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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Author’s response to reviews:

Dear Editor and Reviewers,

We submit the revised version of our manuscript “Vitamin D receptor ApaI polymorphism associated with progression of liver disease in Vietnamese patients chronically infected with Hepatitis B virus” (MGTC-D-19-00085R3).

Thank you very much for considering our manuscript and for providing valuable comments and suggestions. We have carefully considered all comments from the editor and reviewers and revised the manuscript accordingly. All changes in the manuscript are highlighted and specific replies to each reviewer’s comment are detailed in the “Rebuttals to the reviewers” below. We hope that the revised version has improved the manuscript and meets the journal’s requirements for publication. We also believe that our data are of interest for the readership of “BMC Medical Genetics”.

We greatly appreciate your consideration of our manuscript.
Sincerely,
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Dear Dr Hoan,

Your manuscript "Vitamin D receptor Apal polymorphism associated with progression of liver disease in Vietnamese patients chronically infected with Hepatitis B virus" (MGTC-D-19-00085R3) has been assessed by our reviewers. They have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Medical Genetics.

Their reports, together with any other comments, are below. Please also take a moment to check our website at https://www.editorialmanager.com/mgtc/ for any additional comments that were saved as attachments. Please note that as BMC Medical Genetics has a policy of open peer review, you will be able to see the names of the reviewers. If you are able to fully address these points, we would encourage you to submit a revised manuscript to BMC Medical Genetics. Once you have made the necessary corrections, please submit online at: https://www.editorialmanager.com/mgtc/. If you have forgotten your password, please use the 'Send Login Details' link on the login page at https://www.editorialmanager.com/mgtc/. For security reasons, your password will be reset.

Please include a cover letter with a point-by-point response to the comments, describing any additional experiments that were carried out and including a detailed rebuttal of any criticisms or requested revisions that you disagreed with. Please also ensure that all changes to the manuscript are indicated in the text by highlighting or using track changes.

Please also ensure that your revised manuscript conforms to the journal style, which can be found at the Submission Guidelines on the journal homepage. A decision will be made once we have received your revised manuscript, which we expect by 11 Jul 2019.

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Reviewer reports:

Sungho Won (Reviewer 1): In this manuscript, VDR polymorphisms were genotyped by DNA sequencing and in-house validated ARMS assays in 443 HBV infected patients including chronic hepatitis B, liver cirrhosis and hepatocellular carcinoma and in 238 healthy control individuals (HCs). Logistic regression models were applied in order to determine the association of VDR polymorphisms with manifest HBV infection as well as with progression of related liver diseases in different genetic models. Authors found that among the four VDR polymorphisms, ApaI is associated with the clinical outcome and liver disease progression in Vietnamese HBV-infected patients. Manuscript is generally written well but I have some comments.

1. Authors should provide some evidences about the absence of population stratification. Genetic association is very sensitive to population substructure and thus it should be carefully adjusted. Otherwise sample size is small and results are not reliable.
   Answer: We have added this to the revised draft in the discussion, where we provide allele frequencies for Kinh ethnicity
   ‘Kinh ethnicity is a major ethnic population (>87% ~ 77 million) in Vietnam. Based on mtDNA data, all Vietnamese carry South East Asian haplotypes, which show a limited geographic and ethnic stratification. (Pischedda et al. 2017), therefore no population substructure is foreseen’.

2. If race variable is available, authors should adjust its effect by adding it as a covariate. It is not clearly stated how controls were selected.
   Answer: Thank you for the comments. All study subjects (patients and controls) were from Northern Vietnam and belonged to the Kinh ethnicity. Hence, race variable is not considered as a covariate in the analyses. We have added the information on how controls were selected in “Method section” as below
   “In addition, blood samples of 238 healthy blood donors were randomly enrolled from the hospital’s blood bank as control group (HC). They were students in the universities or employers in manufacturing companies located within or nearby Hanoi. All patients and controls came from Northern Vietnam and belonged to the Kinh ethnicity.”

At the same time authors should compare the allele frequencies of controls with public reference data. Please check the minor allele frequencies of 4 SNPs with 1000 Genome project data, etc.
   Answer: We have included these details in the text for Kinh ethnicity as below
The major and minor allele frequencies observed were compared to available data in the 1000 genomes project. The allele frequencies were for 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 alleles frequencies from our study corroborate with available data.

Lai-Ping Wong (Reviewer 2): Authors investigated association of target VDR related SNPs and HBV in Vietnamese subjects, aimed to examine potential contribution of target SNPs to risk and disease progression. Healthy individuals were younger than patients (median age: 30 vs. 51), it is challenging to have ideal matching patients and control in term of age, however mutation burden always correlate with age. Older people tend to have higher mutation burden, any possibility to find control set that have less age gap between patients and controls?

Answer: We strongly agree with the reviewer to that point. In the logistic regression models, we have adjusted for age and gender in order to avoid the confounding impact of these factors to the genetic association of VDR polymorphisms with progression of HBV-related liver diseases.

Despite some findings reported are contradicted compared to other studies in the field, it is good to explain potential clinical values of the findings.

Answer: Based on our findings, we have stated that although there was no association between VDR polymorphisms with HBV infection risk, the ApaI polymorphism might be a genetic factor associated with the clinical outcome and disease progression in Vietnamese HBV infected patients. Additionally, the influences of VDR polymorphisms on the course as well as the pathogenesis of HBV-related liver diseases remain still controversial and need to be explored further. Thus, the potential clinical values of the findings on the role of VDR variant in HBV infection are to provide the additional evidences to the literature rather than to translate into clinical practice at this stage.

Found some typos, proof read the manuscript is necessary.

Answer: The text was corrected for appropriate use of scientific English and language all through the manuscript.