Author's response to reviews

Title: Association between polymorphic glutathione S-transferase (GST) genes, their combination and interaction with tobacco smoke and prostate cancer risk: A case-control study

Authors:

Nicole A Lavender (nalave01@louisville.edu)
Marnita L Benford (mlbenf01@louisville.edu)
Tiva T VanCleave (tmltemp01@louisville.edu)
Guy N Brock (guy.brock@louisville.edu)
Rick A Kittles (rkittles@medicine.bsd.uchicago.edu)
Jason H Moore (jason.h.moore@dartmouth.edu)
David W Hein (dwhein01@gwise.louisville.edu)
La Creis R Kidd (lrkidd01@gwise.louisville.edu)

Version: 2 Date: 6 October 2009

Author's response to reviews: see over
Title: Examination of polymorphic glutathione S-transferase (GST) genes, tobacco smoking and prostate cancer risk among Men of African Descent: A case-control study

Re: Manuscript Ref. No.: 1068280721289828

Dear BioMed Central Editorial Team,

Thank you very much for your email of September 15th and accompanying reviewer comments. We found the reviewer comments very helpful and have revised the manuscript accordingly. Specific responses/changes to each of the reviewer comments is provided in bold font following each of their comments:

Reviewer: PCa Sample size seems a bit low as the incidence rate of PCa was higher in SC in 2008 (Cancer Statistics 2008). Therefore, can MDR analysis justify the low sample size of PCa. Moreover, the sample size is low for LR analysis too

Lavender & Kidd: The reviewer makes an excellent observation. This study was aimed at assessing the single and combined effects GSTM1, GSTT1 and GSTP1 polymorphisms and cigarette smoking on prostate cancer risk. The population used to evaluate these effects was based on men of African descent, rather than residents of South Carolina. However, in a sub-analysis of cases and controls residents of DC we did not observe any difference risk estimates when compared to the entire study population (i.e., DC and South Carolina study participants). There were also no significant differences in the prevalence of the GST variations comparing controls derived from DC and South Carolina.

We had 80% statistical power to observe risk estimates ≥2.4 given a GSTP1 minor allele frequency of 45% and a significance level of 0.05 using logistic regression analysis. We used MDR to address lack of statistical power to evaluate main effects and epistasis models using logistic regression models. MDR is able to overcome sample size issues that often plague conventional methods (e.g., logistic regression models); remaining effective with relatively small sample sizes (i.e., at least 200 cases and 200 controls). Although MDR doesn't allow for adjustments of covariates, it does control for multiple comparisons and spurious risk estimates by using a cross validation and permutation testing scheme as a built-in feature. Our findings emphasize the importance of utilizing a combination of traditional and advanced statistical tools to identify and validate single gene and multi-locus interactions in relation to cancer susceptibility.

Reviewer: As the figures indicated in the study clearly show that 22% of PCa cases had PSA value <4 ng/ml. Is there a possibility that some of the controls with PSA less than 4 ng/ml may have PCa.? The authors should refer to paper of Lobe and Catalona. (2006)

Lavender & Kidd: The reviewer makes an excellent suggestion. In our study, 96% of the controls had a PSA value ≤4.0ng/ml. If we use pure logic, there is a slight possibility that some of the controls with either abnormal ( >4.0ng/ml) or “normal” (i.e., ≤4 ng/ml) PSA levels may actually have prostate cancer that may remain undetected. Controls (5%) who had an abnormal PSA (i.e., PSA >4.0ng/ml) and/or
irregular digital rectal examination DRE underwent multiple core needle biopsies. Participants with a “normal” PSA (i.e., PSA ≤4.0ng/ml) but an abnormal DRE were excluded from participating in the current study. We also excluded individuals who: (1) had an abnormal PSA and/or an irregular DRE, but a normal biopsy; or (2) were diagnosed with benign prostatic hyperplasia. These individuals were excluded because we could not predict with any level of certainty whether these individuals would develop prostate cancer (Methods section, Study population, paragraph 1, lines 3-16, page 5).

As implied within Lobe et al., 2006, even after close inspection of prostate cancer tissue, it is possible to miss a microscopic nodule that can later develop into cancer. If controls in our study population were still misclassified after undergoing a PSA test, DRE, and/or multiple core needle biopsies, then we may expect our calculated risk estimates to underestimate the relationship between the selected GST polymorphisms prostate cancer susceptibility in the current study. But this issue plagues all cancer epidemiology studies. Unfortunately, it is impractical to subject all patients to a radical prostatectomy to permit a more accurate classification by case status (Discussion section, paragraph 4, lines 45-62 page 13-14).

Reviewer: The data in table 2 shows significant difference between case and control in African ancestry. During LR. Did the authors consider correction for this admixture?

Lavender & Kidd: The current study adjusted single loci models for genetic heterogeneity (i.e., population admixture) using logistic regression models. Such adjustment of calculated risk estimates helps to circumvent misclassification of study participants related to self-identified race/ethnicity (SIRE). Our findings suggest that inclusion of West African Ancestry (WAA) did not significantly change the risk estimates relative to unadjusted models; if anything, it makes them more precise to the nearest tenth. (Discussion section, paragraph 5, lines 63-68, page 14).

Reviewer: As stated in the abstract that advanced statistical methods were used like MDR analysis and hierarchical entropy graphs. Hence data related to hierarchy/admixture in the subjects, should be provided in a graphical/tabular form for more transparency and understanding

Lavender & Kidd: MDR is not designed to adjust for covariates. At best, if an interaction is observed it can be analyzed using logistic regression models, which has the capacity to adjust for potential confounders such as admixture. (Methods section, Gene combination effects, lines 63-87, page 7-8)

Reviewer: Result section can be grouped in different subheadings to ease the understanding for the readers

Lavender & Kidd: Previously, the results and discussion were merged into one section and we had a separate conclusion section. The revised article now has separate results, discussion, and conclusion sections.

Reviewer: The title of the article does not seem to be appropriate/confusing, as the study did not demonstrate a significant interaction between smoking and GST gene polymorphisms. Therefore, authors may consider changing the title of the article.

Lavender & Kidd: We altered the article title to better reflect the study findings. The new title now reads, “Examination of polymorphic glutathione S-transferase (GST) genes, tobacco smoking and prostate cancer risk among Men of African Descent: A case-control study”.

Reviewer: It would be idle considering comparing the present data with studies of other population for more lucidity

Lavender & Kidd: Expanded discussion section to the following to include published data from similar studies conducted in different populations (i.e., Caucasians, Asians, Africans). Using data reported by
Agalliu et al and a meta-analysis by Mo et al, the discussion section was extended to present readers with results from similar studies; therefore providing an opportunity for readers to compare our results with other recent findings (Discussion section, paragraph 3, lines 29-40 page 12).

Reviewer: *GSTM3* 3 base pair deletion polymorphism in Intron 6 is another important maker which could have been considered for study using these advanced statistical methods.

**Lavender & Kidd:** The reviewer makes a good suggestion. The *GSTM3* three base pair deletion polymorphism in Intron 6 may be another important maker for prostate cancer risk due to its role in the metabolism and/or detoxification of potentially harmful cigarette components. This base pair deletion may impact *GSTM3* protein expression and therefore reduce the capacity to detoxify harmful compounds found in cigarettes (i.e., polycyclic aromatic hydrocarbons). Mittal et al. 2009 observed that the *GSTM3* polymorphism was associated with a 2.5 fold increase in the risk of developing prostate cancer among a North Indian population of 135 cases and 169 controls (OR = 2.51; p = 0.028). Given this finding, we will attempt to include this marker within a future research project.

Reviewer: There are too many references cited. Try reducing the numbers

**Lavender & Kidd:** We attempted to reduce the number of references cited.

In conclusion, we have addressed each of the reviewer comments and believe the manuscript has been improved considerably.

A reminder that the work described in this manuscript was supported by NIH funds and thus the manuscript should be deposited in PubMed Central upon acceptance.

We look forward to your decision regarding acceptance and publication.

Sincerely Yours,

**Nicole A. Lavender, M.S.**
Ph.D. Candidate
Department of Pharmacology & Toxicology

**La Creis Kidd, Ph.D., M.P.H.**
Assistant Professor
Department of Pharmacology & Toxicology