Title: Breast Tumor Copy Number Aberration Phenotypes and Genomic Instability

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Author's response to reviews: see over
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Dear Sir;

We thank the reviewers for their comments and have revised the manuscript in light of their suggestions. Our detailed responses are below. Among other suggested changes, we included figures in the body of the paper that previously had been supplementary files. Currently, the Statistical Methods are provided as a Supplementary File, because they are quite long, but they could also be incorporated in the Methods section of the paper. We would appreciate your opinion regarding the preferred location of this information.

Regards,
Donna Albertson

Response to reviewers’ comments

Markus Ringner
Minor essential revisions
Discussion of impact of number of clones (resolution) on measures of genomic instability. The arrays used in this study offer ~1.5 Mb resolution across the genome. Clearly some aberrations (i.e. focal deletions and very narrow amplicons) will be missed if they fall between two clones on the array. All copy number transitions will be detected, although the precision of mapping of the boundary will be equal to the clone spacing. This discussion has been included in the Results.

Discretionary Revision
With regard to the number of tumors in the middle branch of the dendrogram, we have clarified the text and stated that the branch contained 16 sporadic tumors.

Petra Nederlof
Major compulsory revisions
More information on breast tumor dataset 2, e.g. patient characteristics. We have indicated in the Methods that the two patient datasets were similar with respect to genomic changes and pathology.

Correlation of telomere length with amplification is not convincing. The association is statistically quite strong, but the correlation may appear by eye to be less convincing because there are overlapping data points for telomere length at the low end of the scale (e.g. number of chromosome arms with amplification less than 2).

Association of expression of DNA damage/repair genes with copy number transitions. It is not clear whether expression was increased or decreased. We have stated more clearly in the text that most associations were positive and therefore enhanced expression of
genes was observed in tumors with greater numbers of copy number transitions or amplifications.

**Copy number loss on 17q includes BRCA1.** We agree with the reviewer that loss of 17q is not frequent in the entire dataset. Nevertheless, this statement was made with respect to the subset of Complex tumors, which compared to other tumors, do show a frequent loss of 17q (see new Figure 4).

**Charles Theillet**

*Major compulsory revisions*

**Consider E2F as working hypothesis.** We agree with the reviewers that associations from studies of tumors do not prove causality. Therefore, as suggested, we have indicated that our observations are consistent with, or support the hypothesis that deregulation of E2F or the Rb pathway contributes to genomic instability in breast tumors. These changes were made in the appropriate parts of the Abstract, Background, Results, Discussion and Conclusions.

**Improved organization of the paper.**

In response to the comments made here and by Dr. Edwards, we have incorporated Supplementary Figures 1-3 in the main body of the paper as Figures 1, 2 and 4, respectively.

Although a rigorous definition of the objective detection of gains and amplifications is given in the Statistical Methods, we have clarified the definition of gains and amplifications in the text by stating that a clone was declared amplified if it belonged to a copy number segment <20 Mb and the increase in ratio exceeded the criterion described in the Methods. We also direct the reader to the individual chromosome copy number profiles in Figure 5A, which provides examples showing the difference between gains and amplifications.

*Minor essential revisions*

**The value of Figure 2.** Figure 2 (new Figure 5) provides information on the more recurrent amplicons. In addition, a more detailed view of amplicons is presented, which illustrates their complexity and distinction from copy number gains. Figure 5b shows that amplicons are present in tumors with varying numbers of low level alterations, and not simply restricted to tumors with many copy number changes.

**Candidate genes in Table 1.** The candidate genes listed in Table 1 were mostly intended to help the reader identify amplicons more readily than is possible from base pair positions. Since it is impractical to list all of the genes within the minimal amplicon, in response to the reviewer, we have updated the Table to include more of the previously identified genes for some of these amplicons (e.g. BAG1 and TACC1 to the FGFR1 amplicon, WISP1 to the MYC amplicon, YEATS4 to the MDM2 amplicon, STARD3, GRB7 and TOP2A to the ERBB2 amplicon and STK6 to the ZNF217 amplicon). We have removed FGF3 from the CCND1 amplicon, since studies have shown that it is not a good
candidate. We have also amended the title of the table to indicate more clearly that the list of candidate oncogenes is not exhaustive.

**Paul Edwards**

*Discretionary Revisions*

Over-statement of conclusions about genetic instability, association does not imply causality. See response to Dr. Theillet’s comments.

We have incorporated Supplementary Figures 1-3 in the main body of the paper as Figures 1, 2 and 4, respectively.