Author's response to reviews

Title: SLC6A3 and body mass index in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

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

We thank the reviewers for their thoughtful comments. A point-by-point response to the additional minor reviewer requests follows. Page numbers are provided in the comment responses referring to these revisions.

Comments from the statistical adviser:

_Further improvement of detail and clarity of the statistical methods section._

We have included some additional information throughout the statistical analysis section to improve the clarity. Specifically, we have also added an explanation of the use of conditional logistical regression (page 8).

“Conditional logistic regression was necessary, as the original study design involved sampling according to these variables. In such a case, conditional logistic regression most appropriately models the sampling frame to obtain proper risk estimates. Attempts to conduct an unconditional logistic regression including all strata terms resulted in non-convergence of the model.”

_Discussion of advantages of using BMI as a continuous variable._

We have added a brief treatment of this to our discussion (page 14):

“We relied on self-report data for height and weight to calculate BMI. This type of self-report data has an inherent degree of misclassification, which can bias results towards the null. In order to minimize this misclassification error, we categorized BMI by established, broad categories. Use of this classification is widely accepted in the literature but does slightly reduce power; the original continuous BMI variable would have provided a slightly more powerful test for association with the genetic polymorphisms.”

**Reviewer 2: Caroline Davis**

1. _Page 3: line 3, “make” should be “makes”._

This typo has been corrected (page 3).
2. In the next paragraph on the same page, “death of heart disease” should be “death from heart disease”.

We have made the suggested revision (page 3).

3. In the added text at the bottom of page 3, there is considerable redundant wording.

We have rewritten this section of the text for clarity and to focus on the most important points (pages 3-4).

“The genetic variation that contributes to susceptibility to obesity in the general population is beginning to be elucidated through candidate gene and genome wide studies focused on obesity and related phenotypes [6]. Recent genome wide association scans have identified regions in the FTO gene [7-10] and variants telomeric to the MC4R gene to be associated with obesity [11, 12]. The genes identified to date likely only identify a small proportion of the genetic variability that influences susceptibility to obesity.”

4. On page 4, the authors claim that SLC6A3 is the most important gene influencing dopamine in the mesolimbic reward pathway. The reasons for this claim need to be explained.

SLC6A3 is the dopamine transporter gene; the dopamine transporter binds the neurotransmitter dopamine and is critical for reuptake of dopamine into the dopaminergic neuron. As such, the dopamine transporter is the major determinant of dopamine clearance at the synapse and is therefore crucial in dopamine signaling. We have written this section to include these points (page 4).

“Dopamine is a key neurotransmitter mediating reward, and therefore, it is relevant to diverse behavioral conditions including obesity. SLC6A3, the dopamine transporter gene, is an important polymorphic gene which controls the reuptake of dopamine in the synapse, in areas of the CNS crucial to reward and learning such as the mesolimbic pathway.”

5. What is meant by “the very first genes studies” on page 4?

SLC6A3 was one of the first genes studied in general population in relation to reward related behaviors, for example, i.e. alcohol (Goldman D, Nature Medicine 1995, 1(7):624-625 and Tiihonen J et al, Nature Medicine 1995, 1(7):654-657) ADHD, (Cook EH et al, Am J Hum Genet 1995, 56(4); 993-998) and smoking (Lerman C et al, Health Psychology 1999, 18(1):14-20), in genetic variation association studies. We have removed the phrase ‘very first’ since this is too general and substituted ‘early genetic association studies,’ which more accurately describes the historic context. Consistent with the reviewer’s earlier comments, we wish to avoid wordiness and increase clarity. As such, we have merely mentioned this and provided the references to support this statement (page 4).
“Early genetic association studies in the general population examined $SLC6A3$ in relation to smoking and alcohol intake [22, 23]...”

6. In the last paragraph on page 4, the authors report associations among various genetic markers. What they found should be explained.

In the current draft of the manuscript, we report an association between polymorphisms in $DRD2$ and obesity. The specific polymorphisms studied are mentioned in this paragraph. We also provide the earlier references to studies in the same population, as well as an earlier study of the same gene, are provided (Morton LM et al, *Pharmacogenet Genomics* 2006, **16**(12):901-910; Noble EP, *Eur Psychiatry* 2000, **15**(2):79-89). We feel that these citations most specifically address the concern of the reviewer; additional explanation would be superfluous and detract from the main points of the Introduction.

7. On page 10, “...change slope were...” should be “...change slope was...”.

Upon inspection of this sentence, we realized that we are discussing the association in relation to two endpoints: percent change in weight AND weight change slope. Simple past tense for a plural noun requires the use of “were,” not “was.” We feel it would be improper grammar to make this change.

8. On page 11, the long sentence beginning “A major hypothesis...” is awkwardly worded and should be rewritten.

We have rewritten this sentence to improve clarity (page 11).

“Dopaminergic function may modulate reward from both food and drugs of abuse. This reward deficiency hypothesis (constitutional anhedonia predisposing individuals to reliance on extrinsic stimuli such as food, nicotine or alcohol) provides the dominant framework in molecular genetic studies of dopaminergic function and its effects on drug addiction (e.g., [30, 31]).”

9. Similarly, on page 12 the sentence beginning “Another recent study...” should be reworded for clarity and to remove awkward wording.

We have rewritten this sentence to improve clarity (page 12).

“Another recent study did not report an association with the $SLC6A3$ VNTR and BMI; however, this study was based on an adolescent cohort, which had large demographic differences from our study [42].”

10. Again on page 13, the sentence beginning “However, dopamine...” needs rewording for clarity.

We have reworded this sentence for clarity (page 13).
“However, dopamine dysregulation is present in anorexia nervosa. For example, increased central dopaminergic activity is observed in weight-recovered anorectics compared to controls [44], and dopamine D2 and D4 receptor polymorphisms have been associated with anorexia [45, 46].”

11. The added text in the Conclusions section is awkwardly worded and needs further clarity.

We have shortened and clarified the Conclusion section to focus more precisely on our findings (pages 14-15).

We hope that these changes are acceptable and look forward to hearing from you.

Sincerely yours,

Elizabeth Azzato
Neil Caporaso