Author’s response to reviews

Title: Development and Validation of Next Generation Sequencing-Based 35-Gene Hereditary Cancer Panel

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To whom it may concern,

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"Development and Validation of Next Generation Sequencing-Based 35-Gene Hereditary Cancer Panel"

We would like to thank you again for reviewing the manuscript. The reviewer’s comments are insightful, which help to improve the manuscript. We greatly appreciate the reviewer’s feedback and please see the followings for our point-by-point responses (the reviewer's comments are in italics):

Comments from Reviewer #1:
1. Grammatical mistake in second sentence of the second paragraph of Background section "However, the development of such clinical evidence for the new genes typically lags behind their discovery of these genes".
   Reply:
   The correction has been made to “However, the development of such clinical evidence for the new genes typically lags behind their discoveries of these genes”.

2. First few paragraphs of Results section contains almost no results, instead it repeats the information already presented in Background and Methods sections. The section should clearly show obtained results and describe the tables. Table 3 should also be described to show the sensitivity and specificity in this section.
   Reply:
   We thank the reviewer for their thoughtful comments. We now removed the information already presented in Background and Methods from Results Section. As per the reviewer’s suggestion, we also now added a summary to describe results. Please see the revised manuscript for the changes.

3. Discussion section should be revised for minor grammatical errors.
   Reply:
   As per the reviewer’s suggestions, we have now corrected the grammatical errors.

Comments from Reviewer #2:

This is a well designed study for validating a laboratory made gene panel testing for hereditary cancer. This is good and necessary for any laboratory that wants to establish such a test, however, it has little value to the scientific community since the accuracy of NGS technology for mutation detection has been already established.
   Reply:
   While the accuracy of NGS technology is established in a general sense, there are certain nuances in NGS technology that need further evaluation. For example, there are some technical challenges in interpreting variants with a complex combination of insertions and deletions as well as sensitivity in the detection of long indels (&gt;10 bps. Complex variants could be reported as multiple distinct neighboring variants and the sensitivity could decrease with long indels. The present validation study demonstrated high accuracy in detecting and interpreting these technically challenging variants. The results of the study would be able to shed lights on further improvements of NGS detection and bioinformatics processing techniques.

Comments from Reviewer #3:

The authors describe the study where they developed and validated a targeted NGS-based test for hereditary cancer risk. The test allowed for the simultaneous analysis of 35 genes that when mutated predispose to several hereditary cancers, including breast, ovarian, prostate, uterine,
colorectal, pancreatic, stomach and melanoma. The manuscript is nicely written with a detail description of the validation process.

Nevertheless, on the market, there are several commercially available NGS-panels covering selected genes; therefore, in my opinion, the article lacks some novelty. On the other hand, it has to be noted that the presented assay was able to detect more challenging variants, including Complex Indel Variant and larger insertions. My only concern toward the manuscript is authors last comment. I can not agree with the statement that "all the known cancer pathogenic variants are within the coding or splicing regions", as more and more often it is suggested that mutations in specific regulatory sequences might play a role in cancer predisposition, development, progression and prognosis. Besides, it is generally accepted that large genomic rearrangements (LRG) might be responsible for some fraction of hereditary cancers; therefore, it would be useful to include LRG analysis in the presented panel and discuss this aspect further in the submitted manuscript.

Reply:

We thank the reviewer for this comment. Large genomic rearrangements indeed attribute to hereditary cancer predisposition but are not routinely detectable in hereditary cancer panel. A paragraph in conclusion is added to highlight the significance of LGRs.