

## **An Epigenome Wide Association Study on Alcohol Consumption: A Monozygotic Twin Study**

Yun Zhu<sup>1</sup>, Eric D. Strachan<sup>2</sup>, Emily Fowler<sup>2</sup>, Tamara J. Bacus<sup>2</sup>, Xinguang Chen<sup>1</sup>, Peter Roy-Byrne<sup>2</sup>, Jinying Zhao<sup>1</sup>

<sup>1</sup>Department of Epidemiology, College of Public Health and Health Professions and School of Medicine, University of Florida

<sup>2</sup>Department of Psychiatry and Behavioral Sciences, School of Medicine, University of Washington

**Background:** Alcohol consumption has been associated with many adverse health outcomes, but the biological mechanisms of alcohol use in human diseases are not fully understood. A wealth of data suggests that epigenetic mechanisms, such as DNA methylation and resulting gene expression, may be implicated in alcohol-induced diseases. However, most previous studies have focused on DNA methylation in a few candidate genes, and the functional importance of altered DNA methylation in relation to gene expression has not been investigated at a genome scale. Moreover, potential confounding by genes and cellular heterogeneity were not controlled in most previous studies.

**Objective:** To identify genes or genomic regions that are responsive to alcohol consumption, and to evaluate the impact of alcohol-induced DNA methylation on gene expression by an epigenomewide association studies (EWAS) in a well matched monozygotic twin sample.

**Methods:** The current study includes 47 monozygotic (MZ) twin pairs (35 female pairs, 12 male pairs, all Caucasian, aged 18 years and above) participating in the Mood and Methylation Study (MMS), an ongoing observational study of major depression and DNA methylation using a MZ twin design. Alcohol consumption was assessed using the Alcohol Use Disorder Identification Test (AUDIT, total score ranges from 0 to 40). Genome-wide DNA methylation level was quantified by the Illumina Infinium HumanMethylation450 BeadChip using genomic DNA isolated from peripheral blood monocytes. Gene expression was assessed using mRNA isolated from monocytes of same twins. Generalized estimate equation was used to test the association of each CpG probe with alcohol consumption (three domains including hazardous alcohol use, dependence symptoms, and harmful alcohol use, as well as overall AUDIT score), adjusting for sex, twin age, smoking and body mass index (BMI). Differentially methylated regions (DMRs) were identified by combining nearby correlated probes using the program comb-p. To evaluate the impact of DNA methylation on gene expression, we calculated partial correlation coefficients (adjusting for age, sex, smoking and BMI) between levels of DNA methylation and gene expression for each gene across all participants. Multiple testing was controlled by false discovery rate.

Functional annotation of putative genes was performed using GO enrichment analysis. Co-methylation modules/networks were also constructed using the Weighted Gene Correlation Network Analysis.

**Results:** Of all 94 twins, 21 had an AUDIT score  $\geq 8$ . The median AUDIT score is 4 (IQR 1-6). A total of 392 CpG probes, clustered into 13 DMRs, were significantly associated with AUDIT score. These regions included genes known to be related to alcohol drinking, such as *GABBR1*, *GABARAP*, *GBX2*, *TDRD1*, *UNK*, *POLR3G* and *ZNF195*. Of note, the *GABBR1* gene was associated with all three domains of the AUDIT ( $p < 2.1 \times 10^{-4}$ ). In addition, we found 173 *cis*- and 288 *trans*-regulating genes associated with alcohol consumption. The DMR genes are nearly 9 times more likely to show differential expression ( $P < 5.2 \times 10^{-4}$ ). Moreover, the identified alcohol-related DMR genes were enriched in GO terms such as alcohol oxidase activity ( $p < 3.8 \times 10^{-6}$ ), fatty alcohol metabolic process ( $p < 2.4 \times 10^{-3}$ ), and one-carbon metabolic process ( $p < 1.9 \times 10^{-3}$ ). Differential connectivity patterns in the co-methylation network were also observed for genes involved in these three GO terms.

**Conclusions:** Alcohol consumption alters DNA methylation and gene expression, independent of genes and other potential confounding factors. Our findings provide novel insight into biological mechanism involved in alcohol use, and may also unravel novel epigenetic pathways through which alcohol drinking contributes to alcohol-related diseases.