Reviewer’s report

Title: Implementation of massive sequencing in the genetic diagnosis of hereditary cancer syndromes: diagnostic performance in the Hereditary Cancer Programme of the Valencia Community (FamCan-NGS)

Version: 0 Date: 18 Sep 2018

Reviewer: Magdalena Ratajska

Reviewer's report:

The authors describe the study where they implemented the multi-gene cancer panel in order to improve the genetic diagnosis in a cohort of non-informative patients with HCSs.

As the result of this study, they identify some pathogenic variants in the genes (BRCA1, BRCA2, MSH2) that have been previously tested with the negative result; additionally based on NGS results authors were able to reclassify several other patients.

The presented study displays potential problems and limitations of standard PCR-based molecular diagnostic, therefore, it could be of interest to genetic counselors and clinicians.

Comments:

1) the authors have a tendency to use very long sentences, with a long subject and/or agent, that negatively affects the clarity of the message. Please consider re-editing of extensively long sentences.

2) Line 88: What the "Universal" stands for?

3) Line 100-103: The aim of the study is a little out-dated, as NGS is a widely accepted approach.

4) In Sup. table 1 authors presented detailed diagnostic criteria. However, in the manuscript, I am not able to find clear information on the inclusion criteria for the study. Based on the first paragraph of the "Material and methods" section, I was able to conclude that the patients had to be negative for tested genes and had a strong family history. Moving the last sentence (line
115-116) just after the 1st paragraph would improve the message. The information about the Ethical Committee agreement should be transferred to the last paragraph of this section.

5) What methods were used to screen selected samples? Was it Sanger sequencing or rather HRM or SSCP (as mentioned later) or a combination of three?

6) I do not see the necessity of providing exact catalog numbers of selected products, the same refers to the list of the genes and SNPs (included in supplementary materials, table 2) this information is available on Illumina webpage.

7) My main concern is limiting the bioinformatical analysis only to vcf and Illumina Variant Studio software. It is generally accepted that some variants (especially) larger deletions/insertions can be omitted by the software. Re-analysing raw data, at least in the negative samples is required.

8) Why the presence of some variants was verified with Sanger sequencing while others were analyzed with alternative NGS panel. Was it a part of a bigger validation study?

9) Authors mentioned two pathogenic variants (NM_015252.3:c.1290+30064G>A, rs721048, NC_000006.12:g.151627231G>A, rs2046210) that have been observed in 1/3 of samples and therefore were excluded from the study. Both variants are denoted as benign according to Varsome, meeting single-stand criteria (BA1). Therefore, authors should put more effort into variants classification.

10) In the results paragraph (line 171-174) the authors listed the most frequently mutated genes. However, it was not clear to me how many mutations were detected in BRCA1, BRCA2, and TP53; based on the table it can be found that there were two variants in each of the genes, however, the information in the text: (2/19, 11% each) is confusing. Accordingly, authors should correct next sentence, in this case staying with the percentage alone would be more transparent.

Editorial comments:
1) Quality of figures (1 and 2) needs to be improved.

2) There is no need for this long abbreviation list, ATM, BRCA1/2, etc. are well-known genes.

3) Table 1: in a case of patients with more than one gene being mutated, please put the gens in alphabetical order, eg.: S22 (APC, MUTYH, TP53). Please update the variant description (on protein level).

In conclusion, this manuscript might be of interest to genetic counselors and clinicians, however, it required editing.

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An article of limited interest

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