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 ≤ 0.05); 52% of these showed increased expression. Cells from alcoholics and controls responded similarly. The genes whose expression changed fell into many pathways: NFκB, neuroinflammation, IL6, IL2, IL8, and dendritic cell maturation pathways were activated, consistent with increased signaling by NFκB, 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 NFκB, TLRs, IL1β and TNFα 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 NFκB, and a decrease in the anti-inflammatory IL10 pathway. Analysis indicated that NFκB, 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-IIIR (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 QIAsymphony 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 μg 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 $\log_2$(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 pre-frontal 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 $p \leq 9x10^{-6}$. 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 ≤ 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, NFκB, 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, NFκB, 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 $p \leq 9x10^{-6}$; 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, $p=1.0x10^{-17}$), 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

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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 ($p<9x10^{-6}$) in the NHGRI GWAS catalog. TPM = highest value of transcripts per million transcripts in any brain region in the GTEx database.

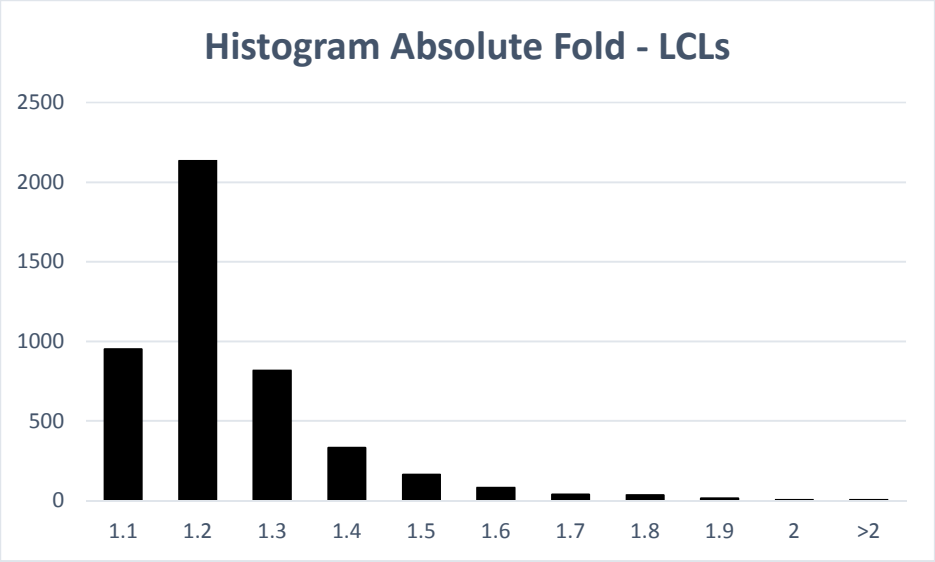

Figure 1. Distribution of Absolute fold changes for treated *vs*. untreated LCLs.

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

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

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

RNF130	ring finger protein 130	1.7	6.79E-08
RPS6KA6	ribosomal protein S6 kinase A6	1.6	1.71E-09
RSAD2	radical S-adenosyl methionine domain containing 2	-1.7	4.51E-41
SCD5	stearoyl-CoA desaturase 5	1.6	6.27E-22
SERPINB6	serpin family B member 6	1.7	1.10E-08
SETD7	SET domain containing lysine methyltransferase 7	2.3	2.39E-32
SFRP1	secreted frizzled related protein 1	1.7	1.88E-07
SLC16A14	solute carrier family 16 member 14	2.1	2.27E-47
SLC16A9	solute carrier family 16 member 9	2.0	1.04E-76
SLC47A1	solute carrier family 47 member 1	1.6	1.30E-09
SLC9A2	solute carrier family 9 member A2	1.6	4.92E-12
SMARCA1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	1.6	6.75E-04
SNPH	syntaphilin	1.9	4.03E-15
SNX7	sorting nexin 7	1.6	1.10E-08
SOAT2	sterol O-acyltransferase 2	-1.6	1.84E-11
SOGA3	SOGA family member 3	1.6	7.35E-15
SRGAP1	SLIT-ROBO Rho GTPase activating protein 1	1.6	3.17E-09
SSTR2	somatostatin receptor 2	1.5	6.79E-27
SSTR3	somatostatin receptor 3	-1.5	1.28E-19
STAT4	signal transducer and activator of transcription 4	1.7	6.96E-29
STK39	serine/threonine kinase 39	1.6	9.98E-08
STXBP1	syntaxin binding protein 1	1.9	1.01E-17
TCL6	T cell leukemia/lymphoma 6	-1.9	1.28E-15
TFCP2L1	transcription factor CP2 like 1	-2.6	7.06E-29
TFPI2	tissue factor pathway inhibitor 2	1.6	1.27E-10
TMEM136	transmembrane protein 136	1.6	6.48E-14
TMEM217	transmembrane protein 217	1.7	2.86E-23
TNFSF4	TNF superfamily member 4	1.8	1.74E-36
TPBG	trophoblast glycoprotein	1.6	4.83E-21
TUFT1	tuftelin 1	1.6	1.02E-18
VASH1	vasohibin 1	1.6	2.04E-12
VAV3	vav guanine nucleotide exchange factor 3	1.8	3.13E-14
VCAM1	vascular cell adhesion molecule 1	1.8	3.76E-30
VPREB3	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*
ACOXL	acyl-CoA oxidase-like	1.5	1.10E-02	
ADCY1	adenylate cyclase 1	-1.7	2.74E-02	1.6
AMICA1	Junction Adhesion Molecule Like (JAML)	1.5	2.56E-02	-1.1
ARHGAP24	Rho GTPase activating protein 24	-1.5	2.54E-02	1.1
ARMCX1	armadillo repeat containing, X-linked 1	-1.5	4.33E-02	1.5
ASB2	ankyrin repeat and SOCS box containing 2	1.9	3.67E-04	
ATP8B1	ATPase phospholipid transporting 8B1	1.9	1.54E-03	
BCL11A	B-cell CLL/lymphoma 11A	-1.6	4.40E-03	-1.2
C3orf14	chromosome 3 open reading frame 14	-1.9	2.28E-02	1.3
CADM1	cell adhesion molecule 1	-1.9	2.84E-02	1.6
CCDC74A	coiled-coil domain containing 74A	-1.7	5.41E-03	1.2
CD200R1	CD200 receptor 1	1.6	4.56E-03	
CDC42BPA	CDC42 binding protein kinase alpha	1.7	2.04E-02	
CLIC2	chloride intracellular channel 2	-2.1	3.63E-04	1.4
CNTLN	centlein	-2.1	2.51E-02	1.2
DLGAP1	DLG associated protein 1	1.5	4.76E-02	
DNAJC6	DnaJ heat shock protein family (Hsp40) member C6	-1.5	4.35E-02	1.1
EFNA5	ephrin A5	-1.6	2.10E-02	1.2
EFR3B	EFR3 homolog B	-1.6	4.22E-02	
EIF1AY	eukaryotic translation initiation factor 1A, Y-linked	-1.9	1.47E-02	
F2R	coagulation factor II thrombin receptor	1.5	2.66E-02	1.3
FAM160A1	family with sequence similarity 160 member A1	-1.5	1.62E-02	
FAM171A1	family with sequence similarity 171 member A1	-1.5	8.95E-03	1.6
FAM26F	family with sequence similarity 26 member F	-1.6	2.49E-02	-1.2
FCRL4	Fc receptor like 4	1.5	4.88E-02	
FCRL5	Fc receptor like 5	1.5	7.27E-03	-1.4

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

RBPMS2	RNA binding protein with multiple splicing 2	1.7	9.90E-03	
RHOU	ras homolog family member U	1.6	4.40E-02	-1.2
RIMS2	regulating synaptic membrane exocytosis 2	1.5	3.99E-02	
SAMD12	sterile alpha motif domain containing 12	1.5	1.50E-02	
SASH1	SAM and SH3 domain containing 1	1.8	4.21E-03	
SEL1L3	SEL1L family member 3	1.6	1.13E-03	-1.4
SEMA6A	semaphorin 6A	1.5	8.52E-03	
SERPINE2	serpin family E member 2	1.5	4.43E-02	
SFRP1	secreted frizzled related protein 1	-1.9	4.56E-02	1.7
SHTN1	shootin 1	-1.7	2.31E-03	1.2
SIX3	SIX homeobox 3	-1.9	1.80E-02	
SLC35F3	solute carrier family 35 member F3	-1.5	4.80E-02	1.5
SNTB1	syntrophin beta 1	1.6	1.58E-03	
STEAP1	STEAP family member 1	1.6	4.65E-02	-1.1
SYNGR1	synaptogyrin 1	1.7	1.20E-02	-1.2
TANC1	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	-1.9	1.81E-03	1.2
TBC1D30	TBC1 domain family member 30	-1.5	4.68E-02	1.5
TC2N	tandem C2 domains, nuclear	-1.6	3.95E-03	1.2
TIE1	tyrosine kinase with immunoglobulin like and EGF like domains 1	2.2	3.43E-04	
TMOD2	tropomodulin 2	-1.5	2.01E-05	1.3
TNFRSF10A	TNF receptor superfamily member 10a	1.5	3.32E-04	
WFDC2	WAP four-disulfide core domain 2	2.4	7.63E-04	-1.5
ZNF300	zinc finger protein 300	-1.8	1.45E-02	
ZNF880	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 ($p<9x10^{-6}$) 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
ANKRD44	ankyrin repeat domain 44	-1.1	1.99E-02	1.6
ARL15	ADP ribosylation factor like GTPase 15	1.2	6.90E-03	12.0
AUTS2	AUTS2, activator of transcription and developmental regulator	1.3	6.50E-22	14.6
B4GALT6	beta-1,4-galactosyltransferase 6	1.3	3.51E-06	28.9
BOD1	biorientation of chromosomes in cell division 1	1.2	1.23E-04	48.0
BPTF	bromodomain PHD finger transcription factor	-1.1	5.35E-03	38.2
C2orf88	chromosome 2 open reading frame 88	-1.1	1.99E-03	4.7
CAMTA1	calmodulin binding transcription activator 1	-1.1	1.55E-03	57.0
CAPN7	calpain 7	1.2	2.56E-04	28.0
CASZ1	castor zinc finger 1	1.3	3.48E-04	0.6
CHL1	cell adhesion molecule L1 like	-1.3	2.20E-12	39.1
CTNNA2	catenin alpha 2	-1.3	5.34E-03	63.7
FABP3	fatty acid binding protein 3	-1.7	3.92E-28	135.4
FAM81A	family with sequence similarity 81 member A	1.1	1.76E-04	32.5
FAM83D	family with sequence similarity 83 member D	1.2	3.06E-06	1.9
GALNT18	polypeptide N-acetylgalactosaminyltransferase 18	1.1	2.43E-02	27.0
GPD1L	glycerol-3-phosphate dehydrogenase 1 like	1.1	9.67E-03	34.1
IGSF9B	immunoglobulin superfamily member 9B	1.3	3.17E-03	16.7
ITPRIPL2	ITPRIP like 2	1.2	4.26E-04	2.0
KCND2	potassium voltage-gated channel subfamily D member 2	1.2	3.38E-07	79.4
LARGE		1.1	6.91E-04	15.3
LINC00158	long intergenic non-protein coding RNA 158	1.2	6.79E-03	1.4
LOC101927697		-1.2	1.12E-03	

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