Reviewer’s report

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

Version: 1 Date: 9 November 2013

Reviewer: Dimitrios Kontis

Reviewer’s report:

This is an interesting and well written paper investigating the association of the methylation of the COMT gene with demographic, genetic and clinical factors which have been reported to interact with COMT activity. Their data are sound and their methods (including statistics) are well described and appropriate. I believe that the manuscript should be appropriately revised in order to be accepted for publication. Please take into consideration the following suggestions:

Major Compulsory Revisions

1. The questions which have been posed by the authors, are not so clear to me. 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. The section: “Factors associated with differential methylation of the COMT gene” should also be re-written. According to my reading, the authors are presenting data on COMT methylation taking into account: a) demographic factors, b) COMT [rs4680] genotype and they then examine the clinical relevance of their data in the context of c) a behavioural phenotype (alcohol use) and d) a disease (breast cancer). However, I have the sense that they are unfortunately mixing these different factors. For instance, alcohol use and COMT genes are not demographic factors (as wrongly shown in the title of table 3). The authors are investigating all the possible combinations of the correlations presented in table 3 (e.g. the association of COMT methylation with alcohol use and COMT genotype across various levels of alcohol use) and, in addition, they also investigate whether these methylation patterns are associated with gene expression in breast cancer. Although the use of different independent factors can be informative and enriches the manuscript, it could also lead to confusion. In order to avoid the latter, the authors should introduce their work and present their findings in a different and more structured way. Specifically, I suggest that they should separately present: a) the associations between COMT methylation and demographic factors, reflecting a possible environment X gene interaction), b) the association between COMT methylation and COMT alleles which could support that COMT methylation is COMT allele-specific, c) the association between COMT methylation and alcohol use including both the findings of table 3 concerning the AUDIT score and those associating methylation patterns with the COMT genotype across the different levels of alcohol use, d) the findings on the association between COMT methylation and COMT expression in breast cancer cells.
2. The authors need to define CpG and change it in the title.

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

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

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

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.

Minor essential revisions

1. The headings of table 3 or the title should change since AUDIT score and COMT Met alleles are not demographic factors.

Discretionary Revisions

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.

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

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)

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

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: No competing interests