Reviewer's report

Title: Methodological Issues in Detecting Gene-Gene Interactions in Breast Cancer Susceptibility: A Population-Based Study in Ontario

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Reviewer: David Goldgar

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General

The manuscript by Briollais et al. discusses some of the issues that are relevant to the search for gene-gene interactions in studies of complex phenotypes. Particular interest is given towards methods designed for higher order interactions such as CART and MDR. The authors illustrate this through examination of a small breast cancer case-control study. Although for the moment it has proven difficult enough to find main effects, I believe this sort of analysis will be more common as the field progresses. However, since we are entering the era of genome-wide association studies using arrays of 600,000 to now 1 million SNPs, it would have been interesting to have some discussion about how the general principals and methods discussed in this paper can apply to this situation; the approach one takes with 20 SNPs is not necessarily what one would do for 600K (e.g., looking for interactions in the absence of main effects). Given that the data used to exemplify the various methods is underpowered for main effects, not to mention interactions, the authors should take care to keep this in mind and not try to make too much of the results, especially since large subsequent studies have not found any roles for most (if not all) of these genes in breast cancer.

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

I believe that the entire attempt to relate the ‘discovered’ interactions to biological pathways is not particularly useful, valid, or particularly interesting. For one thing, until the proposed interactions are independently validated, it is premature to be worrying about pathways. More importantly, the ‘Ingenuity’ software doesn’t see all that ingenious to me. For example many of the genes seem to be in almost all of the pathways (e.g., TNF, ESR, IL10) thus it is not surprising that many of the interactions are found to be in the same pathway. Is this corrected for in the analysis? Perhaps something like a kappa statistic for inter-rater reliability could be used to correct for chance association. One of the genes found in interactions by all three methods, XPD, is not categorized under DNA repair which is surprising since I believe this gene's primary function is base-excision repair in response to UV damage. In any event, I think this part of the paper should be eliminated.

There should be some discussion about the idea of searching for interactions in the absence of main effects. The authors seem to be in favor of this approach; therefore they should give some ideas about how to do this with much larger numbers of SNPs.

Given the limited power in the small study, could the authors comment on the decision to only examine women under age 55? One could hypothesize that much of the common genetic variation such as many of the SNPs studied in this report would be associated with post-menopausal breast cancer. To reduce the sample size by 75% does not seem like a reasonable thing to do in the context of this study.

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

I found Figure 2 difficult (impossible actually) to understand. More explanation in the Figure legend would be helpful, similar to figure 1 legend. By Partition do you mean the largest case-control difference is between the shaded two-locus genotypes and the unshaded?
On page 6, 61/459 case samples seem to have failed in some way. This seems very high to me, especially when 100% of the controls were successfully genotyped. One hopes that there were not some systematic problems that not only effect the failure rate, but also the genotype calls. It would be good to have some idea of the reasons for these failures, and reassurance on the genotype calling quality control.

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: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests