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25/11/2014

Dear Editor,

Please find enclosed the revised manuscript for our study in Microsoft Word format (file name: 14519-review.doc).

Title: Decreased expression of hyperpolarisation-activated nucleotide-gated channel 3 (HCN3) in Hirschsprung's disease

Author: Anne Marie O'Donnell, David Coyle, Prem Puri

Name of Journal: *World Journal of Gastroenterology*

ESPS manuscript number: 14519

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

1. Formatting has been altered according to the specifications suggested.
2. A section entitled "Core Tips" has been incorporated after the abstract.
3. A decomposable form of Figure 2 has been provided which when "ungrouped" will allow movement of the components.
4. Biostatistics statement: As no statistical methods have been employed in this study we have omitted a review of statistical methods.
5. Reviewer 00001391: "In the abstract and in the text please give the exact location of the samples ("colon" is not enough)"
We have clarified the location on the colon from which specimens were collected for experimental purposes.
6. Reviewer 00001391: "In the text give age and sex of patients and controls"
We have listed the sex and age range of the patients whose colonic tissue was utilised in the study.
7. Reviewer 00001391: "For antibodies and immunofluorescence you have used mouse HCN3 and not rabbit as the others HCN. Can you explain why? Do you think this could have influenced your results?"

Thank you for your query. In the experiment we used antibodies which were commercially available for research purposes that had good specificity for the target antigen in question. We found that the monoclonal mouse antibody against HCN3 provided the best specificity of those which we trialled. This should not account for

the difference observed in the expression of HCN3 compared to other HCN isoforms, with the main implication of the use of this antibody being that the host species of the second antibody used in double labelled immunofluorescence had to be from a species other than mouse.

8. Reviewer 00001391: *"Figure 1: is it patients or controls?"*
The composite images in Figure 1 are all from normal controls. The figure legend has been adjusted to reflect this.
9. Reviewer 00001391: *"Discussion: must be more focused on HCN-3"*
Additional background data regarding HCN3 has been included in the discussion
10. Reviewer 00001391: *"Discussion: if HCN channels are involved in regulation and not generation of ICC pacemaker activity please be homogeny in the discussion (p 4 of the discussion)"*
Phrasing has been altered regarding to clarify that HCN channels are involved in the regulation and NOT the generation/initiation of ICC pacemaker currents.
11. Reviewer 00004011: *"Very interesting and well documented study"*
We are delighted with the positive comment from this reviewer.
12. Reference formatting has been corrected

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

Kindest regards,



Anne Marie O'Donnell