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Impact of Long-Term Alcohol Consumption and Relapse on Genome-wide DNA Methylation Changes in Alcohol-Dependent Subjects: A Longitudinal Study

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Background: Genetic factors play an important role in the development and maintenance of alcohol use disorder (AUD). Significant and widespread differences in methylation levels of multiple regions within the genome have been reported between AUD patients and healthy controls in large epigenome-wide association studies (EWASs). Also, within patient populations, methylation changes over time (both during and after withdrawal) have been identified as sensitive indicators for disease activity. The detection of changes in methylation levels is a powerful tool to further explore and understand the biological correlates and underpinnings of AUD. Although there is strong and convincing evidence for differences in methylation of various sites between AUD patients and controls, only few studies assessed changes within patients over longer periods of time while taking into account alcohol consumption, relapse, and abstinence. So far, the longest period assessed as a within-subject design using EWASs was 4 weeks.

Methods: Here, we investigated changes in whole-genome methylation levels within a sample of 69 detoxified AUD patients over a period as long as 12 months for the first time, comparing patients that relapsed within the follow-up period to those that remained abstinent.

Results: Whole-genome methylation patterns of individual CpG sites over time did not differ between abstinent and relapsing patients. However, there was a negative association between global mean methylation at the 12-month follow-up and alcohol consumption within our sample.

Conclusion: Although the present study represents the largest study of methylation levels in a sample of AUD patients with a follow-up period of 1 year and accounting for alcohol consumption and relapse to date, the sample size might still not be large enough to detect genome-wide significant effects. Therefore, large-scale, long-term studies with AUD subjects are needed to determine the utility of DNA methylation for the assessment and monitoring of persons with alcohol use disorders.

Key Words: DNA Methylation, Alcohol Use Disorder, Longitudinal Design, Relapse, Epigenetics, Alcohol Consumption.

CHRONIC EXPOSURE TO alcohol has been associated with far-reaching consequences, including loss of quality of life years, negative effects on mental and physical health (Charlet and Heinz, 2017), and increased mortality (Lozano et al., 2012). Despite the considerable heritability of alcohol use disorder (AUD) proven by adoption and twin studies, there is still a limited number of genetic variants that have been identified in traditional genome-wide association studies (GWASs) as risk factors for AUD, with an estimated 40–60% of genetic factors contributing to the variance in susceptibility to AUD (Deak et al., 2019). In addition to genetic mechanisms, epigenetic regulation—a possible bridge between genetic and environmental factors—has been proposed as one mechanism of interest in neuroadaptation that contributes to the development and maintenance of AUD (Berkel and Pandey, 2017; Hagerty et al., 2016; Hamilton and Nestler, 2019). Epigenetics refer to molecular processes that alter gene expression without altering the deoxyribonucleic acid (DNA) sequence itself (Nestler, 2014). The most commonly accepted mechanisms coded as epigenetic

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mechanisms are DNA methylation, histone modifications, and noncoding RNAs. These mechanisms interact with chromatin, the protein complex that organizes DNA and thus can alter the extent to which genes are accessible to transcription factors. Here, we refer to epigenetic mechanisms after assessing DNA methylation as the most commonly studied epigenetic mechanism in addiction providing a possible bridge between genetic and environmental factors. However, when evaluating the current literature on AUD and methylation changes, we encounter a high inconsistency of published results and an extremely limited number of replications. This might be partly due to varying study designs, heterogeneous cohorts, and unstandardized methodology within this highly dynamic and developing research field (Harlaar and Hutchison, 2013; Zhang and Gelernter, 2017). Furthermore, differences in tissue types used to assess methylation levels might add up to the heterogeneity of results. Although there has been a recent effort in providing cross-tissue and cross-phenotypic approaches using postmortem brain cells, peripheral blood and buccal cells in AUD patients (Hagerty et al., 2016; Lohoff et al., 2018) the majority of studies stems from peripheral blood (Bruckmann et al., 2016; Koller et al., 2019; Liu et al., 2018; Philibert et al., 2018; Philibert et al., 2014a; Philibert et al., 2012) and saliva (Xu et al., 2019), which does not entirely reflect methylation changes in the brain (Rollins et al., 2010). Nevertheless, previous reports stress the value of analyzing peripheral tissue to explore molecular mechanisms and detect new genes, pathways and biomarkers for psychiatric diseases (Pajer et al., 2012; Pedroso et al., 2012).

A considerable number of studies have reported the cross-sectional difference in whole-genome methylation patterns between subjects consuming alcohol (heavy drinkers and AUD patients) and healthy controls (e.g., Bruckmann et al., 2016; Harlaar et al., 2014; Liu et al., 2018; Philibert et al., 2014a; Philibert et al., 2012; Xu et al., 2019; Zhao et al., 2013), with sample sizes ranging from 10/10 (patients/controls) (Zhao et al., 2013) up to n = 13,317 (2018)). The majority of studies revealed differential methylation in a number of genes that are involved in alcohol metabolism, stress immune response, and signal transduction with respect to alcohol consumption. However, replication of specific sites is scarce. One finding recently replicated is for CpG sites in the promoter region of *GDAP* (Bruckmann et al., 2016). Liu and colleagues (2018) assessed DNA methylation as a possible biomarker of alcohol consumption in 13 population-based cohorts (n_{total} = 13,317), identifying 144 CpGs associated with current heavy alcohol consumption. However, the latest study on whole-genome methylation patterns and alcohol consumption (Xu et al., 2019) reported only small effects of alcohol consumption on individual CpG sites, including 64 new CpG sites. Only 6 of the CpG sites previously reported to be associated with AUD, liver function, body mass index, and lipid metabolism could be replicated (n = 1,135).

Longitudinal studies of whole-genome methylation patterns with respect to alcohol consumption are extremely rare: So far, only one study of AUD patients (Bruckmann et al., 2016), 2 studies of healthy drinkers (Philibert et al., 2014a; Philibert et al., 2012), and one of healthy subjects developing AUD after 10 years (Weng et al., 2015) have been published with longitudinal data. Time between baseline and follow-up ranged from 21 days (Bruckmann et al., 2016) to 10 years (Weng et al., 2015), respectively. Philibert and colleagues (2012) assessed changes in methylation patterns in 165 healthy female subjects over a period of 6 months with respect to alcohol consumption. They reported severity-dependent changes in the degree of genome-wide methylation, with 2 regions reaching genome-wide significance. In 2 other studies from the same group (Philibert et al., 2018; Philibert et al., 2014a), changes in whole-genome methylation levels were reported for 66 subjects (33 cases with heavy alcohol consumption, 33 controls) that entered and exited a 30-day inpatient treatment program. At baseline, the case–control comparison revealed a total of 56 CpG sites reaching genome-wide significance. With respect to alcohol-dependent changes over time (max of 25 days), no single CpG site crossed the threshold of genome-wide significance. Interestingly, there was no significant overlap between the CpG sites reported for alcohol consumption and the CpG sites previously reported for smoking (Dogan et al., 2014; Philibert et al., 2014a) or for CpG sites associated with alcohol in healthy women (Philibert et al., 2012). The longest follow-up period was reported for a subgroup of 10 subjects (compared to 10 healthy controls) who developed AUD after 12 years (starting off as healthy participants) (Weng et al., 2015). The authors reported an association between changes in methylation levels of 6 genes and alcohol consumption for this group. However, taking into account that the authors did not correct for multiple testing, this finding should be interpreted with extreme caution until replicated. The most recent longitudinal design assessing whole-genome methylation changes at baseline and 3 weeks after completion of an inpatient alcohol treatment program was reported by Bruckmann and colleagues (2016). The authors performed an EWAS in 49 AUD patients and 47 healthy controls. They report significant differences between *GDAP1* DNA methylation levels in patients before and after alcohol treatment and there was a trend toward a negative association between the mean DNA methylation levels of 3 associated CpG sites and the years of alcohol dependency. *GDAP1* was previously reported as a significant finding in an EWAS of heavy drinkers by Philibert et al. (2014a).

With respect to AUD and alcohol consumption–associated cross-sectional global DNA methylation changes, Zhang and Gelernter (2017) provide an overview of 6 studies published until April 2016, again revealing the inconsistency of published results. Two studies reported an overall increase of methylation with alcohol consumption (Bonsch et al., 2004; Kim et al., 2016), one study an inverse relationship (Zhu et al., 2012), and 3 studies no association at all (Ono et al., 2012; Zhang et al., 2011). Since then, 5 more studies have been published (Bruckmann et al., 2016; Hagerty et al., 2016; Koller et al., 2019; Liu et al., 2018; Xu et al., 2019). Only one of these 4 studies (Koller et al., 2019) reported

changes in global methylation status between cases and controls (with an increase of methylation for AUD patients after withdrawal). The other studies (Bruckmann et al., 2016; Hagerty et al., 2016; Liu et al., 2018; Xu et al., 2019) refer to methylation in specific CpG sites without accounting for global methylation status.

Evaluating the current literature on epigenetic mechanisms in AUD, the urgent need for more longitudinal within-subject designs comparing the effect of alcohol consumption, relapse and abstinence becomes evident. Long-term follow-ups allow investigators to address changes in methylation beyond effects that can be attributed to the epigenetic property of alcohol itself (Zakhari, 2013) and changes that are detectable only during the short period of detoxification and withdrawal (Bruckmann et al., 2016). When comparing DNA methylation differences between AUD cases and controls, it is unclear whether epigenetic differences are already present before alcohol exposure (and should be considered as risk factors for AUD) or whether they are the consequence of chronic alcohol use.

We therefore investigated whole-genome methylation patterns as well as mean methylation levels in 69 well-characterized detoxified alcohol-dependent patients over a period of 1 year and assessed the influence of alcohol consumption, relapse, and abstinence on intraindividual changes in methylation patterns.

MATERIAL AND METHODS

Sample

We assessed 69 subjects suffering from AUD at baseline and after a 1-year follow-up, including blood analysis and detailed information on alcohol consumption. Data were collected as part of the LeAD study (Learning and Alcohol Dependence). The protocol has previously been reported in detail elsewhere (Garbusow et al., 2016; Nebe et al., 2018; Sebold et al., 2019). All patients fulfilled diagnostic criteria for AUD according to ICD-10 and DSM-IV-TR ("Diagnostic and Statistical Manual of Mental Disorders," 2000) for a minimum of 3 years. Patients with history of current or past substance use disorder (except alcohol and nicotine dependence), other major psychiatric disorder (as assessed with the computer-based Composite International Diagnostic Interview, CIDI (Wittchen, 1997)), or neurological disease were excluded. All subjects were free of psychotropic medication known to interact with the central nervous system for at least 4 half-lives (including illegal drugs and detoxification treatment tested by a drug urine). Study participation of the patients took place shortly after detoxification (3–21 days). The Alcohol Dependence Scale (Skinner, 1984), Obsessive Compulsive Drinking Scale (German version; Mann, 2000), and estimated lifetime alcohol consumption (in kg) were assessed for severity of alcohol use (Sobell, 1992). There was no difference between abstaining and relapsing patients in alcohol use severity at inclusion. However, relapsing patients entered the study with a higher number of inpatient detoxification treatments before inclusion ($p < 0.05$) (for a detailed sample description, see Table 1). Smoking status was assessed with the Fagerström Test for Cigarette Dependence (FTCD) (Fagerström, 2012). Within the 12-month period, one abstaining patient and 2 relapsing patients changed smoking status (from smoker to nonsmoker). There was no significant difference in percentage of smoking patients at either baseline or at the 12-month follow-up between abstaining and relapsing patients (Table 2).

Alcohol consumption

After study participation, AUD patients were regularly contacted over a period of 12 months (every 2 weeks during the first 3 months, every 6 weeks from month 3 onward, and every 12 weeks from month 6 onward) to assess alcohol consumption using the alcohol timeline follow-back method (Sobell, 1992). Patients were contacted either via the telephone (on weeks 6, 10, 18, and 36 after baseline) or in a personal assessment (which took place at baseline and on weeks 4, 8, 12, 24, and 48 after baseline). Relapse was defined as consumption of 60 or 40 grams of alcohol on any occasion for males and females, respectively. This definition was used according the WHO (WHO, 2000) criteria of current high-risk versus current low-risk consumption. Personal assessment included alcohol breath tests to validate self-reports. Breathalyzers were used by trained instructors only, who guided patients through the procedure and documented the respective results.

During the follow-up period, 16 patients discontinued participation in the study (15%). In 2 cases, we only had relapse reports from close relatives, which we accepted for classification. Altogether, 38 patients relapsed during the follow-up period, whereas 31 remained abstinent. Within the group of patients classified as abstainers according to WHO criteria, 27 patients reported zero alcohol consumption over the period of 12 months. Four subjects consumed an average of 34.88 (80.55) g on 0.66 (1.56) occasions (see Table 1). Demographic and clinical characteristics of this sample are shown in Tables 1 and 2. As a reliable biological marker for alcohol consumption, gamma-glutamyltransferase (U/l) showed a significant decrease in abstaining patients only.

Analysis

DNA methylation quantification and quality control. Genomic DNA was extracted from peripheral whole-blood samples at baseline and after 12 months from the same subjects. Bisulfite-converted DNA samples were used in the array-based DNAm assay, the Illumina Infinium Human MethylationEPIC BeadChip (Illumina, San Diego, CA) which interrogates DNAm at roughly 850K CpG sites. DNAm profiling was conducted at Hannover Medical Center. For sample distribution on plates and chips with respect to relapse and time point, see Fig. S1.

Raw Illumina EPIC methylation data were preprocessed and converted into beta values using the ChAMP pipeline at baseline and the 12-month follow-up. Subsequent quality control of the sample data included removal of probes with a detection p-value above 0.01. Additionally, probes with a bead count less than 3 and probes with SNPs were removed (Zhou et al., 2017).

Only probes present in both datasets after quality control were included in further analyses, resulting in datasets for the baseline and 12-month follow-up with probes of 740,391 CpG sites of 69 samples. Quality control for batch effects performing singular value decomposition method (SVD) revealed a significant batch effect between the plates, chips, and sample well (see Fig. S2). Batch correction was performed using ComBat as implemented in the sva package (Leek et al., 2012) without the use of a moderating variable after applying probe type normalization with the BMIQ method. A new surrogate variable analysis (SVA) plot showed a considerable reduction of the batch effects after ComBat (see Fig. S2). All subsequent analyses were validated with the untransformed and uncorrected raw beta values to reduce the possibility of introducing false positive results with ComBat and a simulation of the given factor structure regarding the effects of ComBat was carried out in order to rule out a considerable distortion of the test statistics[1].

[1]To further ensure the validity of our sample, we were able to replicate the frequently reported effect of smoking on cg05575921 of the AHRR gene within our sample (see Supplementary Material A).

Table 1. Sample Characteristics

	Abstainer (N = 31; 6 female)				Relapser (N = 38; 4 female)			
	Baseline (BL)		Follow-up (FU12)[a]		Baseline (BL)		Follow-up (FU12)[a]	
	n	*M (SD)*	*n*	*M* (SD)	*n*	*M* (SD)	*n*	*M* (SD)
Smoking (in %)	31	77% (n = 24)	31	81% (n = 25)	38	79% (n = 30)	38	74% (n = 28)
Age (in years)	31	48.51 (11.55)	31	49.46 (11.78)	38	47.24 (9.57)	34	48.21 (9.73)
ADS[b] score	31	13.45 (6.76)	26	10.27 (7.16)	38	14.71 (6.30)	37	11.06 (7.29)
Drinking per occasion past year (in g)	31	173.61 (97.05)	29	34.88 (80.55)	38	212.45 (94.29)	34	163.40 (123.83)
Drinking frequency past year	31	4.29 (1.30)	29	0.66 (1.56)	38	4.66 (0.75)	34	3.79 (1.63)
Drinking per day past year (in g)	31	135.24 (96.09)	29	15.77 (50.15)	38	186.45 (111.53)	34	113.44 (116.55)
Lifetime alcohol intake in kg (pure alcohol)	31	2,081.64 (1416.79)			38	1,973.02 (981.66)		
γ-GT[c] U/l	26	181.92 (273.22)	28	42.09 (33.61)	31	77.05 (68.23)	30	84.54 (115.26)
GOT[d] U/l	26	42.08 (20.13)	28	25.42 (7.66)	31	35.65 (29.94)	30	41.36 (39.91)
GPT[e] U/L	26	59.23 (44.53)	28	27.23 (18.30)	31	34.50 (22.65)	30	42.14 (45.23)
OCDS[f]	31	10.28 (8.60)	31	2.75 (3.31)	36	10.61 (7.21)	38	8.66 (6.76)
Outpatient detoxification	31	26% (n = 8)			38	26% (n = 10)		
Inpatient detoxification	31	87% (n = 27)			38	95% (n = 36)		
Time to relapse (in days)			31				38	111.76 (82.33)

[a]After 12 months.
[b]Alcohol Dependence Scale.
[c]Gamma-glutamyltransferase.
[d]Glutamate-oxaloacetate transaminase.
[e]Glutamate-pyruvate transaminase.
[f]Obsessive Compulsive Drinking Scale.

Table 2. Descriptive Statistics for Group Comparisons at Baseline (BL–BL), at Follow-Up (FU12–FU12), and for Changes Over Time Within Each Group Separately (BL–FU12)

	BL–BL (Abstainer–Relapser)		FU12–FU12 (Abstainer–Relapser)		BL–FU12 (Abstainer)		BL–FU12 (Relapser)	
	t (df)	*p*	*t* (df)	*p*	*t* (df)	*p*	*t* (df)	*p*
Smoking (in %)		1[a]		1[a]		1[a]		1[a]
Age (in years)	0.49 (58.20)	0.63	0.45 (54.40)	0.65				
ADS[b] score	−0.79 (62.24)	0.43	−0.42 (54.44)	0.68	1.71 (52.10)	0.09	2.26 (65.69)	0.03
Drinking per occasion past year (in g)	−1.67 (63.47)	0.10	−4.95 (57.26)	<0.0001	6.04 (57.21)	<0.0001	1.87 (61.39)	0.07
Drinking frequency past year	−1.40 (45.68)	0.17	−7.79 (60.11)	<0.0001	9.76 (54.52)	<0.s	2.84 (45.11)	
0.007			Drinking per day past year (in g)	−2.05 (66.78)	0.05	−4.43 (46.31)	<0.0001	
6.09 (45.85)	<0.0001	2.71	(68.32)	0.009				
Lifetime alcohol intake in kg (pure alcohol)	0.36 (51.67)	0.72						
γ-GT[c] U/l	1.91 (27.62)	0.07	−1.93 (34.22)	0.06	2.59 (25.70)	0.02	−0.31 (46.80)	0.76
GOT[d] U/l	0.96 (52.69)	0.34	−2.15 (31.28)	0.04	3.96 (31.64)	<0.001	−0.63 (53.77)	0.53
GPT[e] U/l	2.57 (35.63)	0.01	−1.67 (38.79)	0.10	3.41 (32.72)	0.002	−0.83 (42.37)	0.41
OCDS[f]	−0.17 (58.86)	0.87	−4.74 (56.00)	<0.0001	4.55 (38.71)	<0.0001	1.20 (70.99)	0.23
Outpatient detoxification	0.64 (11.30)	0.53						
Inpatient detoxification	−2.15 (53.03)	0.04						

[a]Fisher's exact test.
[b]Alcohol Dependence Scale.
[c]Gamma-glutamyltransferase.
[d]Glutamate-oxaloacetate transaminase.
[e]Glutamate-pyruvate transaminase.
[f]Obsessive Compulsive Drinking Scale.

Statistical analysis. The statistical analyses were conducted within the R (v3.6.1) environment and the Bioconductor (v3.9) framework. We performed exploratory genome-wide methylation analysis to identify relevant changes in methylation within our patients over a period of 12 months with respect to drinking behavior and relapse.

First, we checked for differences in overall methylation with a repeated-measures ANOVA from baseline to the 12-month follow-up for relapse versus nonrelapse (at the 12-month follow-up). Additionally, the Spearman rank correlation between alcohol consumption in grams (g) per past year and the mean methylation at the 12-month follow-up was conducted.

Second, we performed a series of *t*-tests for the differences in methylation with respect to relapse/nonrelapse at the 12-month follow-up for every probe (DMP) with a false discovery rate (FDR) of < 5%. In addition to the DMP comparison, an analysis for the identification of differentially methylated regions (DMRs) was carried out with the Bumphunter algorithm implemented in the

ChAMP package (Tian et al., 2017) on the beta value differences in the 12-month follow-up period. An ingenuity pathway analysis was planned for genes with sites significant after considering the FDR. To rule out the effect of alcohol consumption within the sample of abstaining patients (4 patients continued the consumption of alcohol on a low level with an average of 34.88 (80.55) g on 0.66 (1.56) occasions; see Table 1), all t-tests were reanalyzed using a criterion of zero alcohol consumption (for a detailed description, see Supplementary material B).

In an additional approach, we tried to identify relevant CpGs and their corresponding genes in 2 steps: In the first step, we performed a partial correlation of lifetime kg alcohol consumption, age and whole-genome methylation at baseline. The top 1,000 CpG sites correlated with lifetime alcohol intake were then advanced to the second step of the analysis. In this second step, we calculated the difference in methylation between baseline and 1-year follow-up of the previously identified top 1,000 CpG sites and correlated this difference score with g alcohol intake for the 12-month time period between the methylation measurements. FDR correction for the second step was applied to 1,000 calculations to reduce the probability of false negative results. Third, we calculated a series of Spearman's rank correlation coefficients for all probes with the average daily total drinking volume and change in liver enzymes (GPT/GOT) over the observation period. Correlations with a false discovery rate (FDR) of < 5% were defined as significant.

RESULTS

The repeated-measures ANOVA did not show any significant effects on mean methylation level: Neither the effect of time, $F(1, 134) = 2.36$, $p = 0.13$, nor the group effect between the relapse and nonrelapse groups, $F(1, 134) = 0.01$, $p = 0.94$ was significant. There was a nonsignificant but trendwise interaction between time and group, $F(1, 134) = 3.24$, $p = 0.07$), pointing toward a decrease in mean methylation difference for relapsers compared to abstainers.

The Spearman rank correlation between mean methylation at the 12-month follow-up and alcohol consumption (over a period of 12 months) revealed a significant, moderate, and negative ($r_s = -0.30$, $p = 0.01$) association. This again provided evidence for decreasing methylation with increasing alcohol consumption.

A Wilcoxon signed-rank test indicated no difference in kg lifetime alcohol intake at baseline in relation between abstinent (median = 1,806.555) and relapsing (median = 2,017.337), W = 586, $p = 0.98$ patients. Correlation of the top 1,000 CpGs (with highest correlation between lifetime alcohol consumption and baseline methylation (T0)) between difference in methylation (T0–T1) and g alcohol intake (for the 12-month follow-up period) revealed no significant results. The highest negative Pearson product–moment correlation between the methylation-difference score T0–T1 and alcohol consumption during 12-month follow-up was not FDR significant and small, $r(67.00) = -0.21$, 95% CI [−0.43, 0.02], $p = 0.08$, FDR $p = 1.00$) for cg22544563 (SLC35F1). The highest positive Pearson product–moment correlation between the methylation-difference score T0–T1 and alcohol consumption during 12-month follow-up was not significant and small, $r(67.00) = 0.28$, 95% CI [0.05, 0.48], $p < 0.05$, FDR $p = 1.00$) for cg03452160 (RBFOX3). For all correlations of the 1,000 CpGs, see Table S1. The conducted series of *t*-tests to identify differentially methylated probes revealed that none of the 740,391 tested CpG probes exceeded the significance level of FDR < 5% (Fig. 1). The analysis for identification of DMRs also showed no significant result. The range of methylation changes within our cohort was relatively small ($M = -0.008997147$, median = −0.009088333, SD = 0.0389543; for a detailed description, see Table S2). Since no significant CpG probes could be identified, the planned pathway analysis was subsequently not carried out. Reanalyses of *t*-tests for the differences in methylation with respect to relapse/nonrelapse at the 12-month follow-up with a 0/ >0 criterion of alcohol consumption did not lead to more significant results, but the *p*-values tend to increase (for a detailed description, see Supplementary material B and Table S3).

There were no correlations exceeding the significance level of FDR < 5% between the difference in methylation rate for every probe between T1 and T0 and the average daily alcohol consumption during the follow-up period and change in liver enzymes. Subsequent analyses with the untransformed and uncorrected raw beta values yielded validation of the reported results.

DISCUSSION

This is the first study to date investigating the long-term effects of abstinence and alcohol consumption on patients diagnosed with AUD according to DSM-IV-TR. We did not find an association of methylation patterns when comparing individuals with long-term alcohol consumption to those of abstinent individuals suffering from AUD. A trendwise interaction between relapse and time for mean whole-genome methylation and a negative correlation of mean whole-genome methylation with alcohol consumption at the 1-year follow-up could be detected.

To date, only 2 studies with a maximum follow-up period of 30 days systematically investigated the effects of an alcohol treatment program on the epigenome (Bruckmann et al., 2016; Philibert et al., 2014a). In the first study (Philibert et al., 2014a) comparing the methylation levels of heavy drinkers at the beginning of the alcohol treatment and after 4 weeks of treatment, no genes reached epigenome-wide significance. However, 56 CpGs reached epigenome-wide significance when comparing AUD patients to controls at inclusion. The second study (Bruckmann et al., 2016) was able to validate one of the findings (*GDAP1)* as an indicator for disease severity and treatment outcome after a 21-day alcohol treatment program. The authors report 48 differentially methylated CpG sites at $p < 0.1$ and with a DNA methylation difference of> 5%. Findings in *GDAP1* were validated by pyrosequencing. Interestingly, the exclusion of 8 patients who had been abstinent for more than 3 days before hospital admission enhanced the observed effect of differential *GDAP1* methylation between control individuals and

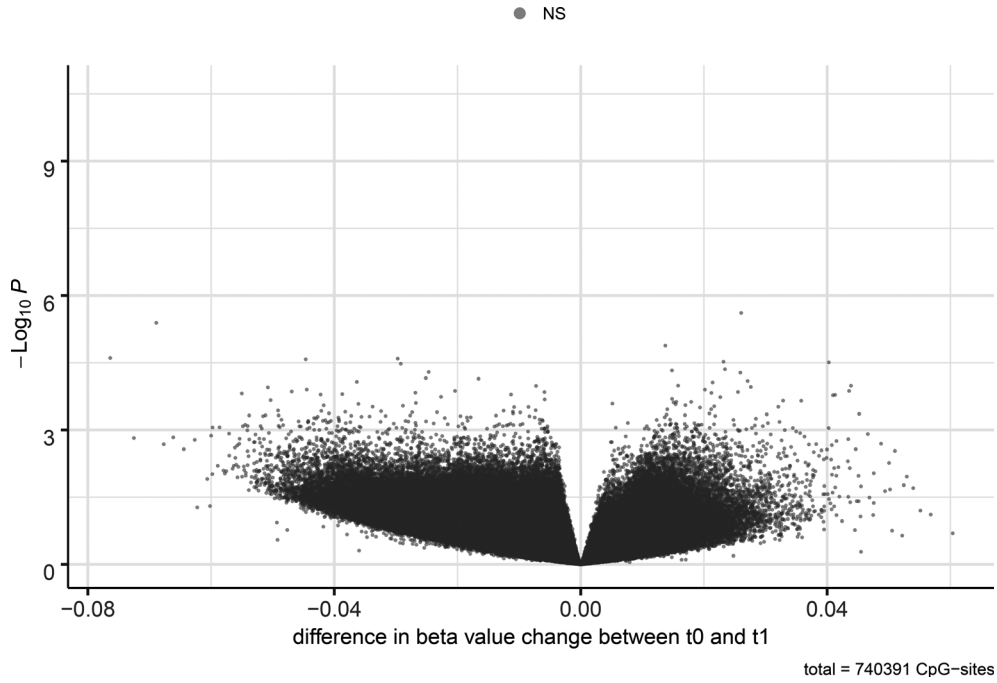

Fig. 1. Volcano plot for the group differences regarding relapse and abstain with -log10 *p*-value on the y-axis and the difference in beta value change between the 2 time points (t0 = baseline; t1 = 12-month follow-up) on the *x*-axis. The volcano plot enables visual identification of CpG sites with large changes between t0 and t1 that are also statistically significant. More specifically, it enables quick visual identification of sites with large beta value change and their respective significance. This subsequently can help to identify the most biologically significant sites. Here, no significant changes in beta value occur.

patients at baseline. Time to last drink and time to relapse until methylation analysis might have been too long to detect the previously described short-term effect of alcohol consumption in AUD patients in our sample. Relevant epigenetic mechanisms have been identified during the period of withdrawal (Biermann et al., 2009; Bruckmann et al., 2016; Hillemacher et al., 2009); however, within our sample, important changes might have already occurred before inclusion. All patients had been abstinent from alcohol for at least 30 days until first relapse (Tables 1 and 2), and all patients had a minimum of 3 years of AUD according to DSM-IV criteria before inclusion. Homogeneity in sense of disease severity within this sample might explain the overall relatively small range of methylation changes comparing abstaining and relapsing patients after the 1-year follow-up. More extreme group comparisons with respect to duration of abstinence versus duration and amount of alcohol consumed might be necessary to detect differences using within-sample designs.

We did find a trendwise increase of mean global methylation levels in abstinent compared to relapsing patients, which is in line with the most recently published results (Koller et al., 2019) but inconsistent with previously reported increase of methylation levels in AUD cases compared to controls (Bonsch et al., 2004; Kim et al., 2016). There was a negative correlation between mean methylation at the 1-year follow-up and alcohol consumption, accordingly. However, a stepwise correlation taking into account age and lifetime alcohol consumption between the methylation-difference score of both time points and 12-month alcohol intake did not yield significant results.

DNA methylation studies of AUD, especially those on whole-genome methylation patterns, are still at an early stage (Zhang and Gelernter, 2017). Most studies have been published in the past 5 years, with the vast majority reporting differences between healthy control individuals and AUD patients (Hagerty et al., 2016; Liu et al., 2018; Xu et al., 2019). There has been great enthusiasm with recent findings in large case–control cohorts (Hagerty et al., 2016; Liu et al., 2018; Xu et al., 2019); however, we need to develop a more detailed picture of the biological processes involved in the detected changes of methylation patterns. Most of what we currently know about epigenetic processes and addiction stems from animal studies, which enable the experimental manipulation of important factors such as the type, extent, and timing of substance exposure (Bekdash et al., 2013; Finegersh and Homanics, 2014a; Finegersh and Homanics, 2014b; Wieting et al., 2019). Besides the epigenetic impact of alcohol itself, (dys)functional learning processes (Garbusow et al., 2014; Sebold et al., 2017), social status, early (stressful) life experience (Sebold et al., 2019b), tolerance toward alcohol exposure, and the high comorbidity with other substance use disorders make this research in human subjects far more challenging. As in many other studies exploring AUD, nicotine dependence has not been excluded (Beck et al., 2012; Garbusow et al., 2016; Garbusow et al., 2018; Schad et al., 2019; Sebold et al., 2019a; Sebold et al., 2017; Sekutowicz et al., 2019; Weinberger et al., 2016). And even though

nicotine dependence is among the most common comorbidities in AUD (Weinberger et al., 2016) results are hence limited in their generalizability to nonsmoking AUD patients. Another limitation of generalizability and replicability might be caused by the usage of a nonzero alcohol consumption as relapse criterion, which here was defined according to the WHO criterion of low- versus high-risk consumption (WHO, 2000). With respect to abstinence rate, in our sample this was relatively high with 45%. Using zero alcohol consumption as abstinence criterion, abstinence rates decrease to 39%. However, results of supplementary analysis using the zero criterion did not substantially differ from our results and a significant decrease in liver enzymes in abstaining patients only might serve as one indicator of the validity of the above reported relapse criterion. This is in line with previously reported designs on AUD using the same criterion (e.g., Beck et al., 2012; Charlet et al., 2014; Garbusow et al., 2016; Garbusow et al., 2018; Schad et al., 2019; Sebold et al., 2017; Sekutowicz et al., 2019; Witkiewitz et al., 2017; Witkiewitz et al., 2020).

When using cross-sectional designs, it remains unclear whether differences between AUD cases and controls were already present before alcohol exposure (and should be considered as risk factors for AUD) or whether they are the consequence of chronic alcohol use. Another well-known limitation of studies assessing epigenetic changes in humans is the tissue analyzed. Tissues commonly accessible and thus of potential benefit for daily clinical use in AUD patients are blood and saliva (as opposed to, e.g., tumor tissue in cancer research), whereas brain tissue (of various regions within the brain) would be the tissue clearly stronger associated with addictive behavior (Hagerty et al., 2016; Lohoff et al., 2018). Some studies have shown that brain cellular heterogeneity may bias DNA methylation patterns (Guintivano et al., 2013). We hence understand peripheral mechanisms of alcohol consumption rather than exploring its neural mechanisms, except to the extent that peripheral measures reflect central activity.

Methodological differences between studies make it extremely difficult to replicate findings and to disentangle false positives from valid differences between groups. Out of 3 studies that assessed methylome-wide changes using within-subject designs (Bruckmann et al., 2016; Philibert et al., 2014a; Weng et al., 2015), one study reported a correlation of methylation changes with alcohol consumption but without accounting for the effect of multiple comparisons (for approximately 28,000 CpG site comparisons in 20 subjects (Weng et al., 2015)). Therefore, validation of results with an alternative method and replication in larger samples is urgently needed. Also, the publication of negative findings must also be encouraged to get a better idea of the average effect sizes. Unfortunately, due to publication bias, small and nonsignificant effects are either often not submitted for publication or have a higher probability of being denied for publication by reviewers or editors (Bakker et al., 2012; John et al., 2012; Schafer and Schwarz, 2019), making it even more difficult to replicate findings.

Although the present study represents the largest study of methylation levels in a sample of AUD patients with a follow-up period of 1 year and accounting for alcohol consumption and relapse to date, the sample size might still not be large enough to detect genome-wide significant effects. Therefore, large-scale, long-term studies on AUD subjects are needed to determine the utility of DNA methylation for the assessment and monitoring of persons with AUDs. These future results might help to identify reliable biomarkers that remit as a function of abstinence and allow to explore the biological correlates and underpinnings of AUD.

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CONFLICT OF INTEREST

All authors have no competing interests of financial or other nature.

AUTHORS' CONTRIBUTIONS

AH, HW, UZ, EF, and IV were responsible for recruitment of alcohol-dependent patients. TZ and EF were responsible for further statistical analyses with support of HF, HW, IV, and UZ. EF and TZ drafted the manuscript. HF, HW, IV, UZ, and AH provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved the final version for publication.

ETHICS APPROVAL

Ethical approval for the study was obtained from the ethics committee of Charité-Universitätsmedizin Berlin (EA1/157/11) and Universitätsklinikum Dresden (EK228072012). Participants received a monetary compensation of 10 /hour for study participation.

DATA AVAILABILITY STATEMENT

This manuscript contains previously unpublished data. The name of the repository and accession number(s) are not available due to patient confidentiality and participant privacy. Methylation array data can simultaneously identify individuals and convey protected health information (2014b). Patients did not provide written informed consent on the publication of individual methylation profiles. Requests to access the datasets should be directed to Tristan Zindler (Zindler.Tristan@mh-hannover.de).

REFERENCES

Bakker M, van Dijk A, Wicherts JM (2012) The rules of the game called psychological science. Perspect Psychol Sci 7:543–554.

Beck A, Wustenberg T, Genauck A, Wrase J, Schlagenhauf F, Smolka MN, Mann K, Heinz A (2012) Effect of brain structure, brain function, and brain connectivity on relapse in alcohol-dependent patients. Arch Gen Psychiatry 69:842–852.

Bekdash RA, Zhang C, Sarkar DK (2013) Gestational choline supplementation normalized fetal alcohol-induced alterations in histone modifications, DNA methylation, and proopiomelanocortin (POMC) gene expression in beta-endorphin-producing POMC neurons of the hypothalamus. Alcohol Clin Exp Res 37:1133–1142.

Berkel TD, Pandey SC (2017) Emerging role of epigenetic mechanisms in alcohol addiction. Alcohol Clin Exp Res 41:666–680.

Biermann T, Reulbach U, Lenz B, Frieling H, Muschler M, Hillemacher T, Kornhuber J, Bleich S (2009) N-methyl-D-aspartate 2b receptor subtype (NR2B) promoter methylation in patients during alcohol withdrawal. J Neural Transm 116:615–622.

Bonsch D, Lenz B, Reulbach U, Kornhuber J, Bleich S (2004) Homocysteine associated genomic DNA hypermethylation in patients with chronic alcoholism. J Neural Transm (Vienna) 111:1611–1616.

Bruckmann C, di Santo A, Karle KN, Batra A, Nieratschker V (2016) Validation of differential GDAP1 DNA methylation in alcohol dependence and its potential function as a biomarker for disease severity and therapy outcome. Epigenetics 11:456–463.

Charlet K, Beck A, Jorde A, Wimmer L, Vollstadt-Klein S, Gallinat J, Walter H, Kiefer F, Heinz A (2014) Increased neural activity during high working memory load predicts low relapse risk in alcohol dependence. Addict Biol 19:402–414.

Charlet K, Heinz A (2017) Harm reduction-a systematic review on effects of alcohol reduction on physical and mental symptoms. Addiction Biology 22:1119–1159.

Deak JD, Miller AP, Gizer IR (2019) Genetics of alcohol use disorder: a review. Curr Opin Psychol 27:56–61.

Dogan MV, Shields B, Cutrona C, Gao L, Gibbons FX, Simons R, Monick M, Brody GH, Tan K, Beach SR, Philibert RA (2014) The effect of smoking on DNA methylation of peripheral blood mononuclear cells from African American women. BMC Genom 15:151.

Fagerström K (2012) Determinants of tobacco use and renaming the FTND to the Fagerström Test for Cigarette Dependence. Nicotine Tob Res 14:75–78.

Finegersh A, Homanics GE (2014a) Acute ethanol alters multiple histone modifications at model gene promoters in the cerebral cortex. Alcohol Clin Exp Res 38:1865–1873.

Finegersh A, Homanics GE (2014b) Paternal alcohol exposure reduces alcohol drinking and increases behavioral sensitivity to alcohol selectively in male offspring. PLoS One 9:e99078.

Garbusow M, Schad DJ, Sebold M, Friedel E, Bernhardt N, Koch SP, Steinacher B, Kathmann N, Geurts DE, Sommer C, Muller DK, Nebe S, Paul S, Wittchen HU, Zimmermann US, Walter H, Smolka MN, Sterzer P, Rapp MA, Huys QJ, Schlagenhauf F, Heinz A (2016) Pavlovian-to-instrumental transfer effects in the nucleus accumbens relate to relapse in alcohol dependence. Addict Biol 21:719–731.

Garbusow M, Sebold M, Beck A, Heinz A (2014) Too difficult to stop: mechanisms facilitating relapse in alcohol dependence. Neuropsychobiology 70:103–110.

Garbusow M, Sommer C, Nebe S, Sebold M, Kuitunen-Paul S, Wittchen HU, Smolka MN, Zimmermann US, Rapp MA, Huys QJM, Schlagenhauf F, Heinz A (2018) Multi-level evidence of general Pavlovian-to-instrumental transfer in alcohol use disorder. Alcohol Clin Exp Res 42:128a.

Guintivano J, Aryee MJ, Kaminsky ZA (2013) A cell epigenotype specific model for the correction of brain cellular heterogeneity bias and its application to age, brain region and major depression. Epigenetics 8:290–302.

Hagerty SL, Bidwell LC, Harlaar N, Hutchison KE (2016) An exploratory association study of alcohol use disorder and DNA methylation. Alcohol Clin Exp Res 40:1633–1640.

Hamilton PJ, Nestler EJ (2019) Epigenetics and addiction. Curr Opin Neurobiol 59:128–136.

Harlaar N, Bryan AD, Thayer RE, Karoly HC, Oien N, Hutchison KE (2014) Methylation of a CpG site near the ALDH1A2 gene is associated with loss of control over drinking and related phenotypes. Alcohol Clin Exp Res 38:713–721.

Harlaar N, Hutchison KE (2013) Alcohol and the methylome: design and analysis considerations for research using human samples. Drug Alcohol Depend 133:305–316.

Hillemacher T, Frieling H, Luber K, Yazici A, Muschler MAN, Lenz B, Wilhelm J, Kornhuber J, Bleich S (2009) Epigenetic regulation and gene expression of vasopressin and atrial natriuretic peptide in alcohol withdrawal. Psychoneuroendocrinology 34:555–560.

John LK, Loewenstein G, Prelec D (2012) Measuring the prevalence of questionable research practices with incentives for truth telling. Psychol Sci 23:524–532.

Kim DS, Kim YH, Lee WK, Na YK, Hong HS (2016) Effect of alcohol consumption on peripheral blood Alu methylation in Korean men. Biomarkers 21:243–248.

Koller G, Zill P, Soyka M, Adorjan K, Weiss C, Kern A, Nguyen-Thien ML, Kamp F, Proebstl L, Krause D, Ruhdorfer S, Bergmann W, Preuss UW (2019) Short-term changes in global methylation and hydroxymethylation during alcohol detoxification. Eur Neuropsychopharmacol 29:897–903.

Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD (2012) The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 28:882–883.

Liu C, Marioni RE, Hedman AK, Pfeiffer L, Tsai P-C, Reynolds LM, Just AC, Duan Q, Boer CG, Tanaka T, Elks CE, Aslibekyan S, Brody JA, Kühnel B, Herder C, Almli LM, Zhi D, Wang Y, Huan T, Yao C, Mendelson MM, Joehanes R, Liang L, Love S-A, Guan W, Shah S, McRae AF, Kretschmer A, Prokisch H, Strauch K, Peters A, Visscher PM, Wray NR, Guo X, Wiggins KL, Smith AK, Binder EB, Ressler KJ, Irvin MR, Absher DM, Hernandez D, Ferrucci L, Bandinelli S, Lohman K, Ding J, Trevisi L, Gustafsson S, Sandling JH, Stolk L, Uitterlinden AG, Yet I, Castillo-Fernandez JE, Spector TD, Schwartz JD, Vokonas P, Lind L, Li Y, Fornage M, Arnett DK, Wareham NJ, Sotoodehnia N, Ong KK, van Meurs JBJ, Conneely KN, Baccarelli AA, Deary IJ, Bell JT, North KE, Liu Y, Waldenberger M, London SJ, Ingelsson E, Levy D (2018) A DNA methylation biomarker of alcohol consumption. Mol Psychiatry 23:422–433.

Lohoff FW, Sorcher JL, Rosen AD, Mauro KL, Fanelli RR, Momenan R, Hodgkinson CA, Vendruscolo LF, Koob GF, Schwandt M, George DT, Jones IS, Holmes A, Zhou Z, Xu MJ, Gao B, Sun H, Phillips MJ, Muench C, Kaminsky ZA (2018) Methylomic profiling and replication implicates deregulation of PCSK9 in alcohol use disorder. Mol Psychiatry 23:1900–1910.

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Abdulhak AB, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch

M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FGR, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Memish ZA, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KMV, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh P-H, Yip P, Zabetian A, Zheng Z-J, Lopez AD, Murray CJL (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2095–2128.

Nebe S, et al.(2018) No association of goal-directed and habitual control with alcohol consumption in young adults. Addiction Biology 23 (1):379–393.

Nestler EJ(2014) Epigenetic mechanisms of drug addiction. Neuropharmacology 76 (Pt B):259–268.

Mann K & Ackermann K(2000) Die OCDS-G: Psychometrische Kennwerte der deutschen Version der Obsessive Compulsive Drinking Scale. Such 46:90–100.

Ono H, Iwasaki M, Kuchiba A, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Ohnami S, Sakamoto H, Yoshida T, Tsugane S (2012) Association of dietary and genetic factors related to one-carbon metabolism with global methylation level of leukocyte DNA. Cancer Sci 103:2159–2164.

Pajer K, Andrus BM, Gardner W, Lourie A, Strange B, Campo J, Bridge J, Blizinsky K, Dennis K, Vedell P, Churchill GA, Redei EE (2012) Discovery of blood transcriptomic markers for depression in animal models and pilot validation in subjects with early-onset major depression. Transl Psychiatry 2:e101.

Pedroso I, Lourdusamy A, Rietschel M, Nothen MM, Cichon S, McGuffin P, Al-Chalabi A, Barnes MR, Breen G (2012) Common genetic variants and gene-expression changes associated with bipolar disorder are overrepresented in brain signaling pathway genes. Biol Psychiatry 72:311–317.

Philibert R, Dogan M, Noel A, Miller S, Krukow B, Papworth E, Cowley J, Knudsen A, Beach SRH, Black D (2018) Genome-wide and digital polymerase chain reaction epigenetic assessments of alcohol consumption. Am J Med Genet B Neuropsychiatr Genet 177:479–488.

Philibert RA, Penaluna B, White T, Shires S, Gunter T, Liesveld J, Erwin C, Hollenbeck N, Osborn T (2014a) A pilot examination of the genome-wide DNA methylation signatures of subjects entering and exiting short-term alcohol dependence treatment programs. Epigenetics 9:1212–1219.

Philibert RA, Plume JM, Gibbons FX, Brody GH, Beach SR (2012) The impact of recent alcohol use on genome wide DNA methylation signatures. Front Genet 3:54.

Philibert RA, Terry N, Erwin C, Philibert WJ, Beach SR, Brody GH (2014b) Methylation array data can simultaneously identify individuals and convey protected health information: an unrecognized ethical concern. Clin Epigenetics 6:28.

Rollins B, Martin MV, Morgan L, Vawter MP (2010) Analysis of whole genome biomarker expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet 153B:919–936.

Schad DJ, Garbusow M, Friedel E, Sommer C, Sebold M, Hagele C, Bernhardt N, Nebe S, Kuitunen-Paul S, Liu SY, Eichmann U, Beck A, Wittchen HU, Walter H, Sterzer P, Zimmermann US, Smolka MN, Schlagenhauf F, Huys QJM, Heinz A, Rapp MA (2019) Neural correlates of instrumental responding in the context of alcohol-related cues index disorder severity and relapse risk. Eur Arch Psychiatry Clin Neurosci 269:295–308.

Schafer T, Schwarz MA (2019) The meaningfulness of effect sizes in psychological research: differences between sub-disciplines and the impact of potential biases. Front Psychol 10:813.

Sebold M, Garbusow M, Jetzschmann P, Schad DJ, Nebe S, Schlagenhauf F, Heinz A, Rapp M, Romanczuk-Seiferth N (2019a) Reward and avoidance learning in the context of aversive environments and possible implications for depressive symptoms. Psychopharmacology 236:2437–2449.

Sebold M, Nebe S, Garbusow M, Guggenmos M, Schad DJ, Beck A, Kuitunen-Paul S, Sommer C, Frank R, Neu P, Zimmermann US, Rapp MA, Smolka MN, Huys QJM, Schlagenhauf F, Heinz A (2017) When habits are dangerous: alcohol expectancies and habitual decision making predict relapse in alcohol dependence. Biol Psychiatry 82:847–856.

Sebold M, Spitta G, Gleich T, Dembler-Stamm T, Butler O, Zacharias K, Aydin S, Garbusow M, Rapp M, Schubert F, Buchert R, Gallinat J, Heinz A (2019b) Stressful life events are associated with striatal dopamine receptor availability in alcohol dependence. J Neural Transmis 126:1127–1134.

Sekutowicz M, Guggenmos M, Kuitunen-Paul S, Garbusow M, Sebold M, Pelz P, Priller J, Wittchen HU, Smolka MN, Zimmermann US, Heinz A, Sterzer P, Schmack K (2019) Neural response patterns during Pavlovian-to-instrumental transfer predict alcohol relapse and young adult drinking. Biol Psychiatry 86:857–863.

Skinner HA, Allen BA (1984) Alcohol dependence syndrome: Measurement and validation. Journal of Abnormal Psychology 91:199–209.

Sobell LC, Sobell MB (1992) Timeline Follow-back: A technique for assessing self–reported ethanol consumption. In J Allen, RZ Litten (Eds.), Measuring Alcohol Consumption: Psychosocial and Biological Methods (pp. 41–72). Totowa, NJ: Humana Press.

Tian Y, Morris TJ, Webster AP, Yang Z, Beck S, Feber A, Teschendorff AE (2017) ChAMP: updated methylation analysis pipeline for Illumina BeadChips. Bioinformatics 33:3982–3984.

Weinberger AH, Funk AP, Goodwin RD (2016) A review of epidemiologic research on smoking behavior among persons with alcohol and illicit substance use disorders. Prev Med 92:148–159.

Weng JT, Wu LS, Lee CS, Hsu PW, Cheng AT (2015) Integrative epigenetic profiling analysis identifies DNA methylation changes associated with chronic alcohol consumption. Comput Biol Med 64:299–306.

WHO. 2000. International guide for MONITORING alcohol consumption and related harm. [Online]. World Health Organisation, Geneva, Switzerland. Available at: https://apps.who.int/iris/bitstream/handle/10665/66529/WHO_MSD_MSB_00.4.pdf;sequence=1 [Accessed].

Wieting J, Rhein M, Hillemacher T, Lichtinghagen R, Hoppe V, Muschen L, Glahn A, Frieling H, Bleich S, Muschler M (2019) DNA methylation of the leptin gene promoter is altered by chronic alcohol exposure in an animal model for alcohol dependence. Eur Addict Res 25:49–55.

Witkiewitz K, Hallgren KA, Kranzler HR, Mann KF, Hasin DS, Falk DE, Litten RZ, O'Malley SS, Anton RF (2017) Clinical validation of reduced alcohol consumption after treatment for alcohol dependence using the World Health Organization risk drinking levels. Alcohol Clin Exp Res 41:179–186.

Witkiewitz K, Heather N, Falk DE, Litten RZ, Hasin DS, Kranzler HR, Mann KF, O'Malley SS, Anton RF (2020) World Health Organization risk drinking level reductions are associated with improved functioning and are sustained among patients with mild, moderate, and severe alcohol dependence in clinical trials in the United States and United Kingdom. Addiction (Abingdon, England). https://doi.org/10.1111/add.15011.

Wittchen H–U, Pfister H (1997) DIA–X–Interviews: Manual Für Screening–Verfahren Und Interview; Interviewheft Längsschnittuntersuchung (DIA–X–Lifetime); Ergänzungsheft (DIA–XLifetime); Interviewheft Querschnittuntersuchung (DIA–X–12 Monate); Ergänzungsheft (DIA–X–12 Monate); PC–Programm Zur Durchführung Des Interviews (Längs– Und Querschnittuntersuchung); Auswer–tungsprogramm (pp. 41–72). Frankfurt, Germany: Swets.

Xu K, Montalvo-Ortiz JL, Zhang X, Southwick SM, Krystal JH, Pietrzak RH, Gelernter J (2019) Epigenome-wide DNA methylation association analysis identified novel loci in peripheral cells for alcohol consumption among European American Male Veterans. Alcohol Clin Exp Res 43:2111–2121.

Zakhari S (2013) Alcohol metabolism and epigenetics changes. Alcohol Res 35:6–16.

Zhang FF, Cardarelli R, Carroll J, Fulda KG, Kaur M, Gonzalez K, Vishwanatha JK, Santella RM, Morabia A (2011) Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood. Epigenetics 6:623–629.

Zhang H, Gelernter J (2017) Review: DNA methylation and alcohol use disorders: progress and challenges. Am J Addict 26:502–515.

Zhao R, Zhang R, Li W, Liao Y, Tang J, Miao Q, Hao W (2013) Genome-wide DNA methylation patterns in discordant sib pairs with alcohol dependence. Asia Pac Psychiatry 5:39–50.

Zhou W, Laird PW, Shen H (2017) Comprehensive characterization, annotation and innovative use of Infinium DNA methylation BeadChip probes. Nucleic Acids Res 45:e22.

Zhu ZZ, Hou L, Bollati V, Tarantini L, Marinelli B, Cantone L, Yang AS, Vokonas P, Lissowska J, Fustinoni S, Pesatori AC, Bonzini M, Apostoli P, Costa G, Bertazzi PA, Chow WH, Schwartz J, Baccarelli A (2012) Predictors of global methylation levels in blood DNA of healthy subjects: a combined analysis. Int J Epidemiol 41:126–139.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Upper panel: Single value decomposition analysis (SVD) using RAW values. Lower panel: Single value decomposition analysis (SVD) using COMBAT corrected values.

Fig. S2. Distribution of all samples (relapser and abstainer) on chips and plates for t0 = baseline and t1 = 12-month follow up.

Table S1. Correlation of the top 1,000 cpgs (with highest correlation between lifetime alcohol consumption and baseline methylation(T0)) between difference in methylation (T0-T1) and g alcohol intake (for the 12 month follow up period)

Table S2. *T*-tests for the differences in methylation with respect to relapse/non-relapse at the 12-month follow-up.

Table S3. Re-anaylses of *t*-tests for the differences in methylation with respect to relapse/non-relapse at the 12-month follow-up with a 0 / >0 criterion of alcohol consumption.

Data S1. Supplementary materials.

Data S2. Figure legends.