February 21, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8429-review.doc).

Title: Early viral kinetics during HCV genotype 6 treatment according to *IL28B* polymorphisms

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

Reviewer number 02462024

Please provide exact numbers of patients with non-CC genotypes. They appear to be very small which may affect the conclusions. Also I am unsure as to the exact counselling benefit for patients since SVR rates are the same with the difference being mainly in RVR rates. I believe that the conclusion needs to change to reflect that though there is a difference in RVR and very early viral decline a meaningful change in final SVR was not observed.

Ans: *We appreciate this comment. The exact numbers of patients with non-CC genotypes has been added in the revised manuscript (page 4, first paragraph) and the conclusion has been modified according to the reviewer's suggestion (page 11, second paragraph).*

Reviewer number 00012513

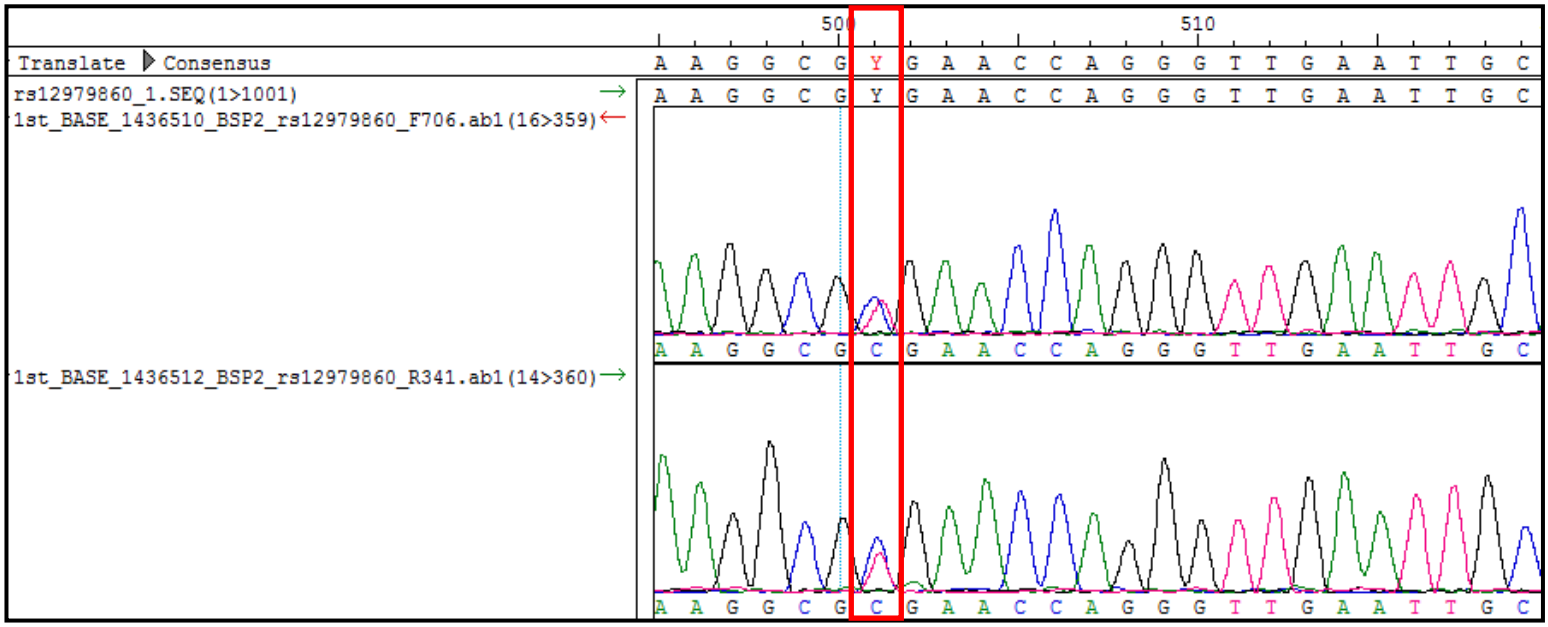
(1) **The method is not clearly explained, there is not any indication of criterion of differentiation between different CC, CT and TT genotypes. It is not understood as a simple process of two PCR reactions without specific probes or RFLP can distinguish between these polymorphisms probes. What do you expect to see in the agarose gels at 2% to distinguish between CC, CT or TT? Perhaps direct sequencing is used? However this is just indicated in the summary not in the**

manuscript. If this is the method, methodological data must be included, because they are not in the manuscript, and some figures must be provided.

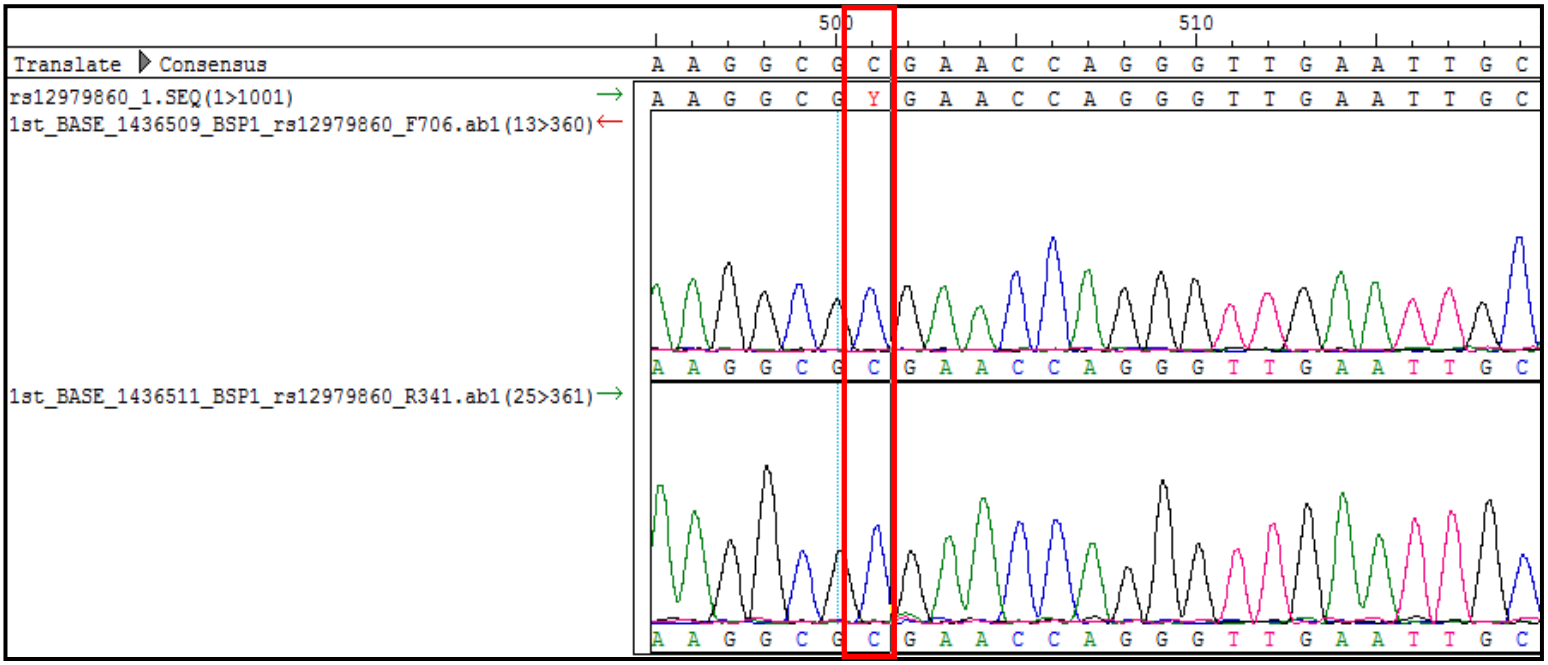
Ans: *We appreciate the comments. The methodology has been clarified in the revised manuscript. This study investigated the rs12979860 genotype by PCR and bidirectional direct sequencing using both forward and reverse primers. The SNP typing of rs12979860 was analyzed based on the chromatograms of nucleotide bases at the SNP position compared with the reference sequence retrieved from Genbank database (page 5, third paragraph and page 6, second paragraph).*

Representative of chromatograms for rs12979860 (heterozygous and homozygous genotypes) are shown in the figures below.

Heterozygous genotype (C/T)



Homozygous genotype (C/C)

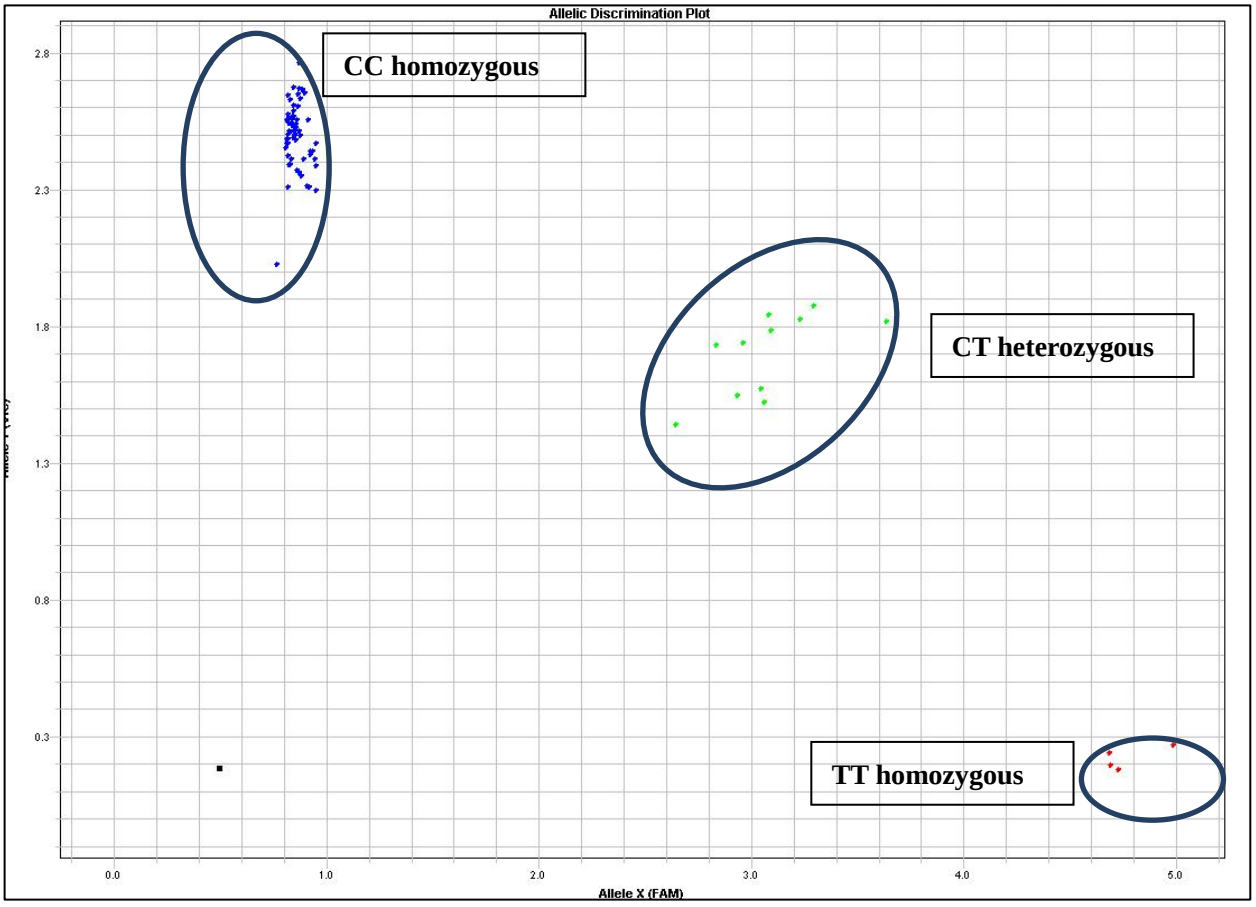


(2) Why a nested PCR is needed for a genomic sequencing? It claims strongly our attention the not use of a real time method such as the commented to compare the method used in the manuscript.

Ans: *In cases that the amount of PBMC was not adequate, plasma samples were used and nested PCR was performed in these samples. (page 6, first paragraph). The method was designed for amplification of short DNA due to relatively low yield of intact human genomic DNA in plasma samples. As mentioned in our previous study, this method exhibited high sensitivity detection even using stored samples (reference #17).*

(3) Although the correct correlation with a validated method indicated no documentary evidence of this correlation is provided or only a figure showing the different patterns of genotypes obtained by the proposed method. It should provide clear documentation of the process of validation of the method with examples to check unambiguity System setting polymorphisms

Ans: *In some cases that the results from sequencing-based assays were not clear, we confirmed the genotyping by TaqMan SNP Genotyping Assays (Assay ID AH8823E) (Applied Biosystems). (page 6, second paragraph). The figure below shows the results obtained from TaqMan SNP Genotyping Assays.*



(4) It is highly risky to conduct a study of genomic material by a double PCR process due to the high probability of contamination. For this motive, the authors must provide evidence of the controls

used to ensure the absence of contamination and related risks should be indicated in the manuscript

Ans**:** *Negative control was included in every PCR amplification to monitor and ensure that there was no contamination during the experiment (page 6, first paragraph).*

(5) When and because plasma DNA or PBMC DNA were used? , these two alternatives results really surprising, when it is usual the use of DNA of EDTA whole blood.

Ans: *As the above-mentioned, plasma samples were used in cases that PBMC was not sufficient. Nested PCR was performed with samples extracted from plasma (low amount of DNA) whereas conventional PCR was performed with samples extracted from PBMCs (higher amount of DNA).*

(6) Currently it is already accepted the existence of seven HCV genotypes.

Ans: *We have revised the manuscript and the corresponding reference (reference #3), as the reviewer's suggestion. (page 4, first paragraph)*

3. References and typesetting were corrected

4. We have added 'Sunchai Payungporn' as an author who have contributed in the experiment design.

Thank you for considering this revised manuscript, we look forward to your kind reply.

Sincerely yours,

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