

Seville, January 15th 2024

Dear Reviewer,

Thank you very much for taking the time to review this manuscript. Please find the detailed responses below and the corresponding revisions/corrections highlighted/in track changes in the re-submitted files.

Point-by-point response to Comments and Suggestions for Authors:

Comments 1: The writing/language can be improved, there are numerous grammar issues in the manuscript. For example, in line 91, “Data collection and preprocessing Expression data” should be corrected as “Collecting and preprocessing expression data”. Please carefully check the entire text from front to back and correct language problems.

Response 1: We agree with this comment. Therefore, we have checked the entire text for grammar and style mistakes, finding every modification in track changes in the revised version of the manuscript. For example, in Figure 1, and in line 107, “Data collection and preprocessing Expression data” was corrected as “Collecting and preprocessing expression data”.

Comments 2: Are there any similar articles in this area before? What is the current research bringing in addition to other ones in this field? Some more references should be added in this part.

Response 2: Biological networks have been applied to a broad spectrum of disease types. However, there are few such studies of rare diseases. In this context, Hirschsprung disease (ORPHA:388) is only one of more than 6000 rare (or orphan) diseases cataloged by the rare (or orphan) diseases cataloged by the Orphanet database. Moreover, we validated a new network-based method for searching for disease-causing genes in rare diseases, different of previously employed, such as Vertex similarity and/or DIGNiFI. We have, accordingly, added some references (new 101 and 102) in this part (in line 606) to emphasize this point.

Comments 3: On the one hand, Figure 1 is not clear; on the other hand, readers cannot intuitively find the six steps claimed by the author in Figure 1.

Response 3: The reviewer is correct. We have, accordingly, modified Figure 1 and its legend in the manuscript to clarify it. For example, we have numbered each step, we have written in bold each step title to help readers to find them and finally we have added the missing arrows which show the direction of the workflow in the additional working packages.

Comments 4: There are some risks in similar analysis to Figure 2: Although a gene is a shared differential gene, it is possible that its up- and down-regulation trends are

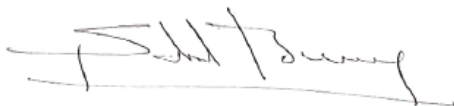
different between two or more groups. Please mark the numbers of up- and down-regulated differentially expressed genes in the Venn diagram respectively.

Response 4: We agree with this comment. Therefore, we have modified Figure 2 A. Moreover, thank you for pointing this out. In this line, we have marked the total numbers of up- and down-regulated differentially expressed genes in the Venn diagram respectively. Additionally, for clarity reasons, we also have included detailed information of the overlap genes that were significantly regulated in more than one sequencing experiment (Figure 2 B), in which we account the total numbers of up- and down-regulated differentially expressed genes in each sequencing experiment and we identified each shared differential expressed gene and its trend of regulation at mRNA and protein level for each expression assay. For example, all shared genes from the overlapped set GSE96854∩GSE98502 are downregulated at mRNA level in both experiments, whereas from the overlapped set GSE96854∩PXD021292 among the four shared different expressed genes are always up-regulated at protein level, only RGS17 is also up-regulated at mRNA level.

Comments 5: The author conducted a more in-depth discussion of the disease from the perspective of integrated transcriptomics, but in the results we did not see a clarification or depiction of the molecular mechanism (such as the display of molecular mechanism diagrams). This is not easy to understand for a bioinformatics type research paper.

Response 5: Agree. We performed the functional enrichment analysis to the HSCR omics expression data and to proposed modules, genes and miRNAs identified as Hirschsprung associated. Sum up, the visualization of significant enriched biological processes, pathways, etc. is shown in Table S2, Figure 4, Table 3, Table S9, Table 5 and Table 6. However, we don't display any individual molecular mechanism diagram because this study is not driven to analyze any specific molecular mechanism in Hirschsprung. Of course, based on the results obtained here, we plan to perform new research in this field to clarify the fundamental processes underpinning this disease at the molecular level. For example, to study the Prolactin signaling pathway and Aldosterone-regulated sodium reabsorption in Hirschsprung by a trans-omics analysis which included metabolomics inputs.

Yours sincerely,



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