Author's response to reviews

Title: Polymorphisms in NF-kappaB Inhibitors and Risk of Epithelial Ovarian Cancer

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Author's response to reviews: see over
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Andrea Bucceri
Scientific Editor
BMC Cancer

Dear Dr. Bucceri,

Thank you for the thoughtful reviews of our manuscript entitled “Polymorphisms in NF-kappaB Inhibitors and Risk of Epithelial Ovarian Cancer”. We appreciate the critical comments made by each reviewer. The manuscript has been revised (modified text is highlighted in yellow); particular issues are addressed below:

Reviewer 1

“It’s not clear why the authors did WGA on DNA samples when the genotyping assay only requires 250 ng of 50 ng/ul for each sample and your source of DNA was blood. Although WGA DNA works well with GoldenGate assay but gDNA is always preferred, and produces better results especially when you are trying to replicate your data.”

WGA was used for the Duke University samples because gDNA had been consumed and was not available for use at the Mayo Clinic where genotyping took place. Prior to use of WGA, we performed and published an extensive evaluation of the WGA genotype quality and consistency with gDNA results in the current experiment. Based on sets of duplicated samples from independent WGA preparations (N=15) and from WGA matched genomic samples (N=124), genotype concordance was > 99.0%. Results are provided in the attached reprint [1]. The text has been updated on Page 5.

“We were the samples from Duke genotyped and clustered separately or all the samples from both places were clustered together?”

We regret this wasn’t clear. Indeed, the samples from Duke University and the Mayo Clinic were genotyped and clustered separately. The text has been updated on Page 6.
“I suggest if possible the authors, genotype the samples from Duke for SNP rs3138050 using gDNA to see if their findings can be replicated and this would also give them more power for analysis.”

We agreed that this would be ideal. Unfortunately, this is not possible given the current availability of gDNA on the included Duke University samples. Conservatively, rs3138050 was excluded for all Duke (WGA) samples because one of the 384-well plates completely failed for this SNP. Because this SNP is suggestive (but not conclusive) in a recessive model among Mayo samples, we aim to include this SNP in analyses of other datasets in the future. At the moment, however, only a conservative interpretation is warranted.

“The discussion does not mention anything about the limitation(s) of the study, and also does not discuss the results and findings, it’s mostly the review of the literature on NF-kappaB.”

The Discussion has been extensively revised to include the limitations of the study, other examinations of inherited variations in NFKBIA and NFKBIB and cancer risk, and future directions. Please see revised Discussion on Pages 9 and 10.

“It would be more informative if the authors would show the locations of the SNPs (markers) within both genes and also the haplotype block(s), and LD structures in a figure.”

We appreciate this suggestion and have added edits to Page 8 with a reference to a new Figure 1 (Page 15) to show linkage disequilibrium among the examined SNPs.

Reviewer 2

“Introduction should be reorganized, especially the correlation between inflammation and ovarian cancer should be concise.”

The Introduction has been extensively reorganized to more concisely identify the correlation between inflammation and ovarian cancer. Specifically, the discussion on the “Incessant Ovulation Hypothesis” postulating that increased lifetime ovulations can potentially lead to errors during replication and carcinogenesis [2-5] has been relocated to the Introduction. Please see the improved text on Pages 3 and 4.

“…The authors should also briefly provide evidence of the importance of NF-κB and its inhibitors in ovarian cancer.”

The current Introduction better addresses this with a review of the NF-κB pathway and ovarian cancer. To date there are no studies examining the association between variants in NFBKIA and NFKBIB and risk of epithelial ovarian cancer. However, others studies have found variations in NFKBIA to be associated with increased risk of melanoma [6], colorectal cancer [7], multiple myeloma [8], and Hodgkin lymphoma [9], and NFKBIB has been found to be significantly upregulated in inflammatory breast cancer patients [10].
The ηB’s role in various cancers and biological relevance in ovarian cancer development indicate the potential importance of the NF-κB inhibitors in ovarian cancer. Revised text is highlighted on Pages 3, 4, and 10.

“Data selection and analyses: from Table 1, we can see significant differences between patients and controls regarding family history, oral contraceptive use and postmenopausal hormone use. Although the difference was adjusted by multiple logistic regression analysis, family history, oral contraceptive use and postmenopausal hormone use are closely related to increased or decreased risk of ovarian cancer (as the authors mentioned in the introduction), thus it is important to exclude analysis bias by stratification analysis.”

Indeed, the expected epidemiologic observations were observed in these data. If family history, oral contraceptive use, and post-menopausal hormone use were also associated with genotype, we may observe a spurious association between genotype and risk of ovarian cancer due to confounding. In other words, we’d observe bias away from the null.

To head off this possibility, we conservatively adjusted for each of these factors in multiple logistic regression models. The effect that adjustment had was minimal (ORs differed by < 0.01), suggesting that the impact of these potential confounders was minimal. We choose, nonetheless, to adjust for these factors, again, to be conservative, and, importantly, we note that because results are primary null, bias away from the null is less of a concern for these results.

The reviewer may be suggesting that a gene-environment interaction could exist; i.e., that any genotype/ovarian cancer association may differ by family history, oral contraceptive use, or post-menopausal hormone use. This is an interesting point, and one which could be addressed by stratified analyses if sample size were adequate. We’ve not done this in the current dataset because of a) low power in sub-groups, and b) a mostly-null main genotype effects consistent with lack of strong sub-group specific association.

“Discussion does not adhere to the relevant question. Actually, the second paragraph in the discussion is a repetition of introduction. The authors should focus on the importance of their findings, limitation of the study and any relevant information provided by other studies if possible.”

We agree with the observation that the Discussion strayed from the relevant question. The repeated text from the Discussion has been relocated to the Introduction (Pages 3 and 4). The second paragraph of the Discussion (Page 10) now addresses more pertinent associations between NFKBIA and NFKBIB and risk of other cancers [6-9] and areas for future study.

“Conclusion should be more concise.”

We thank the reviewers for this comment. We have addressed this issue by revising the conclusion to be more succinct. Please see the text on Page 10.
Reviewer 3

“…clarify why these 19 SNPs were chosen and why other SNPs were excluded from the study.”

We now provide additional background on our SNP selection scheme on Pages 5 and 6 and cite Supplemental Table 1 earlier in the text.

“…were all of the tumor types analyzed papillary serous adenocarcinomas or were there mixed subtypes in the analysis.”

This analysis included women with a variety of sub-types of histologically-confirmed epithelial ovarian cancer recruited into the Mayo Clinic and the Duke University studies. Thus, our analysis hypothesizes that these have a common genetic origin. The histological distributions are now provided in the text on Page 5.

Again, thank you very much for consideration of this manuscript for BMC Cancer. If accepted, we will pay for color-figure printing fees. We look forward to receipt of your decision.

Sincerely,

Ellen L. Goode, Ph.D., M.P.H.

References