Data Driven Discovery of Novel Genes for Alcohol Use Disorder and Neuroscience

S.P. Farris1, C.M. Borghese1, E.A Osterndorff-Kahanek1, Y.A. Blednov1, G.E. Homanics2, R.D. Mayfield1, and R.A. Harris1

1 Waggoner Center for Alcohol and Addiction Research The University of Texas at Austin, Austin, TX, 78712-1095, U.S.A. 2 University of Pittsburgh, Department of Anesthesiology, Pittsburgh, PA 15261

Introduction: The etiology of addiction to alcohol and others drugs of abuse is multifaceted, impacting a series of biological systems that are differentially regulated across individuals. Understanding the regulatory landscape of the human genome for specific diseases is essential for achieving precision medicine. Long non-coding RNAs (lncRNAs) represent the largest class of molecules transcribed from the human genome; however, fewer than 1% of lncRNAs have been biologically characterized. Identifying and testing candidate lncRNAs, in parallel with protein-coding transcripts, is important for understanding the underlying neurobiology of alcohol use disorder (AUD), related psychiatric conditions, and fundamental neurobiology.

Material and Methods: Postmortem human brain tissue from the New South Wales Tissue Resource Centre were used to unbiasedly profile the transcriptome from AUD and matched control subjects (n=400). Bioinformatics analyses of RNA-Seq data was used to determine individual genes and gene networks associated with AUD. More than 12,000 lncRNAs were correlated with disease status. To determine the potential conservation of these lncRNAs across species, 128 vertebrate full-length genomes were assessed for genetic loci sharing physical co-localization. Prioritized human transcripts, based on altered expression of molecular networks in AUD and syntenic conservation, were functionally screened with two-electrode voltage clamp recordings using *Xenopus laevis* oocytes as a model system.

Results: The lncRNA *LINC01559* was among the top differentially expressed genes ($p = 5.27e-04$), but to our knowledge has never been evaluated for molecular function. One gene expression module (GM29) was inversely associated with *LINC01559*, suggesting the expression and biological function of genes within GM29 may be inversely regulated by *LINC01559*. GM29 contained genes expressed within glutamatergic neurons ($p = 1.97e-03$), with significant number of genes known to affect related mouse phenotypes, such as 'abnormal NMDA-mediated synaptic currents' ($p = 8.34e-04$) and 'long-term depression' ($p = 1.61e-03$). *LINC01559* is physically located on chromosome 12 adjacent to glutamate ionotropic receptor NMDA type subunit 2B (*GRIN2B*). To test the hypothesis that *LINC01559* may affect regulation of glutamatergic function, oocytes were co-injected with *LINC01559* and the NMDAR1/NMDAR2B receptor complex. *LINC01559* caused a significant reduction (~80%) in maximal glutamate elicited currents. To test for potential non-specific effects of *LINC01559* on ion-channel expression and function we examined major non-glumatatergic inhibitory receptors GABAA ($\alpha1\beta2\gamma2$) or Glycine ($\alpha1$ GlyR). In contrast to NMDAR1/NMDAR2B, *LINC01559* did not alter maximal currents for non-glutamatergic inhibitory receptors

GABAA (α1β2γ2) or Glycine (α1 GlyR).

Discussion: Prior studies have shown an important role for the excitatory neurotransmitter glutamate in the pathophysiology underlying CNS function and behavior. Through an unbiased, data driven, approach our work demonstrates a conserved alcohol-responsive lncRNA, which selectively controls expression of the glutamatergic system. Using CRISPPR-Cas9 we have now created a mouse model to further interrogate the precise cellular circuits and behavioral systems impacted by this lncRNA. Overall, our research suggests lncRNAs are fundamentally important for shaping the regulatory landscape of molecular systems impacting substance abuse, and may represent an innovative paradigm for designing novel pharmacotherapies in addiction.