Author’s response to reviews

Title: Polymorphisms in the Th17 cell-related RORC gene are associated with spontaneous clearance of HCV in Chinese women

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Author’s response to reviews:

Dear editor,

Thank you for your consideration of our manuscript entitled “Polymorphisms in the Th17 cell-related RORC gene are associated with spontaneous clearance of HCV in Chinese women” for possible publication in “BMC infectious diseases”. We are grateful for the comments from reviewers and editor. Point-by-point responses were attached below. All the revised words and sentences were highlighted in the revised version. Hopefully this revised version is acceptable for publication.

Best regards,

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Response to Editor Comments:

1) In the Declarations section, under the heading "Funding", please declare the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Answer: The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript. We added the declaration under the heading “Funding”.

2) In the Declarations section, under the heading "Authors’ contributions", please state the contributions of Dr Weiping Cai (WC) to the study.

Answer: Dr Weiping Cai (WC) has participated in the study design. We added the declaration under the heading “Authors’ contributions”.

3) Under the heading "Figure legends", please also include the supplementary figure descriptions.

Answer: We added the descriptions of supplementary figures under the heading “Figure legends”. Please see lines 429-442 in the revised version.

Response to Reviewer #1:

Major Comments:

1) Page 5, line 13: the authors mentioned 190 patients, but in abstract section the number of patients mentioned 137. Which is true? 190 or 137?

Answer: The total number of anti-HCV positive females was 190, which included HIVpos women with chronic HCV infection (n=53). Since HIV infection may affect the ability of some individuals to spontaneously resolve their HCV infection, HIVpos women with chronic HCV infection (n=53) were excluded from the final primary study. Therefore, 137 female patients were analyzed in the final primary cohort. We made a change in the description under the heading “Participants” to make it clearer (see highlighted lines 94-98), and Figure 1 was also helpful for understanding the selection progress of primary and sub cohorts.

2) Inclusion and exclusion criteria are missing and are better to be added or rewritten in more plausible form.

Answer: The criteria were described in Figure 1 and in line 94-105 in the text.
3) Page 6, line 2: why have the authors omitted HIVpos women with chronic HCV infections (n=53)? Please explain more. Because the number of samples seems to be enough, I recommended the author examine the relationship between RORC polymorphisms and HIV-positive patients.

Answer: Due to the possibility that some HIV-co-infected individuals who could essentially clear HCV spontaneously in the absence of HIV became chronic HCV carriers, chronically HIV/HCV-co-infected patients were excluded from the study, which we discussed under the heading “Participants” (lines 94-96) and “Discussion” (lines 217-219 in the “Discussion”). As reviewer suggested, we examined the relationship between RORC polymorphisms and HIV-positive patients and found no significant differences in RORC polymorphisms rs9826 and rs1521177 between HIVpos HCV carriers and HIVposHCV resolvers, indicating that HIV infection is likely having a negative influence on HCV spontaneous resolve, the results were showed in TableS5 (lines 153-156).

4) Are the treatment responses clear? If yes, the authors should be evaluate the correlation between these SNPs and treatment responses such as RVR, EVR and SVR. I recommended the author examine the relationship between RORC polymorphisms and treatment responses.

Answer: All the patients in the cohort were recruited from a countryside village and none of them received any types of anti-HCV treatment though HIV-positive patients received first-line ART therapy. The treatment status was stated in the revised manuscript (line 92-93).

5) Details of SOC therapy has to be mentioned in term of dose and schedule.

Answer: As we answered comment 4 above, that HCV+ patients in the cohort did not receive any types of anti-HCV treatment, this comment is not applicable.

6) The results of HWE and LD should be mentioned in the text.

Answer: SNP-specific deviation from Hardy–Weinberg Equilibrium (HWE) in the whole study population was tested using a X2 test in SHEsis software. All the X2 and P values were showed in TableS4. The LD results were showed in Figure S3 and Table S7.

7) The relationship between RORC SNPs and levels of viral load and type of HCV genotype should be provided.

Answer: Thanks for reviewer’s suggestion. Since viral load and HCV genotype of spontaneously resolved individuals cannot be detected, we analyzed the relationship between RORC SNPs and levels of HCV RNA and HCV genotype in HIVneg HCV carriers. There was no relationship between RORC SNPs and HCV viral load (Figure S2) or type of HCV genotype (Table S6) (lines 152-153 and lines 155-156).
8) In contrast to other parts of the manuscript the discussion has been poorly written. I would like suggest that the authors revise it.

Answer: According to reviewer’s advice, we improved the Discussion part. Please see it in revised version.

9) Table 2 and 3: The fact that the 95% CI includes 1 meaning one cannot reject the null hypothesis that OR equals 1. The authors must check the interpretation of those 95% CIs. For example 8.25 (0.96-70.78), 0.57 (0.32-1.03) and 0.52 (0.25-1.08) p=0.040, Please clarify

Answer: Thanks for reviewer’s suggestion. It is indeed that the 95% CI includes 1 meaning one cannot reject the null hypothesis that OR equals 1. For example, in the original version, when we examined the 95% CI of rs9825 TT, we took it as reference category and combined the CC and CT as a non-TT group, but some of the comparisons showed no significant differences which was P>0.05 and its 95% CI included 1. As a result, we showed only the OR (95%CI) which would misunderstand the reader. In the revised manuscript, we deleted OR (95%CI) when we took the Chi-square (X2) and Fisher’s exact tests to evaluate the SNP genotype, and only kept the OR (95%CI) of allele evaluation. We wish this change could make table 2&3 clearer.

Response to Reviewer 2:

Major comments:

1) The authors performed the frequency of allele frequencies of RORC and IFLN3 SNPs in different populations from the 1000 genomes database. However, the possible correlation of these SNPs was not discussed in other global populations (with exception of the Han Chinese population).

Answer: Thanks for reviewer’s good suggestion. We discussed the limitations in the discussion and wish we or other groups can validate the SNPs in other populations to further enrich the results in the future.

2) Is the frequency of rs1521177 T allele correlated with the frequency of rs12979860 C allele? I suggest a correlation analysis to corroborate the results and a topic in the Discussion about this issue.

Answer: Thanks for reviewer’s suggestion. We analyzed the correlation between frequency of rs1521177 T allele and rs12979860 C allele in both cohorts (primary cohort: D’=0.066, r2=0.001; modified cohort: D’=0, r2=0) and no correlation between rs1521177 T allele and rs12979860 C allele was indentified. All the linkage disequilibrium tests result for RORC rs9826/rs1521177 and IFNL3 rs12979860/rs8099917/rs12980275 in the primary cohort were showed in Figure S3.

3) The Supplemental Figure 1 is more informative than Figure 1. The information in the text is enough to understand the favorable IFLN3 sub-cohort.
Answer: Figure 1 showed clearly the inclusion and exclusion criteria of cohort selection in the study while Figure S1 showed there was no differences in distributions of genotypes (%) of HIVneg and HIVpos resolvers. Figure S1 cannot replace Figure 1, however, if we put Figure S1 in the manuscript, its information will be partially overlapped with Table 2. Therefore, we considered it was more proper to keep Figure S1 in supplementary materials.

Minor comments:

4) Page 4, line 15: change the word "race" to ethnicity. Human races do not exist.
Answer: Thanks for reviewer’s comment. We have already corrected it in the revised version.

Response to Reviewer 3:

1) In the revised manuscript the authors should present p-value correction for multiple comparisons and need also present their calculation of statistical power.

Answer: Chi-square (X2) and Fisher’s exact tests were used to examine differences in frequencies of individual SNPs between HCV carriers and spontaneous resolvers. For example, for the rs1521177 GG, TT, GT allele, when compared with each other, Bonferroni way was used to correct p-value (also called q-value) which should be 0.05/3=0.0167. All p-values of each other comparisons are more than 0.0167, so that the data is not shown in the text.

Minor issue:

2) Tables 2 and 3: in the estimation of OR for alleles and genotypes it is recommended to specify the reference category used in the analyzes.

Answer : As we replied to comment 9 of reviewe #1, when we examine the 95% CI of genotype of TT, we took itself as reference category and combined the CC and CT as a non-TT group. Combined with the above comment, in the revised manuscript, we delete the OR (95%CI) when we took the Chi-square (X2) and Fisher’s exact tests to evaluate the SNP genotype, and only kept the OR (95%CI) of allele evaluation. We wish this change could make Table 2&3 clearer.