Submitter Name: Jun-Jie Zhang
Submitted email: zhang288@Indiana.edu
PI Name (if different): Howard Edenberg, Yunlong Liu
PI email (if different): edenberg@iu.edu, yunliu@iu.edu

High-throughput identification of functional variants in gene enhancers associated with alcohol use disorder

Jun-Jie Zhang, Andy Chen, Jill Reiter, Hongyu Gao, Howard Edenberg, and Yunlong Liu[1]

[1] Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

Genome-wide association studies (GWAS) are widely used in the discovery of loci associated with complex traits. To prioritize functional variants within loci, we modified the self-transcribing active regulatory region sequencing (STARR-seq) strategy to reveal which SNPs in the enhancer regions within loci are functional, measuring allele-specific expression in a highly parallel manner. Enhancer-associated SNPs were screened, testing both alleles in both directions in a STARR-seq vector electroporated into SH-SY5Y neuroblastoma cells, followed by deep sequencing. The impact of candidate variants on transcriptional activity was directly evaluated using a generalized linear model by comparing the differences in the allelic ratio between mRNA and plasmid DNA. Results showed that the input SNPs were recovered in the DNA extracted from transfected cells at an alternative/reference allele ratio between 0.4-0.6, implying little or no experimental bias in the assay. We analyzed 280 SNPs from the transcribed mRNA with >20 sequencing reads and with a balanced plasmid DNA allele ratio (0.4-0.6). Eighty-two enhancer-associated SNPs (~9% of total screened) showed significant differences in reporter gene expression between the reference and alternative alleles (FDR<0.05). The top-ranked SNPs (by FDR; homozygous with alternative allele frequency < 0.05 or > 0.95) were located in enhancers of several noncoding RNAs (*HCG17*, *HCG18* and *SNORD18A*) and a component of a transcription factor complex, *GTF2H4*. We have extended this approach to oligodendrocytes. We anticipate that results from these studies will contribute to a better understanding of the mechanisms underlying AUD as well as other neuropathological disorders.