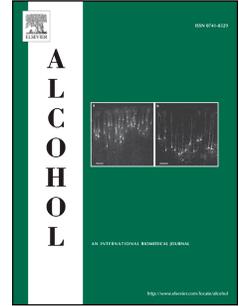


Accepted Manuscript

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PII: S0741-8329(18)30284-2

DOI: <https://doi.org/10.1016/j.alcohol.2019.01.001>

Reference: ALC 6885

To appear in: *Alcohol*

Received Date: 4 October 2018

Revised Date: 21 December 2018

Accepted Date: 3 January 2019

Please cite this article as: McClintick J.N., Tischfield J.A., Deng L., Kapoor M., Xuei X. & Edenberg H.J., Ethanol Activates Immune Response In Lymphoblastoid Cells, *Alcohol* (2019), doi: <https://doi.org/10.1016/j.alcohol.2019.01.001>.

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ETHANOL ACTIVATES IMMUNE RESPONSE IN LYMPHOBLASTOID CELLS

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Abstract

The short-term effects of alcohol on gene expression in brain tissue cannot directly be studied in humans. Because neuroimmune signaling is altered by alcohol, immune cells are a logical, accessible choice to study and might provide biomarkers. RNAseq was used to study the effects of 48 h exposure to ethanol on lymphoblastoid cell lines (LCLs) from 20 alcoholics and 20 controls.

Ethanol exposure resulted in differential expression of 4,456 of the 12,503 genes detectably expressed in the LCLs ($FDR \leq 0.05$); 52% of these showed increased expression. Cells from alcoholics and controls responded similarly. The genes whose expression changed fell into many pathways: $NF\kappa B$, neuroinflammation, IL6, IL2, IL8, and dendritic cell maturation pathways were activated, consistent with increased signaling by $NF\kappa B$, TNF, IL1, IL4, IL18, TLR4, and LPS. Signaling by Interferons A and B decreased, as did EIF2 signaling, phospholipase C signaling and Glycolysis.

Baseline gene expression patterns were similar in LCLs from alcoholics and controls. At relaxed stringency ($p < 0.05$), 465 genes differed, 230 of which were also affected by ethanol. There was a suggestion of compensation because baseline differences (no ethanol) were in the opposite direction of differences due to ethanol exposure in 78% of these genes. Pathways with IL8, phospholipase C, and α -adrenergic signaling were significant. The pattern of expression was consistent with increased signaling by several cytokines including interferons, TLR2 and TLR3 in alcoholics. Expression of genes in the cholesterol biosynthesis pathway, including the rate-limiting enzyme HMGCR, was lower in alcoholics.

LCLs show many effects of ethanol exposure, some of which might provide biomarkers for alcohol use disorders. Identifying genes and pathways altered by ethanol can aid in interpreting

which genes within loci identified by GWAS might play functional roles.

Keywords

alcohol dependence, gene expression, lymphoblastoid cell lines, neuroimmune, RNA sequencing

Abbreviations

AD: Alcohol dependent by DSM-IV criteria

LD: Linkage disequilibrium

eQTL / QTL: (expression) quantitative trait locus

LCLs: Lymphoblastoid cell lines

FDR: false discovery rate

IPA: Ingenuity pathway analysis

Introduction

Alcohol dependence (AD) is a chronic relapsing brain disorder with both environmental and genetic contributions to risk. It is estimated that 40-60% of the difference in risk among individuals is due to genetic variations (Edenberg & Foroud, 2013, 2014; Rietschel & Treutlein, 2013). However, few individual genes have been robustly associated with risk for AD. The largest meta-analysis to date of alcohol dependence in those of European and African Ancestry found only one gene associated with the disorder at genome-wide significance, *ADH1B* (Walters et al., 2018). Another metabolic gene, *ALDH2*, is associated with alcohol dependence in Asians (Edenberg and McClintick, 2018). Many of the variants for alcohol-related traits identified by GWAS are not in coding regions, and might be eQTLs or be in linkage disequilibrium (LD) with them (Gamazon et al., 2018). Transcriptome analyses may help prioritize genes within GWAS loci, identify the eQTLs and pathways affected by ethanol, and help understand mechanisms by which they act.

Alcohol dependent individuals are chronically exposed to large quantities of ethanol. This leads to multiple organ damage, including the liver (Osna, Donohue, & Kharbanda, 2017), brain (Zahr & Pfefferbaum, 2017) and immune system (Szabo & Saha, 2015). Gene expression studies of post mortem human brain tissue can shed light on how the brain is damaged by and adapts to chronic ethanol exposure (Farris, Arasappan, Hunicke-Smith, Harris, & Mayfield, 2015; Flatscher-Bader et al., 2006; Hermann et al., 2017; Mayfield, Ferguson, & Harris, 2013; McClintick et al., 2013). Changes to the brain include direct effects of ethanol and also insults caused by circulating cytokines that can cross the blood-brain barrier (Crews & Vetreno, 2016). These studies identified effects on NF κ B, TLRs, IL1 β and TNF α and thereby point toward neuroimmune signaling as an important effect of chronic ethanol exposure and potential contributor to AD (Crews & Vetreno, 2016; Mayfield et al., 2013; Pascual, Pla, Miñarro, &

Guerri, 2014). Ethanol has been shown to potentiate and prolong the effects of proinflammatory cytokines and microglial activation (Qin et al., 2008). This suggests that immune cells may provide an accessible window into how ethanol affects gene expression. Postmortem brains show the effects of both potentially pre-existing differences between alcoholics and controls and effects of long-term exposure to high levels of alcohol. There are many unrelated variables, however, including cause of death, recency of exposure to ethanol, and post-mortem interval. Lymphoblastoid cell lines (LCLs) can be studied under controlled conditions, and have been used for functional studies that cannot be done with post-mortem brain tissue, such as identifying lithium induced gene expression changes in bipolar patients and controls (Fries et al., 2017). Recent studies have shown strong correlations between blood and brain for *cis* expression QTLs (eQTLs) and methylation QTLs (Qi et al., 2018).

Transcriptome-wide analysis of expression in LCLs from AD and controls may aid the interpretation of variants identified by genetic association studies. Treatment of LCLs with ethanol can reveal direct, relatively short-term effects on cellular function. In a previous microarray study, we examined the effects of 24 h exposure to 75 mM ethanol, which was not toxic to the cells, in LCLs from 21 individuals who met DSM-IV criteria for alcohol dependence and 21 controls (McClintick et al., 2014). The individuals from whom LCLs were created were carefully diagnosed as part of the Collaborative Study on the Genetics of Alcoholism (COGA) (Begleiter et al., 1995). Nearly half of all the expressed genes were affected by ethanol, but most changes were very small; fewer than 20% had absolute fold changes >1.2. Pathways affected included increased pro-inflammatory pathways including IL6, dendritic cell maturation, TNF and NF κ B, and a decrease in the anti-inflammatory IL10 pathway. Analysis indicated that NF κ B, IL6, TNF and other cytokines were likely active, along with TLRs and interferons (McClintick et al., 2014). There was limited power to detect differences between untreated AD

and controls, but decreased IGF1 signaling and increases in protein ubiquitination and hypoxia signaling were identified.

Here, we have analyzed cells from the same individuals exposed to the same concentration of ethanol (75 mM) but for 48 h, to see if changes detected at 24 h are stable over a longer exposure. We also examined the untreated cells for differences in expression between AD and controls using data from both the 48 h and 24 h exposures. Differences between unexposed cells from AD and control individuals could provide insight into the genetics of AD.

Methods

Cell culture and ethanol treatment

Lymphoblastoid cell lines (LCLs) were created by transformation with Epstein-Barr virus of peripheral blood mononuclear cells isolated from subjects recruited as part of the Collaborative Study on the Genetics of Alcoholism (Begleiter et al., 1995) and interviewed with the SSAGA instrument (Bucholz, et al., 1994). LCLs were from 42 individuals, 21 alcohol dependent (AD) and 21 controls. AD was defined as meeting DSM-IV criteria for alcohol dependence (American Psychiatric Association, 1994). Controls were defined as having consumed at least one drink of alcohol but not meeting any of four definitions of alcohol dependence: DSM-IV (American Psychiatric Association 1994), DSM-III-R (American Psychiatric Association, 1987), ICD-10 (World Health Organization, 1993), or Feighner definite alcoholism (Feighner et al., 1972); none were dependent on any illicit drug. These individuals were previously studied using a 24 h exposure to ethanol (McClintick et al., 2014).

2×10^6 LCLs from each of 21 AD and 21 control individuals were seeded in 10 ml of RPMI1640 medium supplemented with 15% FBS, 2 mM glutamine. Cells were cultured in the absence or

presence of 75 mM ethanol for 48 h, at which time cells were harvested and lysed with buffer RLT plus, and RNA extractions were conducted using the QIAasympyphony RNA extraction protocol.

RNA sequencing

Samples were prepared in five balanced batches (4 with 8 pairs, 1 with 10 pairs) with the untreated and treated samples from each subject in the same batch. Samples were processed by polyA capture using Dynabeads mRNA DIRECT Micro Kit (Life Technologies, Carlsbad, CA) starting with 1.5 μ g of total RNA. PolyA RNA was processed using Life Technologies SOLiD™ Total RNAseq kit (Life Technologies, Carlsbad, CA). Sequencing beads were produced separately for each batch using the SOLiD™ EZBead™ System (Life Technologies, Carlsbad, CA). Each batch was sequenced separately on a SOLiD 5500xl DNA sequencer (ThermoFisher/Life Technologies, Carlsbad, CA).

Sequencing reads (75 bases, single reads) were mapped to the human hg19 reference genome using an in-house mapping pipeline (Breese & Liu, 2013) that utilizes bfast-0.7.0a (Homer, Merriman, & Nelson, 2009). In brief, reads were truncated where the average quality fell below 10 within a window size of 5, and then reads of <35 bases were discarded. Reads mapped to rRNA/tRNAs were discarded. The remaining reads were mapped to reference genome hg19 and to a splice-junction library created in-house. The genomic and splice-junction library maps were then merged. Reads mapped to multiple positions in the genome were excluded from further analysis. The gene-based expression levels were calculated using bamutils from NGSUtils, based on the RefSeq gene annotation of hg19. The average number of reads per sample was 28 million. Data deposited in GEO, GEO series <awaiting accession number>.

Differential expression analysis

Reads aligned to known genes were analyzed by edgeR (Robinson, McCarthy, & Smyth, 2010). Counts for each sample were normalized to counts per million reads. To avoid analyzing genes that were not expressed or expressed at near background levels, only genes that had more than 2 counts per million reads in at least 20 samples were retained for analysis. Data were examined by Multidimensional Scaling in the edgeR package (Robinson et al., 2010) and principal components analysis in Partek Genomics Suite (version 6.6, Partek, Inc. St. Louis, Mo) to detect outliers. One outlier (female, alcoholic, untreated) was found (see Supplemental Figure 1), so we removed it and its treated pair from further processing. In addition, we discovered that the demographic info for one of the control samples (male) did not match the phenotype listed for the sample, so it was also removed from analysis, leaving 20 controls and 20 AD. Dispersion estimates (common, trend and tag dispersion) were calculated and used in the analysis by general linear methods in edgeR to identify differentially expressed genes.

Ethanol treatment was analyzed as paired samples (treated and untreated samples from the same individual), which eliminated the need to use gender, phenotype and batch as factors. False discovery rates (FDR) were calculated using the Benjamini & Hochberg (Benjamini & Hochberg, 1995) method within edgeR. EdgeR paired analysis does not do a paired interaction of group (AD vs. control) x exposure, therefore we calculated the CPM treated – CPM untreated for each individual for each gene and used a t-test to see if the mean difference for each gene was equal between alcoholics and controls. This same method was used to test for gender differences in response to alcohol.

Data on untreated cells from a previous microarray experiment that used the Affymetrix HGU133plus2 GeneChip™ analyzed with microarray suite 5.0 software (Affymetrix, San Clara, CA) were available as $\log_2(\text{MAS5 expression})$ (McClintick et al., 2014), GEO (series accession #

GSE52553). These data were matched by gene symbol to the \log_2 (counts per million reads) from the present RNAseq experiment and imported into Partek Genomics Suite version 6.6 (Partek, Inc. St. Louis, Mo). Both experiments had similar ranges of values (Supplemental Figure 2), so the most appropriate approach was to combine the \log_2 RMA (microarray) and \log_2 CPM (sequencing) values, with a term in the ANOVA for experiment type (study) but with no additional normalization. The ANOVA used phenotype (AD, control), study (array, RNAseq) and gender as factors plus the interaction term (phenotype x study). Genes with an absolute fold change <1.2 were removed and genes with $p < 0.05$ for the interaction were removed. FDR was calculated within Partek using the Storey and Tibshirani method (Storey & Tibshirani, 2003).

Pathway and upstream activator analyses

Fold change, p-value and FDR for treatment were imported into Ingenuity Pathway Analysis Fall, September 2018 release (IPA; QIAGEN Inc.). For comparisons of alcohol treated vs. untreated cells, pathway and upstream regulator analyses were carried out on genes with $FDR < 0.05$ and absolute fold ≥ 1.2 , a total of 2318 genes, to keep the number within the 3000 limit suggested by the software vendor. For untreated cells from AD vs. control subjects, 465 genes with $p < 0.05$ and absolute fold ≥ 1.2 were compared; although the low stringency means that false positives will be included in the analysis, random noise should not greatly affect the results since pathways are tested for significance above random sets of genes. Upstream analysis identifies possible upstream regulators that could produce parts of the pattern of results seen in the data. Upstream regulators with absolute z-score ≥ 1.9 were considered to be likely causes of the pattern of gene expression changes.

Comparison to gene expression data from rats and humans

To determine if expression changes in LCLs treated with ethanol mirror changes in the brain and to identify genes with multiple lines of evidence for a relationship to alcohol use disorders,

we assembled two sets of data from microarray experiments on rat models of alcoholism. The first dataset was a composite of expression changes in many brain regions (amygdala and central core of the amygdala, nucleus accumbens and nucleus accumbens shell, dorsal raphe nucleus, periaqueductal grey, ventral tegmental area, ventral hippocampus, and medial prefrontal cortex) of P (alcohol-preferring) rats who had been voluntarily drinking ethanol in various paradigms from 4 to 10 weeks (Bell et al., 2009; McBride et al., 2014; McBride, Kimpel, McClintick, Ding, Hauser, et al., 2013; McBride et al., 2010; McClintick et al., 2015, 2016, 2018; Rodd et al., 2008). The second dataset compiled differences in gene expression in alcohol-naïve animals from pairs of rat lines selected for differences in their voluntary consumption of ethanol (in all cases high drinking lines are listed first): five brain regions from alcohol-naïve inbred P and NP rat hippocampus, amygdala, frontal cortex, striatum and nucleus accumbens (Edenberg et al., 2005; Kimpel et al., 2007), and three regions from 5 pairs of selected lines (P/NP, Had1/Lad1, Had2/Lad2, sP/sNP, AA/ANA, the ventral tegmental area (McBride et al., 2012), central core of the amygdala, and nucleus accumbens shell (McBride, Kimpel, McClintick, Ding, Hyytia, et al., 2013).

In addition to the rat data, we assembled a list of differentially expressed genes from several studies. Gene expression data from human brain regions was obtained from the GTEx portal (version V7; Lonsdale et al., 2013) as median number of transcripts per million (TPM; normalized to gene length). Single tissue eQTL (expression quantitative trait loci) data from GTEx was used to identify eQTLs in EBV-transformed-lymphocytes (LCLs). Gene expression data from post-mortem human brain tissues of AD vs. controls were obtained from both microarray and RNAseq (Farris, Harris, & Ponomarev, 2015; Flatscher-Bader, Harrison, Matsumoto, & Wilce, 2010; Flatscher-Bader et al., 2005; Iwamoto et al., 2004; Kryger & Wilce, 2010; Lewohl et al., 2000; Liu et al., 2004; Liu et al., 2006; Liu, Lewohl, Harris, Dodd, & Mayfield, 2007; Mayfield et al.,

2002; McClintick et al., 2013; Ponomarev, Wang, Zhang, Harris, & Mayfield, 2012) and matched by gene symbol to our gene expression results.

Comparison to GWAS results

We assembled genetic evidence from the NHGRI-EBI GWAS Catalog (April 2018). We selected entries with phenotypes related to alcohol dependence and consumption and $p \leq 9 \times 10^{-6}$. These were matched by gene symbol to the gene expression results described above.

Results and Discussion

We examined gene expression in LCLs from alcohol dependent and control individuals to detect both pre-existing differences and the effects of ethanol. Because much data from brain transcriptome studies suggests that ethanol is associated with changes in neuroimmune genes and pathways (Crews & Vetreno, 2016; Mayfield et al., 2013; McClintick et al., 2013), and because many of the genes expressed in brain are also expressed in LCLs, LCLs are reasonable candidates to study both preexisting differences and effects of ethanol under controlled conditions. Their accessibility is a major advantage for biomarker and functional studies. Demographic information on the subjects whose LCLs were analyzed is in Table 1. The average age of onset for DSMIV AD was 16.8, and they each met 6 or 7 of the DSM-IV criteria. Four of the controls met one criterion, the rest none.

Effects of ethanol treatment

LCLs from alcohol dependent individuals (AD) and controls were exposed to ethanol (or not) for 48 h and the differences in expression were analyzed by RNA sequencing. Cells from these same individuals had previously been used in a 24h exposure to ethanol (McClintick et

al.,2014). We have previously shown that exposure to 75 mM ethanol was not toxic to LCLs, and that the doubling time in the presence of ethanol was very close to that in its absence (27.7 h vs. 27.4 h; McClintick et al., 2014). 12,503 genes were detectably expressed in the LCLs. Ethanol exposure resulted in differential expression of 4456 genes at $FDR \leq 0.05$ (Table 2, Supplemental Table S1), 3716 (83%) of which are also expressed in human brain (median TPM ≥ 2 for at least one brain region in the GTEx V7 data, Supplemental Table S1). Among the 4456 genes, 2332 (52%) showed increased expression. Most of changes were small (Figure 1); 2318 genes (52%) had absolute fold change ≥ 1.2 . There were no significant differences in the effects of ethanol on cells from AD individuals and controls, nor were there significant differences in the effects of ethanol on cells from men and women.

Many genes associated with inflammatory responses, including interleukins and chemokines, were affected by ethanol (Table 2). These fell into many pathways (Supplemental Table S2). Pathways related to inflammation and neuroimmune activation showed increased overall expression in the alcohol-treated cells, including neuroinflammation signaling, Th1 and Th2 activation, NF κ B, interleukin signaling, STAT3, JAKs in cytokine signaling, and T-cell receptors. Higher activation of neuroimmune pathways has been shown in post-mortem brain tissue from alcoholics (Crews, Qin, Sheedy, Vetreno, & Zou, 2013; McClintick et al., 2013) and in animal studies (Blednov et al., 2011; Qin et al., 2008). Chronic activation of neuroimmune pathways is one of the hallmarks of alcohol use disorders (Crews & Vetreno, 2016; Mayfield et al., 2013). Analyses of upstream regulators indicate that the observed changes in expression could be related to ethanol's activation of Toll-like receptors, NF κ B, and TNF. Lipopolysaccharide (LPS) was also listed as a putative regulator, although the LCLs were not exposed to LPS, indicating that the changes were similar to those seen when exposed to LPS, i.e. immune stimulation. In contrast to the 24 h study (McClintick et al., 2014), which showed increased interferon signaling, cells exposed for 48 h show reduced interferon signaling (Supplementary Tables S2 and S3).

Among genes affected by ethanol treatment, *STXBP1* (syntaxin binding protein 1), *PEA15* (phosphoprotein enriched in astrocytes 15), *NPDC1* (neural proliferation, differentiation and control 1), *NRXN3* (neurexin 3), *SYT11* (synaptotagmin 11), *CBLN3* (cerebellin 3 precursor), *SYNGR1* (synaptogyrin 1), synaptophilin, and *SYNPO* (synaptopodin) are highly expressed in brain (Table 2). Several of these produce proteins that are known to reside in the post-synaptic density (Bayes et al., 2011). Ethanol caused a large decrease in the expression of *GRM8* (glutamate metabotropic receptor 8), *SSTR3* (somatostatin receptor 3) and *CHRNB1* (cholinergic receptor nicotinic beta 1 subunit). The *SSTR2* (somatostatin receptor 2) and *CHRNA5* (cholinergic receptor nicotinic alpha 5 subunit) are increased.

We examined other datasets to look for converging evidence supporting a potential role for the genes affected by ethanol in either predisposition to or effects of ethanol. Studies of post mortem brains from AD vs. control individuals have identified 928 of these 4456 genes as differentially expressed in at least one brain region (Supplemental Table S1). Forty-eight genes show some evidence of association with alcohol dependence or consumption in the NHGRI catalog (at $p \leq 9 \times 10^{-6}$; Table 4 and Supplemental table S1). Genes differentially expressed in the brains of P rats consuming large amounts of ethanol over the course of 4 to 10 weeks (Bell et al., 2009; McBride et al., 2014; McBride, Kimpel, McClintick, Ding, Hauser, et al., 2013; McBride et al., 2010; McClintick et al., 2015, 2016, 2018) matched 1214 of the genes affected by ethanol exposure in the LCLs (Supplemental Table S1). Some genes show evidence from several of these analyses. For example, *TSPAN5* (tetraspanin 5) is decreased in the hippocampus (McClintick et al., 2013) and frontal cortex (Liu et al., 2007) of alcoholics, increased in the dorsal raphe nucleus (McClintick et al., 2015) and central core of the amygdala (McBride et al., 2010) of P rats in binge-like models of drinking, and associated with alcohol consumption (Clarke, et al., 2017). *AUTS2* (activator of transcription and developmental regulator) has been associated with alcohol drinking (Schumann et al., 2011) and was

decreased in post-mortem frontal cortex of alcoholics (Liu et al., 2007). *CHL1* (cell adhesion molecule L1 like) was associated with response to trauma and total AUDIT score (Almli et al., 2017) and decreased in post-mortem frontal cortex (Liu et al., 2007).

Baseline differences between LCLs from alcohol-dependent and control subjects

Baseline expression differences between LCLs derived from AD subjects vs. controls could result either from pre-existing genetic differences that affect risk for AD or from effects of the long-term alcohol exposure of the AD subjects from whom they came. Transformation and growth of the LCL would be expected to reduce differences due to the previous drinking history. Differences were, in fact, small and our data both here and in the previous experiment on LCLs from the same individuals (McClintick et al., 2014) were underpowered to identify them. Even combining the data on unexposed cells identified only 9 genes with an FDR < 0.20 (Table 3, Supplemental table S4). To see if there were suggestions of effects, we relaxed the criteria for this combined analysis to $p < 0.05$; 465 genes showed differences ≥ 1.2 -fold. *TMOD2*, *CLIC2*, *NPNT*, *EIF1AY*, *FYB*, *TANC1* and *KCNA1* were expressed at much lower levels in cells from AD, and *WFDC2*, *TIE1*, *ASB2*, *MUC13*, and *FOXA3* were expressed at much higher levels (Table 3).

Among the genes that differed at baseline, 230 were affected by ethanol, 180 of which differed in the opposite direction (Table 3, Supplemental Table S4). This strong bias is not likely due to chance ($180/230$, $p = 1.0 \times 10^{-17}$), and suggests that the heavy drinking history in the AD subjects may have led to changes that compensate for the effects of ethanol.

Some genes that differ between AD and controls have prominent roles in brain function and were identified as either genome-wide or nominally significant by GWAS, making them promising targets for follow-up studies. *SASH1* (SAM and SH3 domain containing 1) is a scaffold protein involved in the TLR4 signaling pathway that has been linked to the inflammation

seen in chronic drinking (Crews et al., 2015; Kelley & Dantzer, 2011) and associated with unipolar depression and alcohol dependence (Zhou et al., 2017). *SIX3*, a homeobox gene and transcriptional regulator important during forebrain development, is associated with alcohol and nicotine codependence (Zuo et al., 2012). *SLC35F3* (solute carrier family 35 member F3) is a probable thiamine transporter expressed at highest levels in brain (GTEx); an intronic variant, rs17514104, is suggestively associated with dependence on at least one substance (alcohol, cannabis, opioid or cocaine) in the COGA sample (Wetherill et al., 2015). *FYB*, FYN binding protein, is associated with the innate immune system and T-cell receptor signaling; rs113497429, in an intron of *FYB*, is suggestively associated with alcohol use disorder symptom count (Gelernter et al., 2015). Two intronic SNPs in *TNFRSF10A*, rs73222599 and rs73222600, were also suggestive with symptom count in African Americans (Gelernter et al., 2015) (Gelernter et al., 2014); *TNFRSF10A* (TNF receptor superfamily member 10a) is a receptor for the ligand Trail (TNF-related apoptosis-inducing ligand) that can mediate neuronal apoptosis (Ryan et al., 2004). *FOXA3* (forkhead box A3), more highly expressed in AD and was decreased by ethanol treatment, plays a role in neuronal death after seizure-induced neuronal injury (Kim et al., 2014).

Two genes with large differences between untreated cells from AD vs. control subjects are involved in formation and maintenance of synapses: *TMOD2* (tropomodulin) and *TANC1* (tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1). *TMOD2* is a negative regulator of neurites and is expressed mostly in neurons (Fath, Fischer, Dehmelt, Halpain, & Fowler, 2011). *TANC1* is in the post synaptic density and may be important for dendritic spine maintenance (Han et al., 2010).

The 465 nominally significant genes mapped to sixty-seven pathways (Supplemental Table S5). Cholesterol biosynthesis was one of the most significant, with lower expression of *HMGCR*, *CYP51A1* and *IDI1*. Chronic ethanol exposure has been shown to decrease cholesterol

synthesis with an associated decrease in myelination in humans (Liu et al., 2006, McClintick et al., 2013) and animals (Alfonso-Loeches et al., 2012; McClintick et al., 2013; Pascual et al., 2014), leading to losses in white matter in the brain (Jacobus & Tapert, 2013). Upstream analysis was consistent with higher activity of TP53, TNF, LPS, TLRs, interferons and interleukins (Supplemental Table S6). In contrast to the effects of ethanol treatment, the analysis indicates Phospholipase C has higher activity in alcoholics.

Among the 465 genes, expression differences between alcohol-naïve rats from 5 selectively bred pairs of high-drinking and low-drinking rats were found for 138 of the genes that differed at baseline, eQTLs from lymphocytes (GTEx) affected 83 of these genes, and 6 were found in the NHGRI catalog (Supplemental Table 4). Three GWAS genes, *CASZ1* (castor zinc finger 1), *MREG* (melanoregulin) and *ST3GAL1* (ST3 beta-galactoside alpha-2,3-sialyltransferase 1), were affected by ethanol with a direction opposite those for AD vs. controls.

Conclusions

The results of this study provide additional data on genes potentially linked either to the development of alcohol dependence or the effects of excessive alcohol. There are, however, limitations. Even though most of the genes we found differentially expressed in the LCLs exposed to ethanol are also expressed in brain, the changes we found might not mirror what happens in the brain. Long-term drinking patterns are highly variable, and not replicated *in vitro*; our goal was to determine if there is molecular evidence of either adaptation or increased perturbation of gene expression after 48 h of exposure, compared to 24 h. Differences at baseline (unexposed cells) were very small, and because we were underpowered to detect many we relaxed our statistical criteria to examine trends.

This study shows that ethanol exposure induces many changes in LCLs, including a robust immune response seen strongly after 24 h exposure, but decreased in intensity, possibly due to a reversal of interferon signaling, after 48 h exposure. Some expression differences between the LCL from AD and controls are likely genetic, since those groups differ in genetic risk for AD. Accessible tissues like LCLs can be useful in interpreting GWAS results, and giving additional support to prioritize genes that don't quite reach genome wide significance for follow-up studies.

Declarations of interest: none

Acknowledgements

The Center for Medical Genomics at the Indiana University School of Medicine performed the RNA sequencing and alignment.

The Collaborative Study on the Genetics of Alcoholism (COGA), Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes 11 different centers: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenberg, J. Nurnberger Jr., T. Foroud); University of Iowa (S. Kuperman, J. Kramer); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, J. Rice, K. Bucholz, A. Agrawal); University of California at San Diego (M. Schuckit); Rutgers University (J. Tischfield, A. Brooks); Department of Biomedical and Health Informatics, The Children's Hospital of Philadelphia; Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA (L. Almasy); Virginia Commonwealth University (D. Dick); Icahn School of Medicine at Mount Sinai (A. Goate); and Howard University (R. Taylor). Other COGA collaborators include L. Bauer (University of Connecticut); J. McClintick, L. Wetherill, X. Xuei, Y. Liu, D. Lai, S. O'Connor, M. Plawecki, S. Lourens (Indiana University); G. Chan (University of Iowa; University of Connecticut); J. Meyers, D. Chorlian, C. Kamarajan, A. Pandey, J. Zhang (SUNY Downstate); J.-C. Wang, M. Kapoor, S. Bertelsen (Icahn School of Medicine at Mount Sinai); A. Anokhin, V. McCutcheon, S. Saccone (Washington University); J. Salvatore, F. Aliev, B. Cho (Virginia Commonwealth University); and M. Kos (University of Texas Rio Grande Valley). A. Parsian and M. Reilly are the NIAAA Staff Collaborators. This national collaborative study is supported by NIH Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA).

The LCLs are stored at RUDCR Infinite Biologics at Rutgers University and are made available to qualified scientists.

The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 04/18/2018 (version 7, Lonsdale et al., 2013).

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Web resources

GTEx portal version V7, accessed on 04/18/2018. (Lonsdale et al., 2013)

www.gtexportal.org/

files:

Gene expression data: [GTEx_Analysis_2016-01-15_v7_RNASEQCv1.1.8_gene_median_tpm.gct.gz](#)

eQTL data: [GTEx_Analysis_v7_eQTL.tar.gz](#)

NHGRI-EBI GWAS Catalog (April 2018);

<https://www.ebi.ac.uk/gwas/docs/file-downloads>

file: [NHGRI_gwas_catalog_v1.0.1-associations_e92_r2018-04-10](#)

Partek Genomics Suite (version 6.6, Partek, Inc. St. Louis, Mo)

<http://www.partek.com/pgs>

Ingenuity Pathway Analysis (Fall, September 2018 release)

www.qiagenbioinformatics.com/products/ingenuitypathway-analysis).

Tables and Figures

Table 1. Demographics of AD and controls used in this project; mean values (standard deviations).

Table 2. Genes differentially expressed after 48 hr. treatment with ethanol; this is a subset with absolute fold ≥ 1.6 plus any genes discussed in the text. The full set of genes is in Supplemental Table S1. FDR = false discovery rate.

Table 3. Genes differentially expressed at baseline between alcohol dependent and control individuals. This is a subset with absolute fold ≥ 1.5 ; the full list is in Supplemental Table S4.
*fold change for ethanol treatment shown only when FDR < 0.05.

Table 4. Differentially expressed genes that matched genes for alcohol dependence or consumption that were significant or nominally significant ($p < 9 \times 10^{-6}$) in the NHGRI GWAS catalog. TPM = highest value of transcripts per million transcripts in any brain region in the GTEx database.

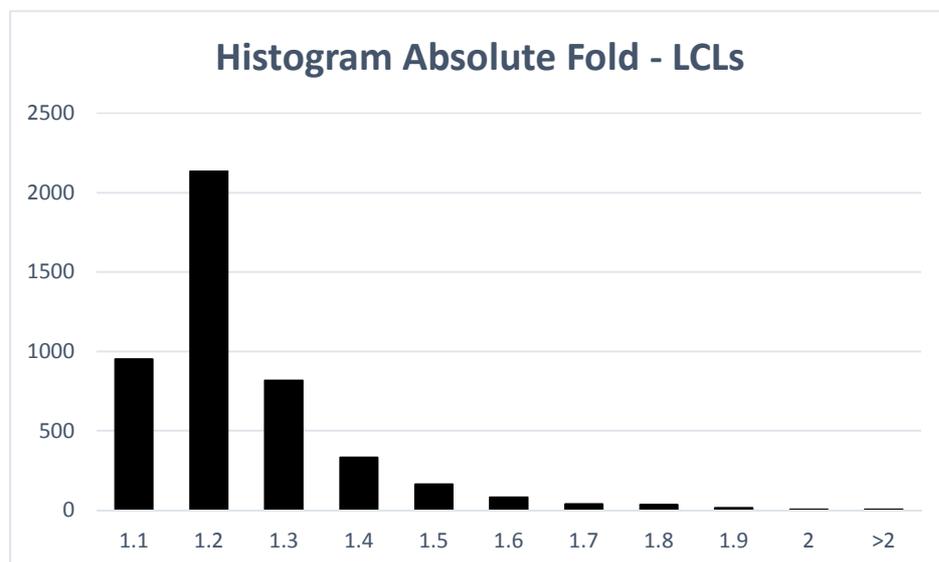


Figure 1. Distribution of Absolute fold changes for treated vs. untreated LCLs.

Supplemental Data

Supplemental Figure 1. MDS plot from edgeR showing outlier 1U. Note that most other Treated (T) and Untreated (U) pairs (same number) group together. 1U does not group with 1T and is separated on the first axis from the rest of the samples. We removed 1U and 1T from further processing.

Supplemental Figure 2. Box plots of log base 2 transformed CPM for RNAseq and RMA for microarray data.

Supplemental Table S1. Full list of differentially expressed genes after LCLs were treated with ethanol for 48h, FDR < 0.05.

Supplemental Table S2. IPA Pathways for genes affected by ethanol treatment of LCLs.

Supplemental Table S3. IPA Upstream analysis of genes affected by ethanol treatment of LCLs.

Supplemental Table S4. Full list of gene expression differences between alcohol dependent and control individuals.

Supplemental Table S5. IPA Pathways for genes that differed between alcohol dependent vs. controls.

Supplemental Table S6. IPA Upstream analysis of genes that differed between alcohol dependent vs. controls.

Table 1. Demographics of AD and controls used in this project; mean values (standard deviations).

	AD	control
Male	12	11
Female	8	9
DSM4 criterion count	6.8 (0.6)	0.2 (0.4)
Age DSM4 onset	16.7 (1.0)	NA
max drinks	35.0 (15.4)	8.2 (3.3)
age	55.4 (7.5)	64.0 (16.7)

Table 2. Genes differentially expressed after 48 hr. treatment with ethanol. S; this is a subset with absolute fold ≥ 1.6 plus any genes discussed in the text. The full set of genes is in Supplemental Table S1. FDR = false discovery rate.

gene symbol	gene name	treated/ untreated fold	treated/ untreated FDR
<i>ABCA1</i>	ATP binding cassette subfamily A member 1	-1.7	1.19E-03
<i>ABCB1</i>	ATP binding cassette subfamily B member 1	2.2	1.44E-30
<i>ACY3</i>	aminoacylase 3	-1.7	1.37E-38
<i>ADCY1</i>	adenylate cyclase 1	1.6	3.43E-15
<i>AFF3</i>	AF4/FMR2 family member 3	1.8	4.70E-15
<i>ANGPTL6</i>	angiopoietin like 6	-1.6	6.17E-16
<i>ANKRD30BL</i>	ankyrin repeat domain 30B like	-1.8	8.23E-04
<i>ANXA3</i>	annexin A3	1.7	6.79E-09
<i>APOBEC3H</i>	apolipoprotein B mRNA editing enzyme catalytic subunit 3H	-1.8	1.65E-28
<i>AQP9</i>	aquaporin 9	1.6	1.13E-15
<i>BAG3</i>	BCL2 associated athanogene 3	1.7	9.60E-12
<i>BCO1</i>	beta-carotene oxygenase 1	-1.6	4.58E-15
<i>BEX2</i>	brain expressed X-linked 2	1.7	4.76E-17
<i>BMP4</i>	bone morphogenetic protein 4	1.6	1.26E-11
<i>C14orf105</i>		-1.6	1.70E-39
<i>C1orf228</i>		-1.8	2.94E-22
<i>CABP1</i>	calcium binding protein 1	-1.6	1.40E-14
<i>CADM1</i>	cell adhesion molecule 1	1.6	7.87E-13
<i>CAV2</i>	caveolin 2	1.8	1.53E-04
<i>CCL22</i>	C-C motif chemokine ligand 22	1.6	2.59E-24
<i>CCR8</i>	C-C motif chemokine receptor 8	1.7	7.21E-22
<i>CD68</i>	CD68 molecule	-1.7	6.70E-29
<i>CDHR1</i>	cadherin related family member 1	1.6	2.04E-36
<i>CEP170B</i>	centrosomal protein 170B	1.6	9.25E-06
<i>CLLU1OS</i>	chronic lymphocytic leukemia up-regulated 1 opposite strand	-1.7	2.40E-16
<i>CPEB1</i>	cytoplasmic polyadenylation element binding protein 1	1.8	2.65E-18
<i>CREB3L1</i>	cAMP responsive element binding protein 3 like 1	1.6	2.48E-12
<i>CXCL10</i>	C-X-C motif chemokine ligand 10	1.8	7.28E-22
<i>CYP1A1</i>	cytochrome P450 family 1 subfamily A member 1	-1.7	8.97E-26
<i>DBNDD1</i>	dysbindin domain containing 1	-1.8	5.51E-25
<i>DDX60L</i>	DExD/H-box 60 like	-1.6	2.59E-25

<i>DNAH14</i>	dynein axonemal heavy chain 14	1.6	1.76E-05
<i>DZIP1</i>	DAZ interacting zinc finger protein 1	1.6	3.99E-32
<i>EIF4E3</i>	eukaryotic translation initiation factor 4E family member 3	1.7	1.99E-08
<i>ELK2AP</i>	ELK2A, member of ETS oncogene family, pseudogene	-1.6	1.69E-05
<i>EPHB2</i>	EPH receptor B2	1.7	2.71E-12
<i>ESPNL</i>	espin like	-1.8	7.91E-25
<i>F5</i>	coagulation factor V	1.8	5.28E-21
<i>FABP3</i>	fatty acid binding protein 3	-1.7	3.92E-28
<i>FAM171A1</i>	family with sequence similarity 171 member A1	1.6	9.17E-11
<i>FCHO2</i>	FCH domain only 2	1.6	6.46E-07
<i>FFAR2</i>	free fatty acid receptor 2	-2.0	2.77E-27
<i>FLRT3</i>	fibronectin leucine rich transmembrane protein 3	1.6	8.03E-08
<i>GBP4</i>	guanylate binding protein 4	1.6	4.35E-20
<i>GJB2</i>	gap junction protein beta 2	1.9	1.21E-27
<i>GNAI1</i>	G protein subunit alpha i1	1.8	4.80E-07
<i>GPER1</i>	G protein-coupled estrogen receptor 1	-1.8	8.88E-24
<i>GPR153</i>	G protein-coupled receptor 153	1.6	5.09E-15
<i>GRM8</i>	glutamate metabotropic receptor 8	-1.8	1.49E-17
<i>HIST1H1C</i>	histone cluster 1 H1 family member c	-1.8	2.06E-19
<i>HRASLS2</i>	HRAS like suppressor 2	-1.7	2.40E-11
<i>IGF1</i>	insulin like growth factor 1	-1.6	3.78E-15
<i>IGFBP2</i>	insulin like growth factor binding protein 2	-1.8	4.83E-07
<i>IGFBP4</i>	insulin like growth factor binding protein 4	1.6	1.93E-14
<i>IL18R1</i>	interleukin 18 receptor 1	1.8	2.68E-68
<i>IL1R2</i>	interleukin 1 receptor type 2	1.6	1.41E-10
<i>JPH4</i>	junctophilin 4	1.7	1.64E-12
<i>KCNMA1</i>	potassium calcium-activated channel subfamily M alpha 1	1.6	7.36E-11
<i>KCNMB2</i>	potassium calcium-activated channel subfamily M regulatory beta subunit 2	-1.8	2.65E-20
<i>KHDRBS3</i>	KH RNA binding domain containing, signal transduction associated 3	1.7	1.30E-09
<i>KIAA0408</i>	KIAA0408	1.7	1.13E-14
<i>KIF7</i>	kinesin family member 7	1.7	2.42E-09

<i>KITLG</i>	KIT ligand	1.8	2.38E-05
<i>LARP6</i>	La ribonucleoprotein domain family member 6	1.7	1.69E-08
<i>LHFP</i>		1.6	9.61E-22
<i>LOC101927412</i>		-1.8	6.80E-13
<i>LOC63930</i>		-1.6	5.69E-10
<i>LRRC25</i>	leucine rich repeat containing 25	-1.8	6.08E-19
<i>MAN1C1</i>	mannosidase alpha class 1C member 1	1.9	1.63E-21
<i>MEOX1</i>	mesenchyme homeobox 1	1.8	2.27E-47
<i>MIR6787</i>	microRNA 6787	-1.6	4.45E-03
<i>MMRN1</i>	multimerin 1	1.6	1.97E-08
<i>MSRB3</i>	methionine sulfoxide reductase B3	1.8	1.03E-06
<i>MYBPC2</i>	myosin binding protein C, fast type	-1.9	1.37E-14
<i>MYO7B</i>	myosin VIIb	-1.6	2.96E-09
<i>NBL1</i>		1.7	1.66E-10
<i>NCS1</i>	neuronal calcium sensor 1	1.8	1.50E-08
<i>NEB</i>	nebulin	-1.8	2.90E-03
<i>NOTCH2</i>	notch 2	1.7	4.95E-37
<i>NPDC1</i>	neural proliferation, differentiation and control 1	1.9	4.55E-11
<i>NPL</i>	N-acetylneuraminase pyruvate lyase	1.6	1.21E-34
<i>NRXN3</i>	neurexin 3	1.6	9.30E-07
<i>PDZD2</i>	PDZ domain containing 2	-1.9	2.21E-11
<i>PHKA1</i>	phosphorylase kinase regulatory subunit alpha 1	1.8	4.55E-09
<i>PLD4</i>	phospholipase D family member 4	-2.2	1.89E-31
<i>PLEKHA5</i>	pleckstrin homology domain containing A5	1.7	6.34E-06
<i>PODXL</i>	podocalyxin like	1.7	1.85E-18
<i>PPFIA4</i>	PTPRF interacting protein alpha 4	-1.7	4.41E-16
<i>PSD3</i>	pleckstrin and Sec7 domain containing 3	-1.6	1.00E-04
<i>PTPRO</i>	protein tyrosine phosphatase, receptor type O	-1.6	4.60E-19
<i>PVR</i>	poliovirus receptor	1.6	9.10E-24
<i>PXDC1</i>	PX domain containing 1	1.7	9.30E-18
<i>RAB15</i>	RAB15, member RAS oncogene family	1.7	6.40E-28
<i>RAB32</i>	RAB32, member RAS oncogene family	1.8	5.74E-18

<i>RNF130</i>	ring finger protein 130	1.7	6.79E-08
<i>RPS6KA6</i>	ribosomal protein S6 kinase A6	1.6	1.71E-09
<i>RSAD2</i>	radical S-adenosyl methionine domain containing 2	-1.7	4.51E-41
<i>SCD5</i>	stearoyl-CoA desaturase 5	1.6	6.27E-22
<i>SERPINB6</i>	serpin family B member 6	1.7	1.10E-08
<i>SETD7</i>	SET domain containing lysine methyltransferase 7	2.3	2.39E-32
<i>SFRP1</i>	secreted frizzled related protein 1	1.7	1.88E-07
<i>SLC16A14</i>	solute carrier family 16 member 14	2.1	2.27E-47
<i>SLC16A9</i>	solute carrier family 16 member 9	2.0	1.04E-76
<i>SLC47A1</i>	solute carrier family 47 member 1	1.6	1.30E-09
<i>SLC9A2</i>	solute carrier family 9 member A2	1.6	4.92E-12
<i>SMARCA1</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	1.6	6.75E-04
<i>SNPH</i>	syntaphilin	1.9	4.03E-15
<i>SNX7</i>	sorting nexin 7	1.6	1.10E-08
<i>SOAT2</i>	sterol O-acyltransferase 2	-1.6	1.84E-11
<i>SOGA3</i>	SOGA family member 3	1.6	7.35E-15
<i>SRGAP1</i>	SLIT-ROBO Rho GTPase activating protein 1	1.6	3.17E-09
<i>SSTR2</i>	somatostatin receptor 2	1.5	6.79E-27
<i>SSTR3</i>	somatostatin receptor 3	-1.5	1.28E-19
<i>STAT4</i>	signal transducer and activator of transcription 4	1.7	6.96E-29
<i>STK39</i>	serine/threonine kinase 39	1.6	9.98E-08
<i>STXBP1</i>	syntaxin binding protein 1	1.9	1.01E-17
<i>TCL6</i>	T cell leukemia/lymphoma 6	-1.9	1.28E-15
<i>TFCP2L1</i>	transcription factor CP2 like 1	-2.6	7.06E-29
<i>TFPI2</i>	tissue factor pathway inhibitor 2	1.6	1.27E-10
<i>TMEM136</i>	transmembrane protein 136	1.6	6.48E-14
<i>TMEM217</i>	transmembrane protein 217	1.7	2.86E-23
<i>TNFSF4</i>	TNF superfamily member 4	1.8	1.74E-36
<i>TPBG</i>	trophoblast glycoprotein	1.6	4.83E-21
<i>TUFT1</i>	tuftelin 1	1.6	1.02E-18
<i>VASH1</i>	vasohibin 1	1.6	2.04E-12
<i>VAV3</i>	vav guanine nucleotide exchange factor 3	1.8	3.13E-14
<i>VCAM1</i>	vascular cell adhesion molecule 1	1.8	3.76E-30
<i>VPREB3</i>	V-set pre-B cell surrogate light chain 3	-1.9	2.07E-12

Table 3. Genes differentially expressed at baseline between alcohol dependent and control individuals. This is a subset with absolute fold ≥ 1.5 ; the full list is in Supplemental Table S4.

*fold change for ethanol treatment shown only when FDR < 0.05.

gene symbol	gene name	AD / Ctl Fold	AD / Ctl pvalue	ethanol fold*
<i>ACOXL</i>	acyl-CoA oxidase-like	1.5	1.10E-02	
<i>ADCY1</i>	adenylate cyclase 1	-1.7	2.74E-02	1.6
<i>AMICA1</i>	Junction Adhesion Molecule Like (JAML)	1.5	2.56E-02	-1.1
<i>ARHGAP24</i>	Rho GTPase activating protein 24	-1.5	2.54E-02	1.1
<i>ARMCX1</i>	armadillo repeat containing, X-linked 1	-1.5	4.33E-02	1.5
<i>ASB2</i>	ankyrin repeat and SOCS box containing 2	1.9	3.67E-04	
<i>ATP8B1</i>	ATPase phospholipid transporting 8B1	1.9	1.54E-03	
<i>BCL11A</i>	B-cell CLL/lymphoma 11A	-1.6	4.40E-03	-1.2
<i>C3orf14</i>	chromosome 3 open reading frame 14	-1.9	2.28E-02	1.3
<i>CADM1</i>	cell adhesion molecule 1	-1.9	2.84E-02	1.6
<i>CCDC74A</i>	coiled-coil domain containing 74A	-1.7	5.41E-03	1.2
<i>CD200R1</i>	CD200 receptor 1	1.6	4.56E-03	
<i>CDC42BPA</i>	CDC42 binding protein kinase alpha	1.7	2.04E-02	
<i>CLIC2</i>	chloride intracellular channel 2	-2.1	3.63E-04	1.4
<i>CNTLN</i>	centlein	-2.1	2.51E-02	1.2
<i>DLGAP1</i>	DLG associated protein 1	1.5	4.76E-02	
<i>DNAJC6</i>	DnaJ heat shock protein family (Hsp40) member C6	-1.5	4.35E-02	1.1
<i>EFNA5</i>	ephrin A5	-1.6	2.10E-02	1.2
<i>EFR3B</i>	EFR3 homolog B	-1.6	4.22E-02	
<i>EIF1AY</i>	eukaryotic translation initiation factor 1A, Y-linked	-1.9	1.47E-02	
<i>F2R</i>	coagulation factor II thrombin receptor	1.5	2.66E-02	1.3
<i>FAM160A1</i>	family with sequence similarity 160 member A1	-1.5	1.62E-02	
<i>FAM171A1</i>	family with sequence similarity 171 member A1	-1.5	8.95E-03	1.6
<i>FAM26F</i>	family with sequence similarity 26 member F	-1.6	2.49E-02	-1.2
<i>FCRL4</i>	Fc receptor like 4	1.5	4.88E-02	
<i>FCRL5</i>	Fc receptor like 5	1.5	7.27E-03	-1.4

<i>FNIP2</i>	folliculin interacting protein 2	1.5	2.55E-02	
<i>FOXA3</i>	forkhead box A3	1.7	7.91E-03	-1.2
<i>FYB</i>	FYN binding protein	-2.1	1.46E-03	1.3
<i>HIC1</i>	HIC ZBTB transcriptional repressor 1	1.5	2.54E-02	-1.3
<i>HMX2</i>	H6 family homeobox 2	-2.0	2.75E-02	1.2
<i>HNF4G</i>	hepatocyte nuclear factor 4 gamma	1.7	2.41E-02	
<i>HNMT</i>	histamine N-methyltransferase	-1.8	3.28E-02	
<i>HNRNPLL</i>	heterogeneous nuclear ribonucleoprotein L like	1.6	6.34E-04	1.3
<i>IL23R</i>	interleukin 23 receptor	1.7	3.70E-02	
<i>INA</i>	internexin neuronal intermediate filament protein alpha	-1.5	2.30E-02	
<i>KCNA1</i>	potassium voltage-gated channel subfamily A member 1	-1.8	1.64E-03	1.2
<i>KCNMB2</i>	potassium calcium-activated channel subfamily M regulatory beta subunit 2	1.5	3.22E-02	-1.8
<i>KIAA0226L</i>	#N/A	1.6	1.72E-02	-1.1
<i>KIAA1549L</i>	KIAA1549 like	-1.5	4.10E-02	1.2
<i>LEF1</i>	lymphoid enhancer binding factor 1	1.6	2.31E-02	1.2
<i>LINC01225</i>	LINC01225 pseudogene	1.8	1.12E-02	
<i>LMO3</i>	LIM domain only 3	1.6	2.75E-02	
<i>LRRC1</i>	leucine rich repeat containing 1	1.5	1.33E-02	1.1
<i>MAATS1</i>	MYCBP associated and testis expressed 1	-1.5	5.69E-03	1.3
<i>MACROD2</i>	MACRO domain containing 2	1.7	4.57E-03	-1.1
<i>MTTP</i>	microsomal triglyceride transfer protein	1.5	2.61E-02	
<i>MUC13</i>	mucin 13, cell surface associated	2.2	1.22E-02	
<i>MYBPC2</i>	myosin binding protein C, fast type	1.6	3.37E-03	-1.9
<i>NCKAP5</i>	NCK associated protein 5	1.7	2.32E-02	-1.3
<i>NOL4</i>	nucleolar protein 4	-1.9	3.98E-02	1.2
<i>NPNT</i>	nephronectin	-2.5	4.08E-03	1.5
<i>PDZD2</i>	PDZ domain containing 2	1.7	2.50E-02	-1.9
<i>PIEZO2</i>	piezo type mechanosensitive ion channel component 2	-1.5	3.06E-02	
<i>PYROXD1</i>	pyridine nucleotide-disulphide oxidoreductase domain 1	1.5	1.99E-02	-1.1
<i>RAB3B</i>	RAB3B, member RAS oncogene family	-1.5	3.15E-02	

<i>RBPM52</i>	RNA binding protein with multiple splicing 2	1.7	9.90E-03	
<i>RHOU</i>	ras homolog family member U	1.6	4.40E-02	-1.2
<i>RIMS2</i>	regulating synaptic membrane exocytosis 2	1.5	3.99E-02	
<i>SAMD12</i>	sterile alpha motif domain containing 12	1.5	1.50E-02	
<i>SASH1</i>	SAM and SH3 domain containing 1	1.8	4.21E-03	
<i>SEL1L3</i>	SEL1L family member 3	1.6	1.13E-03	-1.4
<i>SEMA6A</i>	semaphorin 6A	1.5	8.52E-03	
<i>SERPINE2</i>	serpin family E member 2	1.5	4.43E-02	
<i>SFRP1</i>	secreted frizzled related protein 1	-1.9	4.56E-02	1.7
<i>SHTN1</i>	shootin 1	-1.7	2.31E-03	1.2
<i>SIX3</i>	SIX homeobox 3	-1.9	1.80E-02	
<i>SLC35F3</i>	solute carrier family 35 member F3	-1.5	4.80E-02	1.5
<i>SNTB1</i>	syntrophin beta 1	1.6	1.58E-03	
<i>STEAP1</i>	STEAP family member 1	1.6	4.65E-02	-1.1
<i>SYNGR1</i>	synaptogyrin 1	1.7	1.20E-02	-1.2
<i>TANC1</i>	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	-1.9	1.81E-03	1.2
<i>TBC1D30</i>	TBC1 domain family member 30	-1.5	4.68E-02	1.5
<i>TC2N</i>	tandem C2 domains, nuclear	-1.6	3.95E-03	1.2
<i>TIE1</i>	tyrosine kinase with immunoglobulin like and EGF like domains 1	2.2	3.43E-04	
<i>TMOD2</i>	tropomodulin 2	-1.5	2.01E-05	1.3
<i>TNFRSF10A</i>	TNF receptor superfamily member 10a	1.5	3.32E-04	
<i>WFDC2</i>	WAP four-disulfide core domain 2	2.4	7.63E-04	-1.5
<i>ZNF300</i>	zinc finger protein 300	-1.8	1.45E-02	
<i>ZNF880</i>	zinc finger protein 880	1.5	2.13E-02	-1.1

Table 4. Differentially expressed genes that matched genes for alcohol dependence or consumption that were significant or nominally significant ($p < 9 \times 10^{-6}$) in the NHGRI GWAS catalog. TPM = highest value of transcripts per million transcripts in any brain region in the GTEx database.

gene symbol	gene name	treated/ untreated fold	treated / untreated FDR	TPM GTEx brain
<i>ANKRD44</i>	ankyrin repeat domain 44	-1.1	1.99E-02	1.6
<i>ARL15</i>	ADP ribosylation factor like GTPase 15	1.2	6.90E-03	12.0
<i>AUTS2</i>	AUTS2, activator of transcription and developmental regulator	1.3	6.50E-22	14.6
<i>B4GALT6</i>	beta-1,4-galactosyltransferase 6	1.3	3.51E-06	28.9
<i>BOD1</i>	bioorientation of chromosomes in cell division 1	1.2	1.23E-04	48.0
<i>BPTF</i>	bromodomain PHD finger transcription factor	-1.1	5.35E-03	38.2
<i>C2orf88</i>	chromosome 2 open reading frame 88	-1.1	1.99E-03	4.7
<i>CAMTA1</i>	calmodulin binding transcription activator 1	-1.1	1.55E-03	57.0
<i>CAPN7</i>	calpain 7	1.2	2.56E-04	28.0
<i>CASZ1</i>	castor zinc finger 1	1.3	3.48E-04	0.6
<i>CHL1</i>	cell adhesion molecule L1 like	-1.3	2.20E-12	39.1
<i>CTNNA2</i>	catenin alpha 2	-1.3	5.34E-03	63.7
<i>FABP3</i>	fatty acid binding protein 3	-1.7	3.92E-28	135.4
<i>FAM81A</i>	family with sequence similarity 81 member A	1.1	1.76E-04	32.5
<i>FAM83D</i>	family with sequence similarity 83 member D	1.2	3.06E-06	1.9
<i>GALNT18</i>	polypeptide N- acetylgalactosaminyltransferase 18	1.1	2.43E-02	27.0
<i>GPD1L</i>	glycerol-3-phosphate dehydrogenase 1 like	1.1	9.67E-03	34.1
<i>IGSF9B</i>	immunoglobulin superfamily member 9B	1.3	3.17E-03	16.7
<i>ITPRIPL2</i>	ITPRIP like 2	1.2	4.26E-04	2.0
<i>KCND2</i>	potassium voltage-gated channel subfamily D member 2	1.2	3.38E-07	79.4
<i>LARGE</i>		1.1	6.91E-04	15.3
<i>LINC00158</i>	long intergenic non-protein coding RNA 158	1.2	6.79E-03	1.4
<i>LOC101927697</i>		-1.2	1.12E-03	

<i>MICU3</i>	mitochondrial calcium uptake family member 3	1.3	2.83E-02	60.0
<i>MREG</i>	melanoregulin	1.4	3.57E-18	34.8
<i>NAA30</i>	N(alpha)-acetyltransferase 30, NatC catalytic subunit	1.2	1.37E-04	12.9
<i>NCALD</i>	neurocalcin delta	1.2	9.62E-11	87.8
<i>NEB</i>	nebulin	-1.8	2.90E-03	0.7
<i>NR2C2</i>	nuclear receptor subfamily 2 group C member 2	1.1	8.49E-04	9.8
<i>NR4A2</i>	nuclear receptor subfamily 4 group A member 2	-1.2	4.60E-03	34.0
<i>OAS3</i>	2'-5'-oligoadenylate synthetase 3	-1.3	9.26E-20	3.8
<i>OXTR</i>	oxytocin receptor	1.5	3.91E-30	6.6
<i>PLEKHG1</i>	pleckstrin homology and RhoGEF domain containing G1	1.4	2.57E-10	6.6
<i>PODXL</i>	podocalyxin like	1.7	1.85E-18	29.5
<i>PPP1R16B</i>	protein phosphatase 1 regulatory subunit 16B	1.1	1.32E-03	94.9
<i>RB1</i>	RB transcriptional corepressor 1	-1.2	6.84E-03	18.8
<i>RPS3</i>	ribosomal protein S3	-1.2	2.41E-02	164.7
<i>RUNX3</i>	runt related transcription factor 3	1.2	4.57E-13	0.9
<i>SERINC2</i>	serine incorporator 2	-1.2	1.67E-03	1.8
<i>SMIM20</i>	small integral membrane protein 20	-1.1	2.51E-04	25.6
<i>ST3GAL1</i>	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	-1.3	2.85E-20	7.7
<i>STAG3</i>	stromal antigen 3	-1.3	3.19E-14	11.2
<i>TANK</i>	TRAF family member associated NFKB activator	1.2	6.55E-03	11.0
<i>TNN</i>	tenascin N	-1.5	4.15E-12	0.1
<i>TRIM71</i>	tripartite motif containing 71	-1.2	9.68E-03	1.0
<i>TSPAN5</i>	tetraspanin 5	1.4	2.40E-11	153.0
<i>WDR7</i>	WD repeat domain 7	1.1	1.39E-03	17.6
<i>ZNF697</i>	zinc finger protein 697	1.1	9.26E-05	3.0

Highlights

- 48 h treatment of LCLs with ethanol activates immune signaling.
- Immune signaling increased without exposure to LPS.
- Interferon signaling is decreased in 48h compared to 24h treatment with ethanol.
- At baseline, cholesterol synthesis genes are lower in alcoholics than in controls.
- At baseline, cytokines in are more highly expressed in alcoholics than controls.