Alcohol and tobacco consumption alter hypothalamic pituitary adrenal axis DNA methylation

Meeshanthini V. Dogan[1,2], **Man-Kit Lei**[3], **Steven R.H. Beach**[3], **Gene Brody**[3], and **Robert A. Philibert**[1,2,*]

[1]Department of Biomedical Engineering, University of Iowa, Iowa City, IA, USA

[2]Department of Psychiatry, University of Iowa, Iowa City, IA, USA

[3]Center for Family Research, University of Georgia, Athens, GA, USA

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.

*Corresponding author. Department of Psychiatry, Rm 2-126 MEB, 500 Newton Road, Iowa City, IA, 52242, USA; phone 319-353-4986.
Email addresses:
MVD: meeshanthini-vijayendran@uiowa.edu
MKL: karlo@uga.edu
SRHB: srhbeach@uga.edu
GB: gbrody@uga.edu
RAP: robert-philibert@uiowa.edu

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 to 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 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.

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 450K 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 from the FACHS study (Dogan et al., 2014). The individuals from FACHS who contributed their data are African-American and mostly female (~62%) with an average age in their late 40s. The second cohort is from a study on the epigenetics of aging by Hannum and colleagues (Hannum et al., 2013). These individuals were either Northern European (~73%) or Hispanic (~27%), with an average age in the early 60s. The last cohort (referred to as AlcMeth), consisted of 64 individuals who participated in a commercial case and control study on the epigenetic effects of heavy alcohol consumption (Philibert et al., 2014). These individuals are almost all of Northern European ancestry, mostly male (75%) with their average age being in the mid-40s.

Importantly, both the FACHS and the AlcMeth cohorts have high rates of substance use and comorbid medical disorders.

The average DNA methylation for the smoking biomarker, cg05575921, in the FACHS, Hannum and AlcMeth cohorts were 0.749, 0.821, and 0.814, respectively, while the average for the alcohol biomarker, cg23193759, was 0.171, 0.167, and 0.149, respectively (Table 1). As a reference, methylation in lifetime American non-smokers of Northern European ancestry at cg05575921 is approximately 0.91 while the methylation status at the cg23193759 locus in lifetime non-drinkers of Northern European ancestry is approximately 0.17 (Philibert et al., 2015; Philibert et al., 2014).

We examined the influence of cigarette smoking and alcohol consumption on the DNA methylation of the two frequently examined HPA axis genes, *FKBP5* and *NR3C1*. These genes have 34 and 41 Illumina 450k array methylation probes mapping to them, respectively. Details on the placement of the probes, their mean and standard deviations in all three cohorts are provided in Appendix A. The influence of cigarette consumption, as indicated by demethylation at cg05575921, and alcohol consumption, as indicated by demethylation at cg23193759, in the FACHS, Hannum and AlcMeth cohorts were determined by fitting a linear regression model. The results of this analysis are summarized in Tables 2 and 3. The DNA methylation at FKBP5 was significantly associated with smoking and alcohol consumption in all three cohorts, with the most significant smoking and alcohol association observed in the FACHS ($p<1.86E-06$) and AlcMeth ($p<2.16E-07$) cohorts, respectively. Similarly, for DNA methylation at *NR3C1*, a significant association was only observed in the Hannum cohort with respect to alcohol consumption ($p<0.0009$). This implies that smoking and alcohol has a stronger influence on the DNA methylation of FKBP5 than NR3C1.

The distribution of substance use induced differential methylation at the generic structural level is still not well understood. To better understand this, we examined the effects of smoking and alcohol consumption at each locus in all three cohorts. The results from this analysis are summarized in Appendices B, C and D for the FACHS, Hannum and AlcMeth cohorts, respectively. In all three cohorts, at the NR3C1 gene, only cg03857453 located in the body of the gene was significantly associated with smoking and drinking. The positive regression coefficient at this locus also suggests that, with increasing levels of smoke exposure and drinking (biomarkers hypomethylation), the methylation level at this locus decreases. While the number of significant associations was larger for FKBP5, the only significant CpG site for smoking common to all three cohorts was cg19226017, located at TSS1500. For alcohol consumption, the only two significant associations observed in all three cohorts were at cg03591753 located at the 5'UTR and cg14284211 located in the body of the gene. Once again, the positive regression coefficients at these loci imply that with increasing exposure, methylation level decreases.

4. Discussion

Examinations of the biology of human behavioral disorders are challenging to conduct for a number of reasons. One of those is the potential for unreliable self-report data (Caraballo et

al., 2004; Corbett et al., 2012; Kandel et al., 2006; Webb et al., 2003). The strong effects of substance use on peripheral DNA methylation shown by ourselves and others suggest a potential for the effects of substance use to confound epigenetic analyses. This is particularly true when analyses are not corrected in any way for substance use.

In this study, we investigate the relationship between objective markers of cigarette and alcohol consumption and DNA methylation at two prominent HPA axis genes, *FKBP5* and *NR3C1*, using DNA prepared from blood. The results demonstrate the stronger effects of these substances on the methylation status of the *FKBP5* gene and the strong potential for substance use mediated confounding of DNA methylation analyses. The effects of alcohol consumption on *FKBP5* methylation are not all that surprising. Prior studies have implicated *FKBP5* sequence and expression variation in moderating responses to alcohol use. For example, a recent study by Huang and associates demonstrated that *FKBP5* genetic variation moderated the severity of alcohol withdrawal in both humans and rodents (Huang et al., 2014). Using a genome wide approach, Bell and associates showed that acute alcohol intake in rats was associated with increased transcription of *FKPB5* (Bell et al., 2009). Consequently, while current findings may have a chilling effect on some biomarker analyses of complex behavioral disorders, when taken together with prior genetic variation and gene expression studies, the current results actually suggest the additional need for further epigenetic and genetic examinations of the role of *FKBP5* in moderating alcohol use disorders.

The need to control for substance use effects is probably not limited to studies of the HPA axis. Therefore, the potential for confounding may apply to virtually all epigenetic analyses of psychiatric candidate genes (de Leon and Diaz, 2005; Swendsen et al., 2010). Even so, simply using self-report of alcohol or smoking may not be sufficient to control for the effects of substance use. As compared to the gold standard of cotinine determinations, self-report of smoking is known to be unreliable in some high risk populations (Caraballo et al., 2004; Kandel et al., 2006; Russell et al., 2004). Substance use is not the only variable that needs to be considered as potential confounder in DNA methylation analyses. Age, gender, and body mass index are several variables that are known to have significant effects on genome wide DNA methylation signatures (Almén et al., 2014; Hannum et al., 2013). Fortunately, these variables are generally highly reliably assessed in most data sets. As such, their effects can be readily taken into account. In contrast, the presence or absence of other medical conditions such as type II diabetes, which also has significant genome wide effects (Toperoff et al., 2012), may not always be known, even by the individuals themselves. Hence, some degree of confounding will inevitably be present.

5. Conclusions

In summary, in this communication, we show the broad effects of alcohol and cigarette consumption on DNA methylation at the HPA axis with particularly prominent effects at *FKBP5*. These results highlight the need for controlling for the effects of substance use in epigenetic studies of complex disorders and the need for further studies on the role of the HPA axis in moderating alcohol use disorders.

Author Manuscript

Acknowledgements

Appendices

Appendix A

The placement of NR3C1 and FKBP5 methylation probes with their respective means and standard deviations in the FACHS, Hannum and AlcMeth cohorts.

CpG	Gene	Region	Island Status	FACHS		Hannum		AlcMeth	
				Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
cg12466613	NR3C1	TSS1500		0.895	0.029	0.925	0.038	0.746	0.036
cg07589972	NR3C1	TSS1500		0.869	0.027	0.916	0.031	0.804	0.027
cg26720913	NR3C1	1stExon		0.015	0.007	0.019	0.012	0.019	0.011
cg08818984	NR3C1	1stExon		0.057	0.017	0.052	0.021	0.070	0.020
cg07528216	NR3C1	5'UTR	S_Shelf	0.918	0.018	0.944	0.022	0.865	0.019
cg27345592	NR3C1	5'UTR	S_Shore	0.923	0.017	0.940	0.024	0.851	0.021
cg13648501	NR3C1	5'UTR	S_Shore	0.073	0.020	0.064	0.027	0.097	0.025
cg24026230	NR3C1	5'UTR	S_Shore	0.025	0.008	0.016	0.007	0.024	0.009
cg14558428	NR3C1	5'UTR	Island	0.024	0.008	0.016	0.008	0.018	0.008
cg21702128	NR3C1	TSS1500	Island	0.070	0.008	0.095	0.017	0.069	0.010
cg10847032	NR3C1	TSS1500	Island	0.042	0.008	0.053	0.010	0.048	0.012
cg16335926	NR3C1	TSS1500	Island	0.017	0.005	0.013	0.005	0.013	0.005
cg18849621	NR3C1	TSS1500	Island	0.055	0.013	0.054	0.015	0.059	0.018
cg06968181	NR3C1	TSS1500	Island	0.063	0.028	0.026	0.015	0.028	0.014
cg26464411	NR3C1	TSS1500	Island	0.067	0.028	0.027	0.011	0.034	0.010
cg18068240	NR3C1	5'UTR	Island	0.012	0.006	0.005	0.004	0.010	0.005
cg15645634	NR3C1	5'UTR	Island	0.028	0.008	0.017	0.006	0.025	0.007
cg15910486	NR3C1	5'UTR	Island	0.090	0.021	0.046	0.020	0.055	0.010
cgQ4111177	NR3C1	5'UTR	Island	0.050	0.006	0.073	0.015	0.053	0.009
cg17860381	NR3C1	5'UTR	Island	0.035	0.011	0.014	0.008	0.014	0.005
cg18019515	NR3C1	TSS200	Island	0.011	0.004	0.006	0.004	0.008	0.004
cg11152298	NR3C1	TSS200	Island	0.062	0.006	0.080	0.011	0.064	0.009
cg00629244	NR3C1	TSS200	Island	0.014	0.007	0.007	0.005	0.016	0.007
cg18146873	NR3C1	1stExon	Island	0.034	0.010	0.047	0.019	0.044	0.010
cg20753294	NR3C1	1stExon	Island	0.062	0.020	0.039	0.028	0.055	0.018

CpG	Gene	Region	Island Status	FACHS		Hannum		AlcMeth	
				Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
cg17617527	NR3C1	5'UTR	Island	0.008	0.005	0.005	0.004	0.007	0.004
cg06521673	NR3C1	5'UTR	Island	0.040	0.007	0.043	0.008	0.044	0.008
cg06952416	NR3C1	5'UTR	N_Shore	0.068	0.027	0.041	0.027	0.074	0.032
cg27122725	NR3C1	5'UTR	N_Shore	0.109	0.029	0.088	0.040	0.060	0.028
cg18998365	NR3C1	5'UTR	N_Shore	0.551	0.039	0.580	0.053	0.533	0.040
cg07733851	NR3C1	5'UTR	N_Shore	0.320	0.039	0.354	0.057	0.344	0.037
cg08845721	NR3C1	5'UTR	N_Shore	0.827	0.041	0.891	0.033	0.794	0.031
cg17342132	NR3C1	Body	N_Shore	0.711	0.060	0.865	0.030	0.730	0.056
cg06613263	NR3C1	Body	N_Shelf	0.794	0.044	0.846	0.041	0.749	0.043
cg27107893	NR3C1	Body		0.860	0.035	0.897	0.053	0.743	0.053
cg25535999	NR3C1	Body		0.852	0.024	0.890	0.027	0.800	0.023
cg16586394	NR3C1	Body		0.866	0.026	0.901	0.023	0.837	0.021
cg18484679	NR3C1	Body		0.867	0.024	0.913	0.034	0.786	0.026
cg03857453	NR3C1	Body		0.754	0.051	0.700	0.051	0.757	0.049
cg19457823	NR3C1	Body		0.776	0.073	0.854	0.054	0.701	0.058
cg23273257	NR3C1	3'UTR		0.934	0.015	0.956	0.018	0.887	0.017
cg08915438	FKBP5	TSS1500	N_Shore	0.539	0.050	0.534	0.057	0.549	0.050
cg19226017	FKBP5	TSS1500	N_Shore	0.766	0.040	0.719	0.039	0.722	0.034
cg25114611	FKBP5	TSS1500	S_Shore	0.304	0.034	0.326	0.038	0.296	0.037
cg17030679	FKBP5	5'UTR	S_Shore	0.043	0.009	0.048	0.012	0.044	0.009
cg07485685	FKBP5	5'UTR	Island	0.030	0.009	0.016	0.010	0.017	0.006
cg00610228	FKBP5	5'UTR	Island	0.056	0.007	0.091	0.024	0.060	0.008
cg11845071	FKBP5	5'UTR	Island	0.010	0.006	0.006	0.005	0.011	0.006
cg06937024	FKBP5	5'UTR	N_Shore	0.032	0.015	0.012	0.006	0.008	0.004
cg00052684	FKBP5	5'UTR	N_Shore	0.393	0.062	0.470	0.056	0.338	0.071
cg23416081	FKBP5	5'UTR	N_Shelf	0.313	0.060	0.188	0.058	0.334	0.080
cg15929276	FKBP5	5'UTR		0.194	0.053	0.137	0.052	0.197	0.065
cg03591753	FKBP5	5'UTR	S_Shelf	0.547	0.068	0.472	0.048	0.587	0.065

CpG	Gene	Region	Island Status	FACHS		Hannum		AlcMeth	
				Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
cg08636224	FKBP5	5'UTR	S_Shore	0.896	0.036	0.908	0.017	0.876	0.016
cg00130530	FKBP5	5'UTR	S_Shore	0.612	0.052	0.631	0.040	0.607	0.048
cg20813374	FKBP5	5'UTR	S_Shore	0.411	0.049	0.404	0.040	0.399	0.046
cg01294490	FKBP5	TSS200	S_Shore	0.089	0.017	0.080	0.017	0.100	0.024
cg07843056	FKBP5	TSS200	Island	0.008	0.006	0.007	0.006	0.012	0.008
cg16012111	FKBP5	TSS200	Island	0.058	0.010	0.075	0.017	0.063	0.011
cg10913456	FKBP5	1stExon	Island	0.007	0.004	0.007	0.011	0.007	0.004
cg00140191	FKBP5	5'UTR	Island	0.045	0.014	0.025	0.013	0.028	0.010
cg00862770	FKBP5	5'UTR	Island	0.038	0.007	0.037	0.008	0.045	0.011
cg03546163	FKBP5	5'UTR	N_Shore	0.503	0.074	0.550	0.097	0.423	0.062
cg14642437	FKBP5	5'UTR	N_Shelf	0.844	0.027	0.842	0.035	0.797	0.026
cg17085721	FKBP5	5'UTR		0.856	0.025	0.907	0.020	0.844	0.025
cg19014730	FKBP5	5'UTR		0.651	0.050	0.726	0.055	0.579	0.050
cg07061368	FKBP5	5'UTR		0.813	0.045	0.880	0.042	0.729	0.045
cg08586216	FKBP5	5'UTR		0.916	0.012	0.938	0.014	0.903	0.014
cg16052510	FKBP5	Body		0.729	0.059	0.847	0.049	0.709	0.049
cg14284211	FKBP5	Body		0.246	0.050	0.151	0.047	0.225	0.054
cg07633853	FKBP5	Body		0.159	0.037	0.109	0.050	0.185	0.044
cg10300814	FKBP5	Body		0.881	0.019	0.890	0.020	0.860	0.016
cg06087101	FKBP5	Body		0.412	0.081	0.412	0.073	0.407	0.064
cg02665568	FKBP5	Body		0.865	0.027	0.891	0.032	0.800	0.025

Appendix B

The smoking and alcohol consumption associated regression coefficient, percent variation explained and corrected p-value of DNA methylation probes at the NR3C1 and FKBP5 genes in the FACHS cohort.

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg12466613	KR3C1	−0.0293	0.89	1	−0.1849	3.31	1
cg07589972	KR3C1	0.0516	3.18	1	−0.121	1.63	1
cg26720913	KR3C1	0.009	1.38	1	0.0528	4.55	0.355
cg08818984	KR3C1	0.0127	0.5	1	0.0843	2.01	1
cg07528216	KR3C1	0.023	1.44	1	0.0692	1.21	1
cg27345592	KR3C1	0.0007	0	1	0.0819	1.87	1
cg13648501	KR3C1	−0.0274	1.76	1	−0.1092	2.59	1
cg24026230	KR3C1	−0.0019	0.06	1	−0.0192	0.54	1
cg14558428	KR3C1	−0.0189	4.72	0.253	−0.0275	0.93	1
cg21702128	KR3C1	0.0028	0.12	1	0.0515	3.83	0.651
cg10847032	KR3C1	0.0145	3.25	1	0.0467	3.12	1
cg16335926	KR3C1	−0.0017	0.11	1	−0.0129	0.61	1
cg18849621	KR3C1	0.0245	3.17	1	−0.0191	0.18	1
cg06968181	KR3C1	0.0312	1.14	1	0.0976	1.05	1
cg26464411	KR3C1	−0.0021	0	1	0.0387	0.16	1
cg18068240	KR3C1	−0.0032	0.26	1	−0.0031	0.02	1
cg15645634	KR3C1	−0.0162	3.68	0.742	−0.0264	0.91	1
cg15910486	KR3C1	0.0204	0.87	1	0.0517	0.52	1
cg04111177	KR3C1	0.0084	1.67	1	0.0309	2.1	1
cg17860381	KR3C1	−0.001	0.01	1	−0.0014	0	1
cg18019515	NR3C1	0.0079	2.83	1	0.0181	1.38	1
cg11152298	NR3C1	0.0041	0.37	1	0.034	2.34	1
cg00629244	NR3C1	−0.0004	0	1	0.0124	0.31	1
cg18146873	NR3C1	−0.0073	0.52	1	−0.0139	0.17	1
cg20753294	NR3C1	−0.0048	0.05	1	0.0811	1.38	1

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg17617527	NR3C1	0.0057	1.32	1	0.0008	0	1
cg06521673	NR3C1	−0.002	0.07	1	0.0057	0.05	1
cg06952416	NR3C1	0.0577	4.31	0.418	−0.0699	0.58	1
cg27122725	NR3C1	−0.035	1.28	1	−0.1145	1.31	1
cg18998365	NR3C1	0.0826	4.03	0.517	0.1691	1.57	1
cg07733851	NR3C1	0.1013	6.18	0.057	0.1679	1.58	1
cg08845721	NR3C1	0.0804	3.41	0.981	0.0615	0.19	1
cg17342132	NR3C1	0.0096	0.02	1	0.1149	0.31	1
cg06613263	NR3C1	0.1154	6.14	0.060	0.0001	0	1
cg27107893	NR3C1	0.0561	2.26	1	−0.1607	1.73	1
cg25535999	NR3C1	0.0375	2.26	1	−0.0439	0.29	1
cg16586394	NR3C1	0.0506	3.3	1	−0.2473	7.34	0.018
cg18484679	NR3C1	0.047	3.56	0.839	−0.1255	2.36	1
cg03857453	NR3C1	0.1665	9.45	0.002	0.6602	13.84	2.04E−05
cg19457823	NR3C1	0.0608	0.63	1	−0.7208	8.29	0.007
cg23273257	NR3C1	0.0189	1.47	1	0.0764	2.25	1
cg08915438	FKBP5	0.101	3.74	0.694	0.1853	1.17	1
cg19226017	FKBP5	0.1163	7.47	0.015	0.1348	0.94	1
cg25114611	FKBP5	0.1088	9.06	0.003	0.1554	1.72	1
cg17030679	FKBP5	0.0087	0.88	1	0.0194	0.4	1
cg07485685	FKBP5	0.0124	1.78	1	0.0132	0.19	1
cg00610228	FKBP5	0.0147	3.61	0.795	0.0335	1.75	1
cg11845071	FKBP5	0.0023	0.15	1	−0.0055	0.08	1
cg06937024	FKBP5	0.0078	0.25	1	0.0129	0.06	1
cg00052684	FKBP5	0.042	0.42	1	0.2289	1.15	1
cg23416081	FKBP5	0.1262	4.04	0.513	0.5154	6.27	0.052
cg15929276	FKBP5	0.0464	0.69	1	0.03	0.03	1
cg03591753	FKBP5	0.1609	5.13	0.167	0.8879	14.55	9.50E−06

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg08636224	FKBP5	0.0281	0.56	1	0.0626	0.26	1
cg00130530	FKBP5	0.1664	9.33	0.002	-0.1729	0.94	1
cg20813374	FKBP5	0.1934	14.03	1.66E-05	0.0365	0.05	1
cg01294490	FKBP5	0.0256	1.98	1	0.0533	0.8	1
cg07843056	FKBP5	0.01	2.33	1	-0.0228	0.83	1
cg16012111	FKBP5	0.0005	0	1	0.028	0.67	1
cg10913456	FKBP5	-0.0062	2.15	1	0.002	0.02	1
cg00140191	FKBP5	0.0164	1.26	1	0.0339	0.51	1
cg00862770	FKBP5	-0.007	0.81	1	0.0111	0.19	1
cg03546163	FKBP5	0.1087	1.94	1	0.5202	4.13	0.465
cg14642437	FKBP5	0.0589	4.19	0.438	0.3242	11.83	1.70E-04
cg17085721	FKBP5	0.0208	0.62	1	0.1613	3.54	0.895
cg19014730	FKBP5	0.0608	1.34	1	-0.2119	1.51	1
cg07061368	FKBP5	-0.0093	0.04	1	-0.1963	1.58	1
cg08586216	FKBP5	0.0257	4.08	0.49	0.0995	5.68	0.096
cg16052510	FKBP5	0.0193	0.1	1	0.1872	0.86	1
cg14284211	FKBP5	0.1106	4.41	0.349	0.9157	28.14	1.40E-12
cg07633853	FKBP5	0.0371	0.89	1	0.3157	6.02	0.07
cg10300814	FKBP5	0.048	5.67	0.096	0.1565	5.61	0.102
cg06087101	FKBP5	0.1063	1.55	1	0.1201	0.18	1
cg02665568	FKBP5	-0.0018	0	1	0.1054	1.26	1
cg18726036	FKBP5	-0.016	2.79	1	-0.0121	0.15	1

Appendix C

The smoking and alcohol consumption associated regression coefficient, percent variation explained and corrected p-value of DNA methylation probes at the NR3C1 and FKBP5 genes in the Hannum cohort.

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg12466613	NR3C1	−0.0185	0.13	1	−0.0315	0.08	1
cg07589972	NR3C1	−0.0091	0.05	1	−0.0325	0.13	1
cg26720913	NR3C1	−0.0041	0.07	1	0.0341	0.93	1
cg08818984	NR3C1	0.0151	0.27	1	0.1141	3.24	2.87E-04
cg07528216	NR3C1	0.0079	0.07	1	0.0082	0.01	1
cg27345592	NR3C1	−0.0041	0.01	1	0.0546	0.56	1
cg13648501	NR3C1	0.011	0.08	1	0.105	1.64	0.078
cg24026230	NR3C1	−0.0002	0	1	−0.0086	0.17	1
cg14558428	NR3C1	−0.0092	0.76	1	0.0269	1.32	0.285
cg21702128	NR3C1	0.0165	0.5	1	0.0563	1.25	0.309
cg10847032	NR3C1	−0.0093	0.43	1	−0.0187	0.37	1
cg16335926	NR3C1	−0.0015	0.05	1	0.0001	0	1
cg18849621	NR3C1	−0.003	0.02	1	−0.0153	0.12	1
cg06968181	NR3C1	−0.0094	0.19	1	−0.0301	0.34	1
cg26464411	NR3C1	−0.0064	0.17	1	0.0015	0	1
cg18068240	NR3C1	−0.0017	0.11	1	−0.001	0.01	1
cg15645634	NR3C1	−0.0034	0.16	1	0.0026	0.02	1
cg15910486	NR3C1	−0.031	1.26	0.294	0.0238	0.16	1
cg04111177	NR3C1	0.0013	0	1	0.0425	0.89	1
cg17860381	NR3C1	−0.0012	0.01	1	−0.002	0.01	1
cg18019515	NR3C1	−0.0011	0.04	1	−0.0031	0.06	1
cg11152298	NR3C1	−0.0118	0.6	1	0.0163	0.24	1
cg00629244	NR3C1	0	0	1	−0.0071	0.23	1
cg18146873	NR3C1	0.017	0.42	1	0.0542	0.92	1
cg20753294	NR3C1	−0.0059	0.02	1	−0.0117	0.02	1

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg17617527	NR3C1	0.0013	0.06	1	0.0042	0.15	1
cg06521673	NR3C1	0.0013	0.01	1	0.0121	0.26	1
cg06952416	NR3C1	0.0044	0.01	1	0.0045	0	1
cg27122725	NR3C1	−0.0019	0	1	−0.0138	0.01	1
cg18998365	NR3C1	0.0636	0.75	1	0.2615	2.73	0.002
cg07733851	NR3C1	0.0956	1.46	0.144	0.1562	0.84	1
cg08845721	NR3C1	0.0212	0.21	1	−0.0847	0.74	1
cg17342132	NR3C1	−0.0129	0.09	1	−0.0463	0.26	1
cg06613263	NR3C1	0.004	0	1	−0.1436	1.34	0.222
cg27107893	NR3C1	0.0603	0.67	1	0.0667	0.18	1
cg25535999	NR3C1	0.0259	0.49	1	−0.0373	0.22	1
cg16586394	NR3C1	0.0113	0.12	1	−0.0355	0.26	1
cg18484679	NR3C1	0.0013	0	1	0.0122	0.01	1
cg03857453	NR3C1	0.1438	4.17	9.93E-06	0.4385	8.35	3.35E-12
cg19457823	NR3C1	−0.0095	0.02	1	−0.2218	1.9	0.030
cg23273257	NR3C1	−0.0096	0.15	1	0.0438	0.66	1
cg08915438	FKBP5	0.1474	3.54	9.27E-05	0.2338	1.92	0.028
cg19226017	FKBP5	0.0809	2.25	0.009	0.127	1.2	0.377
cg25114611	FKBP5	0.0532	1.01	0.746	0.2186	3.67	5.69E-05
cg17030679	FKBP5	0.0015	0.01	1	0.018	0.25	1
cg07485685	FKBP5	−0.0116	0.71	1	−0.0043	0.02	1
cg00610228	FKBP5	−0.0071	0.04	1	−0.0113	0.02	1
cg11845071	FKBP5	0.0037	0.32	1	0.0043	0.09	1
cg06937024	FKBP5	−0.0039	0.19	1	−0.0071	0.14	1
cg00052684	FKBP5	0.0646	0.7	1	−0.0778	0.22	1
cg23416081	FKBP5	0.0984	1.49	0.131	0.632	13.2	4.98E-20
cg15929276	FKBP5	0.0313	0.19	1	0.1445	0.87	1
cg03591753	FKBP5	0.0923	1.95	0.025	0.4794	11.34	5.46E-17

CpG	Gene	Smoking				Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg08636224	FKBP5	0.0225	0.96	0.918	0.0145	0.09	1
cg00130530	FKBP5	0.1225	4.89	7.78E-07	-0.0578	0.23	1
cg20813374	FKBP5	0.1194	4.65	1.80E-06	-0.0145	0.01	1
cg01294490	FKBP5	0.0064	0.07	1	0.0433	0.72	1
cg07843056	FKBP5	0.0021	0.06	1	0.0007	0	1
cg16012111	FKBP5	-0.0165	0.5	1	-0.01	0.04	1
cg10913456	FKBP5	-0.0169	1.34	1	-0.0154	0.22	1
cg00140191	FKBP5	-0.0026	0.02	1	-0.0209	0.28	1
cg00862770	FKBP5	0.0023	0.04	1	-0.0003	0	1
cg03546163	FKBP5	0.2096	2.42	0.005	0.5897	4.12	1.21E-05
cg14642437	FKBP5	0.0211	0.19	1	0.0954	0.85	1
cg17085721	FKBP5	0.0164	0.37	1	0.0124	0.04	1
cg19014730	FKBP5	0.0568	0.56	1	0.0198	0.01	1
cg07061368	FKBP5	-0.0337	0.33	1	-0.1549	1.5	0.127
cg08586216	FKBP5	0.0117	0.37	1	-0.0048	0.01	1
cg16052510	FKBP5	0.0247	0.13	1	0.0886	0.37	1
cg14284211	FKBP5	0.0988	2.29	0.007	0.4543	10.43	1.65E-15
cg07633853	FKBP5	0.0459	0.43	1	0.3783	6.35	5.20E-09
cg10300814	FKBP5	0.0282	1.03	0.708	0.0426	0.51	1
cg06087101	FKBP5	0.0759	0.57	1	0.0802	0.14	1
cg02665568	FKBP5	-0.0021	0	1	-0.003	0	1
cg18726036	FKBP5	0.0024	0.02	1	0.0073	0.04	1

Appendix D

The smoking and alcohol consumption associated regression coefficient, percent variation explained and corrected p-value of DNA methylation probes at the NR3C1 and FKBP5 genes in the AlcMeth cohort.

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg12466613	NR3C1	−0.0406	2.16	1	0.0632	0.24	1
cg07589972	NR3C1	−0.0412	3.87	1	−0.3280	11.24	0.507
cg26720913	NR3C1	−0.0052	0.40	1	0.0117	0.09	1
cg08818984	NR3C1	0.0064	0.17	1	0.0347	0.23	1
cg07528216	NR3C1	−0.0062	0.18	1	−0.0137	0.04	1
cg27345592	NR3C1	0.0032	0.04	1	0.0756	0.99	1
cg13648501	NR3C1	0.0280	2.13	1	0.0771	0.74	1
cg24026230	NR3C1	−0.0025	0.13	1	−0.0388	1.48	1
cg14558428	NR3C1	−0.0083	1.71	1	0.0692	5.55	1
cg21702128	NR3C1	0.0018	0.05	1	0.1088	8.82	1
cg10847032	NR3C1	−0.0229	5.73	1	−0.0036	0.01	1
cg16335926	NR3C1	0.0038	1.10	1	−0.0270	2.47	1
cg18849621	NR3C1	0.0087	0.39	1	0.0724	1.25	1
cg06968181	NR3C1	0.0173	2.52	1	0.0050	0.01	1
cg26464411	NR3C1	0.0126	2.60	1	0.0872	5.67	1
cg18068240	NR3C1	0.0013	0.12	1	−0.0184	1.03	1
cg15645634	NR3C1	0.0064	1.56	1	0.0284	1.41	1
cg15910486	NR3C1	0.0191	5.97	1	−0.0369	1.02	1
cg04111177	NR3C1	0.0021	0.10	1	0.0838	7.49	1
cg17860381	NR3C1	−0.0018	0.22	1	−0.0093	0.27	1
cg18019515	NR3C1	0.0004	0.01	1	0.0279	3.66	1
cg11152298	NR3C1	0.0087	1.60	1	0.0867	7.32	1
cg00629244	NR3C1	0.0040	0.57	1	0.0690	7.89	1
cg18146873	NR3C1	−0.0004	0.00	1	0.0696	3.50	1
cg20753294	NR3C1	0.0218	2.56	1	−0.0757	1.42	1

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg17617527	NR3C1	0.0050	2.33	1	−0.0230	2.20	1
cg06521673	NR3C1	0.0034	0.31	1	0.0754	6.89	1
cg06952416	NR3C1	0.0029	0.01	1	0.1002	0.76	1
cg27122725	NR3C1	0.0410	3.60	1	0.2075	4.23	1
cg18998365	NR3C1	0.1019	11.00	0.558	−0.0451	0.10	1
cg07733851	NR3C1	0.0118	0.17	1	−0.0026	0	1
cg08845721	NR3C1	−0.0681	8.39	1	−0.3822	12.11	0.363
cg17342132	NR3C1	−0.1458	11.39	0.48	−0.8965	19.69	0.018
cg06613263	NR3C1	−0.0876	6.98	1	−0.2386	2.37	1
cg27107893	NR3C1	0.0620	2.11	1	−0.1326	0.50	1
cg25535999	NR3C1	−0.0013	0.01	1	−0.0577	0.50	1
cg16586394	NR3C1	−0.0224	1.85	1	−0.1861	5.84	1
cg18484679	NR3C1	−0.0367	3.25	1	−0.0745	0.61	1
cg03857453	NR3C1	0.2200	33.40	4.28E-05	0.8831	24.64	0.002
cg19457823	NR3C1	−0.1079	5.92	1	−0.3183	2.36	1
cg23273257	NR3C1	−0.0045	0.12	1	−0.0841	1.92	1
cg08915438	FKBP5	0.2007	27.75	0.001	0.8771	24.26	0.003
cg19226017	FKBP5	0.1634	39.96	1.59E-06	0.5490	20.64	0.012
cg25114611	FKBP5	0.1618	32.63	6.19E-05	0.7582	32.80	5.71E-05
cg17030679	FKBP5	0.0038	0.29	1	0.0992	9.25	1
cg07485685	FKBP5	−0.0014	0.10	1	−0.0070	0.12	1
cg00610228	FKBP5	0.0061	0.90	1	0.0736	5.95	1
cg11845071	FKBP5	−0.0034	0.44	1	0.0117	0.26	1
cg06937024	FKBP5	0.0015	0.26	1	0.0301	4.52	1
cg00052684	FKBP5	0.0171	0.10	1	1.0074	14.90	0.134
cg23416081	FKBP5	0.3538	33.14	4.87E-05	1.6091	31.38	1.11E-04
cg15929276	FKBP5	0.1965	15.46	0.098	0.1888	0.65	1
cg03591753	FKBP5	0.2667	28.59	4.00E-04	1.1008	22.30	0.006

CpG	Gene	Smoking			Alcohol Consumption		
		Regression Coefficient	% Variation Explained	Corrected P-value	Regression Coefficient	% Variation Explained	Corrected P-value
cg08636224	FKBP5	0.0353	8.18	1	0.0961	2.78	1
cg00130530	FKBP5	−0.0048	0.02	1	0.2608	2.24	1
cg20813374	FKBP5	0.0990	7.73	1	0.5843	12.33	0.334
cg01294490	FKBP5	0.0118	0.40	1	0.3264	13.85	0.184
cg07843056	FKBP5	0.0021	0.12	1	0.0242	0.74	1
cg16012111	FKBP5	−0.0062	0.50	1	0.0633	2.35	1
cg10913456	FKBP5	0.0035	1.54	1	0.0143	1.24	1
cg00140191	FKBP5	−0.0040	0.25	1	−0.0956	6.67	1
cg00862770	FKBP5	0.0057	0.47	1	0.0728	3.55	1
cg03546163	FKBP5	0.1800	14.08	0.169	0.9168	16.72	0.059
cg14642437	FKBP5	0.0608	9.48	1	0.3563	14.90	0.122
cg17085721	FKBP5	−0.0204	1.11	1	−0.1119	1.53	1
cg19014730	FKBP5	−0.0348	0.80	1	0.0732	0.16	1
cg07061368	FKBP5	−0.0869	6.22	1	−0.4238	6.77	1
cg08586216	FKBP5	−0.0039	0.13	1	−0.0458	0.82	1
cg16052510	FKBP5	0.0281	0.56	1	0.4122	5.55	1
cg14284211	FKBP5	0.1913	21.58	0.008	1.2158	39.91	1.63E-06
cg07633853	FKBP5	0.0511	2.39	1	0.4451	8.53	1
cg10300814	FKBP5	0.0449	13.58	0.205	0.1495	6.90	1
cg06087101	FKBP5	0.0778	2.48	1	0.0003	0	1
cg02665568	FKBP5	−0.0118	0.37	1	0.0034	0	1
cg18726036	FKBP5	0.0157	1.71	1	−0.0348	0.38	1

References

Almén MS, Nilsson EK, Jacobsson JA, Kalnina I, Klovins J, Fredriksson R, Schiöth HB. Genome-wide analysis reveals DNA methylation markers that vary with both age and obesity. Gene. 2014; 548:61–67. [PubMed: 25010727]

Bell RL, Kimpel MW, McClintick JN, Strother WN, Carr LG, Liang T, Rodd ZA, Mayfield RD, Edenberg HJ, McBride WJ. Gene expression changes in the nucleus accumbens of alcohol-preferring rats following chronic ethanol consumption. Pharmacol. Biochem. Behav. 2009; 94:131–147. [PubMed: 19666046]

Brauers E, Vester U, Zerres K, Eggermann T. Search for mutations in SLC1A5 (19q13) in cystinuria patients. J. Inherited Metab. Dis. 2005; 28:1169–1171. [PubMed: 16435221]

Breitling LP, Salzmann K, Rothenbacher D, Burwinkel B, Brenner H. Smoking, F2RL3 methylation, and prognosis in stable coronary heart disease. Eur. Heart J. 2012; 33:2841–2848. [PubMed: 22511653]

Burgess DJ, Fu SS, van Ryn M. Potential Unintended Consequences of Tobacco-Control Policies on Mothers Who Smoke: A Review of the Literature. Am. J. Prev. Med. 2009; 37:S151–S158. [PubMed: 19591755]

Caraballo RS, Giovino GA, Pechacek TF. Self-reported cigarette smoking vs. serum cotinine among U.S. adolescents. Nicotine Tobacco Res. 2004; 6:19–25.

Caraballo RS, Giovino GA, Pechacek TF, Mowery PD. Factors associated with discrepancies between self-reports on cigarette smoking and measured serum cotinine levels among persons aged 17 years or older: Third National Health and Nutrition Examination Survey, 1988–1994. Am. J. Epidemiol. 2001; 153:807–814. [PubMed: 11296155]

Corbett C, Armstrong MJ, Neuberger J. Tobacco Smoking and Solid Organ Transplantation. Transplantation. 2012; 94:979–987. 910.1097/TP.1090b1013e318263ad318265b. [PubMed: 23169222]

Daban C, Vieta E, Mackin P, Young AH. Hypothalamic-pituitary-adrenal Axis and Bipolar Disorder. Psychiatr. Clin. North Am. 2005; 28:469–480. [PubMed: 15826743]

de Leon J, Diaz FJ. A meta-analysis of worldwide studies demonstrates an association between schizophrenia and tobacco smoking behaviors. Schizophr. Res. 2005; 76:135–157. [PubMed: 15949648]

Dogan MV, Shields B, Cutrona C, Gao L, Gibbons FX, Simons R, Monick M, Brody G, Tan K, Philibert R. The effect of smoking on DNA methylation of peripheral blood mononuclear cells from African American women. BMC Genomics. 2014; 15:151. [PubMed: 24559495]

Erim Y, Böttcher M, Dahmen U, Beck O, Broelsch CE, Helander A. Urinary ethyl glucuronide testing detects alcohol consumption in alcoholic liver disease patients awaiting liver transplantation. Liver Transpl. 2007; 13:757–761. [PubMed: 17457868]

Florescu A, Ferrence R, Einarson T, Selby P, Soldin O, Koren G. Methods for Quantification of Exposure to Cigarette Smoking and Environmental Tobacco Smoke: Focus on Developmental Toxicology. Ther. Drug Monit. 2009; 31:14–30. [PubMed: 19125149]

Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan J-B, Gao Y, Deconde R, Chen M, Rajapakse I, Friend S, Ideker T, Zhang K. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. Mol. Cell. 2013; 49:359–367. [PubMed: 23177740]

Huang M-C, Schwandt ML, Chester JA, Kirchhoff AM, Kao C-F, Liang T, Tapocik JD, Ramchandani VA, George DT, Hodgkinson CA, Goldman D, Heilig M. FKBP5 Moderates Alcohol Withdrawal Severity: Human Genetic Association and Functional Validation in Knockout Mice. Neuropsychopharmacology. 2014; 39:2029–2038. [PubMed: 24603855]

Joubert BR, Håberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, Huang Z, Hoyo C, Midttun Ø, Cupul-Uicab LA, Ueland PM, Wu MC, Nystad W, Bell DA, Peddada SD, London SJ. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ. Health Perspect. 2012; 120:1425–1431. [PubMed: 22851337]

Kandel DB, Schaffran C, Griesler PC, Hu M-C, Davies M, Benowitz N. Salivary Cotinine Concentration Versus Self-Reported Cigarette Smoking: Three Patterns of Inconsistency in Adolescence. Nicotine Tobacco Res. 2006; 8:525–537.

Kessler RC, Crum RM, Warner LA, Nelson CB, Schulenberg J, Anthony JC. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the National Comorbidity Survey. Arch. Gen. Psychiatry. 1997; 54:313–321. [PubMed: 9107147]

Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TWW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat. Neurosci. 2013; 16:33–41. [PubMed: 23201972]

Monick MM, Beach SR, Plume J, Sears R, Gerrard M, Brody GH, Philibert RA. Coordinated changes in AHRR methylation in lymphoblasts and pulmonary macrophages from smokers. Am. J. Med. Genet. B Neuropsychiatr. Genet. 2012; 159B:141–151. [PubMed: 22232023]

Non AL, Binder AM, Barault L, Rancourt RC, Kubzansky LD, Michels KB. DNA methylation of stress-related genes and LINE-1 repetitive elements across the healthy human placenta. Placenta. 2012; 33:183–187. [PubMed: 22222044]

Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics. 2008; 3:97–106. [PubMed: 18536531]

Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 2008; 31:464–468. [PubMed: 18675469]

Perroud N, Paoloni-Giacobino A, Prada P, Olie E, Salzmann A, Nicastro R, Guillaume S, Mouthon D, Stouder C, Dieben K, Huguelet P, Courtet P, Malafosse A. Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. Transl Psychiatry. 2011; 1:e59. [PubMed: 22832351]

Philibert R, Hollenbeck N, Andersen E, Osborn T, Gerrard M, Gibbons R, Wang K. A Quantitative Epigenetic Approach for the Assessment of Cigarette Consumption. Front. Psychol. 2015; 6:656. [PubMed: 26082730]

Philibert R, Penaluna B, White T, Shires S, Gunter TD, Liesveld J, Erwin C, Hollenbeck N, Osborn T. A pilot examination of the genome-wide DNA methylation signatures of subjects entering and exiting short-term alcohol dependence treatment programs. Epigenetics. 2014; 9:1–7. [PubMed: 24739672]

Russell TV, Crawford MA, Woodby LL. Measurements for active cigarette smoke exposure in prevalence and cessation studies: Why simply asking pregnant women isn't enough. Nicotine Tobacco Res. 2004; 6:S141–S151.

Swendsen J, Conway KP, Degenhardt L, Glantz M, Jin R, Merikangas KR, Sampson N, Kessler RC. Mental disorders as risk factors for substance use, abuse and dependence: results from the 10-year follow-up of the National Comorbidity Survey. Addiction. 2010; 105:1117–1128. [PubMed: 20331554]

Tavakoli HR, Hull M, Okasinski LM. Review of current clinical biomarkers for the detection of alcohol dependence. Innov. Clin. Neurosci. 2011; 8:26.

Team, RC. R: A language and environment for statistical computing, R Foundation for Statistical Computing. Vienna, Austria: 2012.

Toperoff G, Aran D, Kark JD, Rosenberg M, Dubnikov T, Nissan B, Wainstein J, Friedlander Y, Levy-Lahad E, Glaser B, Hellman A. Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. Hum. Mol. Genet. 2012; 21:371–383. [PubMed: 21994764]

Webb DA, Boyd NR, Messina D, Windsor RA. The discrepancy between self-reported smoking status and urine continine levels among women enrolled in prenatal care at four publicly funded clinical sites. J. Public Health Manag. Pract. 2003; 9:322–325. [PubMed: 12836515]

Whitford JL, Widner SC, Mellick D, Elkins RL. Self-Report of Drinking Compared to Objective Markers of Alcohol Consumption. The American Journal of Drug and Alcohol Abuse. 2009; 35:55–58. [PubMed: 19322728]

Zeilinger S, Kühnel B, Klopp N, Baurecht H, Kleinschmidt A, Gieger C, Weidinger S, Lattka E, Adamski J, Peters A, Strauch K, Waldenberger M, Illig T. Tobacco smoking leads to extensive genome-wide changes in DNA methylation. PLoS One. 2013; 8:e63812. [PubMed: 23691101]

Zhang Y, Yang R, Burwinkel B, Breitling LP, Holleczek B, Schöttker B, Brenner H. F2RL3 methylation in blood DNA is a strong predictor of mortality. Int. J. Epidemiol. 2014; 43:1215–1225. [PubMed: 24510982]

Highlights

- Objective DNA methylation biomarkers can quantify smoking and alcohol consumption.

- Substance use affects HPA axis DNA methylation.

- Influence of substance use is more prominent at FKBP5 than NR3C1.

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

Table 2

Summary of the linear regression parameters in the FACHS, Hannum and AlcMeth cohorts at FKBP5

	Smoking	Alcohol
FACHS		
Regression coefficient	0.058	0.145
Percent variation explained by model (%)	12.03	6.86
p-value	1.85E-06	3.81E-04
Hannum		
Regression coefficient	0.047	0.129
Percent variation explained by model (%)	2.30	3.76
p-value	9.54E-05	5.46E-07
AlcMeth		
Regression coefficient	0.057	0.294
Percent variation explained by model (%)	28.63	35.41
p-value	5.23E-06	2.15E-07

Table 3

Summary of the linear regression parameters in the FACHS, Hannum and AlcMeth cohorts at NR3C1

	Smoking	Alcohol
FACHS		
Regression coefficient	0.009	−0.017
Percent variation explained by model (%)	0.97	0.32
p-value	0.19	0.45
Hannum		
Regression coefficient	0.017	0.064
Percent variation explained by model (%)	0.52	1.68
p-value	0.06	8.76E-04
AlcMeth		
Regression coefficient	0.009	−0.001
Percent variation explained by model (%)	2.88	6.75E-06
p-value	0.18	0.98