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Alcohol and tobacco consumption alter hypothalamic pituitary adrenal axis DNA methylation

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ABSTRACT

Alcohol and cigarette consumption have profound effects on genome wide DNA methylation and are common, often cryptic, comorbid features of many psychiatric disorders. This cryptic consumption is a possible impediment to understanding the biology of certain psychiatric disorders because if the effects of substance use are not taken into account, their presence may confound efforts to identify effects of other behavioral disorders. Since the hypothalamic pituitary adrenal (HPA) axis is known to be dysregulated in these disorders, we examined the potential for confounding effects of alcohol and cigarette consumption by examining their effects on peripheral DNA methylation at two key HPA axis genes, NR3C1 and FKBP5.

We found that the influence of alcohol and smoke exposure is more prominent at the FKBP5 gene than the NR3C1 gene. Furthermore, in both genes, loci that were consistently significantly associated with smoking and alcohol consumption demethylated with increasing exposure.

We conclude that epigenetic studies of complex disorders involving the HPA axis need to carefully control for the effects of substance use in order to minimize the possibility of type I and type II errors.

1. Introduction

One of the largest challenges to the development of an exact understanding of the molecular pathophysiology of individual psychiatric illnesses is that psychiatric disorders are frequently comorbid with one another. For example, according to the National Comorbidity Survey (NCS), subjects with major depression are 3–4 times more likely to also have alcohol dependence than those without depression (Kessler et al., 1997). In addition, those with depression are also more likely than most to experience other forms of substance use as well. This high co-morbidity of depression with substance use disorders is not unique. High rates of substance use disorders are found in most anxiety (e.g., panic disorder), psychotic disorders (e.g., schizophrenia) and other mood disorders (e.g., bipolar). Therefore, investigations seeking to isolate molecular signatures for processes associated with non-substance use disorders need to be concerned with the potential effects of co-morbid substance use among their subjects.

This is particularly true for alcohol and tobacco use disorders. Over the past several years, a number of studies have demonstrated the significant effects of cigarette consumption, and more recently alcohol consumption, on genome wide DNA methylation (Breitling et al., 2012; Dogan et al., 2014; Joubert et al., 2012; Monick et al., 2012; Philibert et al., 2014; Zeilinger et al., 2013). In particular, the genes whose methylation patterns are differentially affected by cigarette consumption preferentially map to gene networks implicated in stroke and heart disease (Dogan et al., 2014; Zhang et al., 2014). Furthermore, these and other studies have identified at least two smoke exposure associated epigenetic biomarkers (*AHRR* and *F2RL3*) with potential utility for the prevention and treatment of medical illness (Dogan et al., 2014; Philibert et al., 2015; Zhang et al., 2014).

Whether these effects of substance use also map to pathways relevant to the development of other psychiatric disorders is not as well understood. One particular process of interest that could be affected by substance use is the biological response to adversity. Adversity is associated with dysregulation of the hypothalamic pituitary adrenal (HPA) axis and is observed in those with psychiatric disorders including bipolar disorder and depression (Daban

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et al., 2005; Pariante and Lightman, 2008). Studies have suggested that stress alters DNA methylation at two key HPA axis genes: the glucocorticoid receptor (*NR3C1*) and its regulator, FK506 binding protein 5 (*FKBP5*) (Klengel et al., 2013; Non et al., 2012; Oberlander et al., 2008; Perroud et al., 2011).

While most investigators appreciate the need for adjusting the effects of substance use on adversity associated methylation changes, controlling for the exact extent of substance use in research subjects is difficult for at least two reasons. First, due to stigmatization and other adverse outcomes, self-report of smoking and drinking in high risk populations is often unreliable (Burgess et al., 2009; Caraballo et al., 2001; Erim et al., 2007; Russell et al., 2004; Whitford et al., 2009). Second, even if studies utilize biochemical verification of substance use status, current biological measures are known to have limited sensitivity (Florescu et al., 2009; Tavakoli et al., 2011). Hence, should the effects of cigarette or alcohol consumption influence the degree of DNA methylation at a locus of interest for these disorders, both type I and type II errors could arise.

This potential for confounding for genes in the HPA axis is not a theoretical issue. In our recent genome wide study of the effects of heavy alcohol consumption on DNA methylation, we identified a total of 8636 CpG residues whose methylation status were significantly associated with heavy alcohol intake (Philibert et al., 2014). With respect to the 1000 most significant CpG residues, 250 of these probes mapped to intergenic areas while 750 mapped to a total of 653 unique genes. Surprisingly, two genes had five genome wide significant associations mapping to their loci. The first was *SLC1A5*, a neutral amino acid transporter (Brauers et al., 2005). The second was *FKBP5*. When these recent results are taken together with our prior understanding of the co-morbidity of alcohol use disorder with psychiatric disorders, they suggest a need to better understand the potential for substance use to confound DNA methylation measurements at commonly studied candidate gene loci.

Therefore, in this communication, we take advantage of recently identified substance use methylation biomarkers and methylation data from three independent cohorts to examine the relationship of alcohol and cigarette consumption to DNA methylation at two key genes in the HPA axis, *FKBP5* and *NR3C1*.

2. Materials and methods

2.1. Informed consent

The protocols and procedures conducted in each study were approved by their respective Institutional Review Boards. The consent form, procedures, and protocols pertaining to the Family and Community Health Study (FACHS) study were approved by the Institutional Review Board at the University of Iowa, the University of Georgia and Iowa State University (Dogan et al., 2014). The Hannum study was approved by the Institutional Review Boards at the University of San Diego, the University of Southern California and West China Hospital (Hannum et al., 2013). The AlcMeth study was approved by the University of Iowa Institutional Review Board (Philibert et al., 2014).

2.2. Human subjects

The individuals included in this study were from the Family and Community Health Study (FACHS) cohort, an aging study (Hannum) and a study on methylation changes associated with alcohol consumption (AlcMeth). These cohorts have been described in previous studies (Dogan et al., 2014; Hannum et al., 2013; Philibert et al., 2014). The FACHS, Hannum and AlcMeth cohorts consisted of 180, 656 and 64 individuals, respectively. The demographics of these subjects are summarized in Table 1. On average, individuals in the Hannum cohort were over ten years older than those in the FACHS and AlcMeth cohorts.

Table 1
Demographic and methylation characteristics of subjects from the FACHS, Hannum and AlcMeth cohorts participating in the study.

	FACHS	Hannum	AlcMeth
n	180	656	64
Age	48.9 ± 8.6	63.4 ± 14.8	46.2 ± 7.8
Gender			
Male	79	–	49
Female	111	–	15
Ethnicity			
Caucasian		482	60
Hispanic		174	1
African American	180		3
Average methylation cg05575921	0.749 ± 0.10	0.821 ± 0.07	0.814 ± 0.13
Average methylation cg23193759	0.171 ± 0.03	0.167 ± 0.03	0.149 ± 0.03

2.3. Genome-wide DNA methylation profiling

Peripheral blood mononuclear cell DNA methylation from the FACHS and AlcMeth cohorts and whole blood DNA methylation from the Hannum cohort was profiled using the Illumina (San Diego, CA) Infinium HumanMethylation450 BeadChip. The methylation data of all three cohorts are publically available and can be obtained from the Gene Expression Omnibus (GEO) database: GSE35059 and GSE59550 for FACHS, GSE40279 for Hannum and GSE57853 for AlcMeth. Beta values were derived using the Illumina Genome Studio software.

2.4. Analyses

For all analyses, the methylation at cg05575921 and cg23193759 were used as objective biomarkers to quantify smoking and alcohol consumption, respectively. Cg05575921 is located in intron 3 of the aryl hydrocarbon receptor repressor (*AHRR*) gene whereas cg23193759 is located on chromosome 10 open reading frame 35. The strong correlation between smoke exposure and methylation changes at cg05575921 is well established and has been consistently replicated (Philibert et al., 2015). While the relationship between alcohol consumption and cg23193759 methylation was only established recently, this locus has been shown to be the most differentially methylated with respect to alcohol use (Philibert et al., 2014). Both loci demethylate with increasing exposure.

There are 41 and 34 CpG sites contained within the Illumina 450 K array for the NR3C1 and FKBP5 genes, respectively. Firstly, to determine the influence of smoking (represented by methylation at cg05575921) and alcohol (represented by methylation at cg23193759) on these genes, the average methylation at all loci within each gene was regressed against the biomarkers. Subsequently, to understand if the effects of alcohol and smoking consumption are concentrated at specific regions of the gene, a linear regression model was fitted for each of the 75 loci. Specifically, the methylation of the locus was regressed against each biomarker individually. From all fitted regression models, the regression coefficient, β, the coefficient of determination, R^2, and the p-value were extracted. Correction for multiple comparisons was conducted by multiplying each p-value with 75. All analyses were performed in *R* (Team, 2012).

3. Results

The data for this study was derived from three independent cohorts (Table 1). The first cohort consisted of 180 individuals