Author's response to reviews

Title: Polymorphisms cMyc-N11S and p27-V109G and Breast Cancer Risk and Prognosis

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Version: 2 Date: 7 March 2007

Author's response to reviews: see over
Dear Dr. Edmunds:

Please find our revised manuscript "Polymorphisms cMyc-N11S and p27-V109G and Breast Cancer Risk and Prognosis" enclosed for your further consideration. We have made revisions to the manuscript, per Reviewer comments, and we feel the paper is stronger as a result. Below, we highlight the revisions and comment on some of the reviewer suggestions. Thank you for your interest in our work.

SPECIFIC COMMENTS

Reviewer 1: Michael Wirtenberger

Major comment #1:
We agree that power calculations are important in order to appropriately design studies. Post-hoc power calculations are however less useful, but may give some indication as to the strength of the study design, although confidence limits are probably more important to note when interpreting the results (Hertz-Picciotto and Neutra 1994). Using Quanto, we show that we do have sufficient statistical power for the cMyc polymorphism with our study population of 1,115 cases and 710 controls to detect an odds ratio of 1.5 with approximately 75% power assuming an allele frequency of 0.05 and the dominant model. Therefore, we agree with the reviewer that there is limited statistical power to detect smaller effects, but point out that, to our knowledge, we have genotyped the largest sample of individuals for this polymorphism. The Wirtenberger study genotyped more controls (n = 1096) but fewer cases (n = 705) than this study, which is about the same statistical power for their combined analyses (not stratified by country). We provide these calculations on page 8 and acknowledge our limited statistical power to detect effects less than 1.5. Further we emphasize the confidence limits when referring to the point estimates in the results and discussion sections (page 12). We also clarify potential reasons why our study does not yield the same results as did the reviewer’s study other than sample size (page 10-11).

Major comment #2:
The reviewer suggested that we state the total number of individuals with BRCA1/2 mutation (n = 75; 7.8%) and we have revised the text accordingly (page 8). We also agree that we should provide a clearer explanation of the potential implications on the results with cMyc in the discussion especially with reference to the Wirtenberger study who specifically examined non-BRCA1/2 carriers (page 10-11).
**Minor comment #1**
We agree with the reviewer that the material and methods is comprehensive, but have decided not to shorten this section since several commentaries about the publication of SNP studies have pointed out that this detail re: SNP selection, analytic validity of genotyping, selection of subjects and analysis, is important in critical appraisal of the study by future readers (Little, Bradley et al. 2002; Rebbeck, Ambrosone et al. 2004; Rebbeck, Martinez et al. 2004). We have shortened the introduction as suggested by Reviewer #4 in order to present a more concise paper (page 3).

**Minor comment #2**
The paragraph on page 4 was re-written for better clarity.

**Minor comment #3**
We have substituted the words “may be associated” with “show a significant association” on page 8 lines 9 and 12.

**Minor comment #4**
We agree with the reviewer to exclude Table 3 since the non-significant results are stated in the text.

**Reviewer 2: Katri Heikkinen**

**Minor Comment #1**
We have outlined how the subjects were recruited into both case-control and prognostic studies including response rates. There were 1,115 cases and 710 controls included in the case-control study genotyped for cMyc-N11S and p27-V109G polymorphisms. Because of the inclusion criteria to be followed prospectively in the Ontario Familial Breast Cancer Registry, as described in another recently published manuscript (Figueiredo, Ennis et al. 2006), not all cases included in the case-control study could be included for the prognostic study. We made a typographical error in reporting 260 as the overlap, and thank the reviewer for asking us to clarify. The 967 is simply a subset of the 1,115 cases included in the case-control study (page 5).

**Minor Comment #2**
On page 10, second paragraph, we have included the codon number (V109) to the V allele to be more informative.

**Reviewer 3: Arto Mannermaa**

**Major Comment #1:**
We agree with the reviewer that the text should be revised and shortened to describe just the non-significant results presented in Tables 3. We believe that Tables 4 and 5 (now Tables 3 and 4) are
important contributions since part of aim of this study was to assess the prognostic effects of these polymorphisms and therefore have chosen to leave them in the manuscript (note that Reviewers #1, #2, #4 did not request to remove these tables). Very few studies have examined the association between tumor features and polymorphisms but this is an important research question as discussed in other publications such as (Shatalova, Walther et al. 2005). As well, the role of SNPs in prognosis is emerging as an important field as highlighted last year by the AACR sponsored meeting on epidemiology of cancer prognosis: traditional and molecular predictors of treatment response and survival (Ambrosone, Rebbeck et al. 2006). Although we show no prognostic effect of these two nsSNPs, the publication of null results is important for further collaborative efforts (such as meta-analyses) as discussed by other researchers (Shields 2000), and this is a well-designed study with systematically collected prospective data from well characterized group of women in the Ontario Familial Breast Cancer Registry which is part of the NIH-funded Breast Cancer Family Registry.

**Major Comment #2:**
The results are presented without correction for multiple testing, which is currently still the standard in epidemiologic research for studies investigating a limited number of a priori defined hypotheses. Nevertheless, we acknowledge the issue of multiple testing, and have presented the work in a manner that will allow the reader to evaluate the results with consideration of all the hypotheses being tested. Our comment in the discussion was referring to Table 3 (stratified on family history and age of onset) which we agree with all the reviewers should be excluded from the paper. Therefore, this sentence has been removed.

**Minor Comment #1:**
We agree that using HapMap data we could identify SNPs in linkage disequilibrium with those investigated in this study. We did look at the HapMap data and found that none of the HapMap SNPs in LD ($r^2>0.80$) with either nsSNP, have been studied in the published literature, and therefore this will need to be followed up by other studies. Nevertheless, we believe that the reviewer made a good comment and agree that since the results overall do not suggest that these nsSNPs are strong factors influencing risk or prognosis, although p27-V109G may be associated with a small protective effect (and any other nsps in strong LD) and we therefore add a sentence to highlight that any SNPs in LD with this polymorphism should be investigated further and even better would be to comprehensive examine the entire gene such as with a htSNP or tagSNP approach (page 12).

**Minor Comment #2:**
We have clarified the description of the cases and controls on page 4-5. Our point is that the Ontario Familial Breast Cancer registry used a multi-stage sampling scheme to enrich the population for genetically predisposed individuals using criteria known at the time to be associated with a higher risk of breast cancer (i.e., young age, family history, multiple primaries, Ashkenazi ethnicity). We wanted to be clear with our readers about the characteristics of our study population and therefore explicitly stated (in brief) their scheme and refer to a previous publication.
Reviewer 4: Hiltrud H Brauch

Major Comment #1:
As suggested by the reviewer, we have more clearly articulated the results as presented in Tables 2 and 4. Initially, our concern was over-interpretation of results and therefore we considered presentation of all the results in table format without a lengthy discussion of the results to be the most appropriate to allow readers to assess the evidence themselves. Overall we believe there is no real effect (at least a strong effect) of either nsSNP, although we agree that there is a significant protective effect of the p27-G109 allele. We have excluded comments about the stratified analysis which are based on smaller numbers. The text in the results section was revised accordingly (page 8-9).

Major Comment #2:
The introduction was shortened as suggested. We exclude our points about the function of cMyc and p27 in cell cycle as well as the functional aspects of the SNPs (which was already pointed out in the discussion) and some of the detail provided by other studies (i.e., p-values). However, we retained some detail in order to present the background literature appropriately (page 3).

Major Comment #3:
We agree with the reviewer and have decided not to include this sentence as they were derived from low numbers and may be misleading.

Major Comment #4:
The comments of the reviewer were considered and the following changes were made to appropriate weight the findings of this study (pages 9-12). We agree that the results for p27 should be presented first followed by discussion of the null results with respect to cMyc. We have also shortened our discussion about the functional aspects of these SNPs, but retain some points about this aspect. Several studies of polymorphisms have also discussed functional work, and combining the functional aspects with epidemiological studies has been appreciated by other investigators since it’s important to consider the underlying biological mechanisms when interpreting epidemiological research (Little, Bradley et al. 2002; Rebbeck, Ambrosone et al. 2004; Rebbeck, Martinez et al. 2004).

Major Comment #5:
The title has been changed.

Thank-you again for considering our work.

Sincerely,

Jane Figueiredo
References


