Reviewer’s report

**Title:** Comprehensive analysis of RET common and rare variants in a series of Spanish Hirschsprung patients confirms a synergistic effect of both kinds of events

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**Reviewer:** Robert Hofstra

Reviewer’s report:

The paper by Rocio Nunez-Torres describe the analyses of RET coding and non-coding mutations in a large cohort of HSCR patients (282 patients).

They authors expanded their original cohort as described earlier by 176 patients. In total they identified 29 RET coding mutations in sporadic cases (11.1%) and 3 mutations in 16 familial cases (18.8%). Obviously, the frequency of the non-coding variants is much higher and often these non-coding variants are found in combination coding variants. Interestingly, they observed that these non-coding mutation are mostly in trans with a coding mutation, as was the case in 91% of such cases.

Moreover, 13% of the patients has neither coding nor (known) non-coding RET variants.

**Comments**

As the title suggest the data presented in this manuscript confirms the data reported earlier by Emison et al 2010. This was a study on a larger set of patients, including part of the patients analysed in this study.

**Statistical analysis**

- Transmission and haplotype data are not presented
- the degrees of freedom for the X2 should be given
- several significant differences are presented in this study. However these differences seem to be based on allelic proportion between the groups (on P-values). However, the differences in P values between groups are highly related to the sample size differences not on allelic distribution differences between groups. Therefore, it should be made clear that the different results are not to be interpreted as differences between groups.

**What is new?**

An omission in this manuscript is a thorough comparison between the previous studies and this one. Data from the older studies should be presented and compared with the new data. Now we cannot compare the data and have no clue
what is new and what is not.

What seems to be new is the trans position of the non-coding intron 1 variant.

Minor comments

How many homozygous cases were found? Were none of the coding mutation carriers homozygous for the intro-1 variant?

Page 5: ….which explains ~2% of hereditability (should be 'heritability') or 20 fold greater that the contribution to risk by coding mutations.

If I understand this correctly, this suggests that the coding mutations, which are found in around 12% of the cases, have hardly any impact on the disease. Is that correct?

In the conclusions it is stated: A proportion of cases without RET mutation might be explained by mutational events in another still unidentified HSCR loci etc.

As the variants found in RET only explain 2% of the heritability (as stated) there must be be additional mutations in all cases (not only the 13%), assuming a heritability of over 80% in HSCR.

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

'I declare that I have no competing interests'