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RE: Prenatal smoke exposure, DNA methylation and a link between DRD1 and lung cancer

We thank Ryan and Robles for their interesting comment on our finding that prenatal smoke exposure was associated with DNA methylation levels in adulthood at a CpG site annotated to *DRD1* (1). In their previous study (2), Ryan and Robles found evidence for a gene-environment interaction between a single nucleotide polymorphism (SNP) in *DRD1* and secondhand smoke exposure in relation to lung cancer. They speculate that their observed interaction could be mediated by DNA methylation at *DRD1*. The SNP they mention (rs686) is located 245,766 base pairs downstream of the identified CpG site (cg22807681) in *DRD1*.

In the same study used to perform the main analysis of DNA methylation levels in adulthood in relation to prenatal smoke exposure (women from the ARIES sub-study of the Avon Longitudinal Study of Parents and Children) (1), we were able to investigate whether rs686 is a methylation quantitative trait loci (mQTL) for cg22807681 as suggested by Ryan and Robles. As was done previously (3), *DRD1* methylation (cg22807681) was rank-normalised to remove outliers and regressed on the following covariates: age, the top ten ancestry principal components, bisulphite conversion batch and estimated white blood cell counts (using an algorithm based on differential methylation between cell types (4)). Residuals were then taken forward and SNP effects were obtained using exact linear regression. In 846 women with genetic data available and DNA methylation quantified on the Illumina 450K array from blood samples taken at the antenatal time point in ARIES (5), we did not find evidence of an association between rs686 and cg22807681 (b = 0.0046 SD increase in methylation (95% CI -0.0018, 0.0112; p-value = 0.16) per copy of the G allele).

We were also able to investigate whether DNA methylation at cg22807681 was associated with lung cancer, stratified by smoking status, using data from a recent epigenome-wide association study meta-analysis conducted using Illumina 450K DNA methylation derived from blood samples in cases and controls (6). We found no convincing evidence for associations between DNA methylation at this site and lung cancer, even when stratified by never, former and current smoking status (**Figure 1**). However, we were unable to specifically investigate secondhand smoke exposure in this analysis.

Finally, using three other mQTLs that are robustly ($p<1x10^{-7}$) and independently ($r^2<0.01$) associated with cg22807681 methylation in ARIES (rs1363681, rs1549225 and rs4868502), we explored whether there is any evidence that methylation at cg22807681 has a causal effect on lung cancer, using a two-sample Mendelian randomization approach (7) and genetic data from the ILCCO-TRICL consortium (29,863 cases and 55,586 controls) (8). Again, we found little evidence to suggest a causal role for DNA methylation at this CpG site in relation to lung cancer, although effect estimates were larger among never smokers (**Figure 2**). Further work to better understand the apparent interaction between genetic variation at *DRD1* and secondhand smoke exposure in relation to lung cancer is required.

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