**Reviewer's report**

**Title:** Exome sequencing of primary breast cancers with paired metastatic lesions reveals metastasis-enriched mutations in the A-kinase anchoring protein family (AKAPs)

**Version:** 1  **Date:** 05 May 2017

**Reviewer:** Maurizio Callari

**Reviewer's report:**

The authors have made an effort to address the previous concerns, significantly improving the manuscript organisation. The main issue I see in the manuscript is the follow: The leading finding of the study is an enrichment of mutations in AKAP gene family in metastatic lesions compared with primary tumours; however, this observation is not validated in the second cohort (ns Fisher's test), although the size is twice the size of the discovery cohort. In my opinion this limit the relevance of the study. Similar studies in larger cohorts have been recently published (e.g. PMID 28027327) and data are available. I would suggest exploring the mutational status of AKAP genes in that cohort in order to establish whether mutations in AKAP genes are really overrepresented in metastatic lesions.

Other points I don't think have been fully addressed:

- About the high number of mutations in the metastatic lesions and the low number of overlapping mutations in cohort 1 the authors state: "Despite this drawback of the bioinformatic pipeline for cohort 1, no main conclusions were made based on these results. All shared mutations, both exome wide and in cancer genes we manually scrutinized". I respectfully disagree with this because mutations in AKAP genes (the main conclusion) are in the large set of not shared mutations. Is the 3 to 4 fold increase in the number of mutations in the metastases confirmed in the second cohort?

- The authors have now specified the source material, however I would specify how FNACs were processed and whether they were fixed in some way. If they were FFPE, the C>T/G>A concern would still be valid.

- Please do not define the two bioinformatic pipelines as "slightly" different since they are completely different approaches.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

Quality of written English
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