Reviewer’s report

Title: The impact of down-regulated SK3 expressions on Hirschsprung disease

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Reviewer: Aldamaria Puliti

Reviewer's report:

The manuscript deals with the persistence of bowel symptoms after a properly performed pull-through operation, an important point for Hirschsprung disease affected patients.

The Authors analysed by real-time PCR the expression level of SK3 RNA in aganglionic and ganglionic colon regions of 14 HSCR patients compared to the ganglionic colon of 6 controls. Data indicate that in all analysed patients the expression of SK3 is reduced with respect to controls which leaded the Authors to sustain a role of SK3 in the persistent bowel symptoms.

To explain these dysmotility problems, it has been previously hypothesised that ganglionic colon, just proximal to the transition zone, could have an anomalous inhibitory neurotransmission, and that SK3 channel expression from PDGFRα+ cells could be implicated. Data in support of this hypothesis have been recently published by Coyle and collaborators (Journal of Pediatric Surgery, 2015). This point deserves much more investigation, as extending the number of investigated patients. In this view, the manuscript presented by Gunadi and collaborators represents an important work, of interest for pediatric surgery.

However, the Authors do not provide any evidence on a genetic contribution of the SK3 gene on the HSCR development. They do not report on the genetic possible causes of HSCR in the analysed patients (e.g. RET screening). They do not investigate genetic variations that could affect SK3 expression, and might support SK3 as susceptibility gene or to have a direct role in persistence of bowel symptoms. Indeed, the conclusion that SK3 expression could be an important marker for genetic counselling is too speculative.

In fact my great concern is on the subject of the research performed by the Authors which, in my opinion, seems to be not in the "Aims and Scope" of the BMC Medical Genetics Journal, probably of interest for a surgery journal.

Indeed, in the present form the manuscript is not acceptable. Hereafter some suggested points that could improve the quality of the manuscript.
Major points

- Methods: RT-PCR: please indicate the quantity of RNA used for cDNA synthesis.

- Methods: RT-PCR: please detail that "……. to determine the SK3 mRNA expression level in ganglionic and aganglionic colon from HSCR patients normalising to GAPDH and relative to ganglionic colon from control individuals."

- Table 1: please split into two separate columns the number of patients (not %), and age.

- Table 1: if possible, add a column indicating the genotype of the patient (RET/other gene mutation?)

- Table 1: add a column with symptoms present in the patient, if any

- Table 2: please replace "Mean difference" with ΔΔCt

- Table 2: please insert a column reporting the fold change

- Table 2: please complete the legend *, p<……….versus control

- Figure: I suggest to modify the figure to better evidence the difference between ganglionic and aganglionic colon (by enlarging the graph between 0 to 0.3, and reporting the control colon with a break). SD are not present.

- Figure legend: please add a sentence describing data, as "data represent the means+/ SD of SK3 expression normalized to GAPDH and relative to control tissues).

- It might be of interest to perform a real-time PCR to test the level of expression of PDGFRA as a control and a marker for the PDGFRA+ cells, supposed to express SK3 and to have a role in persistence of bowel symptoms

- Please comment on the difference of percentages of patients with reduced SK3 expression found in the present research with previous publications. To note, Coyle and coll. found a SK3 down-regulation in only half of analysed patients. Differences maybe attributed also to differences in the genetic background of analysed populations (Asiatic vs Caucasian), different surgery procedure, others.

- Discussion lines 171-174. This sentence is too speculative. Please note, Your data do not contribute with any genetic data relevant to the genetic counselling. In my opinion, it is
appropriate to conclude that the results reported may contribute to extending the knowledge on mechanisms causing the persistence of bowel symptoms. Please, modify this sentence.

- Discussion, line 174-176. The explication on the possibility of continuing intestinal symptoms does not properly concern the "genetic counselling", more appropriate a "surgery counselling", please, modify this sentence.

Minor points:
- Methods, line 105: please use the term "SK3 expression", instead of SK3 expressions, was…., the same in line 131
- Results: line 123: because of
- Discussion: line 169: please eliminate one "that"

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Please indicate the quality of language in the manuscript:

Needs some language corrections before being published
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