Human alcohol brain methylation and biomarker discovery project

Shaunna L Clark[1], Robin F Chan[1], Andrey A Shabalin[1], Lin Ying Xie[1], Min Zhao[1], Gerard van Grootheest[2], Patrik K.E. Magnusson, Christina M. Hultman, William E. Copeland, E. Jane Costello, Brenda W Penninx[2], Karolina A Aberg[1], Edwin JCG van den Oord[1]

[1] Center for Biomarker Research and Precision Medicine, Virginia Commonwealth University, Richmond, VA, USA; [2] Department of Psychiatry, VU University Medical Center / GGZ inGeest, Amsterdam, the Netherlands; [3] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; [4] Department of Psychiatry, Duke University Medical Center, Durham, North Carolina, USA

Although the adverse effects of alcohol are known, the molecular events that underlie these effects remain unclear. Mounting evidence suggests a role for DNA methylation. Because methylation sites can be measured cost-effectively in easy to collect genomic DNA, they are potentially powerful biomarkers that can be used in clinical settings to improve diagnosis, prognosis and monitor response to treatment. The goal of this project is to perform a comprehensive study of the human alcohol brain methylome and develop methylation biomarkers, called methylation risk scores (MRS), that track alcohol associated changes in brain.

One of the main challenges involves the complexity of the brain methylome which involves both methylation and hydroxymethylation. To address this challenge, we performed a comprehensive screen of both the methylome and hydroxymethylome in 50 post-mortem prefrontal cortex tissue samples from alcohol dependent cases and controls. Our top individual methylation and hydroxymentylation sites were located in *KLF12* ($p=4.5x10^{-08}$) and *SCAPER* ($p=5.7x10^{-08}$), respectively. We then used transcriptome sequencing data from the same samples to study functional consequences of associated methylation changes.

To create our blood-based MRS, we will utilize three existing large-scale blood methylation studies (combined N= ~3,300). First, we determined that there was significant overlapping alcohol associated methylation changes in the brain and the blood datasets ($p = 2.9x10^{-04}$). As the predictive power of biomarkers may be enhanced by combining multiple associated methylation sites, we created the MRSs using elastic net regression in combination with *k*-fold cross validation to protect against over fitting and ensure unbiased estimates of predictive power. Specifically, we used the alcohol associated methylation sites in brain, and performed our elastic net with k-fold cross-validation procedure on each of the blood datasets. Preliminary results indicate that our MRS of 25,000 methylation sites explains ~5.6% of the variation in drinking frequency.