Reviewer’s report

Title: CYP1A1 methylation mediates the effect of smoking and occupational polycyclic aromatic hydrocarbons co-exposure on oxidative DNA damage among Chinese coke-oven workers

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Reviewer: Isabel Alvarado-Cruz

Reviewer's report:

The authors present a well-organized manuscript that explores a literature-backed hypothesis that polycyclic aromatic hydrocarbons impact gene-specific methylation of CYP1A1 gene as a mediator of oxidative DNA damage in a relevant coke workers cohort. While the authors present associations and potential interactive effects, there are some minor and more major concerns that limit the impact of the presented findings.

1) The authors refer to the effect of co-exposure to PAHs on altering epigenetic features in coke workers. However, in the Methods sections, it is unclear how the effect of smoking was obtained in terms of the statistical analysis. In any case, to establish a co-exposure between coke and tobacco, first, it is crucial to precisely characterize the smoking status. This could be done through a specific marker as urinary cotinine levels given that even between smokers the PAHs exposure levels can exhibit high variability.

2) Authors suggested that CYP1A1 DNA methylation is a mediator of the observed oxidative damage. Nevertheless, previous literature showed that CYP1A1 expression is induced by PAHs (See 10.1111/j.1349-7006.2004.tb03162.x). Therefore, is expected that CYP1A1 promoter methylation should be reduced in order to allow the assembling of the transcription machinery to express this gene in a way that not necessary involves a disruption in the DNA methylation machinery by PAHs. In addition, CYP1A1 it is not the only isoform or enzyme involved in ROS production by PAHs metabolism, in fact, the concomitant cycle between Aldo-keto reductases (AKRs, see 10.1021/tx500298n) and PAH o-quinones is a main producer of ROS during by PAHs.

3) The observed 1-OHP levels require a wider discussion based on the benchmarks proposed for coke-oven workers by Jongeneelen 2001. In Jongeneelen's work, the occupational and non-occupational 1-OHP levels, as well as the levels for smokers and non-smokers, has been established. A major concern is the fact that 1-OHP concentrations reported by the authors could fall within the range of non-genotoxic effects. How the authors can explain the 1-OHP levels and its correlation with oxidative damage?
4) Diet's contribution (grilled meat consumption) to PAHs exposure was not considered by the authors as a potential confounder for the models (please see 10.1016/j.foodchem.2015.12.017). Is there any reason to not include this parameter in the analysis?

5) The half time for the urinary PAHs measured should be discussed to clarify the exposure timeframe and its relationship with DNA methylation and oxidative damage. Are these biomarkers represent an acute or chronic exposure?

6) DNA methylation outcome was evaluated through pyrosequencing; however, the methods did not describe the quality controls used (methylated DNA, bisulfite conversion controls) neither the method's sensitivity. Since the reported sensitivity for pyrosequencing is above 5%, these aspects should be addressed properly and interpreted accordingly given the low methylation levels reported in this study.

7) Recent evidence support that DNA methylation levels/patterns are specific for each cell type in the blood (e.g. lymphocytes vs monocytes vs neutrophils etc). Thus, region-specific DNA methylation between white blood cell subtypes can confound the outcome of whole blood DNA methylation measurements (see 10.1371/journal.pone.0046705). How the authors can solve the potential interferences by white blood cell subtype?

8) The authors should address whether the oxidative damage is interpreted since the 8-OHdG detected in urine are a proxy of the lesions that were properly repaired. Therefore, the oxidative damage variable is an indirect measurement of the oxidative damage produced that did not end in base transversion of DNA. A deep discussion should be addressed.

Minor concerns: There are some typos along with the document as well as some grammar issues that upon revision.

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