Author's response to reviews

Title: Carboxypeptidase 4 gene variants and early-onset intermediate-to-high risk prostate cancer

Authors:

Phillip L Ross (plross@usa.net)
Iona Cheng (chengi@humgen.ucsf.edu)
Xin Liu (XnLiu@childrensmemorial.org)
Mine S Cicek (Cicek.Mine@mayo.edu)
Peter R Carroll (PCarroll@urology.ucsf.edu)
Graham Casey (gcasey@usc.edu)
John S Witte (jwitte@ucsf.edu)

Version: 2 Date: 17 December 2008

Author's response to reviews: see over
December 17, 2008

Melissa Norton, MD
Editor-in-Chief
BMC Cancer

Dear Dr. Norton,

Attached please find the revised manuscript “Caboxypeptidase 4 gene variants and early-onset intermediate-to-high risk prostate cancer” submitted for publication in BMC Cancer (MS: 2000117818219529), and responses to specific comments made by the Reviewers. In our revised manuscript we have addressed all of these comments as described in the point-by-point responses attached. We hope that you will now find the manuscript acceptable for publication.

Sincerely,

John S. Witte, Ph.D.
Professor of Epidemiology & Biostatistics
Responses to specific comments from Reviewer 1

“...it is important for the authors to investigate if there is any association between high-risk prostate cancer risk and CPA4 variants. Since multiple lines of evidence indicate that for Gleason 7 4+3 is different from 3+4, it might be good to separate out the Gleason 7 (3+4) from Gleason 7 (4+3), 8-10 in the analysis to make sure that the result observed is not driven only by the intermediate-risk prostate cancers.”

Response: This is an excellent point, and we have re-analyzed our data stratified by whether the cases had prostate cancer with Gleason 3+4 (or less) versus cases with Gleason 4+3 (or higher). We found that the association between the rs2172492 TT genotype and prostate cancer among earlier onset cases (<66 years) was strengthened among men with the most advanced disease. Specifically, comparing men with the TT genotype versus those with the GG genotype, in the more advanced disease the odds ratio = 2.48 (95% CI: 1.14-5.40, p=0.02), whereas in the less advanced disease the odds ratio = 1.68 (95% CI: 0.87-3.27, p=0.12). We note this intriguing finding in our revised manuscript.

“When choosing the haplotype tagging SNPs, how many LD blocks covered the CPA4 coding region - just one or more than one? How much of the promoter and downstream region are included in the blocks/genomic area utilized? One SNP was excluded due to genotyping assay difficulties. Is there another SNP which could capture the same genetic information as that SNP? Otherwise the full haplotype information of that region is lost.”

Response: The entire 31 kb CPA4 gene has only two blocks among European Americans, with one large block spanning 24 kb. Five of the SNPs typed in our data define this large haplotype block. The other SNP covers a region 1.6 kb upstream of CPA4, though none of the downstream region was covered. The synonymous SNP (rs2306848) which was excluded due to difficulty in genotyping assay is correlated with the rs1488009 SNP (r$^2$=1) we genotyped, so we were able retain the corresponding haplotype information for that region. We now clarify these points in the paper.

“For the haplotype analysis, what frequency defined a common haplotype in the analysis? Which program did you use from the case-control analysis? Haplovview?”

Response: In our revision we clarify that common haplotypes were defined as having $>5\%$ frequency, and the tagSNP software was used for haplotype estimation for case-control analysis.

“The authors indicate that carrying two copies of the haplotype with the rs2171492 T allele was weakly associated with prostate cancer risk. What was the comparison group? All other haplotypes or just individuals without a copy of the common rs2171492 T allele haplotype. This issue is important as the authors claim that because only one common haplotype contains the T allele (at what freq?) and that the haplotype association was weaker than the SNP alone that “the SNP itself may directly impact development of the more aggressive forms of prostate cancer.” This would be quite a strong statement if the comparison groups are not the same as in the SNP alone analysis. It would be good to see both rs2171492 TT vs TG/GG, and to get a clearer explanation of what the comparison group was for the haplotype analysis.”

Response: We clarify that the comparison for the two copies of the haplotype with the rs2171492 T allele was no copy of this haplotype, which is directly analogous to our genotype-level comparison for this SNP. The frequency of this haplotype was 35.1% and
15.9% among Caucasians and African Americans, respectively. Making a comparison back to any copies of the haplotype (or similarly rs2171492 TT versus TG/GG) would strengthen our results. Nevertheless, we feel it is appropriate to temper our previously strong statement about whether this SNP itself directly impacts prostate cancer.

“Finally, were there any blind duplicates in the genotyping and if so please state rate of duplication?”

Response: Yes, we included 2% duplicates, and observed a 100% concordance rate.
Responses to specific comments from Reviewer 2

“Given that the hypothesis is that CPA4 may enhance progression of disease, it would be worthwhile to examine the associations with a polychotomous outcome. In other words, examine the genotype frequency in SNPs comparing each group (high-risk, intermediate-risk) to the control group. If the hypothesis holds true, we would expect to see a higher frequency of the “TT” genotype among high-risk cases. Despite sample size constraints, this would be worthwhile to explore.”

Response: We undertook a polytomous logistic regression to explore this, and found that the TT genotype was indeed more strongly associated with higher risk disease (see response to first question from Reviewer 1).

“SNP selection was based on a strategy of selected those with a MAF of > 5% in the HapMap database in the EA (Caucasian) sample. Was the expression of selected SNPs different in the African American sample? It would be worthwhile to comment given AA men comprise 18% of the study sample. Were any of the SNPs in LD with one another?”

Response: We have revised Table 1 to include SNP allele frequency distributions among African Americans and Caucasians. The distributions of minor allele frequencies were relatively similar between African Americans and Caucasians. In addition, we have included Supplemental Table 1, which presents the LD ($r^2$ and $D'$) between SNPS for African Americans and Caucasians. There was limited LD among the SNPs, as expected since they were selected to tag regions.

Table 4 should be included within the manuscript and not appended as a supplement as the major findings of the paper are reported in the table.”

Response: We agree and have now included Table 4 as part of the main paper.

Minor note: ‘Carboxypeptidase’ is misspelled in the TITLE and the first sentence of the abstract as well as ‘metallocarboxypeptidase’.

Response: We have corrected these errors.