Reviewer's report

Title: A new molecular breast cancer subclass defined from a large scale real-time quantitative RT-PCR study

Reviewer: Robert B Clarke

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General
The paper describes the application of qRT-PCR for measuring the expression of 47 genes in 199 primary breast tumours and 6 normal breast tissues. Results are then used to cluster tumours into 13 sub-types by their similarity in gene expression. Some represented previously identified sub-types while other previous classes were distributed across several of these new divisions. In particular, a new sub-type emerged from their analysis that had a very low rate of recurrence. It is not clear from figure 1 is how they selected 13 subtypes from the clustering, rather than another number which would also be significant in the Chi2 analysis. However, subtype 8 does appear to have some interesting properties, although the implications of this for clinical practice are not discussed in enough detail.

Overall it is a useful study demonstrating that a relatively small set of genes can usefully sub-divide the tumours, while the approach is not particularly novel it does highlight that using different approaches (and cohorts) will identify different sub-types.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1) Ages at diagnosis vary from 27-92 years (one of the widest), and many different treatment combinations (Table S1). Was recurrence of the different subtypes biased by the treatment method in this cohort?

2) Why did they select 97 and 12 tumours from the van't Veer and Sorlie datasets and on what basis were the particular samples selected?

3) Using 18S as a control gene this seems a little old fashioned. It is normally recommended to test a panel of at least 5 potential housekeepers and then to normalize to a pool of the best 3. Secondly, it is not stated how specificity of RT-PCR reactions was assessed: were gels run or melting point analysis assessed?

4) The authors make the point that their 47-gene set is largely distinct from the 500-gene sorlie/perou intrinsic subset, however they seem surprised that their subset was unable to discriminate between luminal A and luminal B tumours.

5) The comments stating the benefits of qRT-PCR are quite valid, although they do not acknowledge the weaknesses of this approach compared to microarrays for classification and gene discovery/validation.

6) The rationale for choosing a set of genes based upon previous studies and hoping that they will identify new subtypes ('according to hormone-susceptibility and aggressiveness') is rather weak. Is it really unsupervised hierarchical clustering of qRT-PCR data, when the genes have already been chosen?

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

Page 9, line 19: The level of over-expression should be tested stataistically
Figure 3 – the quality of this figure is poor and the Y axis could be shortened to 0.4-1 in order the emphasise the differences in recurrence-free survival.

Discretionary Revisions (which the author can choose to ignore)
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: Needs some language corrections before being published

Statistical review: No