August 07, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3158-review.doc).

Title: Evaluation of the colorectal cancer risk conferred by rare UNC5C alleles

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3158

The manuscript has been improved according to the suggestions of reviewers and editor:
1 Format has been updated, and modifications are highlighted in yellow.

2 Revision has been made according to the suggestions of the reviewer
(1) 00004187
(2) 00502983
(3) 02461836=00180990

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Sébastien KÜRY

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Point-by-point response to reviewers' comments regarding manuscript 3158 entitled "Evaluation of the colorectal cancer risk conferred by rare UNC5C alleles"

It is worth noting that, the version submitted before review was corrected by a professional English language editing company (see the certificate enclosed); the initial title "Rare *UNC5C* alleles do not confer high risk for colorectal cancer" was changed at the last minute before submission, as we believed that it was too controversial. Yet, in order to answer to the overall request of minor language polishing, we were helped by our collaborator Shuo Jiao for the preparation of the present revised version. As a native English speaker, and as a scientist involved in the genetics of colorectal cancer, he actively participated in the draft of this revised version of the manuscript and helped us to improve our work by suggesting relevant modifications. Due to his significant contribution, we decided to include him to the final list of authors.

Reviewer #00004187: *The work is good and I recommend its publication. Better define groups of 120 and 58 patients Add a Table. ? You must specify the 120 unrelated patients with hereditary. How many had Amsterdam II criteria, Bethesda, first-degree relatives with CRC? In each subgroup How many had MSI or MSS or not analyzed. All MSI became germline mutations study? ? Clarify patients 58. What types and number of polyps had the 35 patients? Had MUTYH-PAF?? Of the other 23 How many Amsterdam II criteria met, Bethesda or had first-degree relatives with CRC? In each subgroup had few MSI or MSS or not analyzed. All MSI became germline mutations study? 120 58 Amsterdam - MSI o Mutation o No mutation o Unknown - MSS - Unknown Bethesda - MSI o Mutation o No mutation o Unknown - MSS - Unknown First-degree relatives with CRC - MSI o Mutation o No mutation o Unknown - MSS - Unknown Polyps (> 10 polyps) o Mutyh o Positive o Negative o Unknown o FAP o Positive o Negative o Unknown - MSI o Mutation o No mutation o Unknown -MSS - Unknown ? In results, do not add 120 (37 +50 +34 = 121). The family with 3 diffuse gastric cancer or intestinal Was? Defining what is "Likely Lynch syndrome" and "syndrome X" In the abstract to express the confidence intervals*

We thank the reviewer for the overall statement and respond to the comments below.

We added a table inspired from the model proposed by the reviewer, which classify the 310 familial CRC cases of the study and define the three subgroups. We agree indeed that it is probably easier to follow than in our previous version. In the meantime, to avoid redundancy with the main text, we suppressed the related paragraph in the methods section of the manuscript.
Tables numbering was modified accordingly.
We corrected the error left in the text regarding the subgroup of 120 patients (37 +50 +34 = 121) due to the exclusion of one of the patients after a first round of analysis.
Finally, we suppressed the notion of "syndrome X" from our manuscript, as it did not add any value to our message and could only create confusion in the reader's mind. Therefore, we did not need to define the terms "Likely Lynch syndrome" and "syndrome X", as suggested by the reviewer.

Reviewer #00502983: *Evaluation of the colorectal cancer risk conferred by rare UNC5C alleles ", by KÜRY et al. July 8th 2013 This paper attempt to replicate a study from Coisseux et al. who inferred a major role of the UNC5C gene in the predisposition to familial forms of colorectal cancer (CRC) based on higher frequency of the A628K variant in the patient population than in a control population. In this paper, with samples from independent patient populations, authors conclude that variation observed in exon 11 UNC5C alleles confer only a low risk for both familial and sporadic forms of CRC. These results imply medical and technical implication. I therefore think that this study is appropriate for publication.*

We thank the reviewer for the positive overall statement on our article.

Reviewer #02461836=Reviewer #00180990: *This manuscript presented a well performed study assessing the role of rare genetic variant in the UNC5C gene, in particular the A628K missense mutation in 11 exon of UNC5C in familial colorectal cancer genetic predisposition. The authors also evaluated the relation between this rare variant and sporadic CRC. The methodology used in this investigation is suitable for reaching the results and it is well described; the findings are clearly presented; the analysis and interpretation of data seem appropriate and competent, but too wasteful. The study presented interesting and seemingly controversial results, concerning role of A628K substitution in UNC5C receptor gene that has been studied before from Coissieux et al, 2012. No doubt that expression of the netrin-1 dependence receptor UNC5C is reduced in many colorectal tumors and has a role in triggering apoptosis which prevent tumor cell survival. An explanation of differences in the results of Coissieux et al, and data presented here could be due to statistically interpretation. Indeed rare variant frequencies reported by the two investigators teams are approximately close: range 0.1 to 0.9 of Coissieux et al. and range 0.18 to 0.56 of Kury et al. Moreover in too studies the frequency of variant is enhanced in CRC then control, particularly for familial CRC. All these frequency are too small and less than 1% which show that A628K missense is more like mutation then polymorphic variant. It should be noted that statistical analysis is designed preferentially for normally distributed polymorphic allele. I believe that the authors should discuss their results as not preclude the significance of the mutation as a rare genetic anomaly distributed in distinct family. The author should take in consideration the above mentioned and appropriate interpreted the data observed in their study. Section Discussion and related areas require substantial processing in connection with the foregoing.*

We thank the reviewer for the overall statement and respond to the comments below.

We tried as much as possible to smooth the controversial aspect of the previous version of our manuscript, more especially in our discussion. We stressed the difficulties to identify rare variants potentially predisposing to CRC and to demonstrate their effect on the risk of CRC, because of the analytical methods used and of the barely avoidable heterogeneity in the groups of patients studied.
We agree that our results are not fundamentally that different from the ones of Coissieux and *al.*, and that the main difference lies in the interpretation of the results. We kept however our main message, which is that it is still too premature to apply the authors' finding to routine diagnostic procedures, as it could have suggested. Obviously, our study, like Coissieux et al.'s one, call for further investigation on larger populations or on more homogeneous groups of patients following a refined clinical definition of the cancer(s) possibly due to *UNC5C* mutations.