

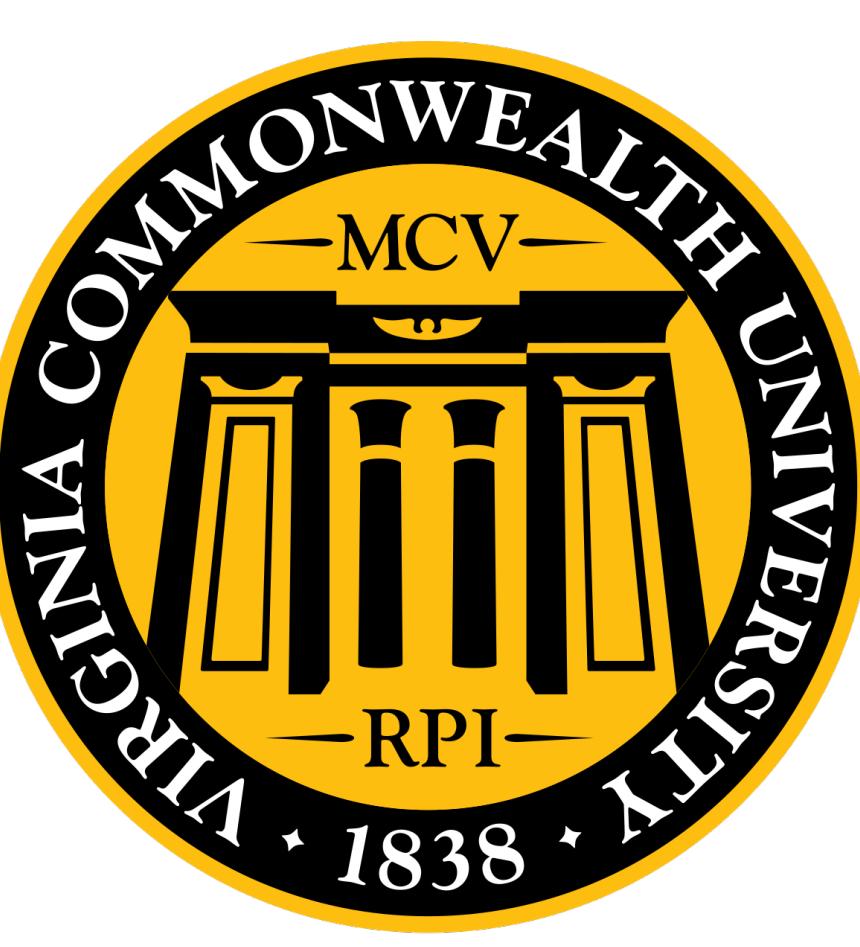
Genetic and transcriptomic analyses in the Diversity Outbred mouse identify *Car8* as a candidate gene for ethanol consumption

Zachary Tatom^{1,2}, Michael Miles^{1,2,3}

¹ Human and Molecular Genetics, Virginia Commonwealth University

² Alcohol Research Center, Virginia Commonwealth University

³ Pharmacology and Toxicology, Virginia Commonwealth University



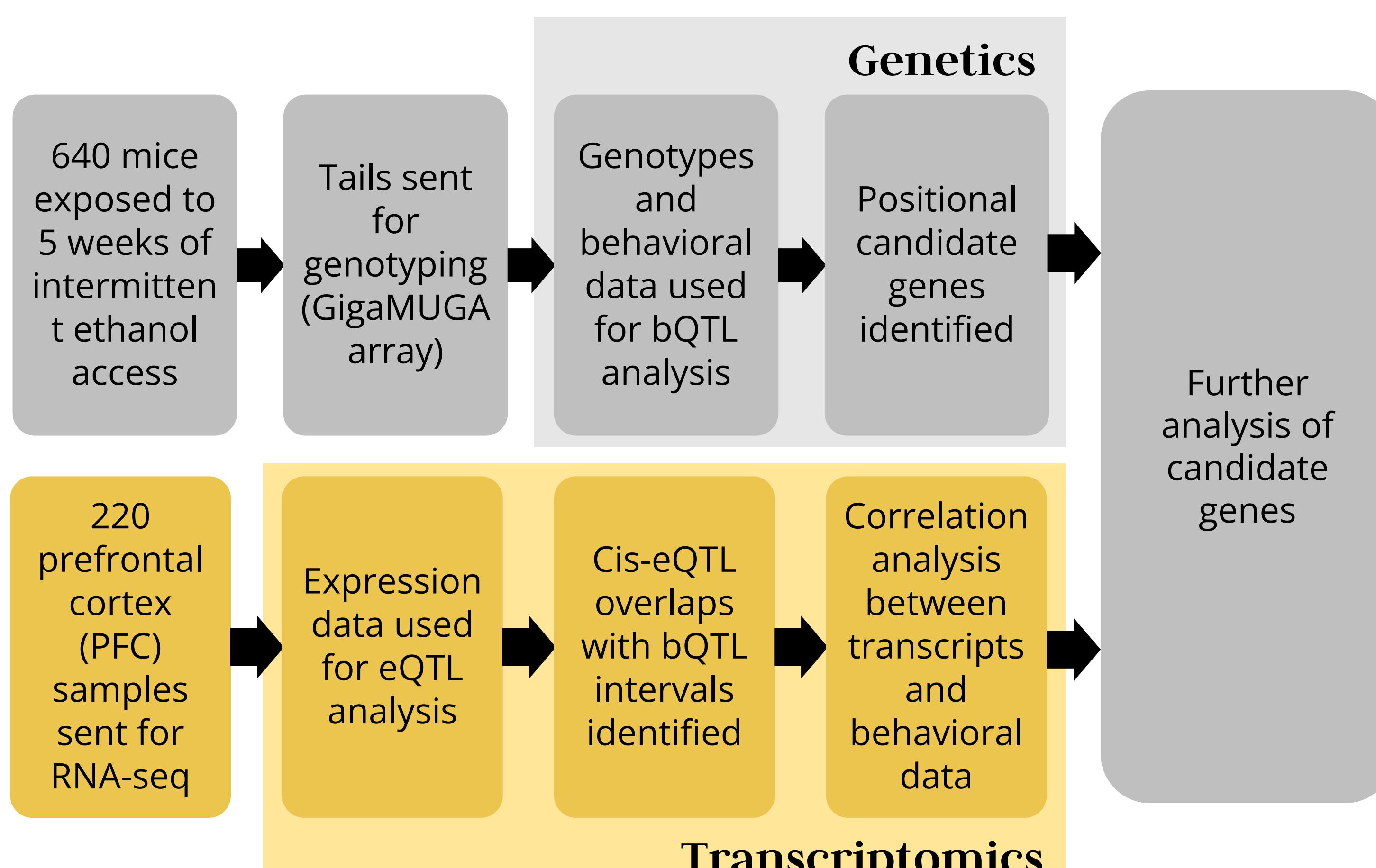
Abstract

636 male Diversity Outbred (DO) were exposed to 5 weeks of intermittent ethanol access (IEA) using a three-bottle choice paradigm. Ethanol consumption data were then used to identify 3 significant and 12 suggestive quantitative trait loci (QTL) for ethanol consumption behaviors. RNA-seq data were collected from prefrontal cortex (PFC) samples from 220 mice from this population. Here, we present findings from analyses of these transcriptomics data, including identification of Carbonic anhydrase 8 (*Car8*) as a positional candidate gene within a significant bQTL for ethanol consumption on chromosome 4 with a significant cis-expression QTL and a significant negative correlation with ethanol consumption. These findings suggest that *Car8* expression may play a key role in regulating progressive ethanol consumption.

Background

Alcohol use disorder (AUD), characterized by an inability to control drinking and a progressive increase in ethanol consumption over time, is extremely prevalent and poses a significant global healthcare burden. Genetic factors have been estimated to contribute to 50% of the risk for AUD, yet identification of causal genetic variants and their mechanistic roles remains a major challenge due to the complex genetic architecture underlying ethanol consumption. In addition to genetic polymorphisms leading to consequential functional changes in protein structure, an additional mechanism by which genetic variants are hypothesized to affect ethanol consumption is through regulation of gene expression. Due to the complex nature of AUD, an integrative approach combining systems genetics with transcriptomics data is necessary to make inferences capturing the full breadth of the genetic mechanisms involved in progressive ethanol consumption.

The DO mouse model was developed from 8 founder strains for the purpose of high-resolution genetic mapping. Our lab has previously collected ethanol consumption and genotypic data on 636 DO mice over the course of 4 weeks of intermittent ethanol access via three-bottle choice, identifying a highly variable distribution of progressive ethanol consumption. These data were used to identify behavioral quantitative trait loci (bQTL) and positional candidate genes within those loci. We hypothesize that integration of transcriptomics data from the same mice will allow us to identify candidate genes and gene networks related to ethanol consumption. Prefrontal cortex samples from 200 ethanol-drinking and 20 ethanol-naïve DO mice were sent for RNA-seq. Here, we analyze the resulting transcriptomic data to support positional candidate genes from bQTL analysis and identify additional candidate genes.



Phenotype	Chromosome	Peak LOD	Position (Mbp)	95% CI (Mbp)	Genes in CI	Genes in CI with cis-eQTL >5
Last Week 30% EtOH Choice (vs. 15% EtOH)	3	8.63	108.22	105.92-109.69	102	39
Last Week Mean EtOH Consumption	4	8.23	8.41	8.24-9.28	11	1
First Week EtOH Preference (vs. water)	12	7.52	79.35	78.09-79.91	19	8

Table 1. bQTL analysis of ethanol consumption in DO mice identifies 3 significant loci ($p < 0.05$). R/QTL2 was used to identify bQTLs and estimate 95% Bayesian confidence intervals. Diversity in this population enables very fine genetic mapping, with two significant QTLs < 2 Mbp in length. Cis-eQTLs which overlapped bQTL CIs were identified, with only a single gene, *Car8*, identified for the chromosome 4 bQTL for last week mean EtOH consumption.

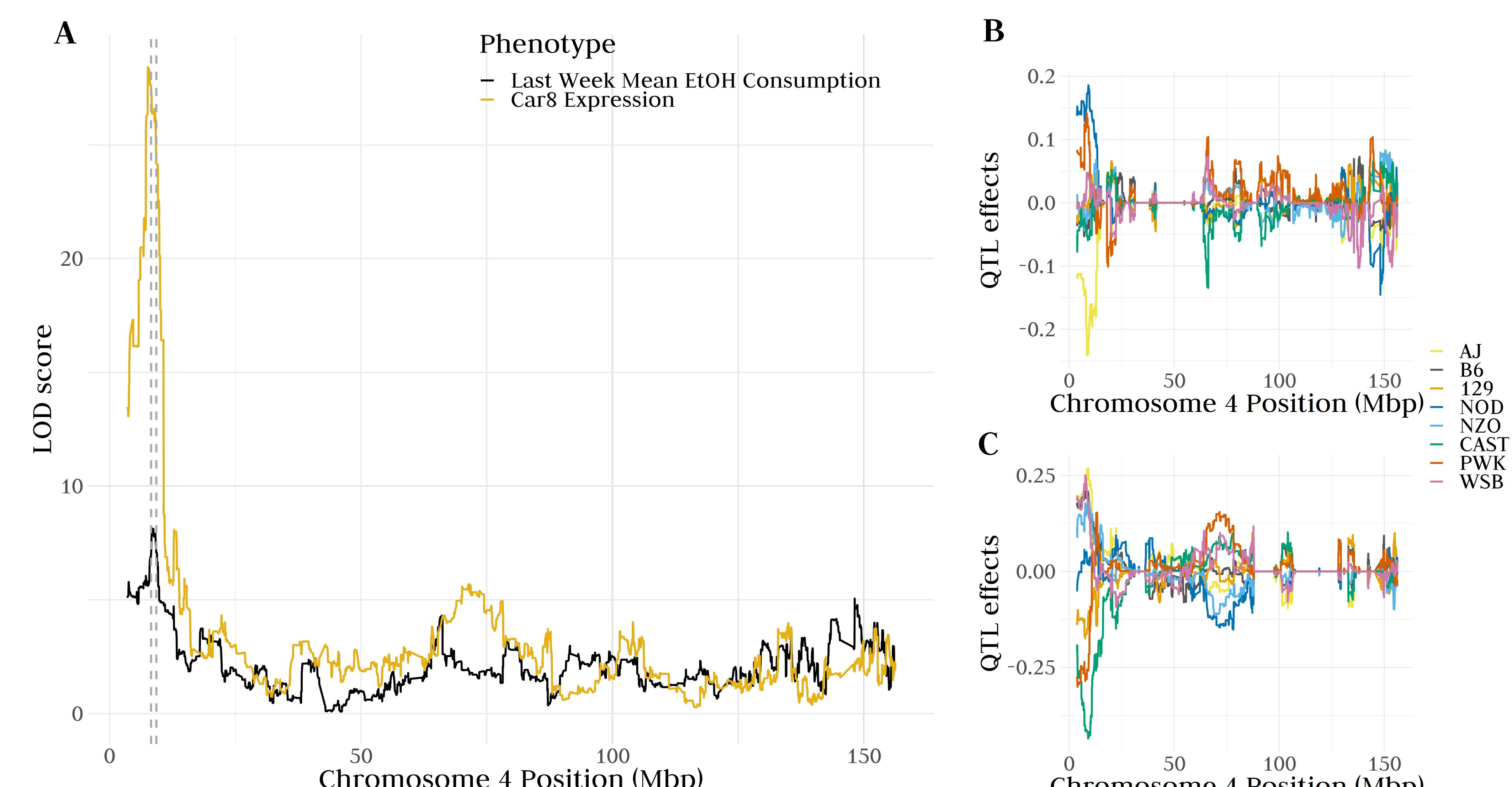


Figure 1. *Car8* eQTL overlaps with bQTL on chromosome 4 for last week mean EtOH consumption, and haplotype analysis suggests opposing roles of A/J and PWK alleles. A significant bQTL for last week mean EtOH consumption (yellow) and a cis-eQTL for *Car8* (black) were both identified on chromosome 4; dashed lines indicate 95% Bayesian CI (A). The Genetics package in R was used to estimate linkage disequilibrium between the peak bQTL marker (UNC6694526) and all markers within the *Car8* eQTL ($n = 79$); 72 of these markers were in strong LD with the peak bQTL marker ($D^* > 0.8$). Haplotype analysis was conducted using R/QTL2 to estimate contributions of founder strain alleles on both last week mean EtOH consumption (B) and *Car8* variant-stabilized transcript count (C). A/J alleles (yellow) appear to correspond with reduced last week mean EtOH consumption and increased *Car8* expression; PWK alleles (red) appear to have the opposite effect.

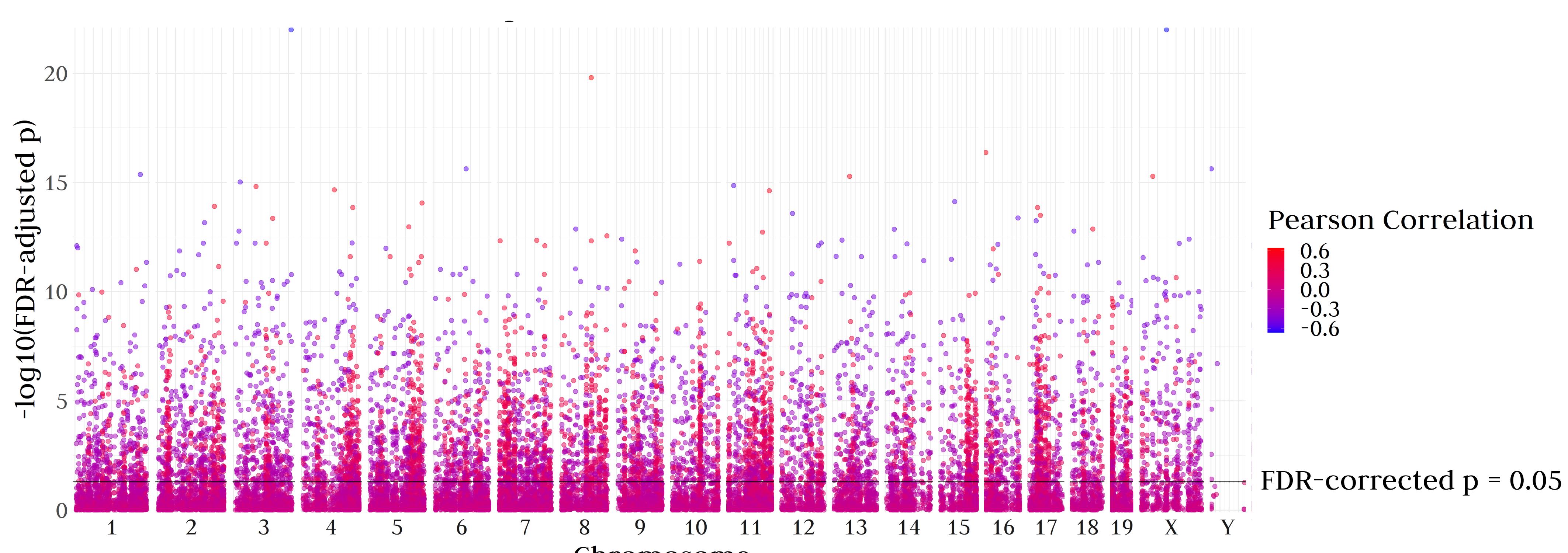


Figure 2. Correlation analysis with last week EtOH consumption identifies 7,453 significantly-correlated transcripts. Pearson correlations between variant-stabilized transcript count and EtOH consumption phenotypes were calculated. Benjamini-Hochberg false-discovery rate correction was used to identify significant transcripts ($p < 0.05$).

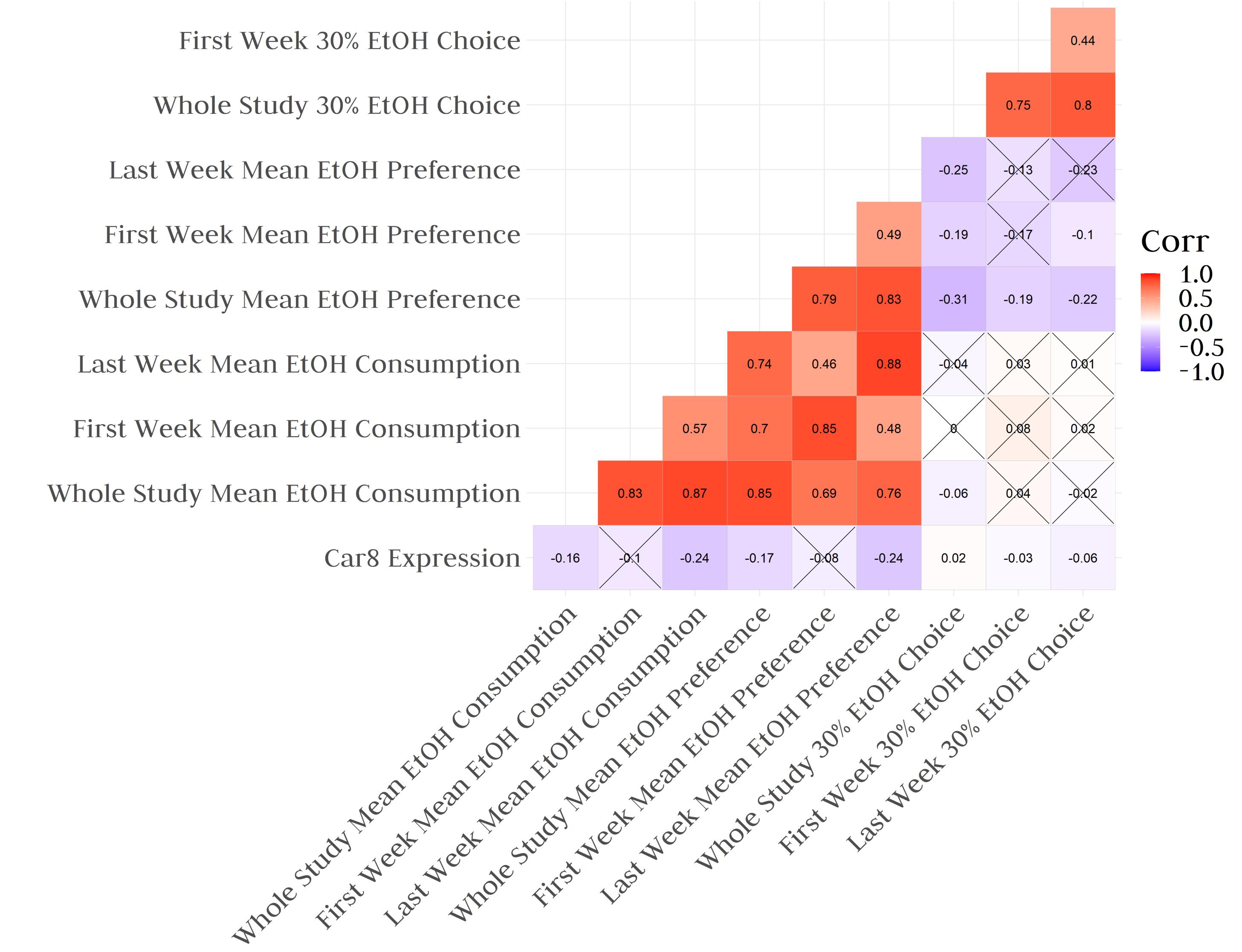


Figure 3. *Car8* variant-stabilized transcript count negatively correlates with EtOH consumption phenotypes. Pearson correlations between variant-stabilized transcript counts and EtOH consumption phenotypes were calculated; correlations which do not meet the $p < 0.05$ level of significance are denoted with a cross. *Car8* expression was significantly negatively correlated with most of these phenotypes, but most strongly with last week mean EtOH consumption (-0.24, $p = 1.74e-04$) and preference (-0.24, $p = 5.05e-05$).

Gene	Pearson Correlation	FDR p-value	Chromosome	Notes
Rpl10	-0.669	1.02e-22	X	
Zranb2	-0.667	1.02e-22	3	GWAS for AUDIT-P, AUD
Prkaca	0.642	1.52e-20	8	

Table 2. Last week mean EtOH consumption correlation analysis identifies additional candidate genes not located within bQTL CIs. Benjamini-Hochberg false-discovery rate correction was used to identify significant correlations.

Conclusions

- Overlapping bQTL for last week EtOH consumption and eQTL for *Car8* demonstrate that variants in the same genetic loci are responsible for variance in both phenotypes.
- Significant correlations between *Car8* expression in PFC and EtOH consumption and preference provide further evidence that *Car8* expression may play a role in modulating EtOH consumption in mice.
- Car8* expression in PFC was negatively correlated with EtOH consumption; planned studies include genetic modulation of *Car8* expression in PFC using viral vectors for overexpression and knockdown.
- Other genes, such as *Zranb2*, had stronger correlations with EtOH consumption and preference than bQTL positional candidates; continued analysis of transcriptomics data from DO mouse PFC may highlight additional candidate genes.

Acknowledgments

The authors of this poster would like to thank Dr. Kristin Mignogna and Lorna McLeod for their work in collecting data from the DO mice and initial analysis of ethanol-related phenotypes. Finally, the Virginia Commonwealth University Department of Pharmacology and Toxicology and the VCU Alcohol Research Center for their support. Supported by NIAAA grant P50AA022537.

Primary contact: tatomz@vcu.edu