, whether "L" and "I" mixed type can be considered as an intermediate process of variation, and how variation is generated, these need to be further discussed.**

2) As reviewer 3 recommended, we further descried the variant types in our discussion section (p. 13) as below:

"A total of 24 (27.6%) of the 87 positively detected samples were identified in a mixed manner, and L1, in most cases of mixed infections, was dominant or codominant over I or L2. These findings further support our hypothesis that I or L2 may be a variant of the L1 type rather than an independent

polymorphism. However, to clarify whether mixed infection in a patient is due to simple mutation of L1 to L2 or I type or superinfection of another type, further quasispecies analysis should be investigated in the future."

3) The subjects included in this manuscript are all patients at the initial stage of drug use, and are samples from one medical institution Whether the results obtained are representative is also a question to be discussed.

3) We agree with the comments raised by reviewer 3. Therefore, we described the limitation of this study in discussion section (p. 13) as below:
"The limitation of this study is that all the samples included were obtained from patients at the initial stage of drug use and are from one medical institution. To determine the exact clinical significance of L1, L2 and I infections or mixed infections in genotype C2-infected chronic patients, our LNA-based RT-PCR assays should be applied to a larger population-based cohort of multicenter registries in future studies."

4) There may be some mistakes in the sentence that our LNA based RT-PCR assays showed that WT, 'L1' type (n=68, 78.2 %) is found in our cohort with the highest frequency, followed by 'I' type (n=12, 13.8%) and 'L2' type (n=3, 3.4%) (Table 3) in the discussion. We know from the manuscript that in the positive detected 87 samples, 63 and 24 samples were identified either singly or in a mixed manner, respectively. Of the 63 samples identified singly, the prevalence of 'L1' type, 'I' type and 'L2' was 82.5% (n=52), 12.7% (n=8) and 4.8% (n=3), respectively. Of the 24 mixed form samples, the prevalence of samples with the almost same ratio of L1 and I (co-dominant cases) was 29.2% (n=7). The prevalence of L1 (L1+I or L1+L2) and I dominant (L1+I) cases was 54.2% (n=13) and 16.7% (n=4), respectively. So the number of L1 cases should be 65 (52+13).

4) As reviewer 3 suggested, we have corrected the mistake in our discussion section, it should be "L1' type (n=65, 74.7%), 'I' type (n=12, 13.8%), and 'L2'

type (n=3, 3.4%).

5) In addition, we performed further language polishing, so that our revised manuscript will meet the publication requirement (Grade A).