Peer Review File

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Reviewer A

This manuscript is an editorial commentary about the study from Pyle et al. about genetic predisposition to testicular germ cell tumours (GCT), where the authors try to identify associations with variants of several genes, which could suggest a heritability. They found some new specific variants and suggest, that they could contribute to the risk of testicular cancer, if inherited together.

The authors of this commentary report the results of some previously studies, which detected only a few common genetic variations, with low to moderate penetrance, associated with predisposition to GCT.

After that, they analyze the study of Pyle at al. and underline the importance of having identified a possible polygenic etiology of GCT, with new candidate genes.

This editorial commentary is very interesting and the content is clearly and appropriately communicated: it summarises the most important studies on this subject and provides a critical and constructive commentary on the work of Pyle et al.

I have no suggestions or corrections to recommend

Reply: We thank the reviewer for this nice comment.

Reviewer B

I commend the authors for their effort in writing this editorial discussing the genetic predisposition of testicular germ cell tumors based on Pyle et al, article.

I have minor comments to address, before the paper is ready for publication.

Comment 1: BRCA1 and BRCA2 are not only genes identified in breast cancer, but they have a high penetrance in prostate cancer, which is closer to our field. I would add this to the editorial and add a reference.

Reply 1: We thank the reviewer for this suggestion. We have added the information with a reference (reference number 3) as suggested (line 19).

Changes in the text 1 (line 18-19): Despite the high heritability of TGCTs (1), no genes (like BRCA1 and BRCA2 in breast and prostate cancer (3)) with high-penetrance predisposition

have been identified.

Comment 2: Did Pyle et al, assessed loss or wild type allele mutations of CHEK2, this would be interesting to discuss as the first two paragraphs of your editorial are based on this genetic mutation.

Reply 2: The author mentioned population allele frequencies, but no information was provided on loss or wild-type allele mutations of candidate genes, including *CHEK2*.

Comment 3: I would rephrase the last sentence of the last paragraph: "Yet, TGCTs 106 remain complex, further research is needed to understand the heritability of TGCTs."
The authors aptly suggest exploring epigenetic alterations and extending research across diverse populations. While the study contributes to a promising polygenic etiology, further investigations are crucial for a comprehensive comprehension of TGCTs heritability and personalized treatment targets.

Reply 3: We thank the reviewer for this suggestion. Due to readability and overall flow of this paragraph, we replaced the last sentence of our ms with an edited version of the reviewers sentence**.**

Changes in the text 3 (line 105-106):

Original sentence (now deleted) ~~. Yet, TGCTs remain complex, further research is needed to understand the heritability of TGCTs.~~

New sentence:

Although the study contributes to a promising polygenic etiology, further research is needed to comprehensively understand the heritability of TGCTs.

Reviewer C

The present editorial commentary includes accurate, insightful viewpoints and constructive opinions on the challenging topic, germline predisposition of testicular germ cell tumors.

Comment 1:
I recommend that it could be accepted for publication, except only one minor comment; "Zp4" should be "ZP4" in line 60.

Reply 1: We thank the reviewer for this suggestion. The typo has been corrected as suggested.

Changes in the text 1 (line 60): However, it appears that only ten genes, including *ZP4*, *NIN* and *QRSL1* with pLoF variants had the most significant association with TGCTs risk.

Reviewer D

I have read your manuscript with interest and found it well written and organized.

Comment 1:

I only have a minor concern, detailed below:

-the introduction should be improved describing TGCTs classification and available mouse models of germ cell tumors:

Guida E, Tassinari V, Colopi A, Todaro F, Cesarini V, Jannini B, Pellegrini M, Botti F, Rossi G, Rossi P, Jannini EA, Dolci S. MAPK activation drives male and female mouse teratocarcinomas from late primordial germ cells. J Cell Sci. 2022 Apr 15;135(8):jcs259375. doi: 10.1242/jcs.259375. Epub 2022 Apr 20. PMID: 35297490.

Pierpont, T. M., Lyndaker, A. M., Anderson, C. M., Jin, Q., Moore, E. S., Roden, J. L., Braxton, A., Bagepalli, L., Kataria, N., Hu, H. Z., et al. (2017). Chemotherapy-induced depletion of OCT4-positive cancer stem cells in a mouse model of malignant testicular cancer. Cell Rep 21, 1896-1909. https://doi.org/10.1016/j.celrep.2017.10.078

Todaro F, Campolo F, Barrios F, Pellegrini M, Di Cesare S, Tessarollo L, Rossi P, Jannini EA, Dolci S. Regulation of Kit Expression in Early Mouse Embryos and ES Cells. Stem Cells. 2019 Mar;37(3):332-344. doi: 10.1002/stem.2960. Epub 2019 Jan 28. PMID: 30566254; PMCID: PMC8265211.

Reply 1: We thank the reviewer for the constructive suggestions. However, with all due respect, we like to point out, that murine models are not the focus of the article on which we are commenting. We also think that in the context of describing new (heritable)mutations predisposing to GCT the murine studies are not informative per se. Finally, there is the problem of limitations in word count and number of references which does not allow to introduce, discuss and compare the results of the murine models in this context here. It will be definitely very interesting to review all animal models published, as they will also grant insight in evolutionary differences of germ cell specification master regulators and their consequences for the occurrence of germ cell tumors.

Comment 2:

-the main body of the text should be reviewed since some typos have been found

Reply 2: The full text was carefully revised, and typos have been corrected.

Comment 3:
-some details within the discussion are missing (for example lines 53-54 cancer types are not specified)

Reply 3: Due to the format and limited length of the text, it is unlikely to provide detailed information about how specific genes (e.g. PIM1) are involved in specific cancers.
Nevertheless, the discussion section of the main article that we are commenting on already provided detailed information on individual genes (including PIM1) and their roles in various cancers.

Comment 4:
-the text should be improved with a graphic representation of the main results

Reply 4: We would like to point out, that the original paper contains several figures to facilitate understanding of the workflow and results. We do not think that a further schematic of their main result is necessary.