Author’s response to reviews

Title: Vitamin D receptor ApaI polymorphism associated with progression of liver disease in Vietnamese patients chronically infected with Hepatitis B virus

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Dear Editor,

We would like to submit the revised version of our manuscript MGTC-D-19-00085R4 " Vitamin D receptor ApaI polymorphism associated with progression of liver disease in Vietnamese patients chronically infected with Hepatitis B virus " to be considered for publication in BMC Medical Genetics journal. We thank the editor for providing valuable comments and suggestions. We have carefully considered the comments and have changed some critical points in the manuscript. We believe that the manuscript has been improved. All changes in the text were highlighted and rebuttals to the queries are interleaved as below.

Thank you very much for your time and consideration.
With best regards

Dr. Nghiem Xuan Hoan
Institute of Clinical Infectious Diseases
Editor Comments:
please discuss the possibility of population stratification and feasibility of correction as limitation of the study.

Thanks for the suggestion dear editor. We have answered the queries that has been raised and have discussed the limitations of the study in the discussion section.

Reviewer reports:

Sungho Won (Reviewer 1):

My comments are about the population stratification. Authors insist that all subjects are from the same ethnicity and no population stratification adjustment is necessary. However I cannot agree on this response. For instance, population stratification is observed even if there is a single ethnicity. Even for the same ethnicity, the minor allele frequencies can differ and thus its adjustment is almost always very important. Authors can consider the EIGENSTRAT approach (https://www.nature.com/articles/ng1847). Alternatively authors can use the family-based samples (https://www.nature.com/articles/5200625.pdf?origin=ppub)

Thank you for your suggestion. We understand population stratification can be a cause for false positive results in genetic association studies. We would have performed a “population stratification” if the gene under study showed a marked variation in allele frequency across subgroups of the population. This study did not have any sub groups, as it represented only one ethnicity. Also, based on principal component analysis using our genotype data, the population structure assigned all individuals in one cluster, that additionally supports the data from HAPMAP and 1000 genomes project.

This study in particular was a case-control study and we have not embarked into family based samples to test for any transmission disequilibrium, where one can compare frequencies of transmission of two alleles from heterozygote parents to an affected offspring. Moreover, the genotype frequencies of the SNPs among HBV patients and HCs were in Hardy-Weinberg equilibrium (P>0.05).

The major and minor allele frequencies observed in this study were compared to available data in the 1000 genomes project. The allele frequencies were ApaI [rs7975232: Major allele C=0.7424; Minor allele A=0.2576]; FokI [rs2228570: Major allele G=0.5859; Minor allele A=0.4141]; Taq1 [rs731236: Major allele T=0.9596; Minor allele C=0.0404] and for BsmI [rs1544410 Major allele C=0.9596; Minor allele T=0.0404]. The observed minor allele frequencies from this study corroborated well with the available data.
Lai-Ping Wong (Reviewer 2): The revised version is much improved compared to the previous. With curiosity why vitamin D levels only measured for 50 out of 238 HC subjects?

The vitamin D serum levels was measured in 50/238 HCs and in 286 HBV patients (CHB=92, LC= 89, HCC=105). The measurement of serum vitamin D levels was a secondary objective of the study to correlate any findings with observed distribution among the HBV patient groups. In addition, we had a constraint in procurement of kits with limited budget. The serum vitamin D levels were also considered as a covariate in all analysis.

We have included this as a limitation of the study in the discussion as ‘One limitation of the study is that Vitamin D levels was measured only in fewer healthy controls compared to a large group of HBV patients’

Akram Alyass (Reviewer 3): The study by Hoan and colleagues assesses the relationship between VDR polymorphisms and the progression of liver disease in HBV cases. The authors have provided appropriate responses to the reviewers' comments. The study is well-executed, and I have enjoyed reading the article. I have no significant remarks and would like to thank the authors for their work. I only have one minor comment.

1. The term "multivariate" logistic regression is confused with "multivariable" logistic regression. Please replace the term "multivariate" with "multivariable"

Thank you for this suggestion. The R package runs a multivariate logistic regression analysis (the commonly agreed and consensus term in statistics), which considers multiple confounding factors that are the different variables. We will consider using this accepted terminology ‘multivariate’ in the light of reader’s perspective.