Reviewer's report

Title: Frequencies and prognostic impact of six amplifications in breast cancer

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Reviewer: Larissa Savelyeva

Reviewer's report:

General

This manuscript describes the study of six chromosomal regions of amplification in breast cancer and establishes their prognostic relevance and correlation with clinical outcome. This is an interesting study, where the authors also estimate the associations and frequencies of co-amplification between non-syntenic chromosomal regions. The manuscript is well presented, however, there are some points that should be improved.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The first part of the result chapter is difficult to follow, the experimental strategy and procedure is unclear and has to be written in more details:

1. The first paragraph of the results part: “We defined two groups of amplified regions…”. If I understood correctly, each BAC pool was analysed separately on all 547 samples. Or the samples were divided into two groups for experimental convenience and analyzed with two sets of the probes? The reason and criteria for definition of the two groups of amplified regions is unclear and need to be explained.

2. Next paragraph presents the frequencies of amplification in percentage for each analyzed region. What was taken for 100%? Are these values referred to all 547 samples including uninformative cases or only informative cases have been evaluated for each region? This point has to be clarified.

3. In some cases the sampling strategy and design is confusing. Especially for regions designed as 20q13T (amplified at least at one of the three subregions) and 20q13Co (co-amplification of at least two of three loci). If, for example, two subregions have been amplified, is this 20q13T or 20q13Co? It would be helpful for reader to have the figure with the schematic representation of the partially/fully overlapping regions of 8p11-12T and 8p11-12*, as well as 20q13T and 20q13Co.

4. Supplementary Table 1 and Table 2. What does the “number of the tumors analyzed” mean? Are these only informative cases for each region and that is why these numbers are different for each region analyzed? The short note bellow the Tables would be helpful. In particularly for Table 2, where the sum of the number of tumors analyzed for the same region, as well as value of intact/amp tumors of the same region are different throughout the table. Interpretation of the results is difficult without that explanation.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Pg 10 “…using R.2.3.0 statistical software”, here Web resources is needed.

2. Pg 11 “…10% for 17q12”, but in abstract on pg .3 “…of 17p12 in 9.9%”, values should be presented in a similar way.

3. Pg 15 “However, few studies have looked at multiple amplifications…”, here the references are needed.

4. Pg.17 “The 11q13 region is a relatively frequent event in breast tumors”, I guess, the word “amplification” is missing.

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Discretionary Revisions (which the author can choose to ignore)

For interpretation of observed results would be very informative to make clear the difference between “real” amplifications caused by certain molecular mechanisms (resulting in formation of DM/HSR or occurred by BFB cycles), and gains of chromosomal material, which are due to the general genomic imbalances in cancer cells. Since in the study the region was considered as amplified with the probe signal number more then 5 and no control probes (for example centromeric) were used, I assume that not only amplifications, but also the copy number gains (including whole chromosomes/chromosome arms) are included in the
calculations. It would be helpful for readers to include this point in discussion section. In this context, the ref [3, Struski et al] is inappropriate, because it presents CGH data addressed to chromosomal gains, and not amplifications. The same terminology problem concerns the sentence on the Pg 17 “Amplification of the short arm of chromosome 12, mostly as isochromosome..”, the isochromosome is a chromosomal rearrangement, which is originated from a distinct mechanism of genetic instability and not resulted from the classical mechanisms of amplification, therefore “overrepresentation” would be the correct term in this case.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes

**Declaration of competing interests:**

I declare that I have no competing interests' below.