Reviewer's report

Title: A novel study of Copy Number Variations in Hirschsprung disease using Multiple Ligation-dependent Probe Amplification (MLPA) technique.

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Reviewer: Alessio Pini Prato

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The paper describes an alternative method to detect Copy Number Variants (CNV) of coding regions of some well known Hirschsprung's (HSCR) genes, including Ret. A wide screening for CNV of the coding regions of Ret, GDNF, END3, and ZFHX1B was performed.

The paper is well written and clear. It is straight-forward and focus on a well defined issue. Although it does not provide such useful information to the reader (only negative results from a provisional experience), it can be of use to the genetist who studies the disease.

Nonetheless, there are a few concerns regarding study design, results, and conclusions.

MINOR ESSENTIAL REVISIONS:

Study design / Methodology section:

1) The Authors should explain more clearly why they chose those genes. In fact, if we exclude Ret gene, the others are involved only in a minority of the patients. In particular, ZFHX1B is specific for the Mowat-Wilson syndrome, which is extremely rare.

2) Basing on previous considerations, the Authors should indicate how many of their patients have a syndromic HSCR and how many have a isolated form of the disease. This is of utmost importance when discussing the results, particularly if certains syndromes such as Down, Ondine, and Waardenburg-Shah are present in their series of patients. In fact those syndromes are well known to be determined by mutations of other genes but those screened by the Authors. Were patients with those syndromes excluded or not?

Conclusions section:

1) Although I grossly agree with the conclusions by the Authors, I think that it would be wiser to state that MLPA studies did not allow to detect CNV of the coding regions of Ret and other three genes in a selected HSCR population. On the other hand I do perfectly agree that this study reliably excluded CNV as common molecular cause of HSCR.

DISCRETIONARY REVISIONS:
Results section:

1) I assume that the mutation involving the EDN3 gene was carried in heterozygosis but this was not stated in the text. If this is the case, a sentence should be added to clarify this issue.

Conclusions section:

1) As stated by the Authors, the study did not include non-coding Ret regions and a number of important syndromic HSCR genes. Basing on this consideration, I would strongly appreciate further studies on this regard. In particular, it would be of great interest to screen those patients with clinical features suggestive for a well defined syndrome who turned out not to have any known mutation. An example could be to apply MLPA or CGH-array to those patients with HSCR associated congenital central hypoventilation syndrome but no PHOX2B mutation that could carry CNV on the PHOX2B gene. This would increase the diagnostic power of genetic screening of these syndromes.

2) This is a valuable study. Nonetheless, in the future, I would select the patients to apply the screening method for CNV in order to avoid useless and time-consuming studies.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests