Author's response to reviews

Title: Comprehensive interrogation of CpG island methylation in the gene encoding COMT, a key estrogen and catecholamine regulator.

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Author's response to reviews: see over
December 28, 2013  
Dr. Tim Sands  
Editor, BMC Medical Genomics

Dear Dr. Sleiman,

We have uploaded a substantially revised copy of our manuscript; “Comprehensive interrogation of CpG islands in the gene encoding COMT, a key estrogen and catecholamine regulator” (MS: 5146966631096272).

In this revision, we have addressed each of the reviewer’s concerns carefully, and provided a point-by-point reply describing our response to their many thoughtful and extremely helpful comments and suggestions. We feel that we have strengthened our manuscript in addressing these concerns. All revisions to the paper have been identified by their page number and are indicated in RED font within the text. We have also uploaded an unmarked copy of the revised manuscript.

We appreciate the opportunity to revise and resubmit our manuscript, and we hope that you find that we have adequately addressed the concerns of the reviewers. We look forward to hearing from you.

Thank you for your consideration.

With kind regards,

Theresa Swift-Scanlan & Charlotte Boettiger

Reviewer Comments

Major Compulsory Revisions

Reviewer #1:

1a. “In my view, the abstract and the last part of the introduction (Background) need to be changed in order to convey more efficiently the research questions and the findings of the paper.”

Author’s Response: Per Reviewer 1’s suggestion, we have revised the Abstract to more efficiently convey the research questions and findings of the paper. We have also revised the last part of the Background/Introduction in accordance with the Reviewer’s suggestions (p. 6-8).
1b. “The [Results] section: “Factors associated with differential methylation of the COMT gene” should also be re-written.”

Author’s Response: Per Reviewer 1’s suggestion, we have rewritten this section of the Results (p. 9-11).

2. “The authors need to define CpG and change it in the title”

Author’s Response: The term “CpG” is well-established in the literature as referring to a C-phosphate-G (a C bound by a phosphodiester bond) dinucleotide sequence on the same strand of DNA, in contrast to a C≡G bond on opposing strands of DNA. Nevertheless, we have defined “CpG” on p. 6-7 “recent evidence suggests that epigenetic changes, particularly DNA methylation of CpG (e.g., “C—phosphate—G” on the same DNA strand) dinucleotide sequences (CpGs) in the COMT gene, may also have an important impact on COMT function [1, 2].” Therefore, we have left CpG in the title as it is a commonly understood and frequently used acronym.

3. “The authors should not include their results in the background (background last lines)”

Author’s Response: We have removed the sentence from the Introduction “With the exception of the CpG island in the 5’UTR and 1st exon, all other CpG islands were strongly methylated with a dynamic range of methylation typically between 50-90%” and placed it in the Results section on p. 8.

4. “Why did the authors interrogate 13 amplicons? How did they choose them? This needs to be clarified.”

Author’s Response: In the Methods section under “Quantifying COMT methylation using the EpiTYPER MassARRAY” (p.17) we clarified the following: “The 13 amplicons were chosen because they either assayed a CpG island as defined by CpG content greater than 50% spanning 300 bp or more, and/or because they allowed interrogation of CpG sequences located in important regulatory regions of the COMT gene.”

5. “Third paragraph: The authors should mention how the selected the three amplicons which are presented in Figures 3A-3C. Was it randomly?”

Author’s Response: We have clarified the reason for our selection on p. 8: “Figures 3A-3C are “epigrams” which serve to illustrate percent methylation variance per CpG unit across three selected amplicons; COMT_001, 005 and 006. These amplicons were chosen because they showed CpG unit methylation variability across sample types.

6. “In the discussion, the authors should mention that their results cannot establish causality and are of an exploratory nature. Their interpretation should be more conservative. It should also be mentioned that their findings using breast cancer cells could be specific to the disorder and cannot be generalized to healthy individuals.”
or other disorders.”

**Author’s Response:** We had already noted on p. 14; “While COMT_006 methylation was strongly inversely correlated with COMT expression, one limitation of our study is that we did not have corresponding RNA from our healthy adult saliva-derived DNA samples with which to perform RT-PCR,...”

However, we have also added another sentence on p. 15 to clarify the important point that loci specific methylation changes can be exquisitely tissue specific, and therefore findings in one tissue cannot be generalized to another tissue; “Therefore, our correlations with COMT expression and methylation at specific COMT CpG loci in breast cell lines may not generalize to any other tissue or sample type.” We have also added that; “Future studies......in multiple human tissues in both affected and healthy individuals will help resolve the relationship between COMT methylation at particular loci and expression of distinct transcript variants.”

**Reviewer #2:**

In ‘Summary’:

“In general, describe more the results than the background”

**Author’s Response:** Per Reviewer 2’s suggestion, in the Summary/Abstract, we have reduced the background information and expanded the description of our results.

“1st paragraph: consider whether the subjects are healthy when some of them are heavy drinkers.”

**Author’s Response:** The saliva samples were collected from a non-clinical community sample of self-identified social drinkers with no known history of neurologic or psychiatric illness, including substance use disorders. We have modified our description of this sample in the Introduction (p. 7).

In ‘Background’:

“Is it possible to explain why ValMet158 is considered the main functional variant of COMT, especially, when the associations between different conditions and the 158val allele have been ‘only’ modest and often inconsistent”

**Author’s Response:** The polymorphism is considered the main functional variant given its location within exon 4 and the fact that Val^{158}Met has been associated with numerous disease states, which is explicated in detail later on p. 5-6. For clarity however, we have added changed the “main functional variant” to the “most studied variant” and added additional information on p. 4: “A great deal of research has focused on a common, functional, single nucleotide polymorphism (SNP) of COMT, Val^{158}Met (rs4680) that is the most studied variant, due to its location within the exon 4 coding region. Specifically, the substitution of a methionine (Met) for a valine (Val) at position 158 results in three- to four-fold reduced activity of the COMT enzyme due to reduced protein stability [13, 15].”

“Alcohol use was estimated using self-reports. In the results, it is mentioned that the samples were balanced in terms of light or heavy drinking. Here in ‘Background’ and also elsewhere, it is said that the subjects were healthy. Is this the case also for
heavy drinking? Was there any effects of different drinking behaviors and were the samples enough large to observe this kind of difference?”

**Author’s Response:** As noted above, the saliva samples were collected from a non-clinical community sample of self-identified social drinkers with no known history of neurologic or psychiatric illness, including substance use disorders. Although the AUDIT scores of approximately half of the samples were in the range that indicates “possible hazardous drinking”, the heavy drinking participants were apparently healthy. As noted in the Results (p. 11), we observed no association between AUDIT score and COMT methylation, except at one site in the Met allele carriers. As we now note on p. 11: “Qualitatively similar results were obtained when participants were dichotomized according to whether they were “possible hazardous drinkers” or not, based on AUDIT scores [60] (data not shown).” It is true however that negative findings should be interpreted with caution in this small sample.

In ‘Results’:
“1st paragraf:
- Explain the potential effects of light and heavy drinking on the results.”

**Author’s Response:** We now make mention of expected effects of heavy alcohol use on methylation (p. 10-11), however, we feel that more extensive commentary is more appropriate to the Discussion, where we have added a section regarding alcohol use and methylation (p. 13-14).

In ‘Discussion’:
“3rd paragraf:
“- As already earlier, discuss the question of drinking effects here. Are there differences when compared light and heavy drinkers in the present study?”

**Author’s Response:** As noted above, we have added a section to the Discussion addressing this point (p. 13-14).

“- Is it possible, even this was included in the study design, that the sex and/or ethnicity had effects on the general results?”

**Author’s Response:** All reported correlations with the other factors reflect partial correlations present after accounting for any effects of sex and ethnicity.

**Minor Essential Revisions**

**Reviewer #1:**
1. The headings of table 3 or the title should change since AUDIT score and COMT Met alleles are not demographic factors.”
**Author’s Response:** We have changed the title of Table 3 ("COMT methylation in healthy human subjects versus individual factors") to more accurately reflect the contents.

**Reviewer #2:**
"In 'Summary':"
"2nd paragraph:
- socioeconomic status > add abbreviation in brackets (SES)"

**Author’s Response:** We have made the recommended change.

"In 'Background': 2nd paragraph: - Open 'PSTD'"

**Author’s Response:** We have replaced the acronym “PTSD” on p. 5 with “posttraumatic stress disorder.”

"In 'Discussion':
3rd paragraph:
- Change '...other factors, While...' to '...factors, while...'"

**Author’s Response:** We have corrected this typo.

"In Figure legends
Figure 3
- in the brackets, (Figure 1), change to Fig. 1."

**Author’s Response:** We have made the recommended change.

"In Figures
- Figure 3
- remove Swift-Scanlan et al., - Change to 'Figure 3' (similarly than in other figures)"

**Author’s Response:** We have made the recommended change.

"In Tables:
Table 4, legend:
- FWE, - change this to FWE,"

**Author’s Response:** We have corrected this typo.
Discretionary Revisions

Reviewer #1:
1. “In the abstract, the authors should remove their comment on the COMT’s role in methylation biology, because it is confusing when it is included in the same phrase which refers to the impact of COMT DNA methylation on COMT’s genetic expression. Otherwise, they should explain the difference between methylation of the COMT gene which is the topic of the paper and COMT gene-induced methylation of other targets.”

Author’s Response: This is an important point and one that we have clarified in the Abstract/Summary section. While we believe it is important to summarize the multiple roles of COMT, including its role in methylation biology as a methyltransferase, we have clarified that our study only addresses the association of COMT methylation on COMT gene expression; “Based on COMT’s numerous biological roles and recent studies suggesting that methylation of the COMT gene impacts COMT gene expression,...”

2. “In the background, they should present more literature findings on the association of COMT with alcohol and breast cancer.”

Author’s Response: We now present more literature findings on the association of COMT with alcohol (p. 5), and on COMT associations with breast cancer on p. 5-6

3. “The authors could also mention the evidence of an interaction of COMT with genetic factors influencing methylation (such as MTHFR gene) on cognition in schizophrenia (Roffman et al Neuropsychiatric Genetics 2008, Kontis et al Neuroscience Letters 2013)”

Author’s Response: In the background information on p. 4-5, we now cite literature on a number of genotype x genotype analyses of COMT polymorphisms in combination with other gene loci in both neurologic diseases and in cancer, including the recommended Roffman & Kontis references.

4. “Results second paragraph: VV, MV, MM, please change into Val/Val, Met/Val, Met/Met respectively.”

Author’s Response: We have made the recommended change (p.8).

Reviewer #2:
“In ’Background’:”
“1st paragraph:- Is there any additional reference for 'affective'”
Author’s Response: We have added an additional reference for “affective” disorders (p. 4, ref #6):

“3rd paragraph: Describe more the role of COMT in catecholamine and catecholestrogen metabolism and physiology, especially in relationship to the regulation of COMT gene, if possible”

Author’s Response: We have added further description of the expected functional consequences of methylating the COMT gene (p. 14-15), together with additional information on p. 6-7.