Sex Differences in the Brain Transcriptome Related to Alcohol Effects and Alcohol Use Disorder

Supplement 1

INTERPRETING SEX DIFFERENCES IN THE TRANSCRIPTOME WITH INTEGRATIVE FUNCTIONAL GENOMICS

Once identified, it is important to interpret the nature and cause of sex differences in alcohol induced transcriptional response, causal genetic variation and other mechanistic sex differences. Comparison of these results to existing functional genomics results provides insight into whether the effects observed are associated with constitutive sex differences or those that are induced or influenced by hormonal cyclicity. Integrative functional genomics analysis brings together diverse data from multiple species and experiment types to find convergent evidence for the roles of genes in related biological functions. Heterogeneous functional genomic data, representing the results of highly specific experimental studies, the curated annotations of gene functions and a wealth of curated pathway data, provide insight into the relations among gene products and facets of disease biology or behavior. This integrative approach is well suited to finding the basis of sex differences in brain and behavior and their consequences for AUD by combining data from studies of alcohol-related transcriptional variation with data on sex differences in gene expression, molecular mechanisms of sex differences and reproductive cyclicity and other genome-wide studies of sex differences. Heterogeneous functional genomic data, representing the results of highly specific experimental studies, the curated annotations of gene functions and a wealth of curated pathway data, provide insight into the relations among gene products and facets of disease biology or behavior. These data are integrated in bioinformatics resources, such as GeneWeaver (1), which allow researchers to combine, compare and contrast the results of many genomic experiments.

There are several paths to inquiry that allow researchers to answer questions such as “Is the transcriptional response to alcohol likely to be affected by sex differences?” “Are sex differences in the brain likely to result in differential response to alcohol?” “Is an observed phenotypic sex difference related to hormonal cyclicity or constitutive developmental sex differences?” The comparison of genomic studies of alcohol response and susceptibility to AUD in humans and model organism studies provides a means to address these questions.

In differential expression studies, one may start with transcriptional response to alcohol or transcriptional correlates of vulnerability to alcohol use. The former has been examined in many mouse, rat and drosophila studies. The latter has been studied in post mortem human brains, selected mouse and rat strains, and in genetic reference populations. The resulting sets of genes can be compared to genes that are differentially expressed in the male and female brain (e.g., (2)). In a screen of thousands of mouse gene deletion mutations, on multiple physiological, morphological and behavioral traits, many gene deletions were associated with phenotypic effects (3). Using integrative functional genomics in GeneWeaver, a number of these were implicated in the control and maintenance of reproductive cycles. Sex differences in the trajectory of alcohol use disorder are attributed to the complex interplay of biopsychosocial processes, all of which have some manifestation in brain circuitry and molecular function. Integrative functional genomics provides a means to identify these processes, so that the causal mechanisms and considerations for prevention and therapeutics in the sexes can be better understood. Comparing a set of 336 differentially expressed genes in the hippocampus of B6 mice drinking ethanol to intoxication (3) with the 902 occipital cortex genes known to be differentially expressed in human brains of both sexes (4), reveals a set of 53 orthologous genes that can be considered alcohol responsive and differentially expressed between the sexes (Figure S1A). This set of genes is 16-fold enriched for

genes with a GO molecular function of structural constituent of the ribosome (FDR 1.6 x 10-3) and a majority (17 genes) have a GO molecular function of binding (Figure S1B). In this case, the sex-specific difference in alcohol response in the brain can be attributed to constitutive developmental sex differences rather than mechanisms of hormonal associated transcript cyclicity. In other cases, behavioral and physiological traits vary across the sexes in a manner attributable to effects on reproductive mechanisms. For example, in a screen of thousands of mouse gene deletion mutations, on multiple physiological, morphological and behavioral traits, many gene deletions were associated with phenotypic physiological and behavioral effects, and of these, many were found to be associated with the control and maintenance of reproductive cycles (3). Using integrative functional genomics in GeneWeaver, a number of these were implicated in the control and maintenance of reproductive cycles.

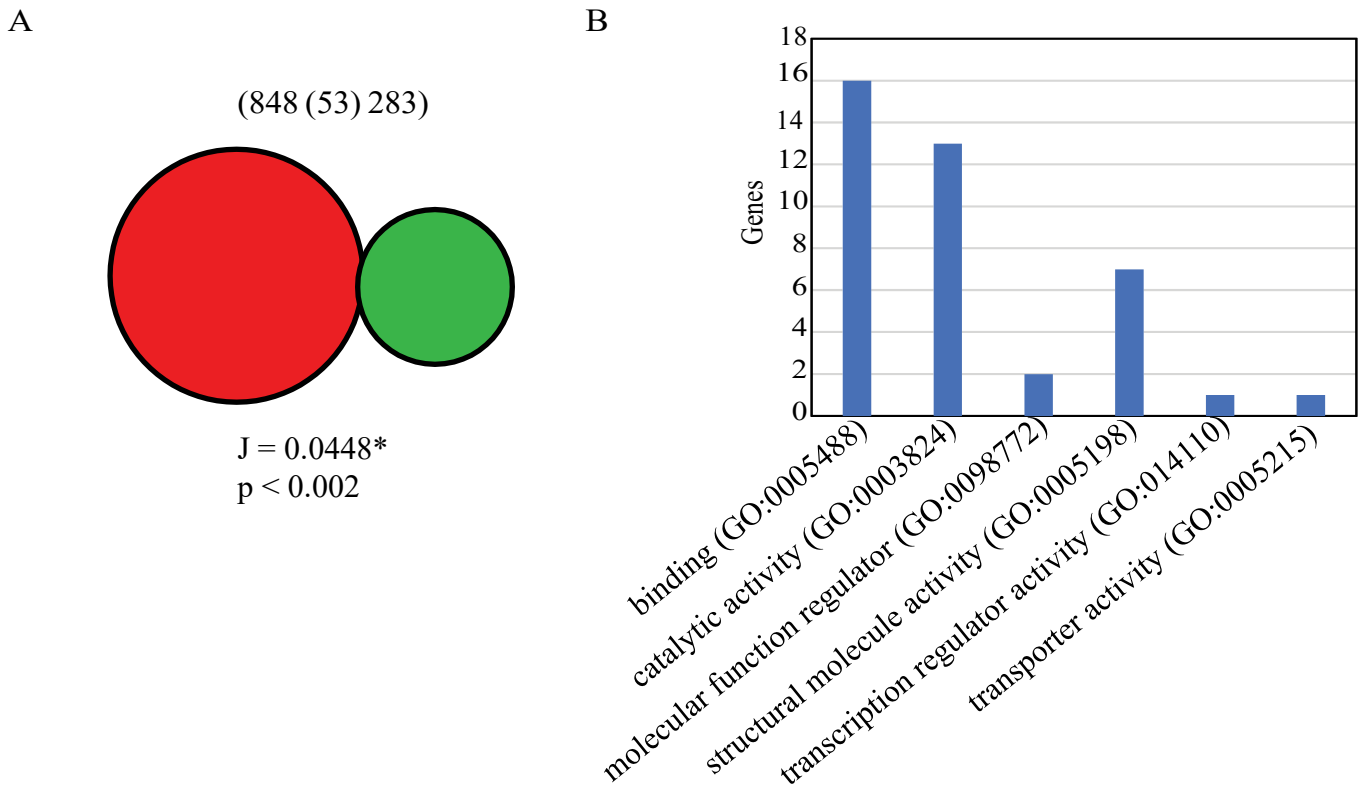

Figure S1. Outcome of integrative functional genomics analysis. (A) Jaccard similarity of 902 genes (red) differentially expressed between the sexes in human brains (occipital cortex) and 336 genes (green) differentially expressed in response to drinking to intoxication in mice (hippocampus), there is a significant ($p<0.002$) overlap of 53 genes. (B) Further characterization of these 53 genes using PANTHER reveals overrepresentation GO Molecular Functions at the intersection of alcohol intoxication and sex differences, indicating transcriptional responses, transporter activity and other potential sex differences that may be mimicked or co-opted by alcohol use and could explain the differences in drinking trajectories between the sexes.

SUPPLEMENTAL REFERENCES

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2. Reinius B, Saetre P, Leonard JA, Blekhman R, Merino-Martinez R, Gilad Y, Jazin E (2008): An evolutionarily conserved sexual signature in the primate brain. *PLoS Genet* 4: e1000100.

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