

PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 80836

Title: Locked nucleic acid real-time polymerase chain reaction method identifying two polymorphisms of hepatitis B virus genotype infections, rt269L and rt269I

Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 06363546

Position: Peer Reviewer

Academic degree: N/A

Professional title: N/A

Reviewer's Country/Territory: China

Author's Country/Territory: South Korea

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Reviewer chosen by: AI Technique

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Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input checked="" type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Peer-reviewer statements	Peer-Review: [<input checked="" type="checkbox"/>] Anonymous [<input type="checkbox"/>] Onymous
	Conflicts-of-Interest: [<input type="checkbox"/>] Yes [<input checked="" type="checkbox"/>] No

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).

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Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



**Baishideng
Publishing
Group**

7041 Koll Center Parkway, Suite
160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
<https://www.wjgnet.com>

Peer-reviewer statements	Peer-Review: [<input checked="" type="checkbox"/>] Anonymous [<input type="checkbox"/>] Onymous
	Conflicts-of-Interest: [<input type="checkbox"/>] Yes [<input checked="" type="checkbox"/>] No

SPECIFIC COMMENTS TO AUTHORS

No comments.

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Group**

7041 Koll Center Parkway, Suite
160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
<https://www.wjgnet.com>

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	Conflicts-of-Interest: [<input type="checkbox"/>] Yes [<input checked="" type="checkbox"/>] No

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.