Ethanol treatment of lymphoblastoid cell lines from alcoholics and non–alcoholics causes many subtle changes in gene expression

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A B S T R A C T

To elucidate the effects of a controlled exposure to ethanol on gene expression, we studied lymphoblastoid cell lines (LCLs) from 21 alcoholics and 21 controls. We cultured each cell line for 24 h with and without 75 mM ethanol and measured gene expression using microarrays. Differences in expression between LCLs from alcoholics and controls included 13 genes previously identified as associated with alcoholism or related traits, including KCNA3, DICER1, ZNF415, CAT, SLC9A9, and PPARGC1B. The paired design allowed us to detect very small changes due to ethanol treatment: ethanol altered the expression of 37% of the probe sets (51% of the unique named genes) expressed in these LCLs, most by modest amounts. Ninety-nine percent of the named genes expressed in the LCLs were also expressed in brain. Key pathways affected by ethanol include cytokine, TNF, and NFκB signaling. Among the genes affected by ethanol were ANK3, EPHB1, SLC1A1, SLC9A9, NRDI, and SH3BP5, which were reported to be associated with alcoholism or related phenotypes in 2 genome-wide association studies. Genes that either differed in expression between alcoholics and controls or were affected by ethanol exposure are candidates for further study.

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Introduction

Alcoholism is a major health problem around the world (World Health Organization, 2011). It is a complex disease with both genetic and environmental contributions to risk, and the interplay between genes and environment is likely to be important (Edenberg & Foroud, 2006; Enoch, 2012; Meyers & Dick, 2010; Rietschel & Treutlein, 2013). Alcoholism and alcoholic organ damage are consequences of repeated exposures to high levels of ethanol over long periods (Koob & Le Moal, 2005; Laakso et al., 2000; Parry, Patra, & Rehm, 2011). Understanding how cells and organs are affected by ethanol can provide clues about mechanisms of toxicity and protection. Studies of gene expression can also complement linkage and association studies, by pointing to genes that differ in basal expression between alcoholics and controls and also to genes whose expression is altered temporarily or permanently by ethanol exposure. Nicolae et al. (2010) showed that trait-associated single nucleotide polymorphisms (SNPs) are more likely to affect gene expression in LCLs (i.e., to be expression quantitative trait loci [eQTLs]), and that application of this information can enhance discovery of trait-associated SNPs for complex phenotypes.

Gene expression has been profiled in post-mortem human brain from alcoholics and controls (Flatscher-Bader et al., 2005; Iwamoto et al., 2004; Liu, Lewohl, Harris, Dodd, & Mayfield, 2007; Liu et al., 2006; Mayfield et al., 2002; McClintick et al., 2013). Those data, while important, do not allow one to disentangle the effects of long-term alcohol exposure and pre-existing expression differences. Animal models have been used to detect both innate differences in gene expression (Edenberg et al., 2005; Kimpel et al., 2007) and differences due to alcohol consumption (Rodd et al., 2008). However, for studies of living humans an accessible tissue such as blood or a cell culture surrogate such as Epstein–Barr virus (EBV) transformed LCLs can be of great value. Thibault, Hassan, and Miles (2005) concluded that in vitro assays in human cell lines are valuable for identifying changes in expression profiles upon exposure to ethanol and other drugs of addiction. Gene expression profiles of LCLs are most like the B cells from which they were derived (Min et al., 2010). They can provide insights into immune response
mechanisms that play an important role in alcoholism and its effects on the brain (Crews, Zou, & Qin, 2011; Mayfield, Ferguson, & Harris, 2013; McClintick et al., 2013). A recent study has shown substantial overlap in expression between blood and many tissues, including many regions of the brain (Sullivan, Fan, & Perou, 2006; Wright et al., 2014), suggesting they also provide a window on many otherwise inaccessible processes. LCLs have been used in the study of other complex diseases, including autism. Nishimura et al. (2007) used expression profiling of LCLs from patients affected with autism and compared the results to controls to find different sets of dysregulated genes for 2 different subtypes of autism.

We have analyzed both basal gene expression and the effects of ethanol on gene expression in LCLs from 21 alcoholics and 21 controls. We have detected differences in gene expression between LCLs from alcoholics and controls and differences caused by the ethanol exposure. Most of the effects of ethanol were modest, but the effects highlighted pathways that have changes in many genes. We have also examined the overlap between the differences we detect in LCL gene expression and the results of expression studies in brain and with data from genome-wide association studies (GWAS) to identify and prioritize promising candidate genes for association and functional studies.

**Methods**

**Cell growth**

Immortalized lymphoblastoid cell lines (LCLs) were created from peripheral blood mononuclear cells isolated from subjects recruited as part of the Collaborative Study on the Genetics of Alcoholism (Begleiter et al., 1995; Bierut et al., 2010; Edenberg & Foroud, 2006). Immortalization was by transformation with Epstein–Barr virus and early passage (>12) cultures were used. In a test of the effects of ethanol on cell growth, 2 × 10⁶ LCLs from each of 3 individuals were cultured in the presence of 0, 50, 75, or 100 mM ethanol in 10 mL RPMI1640 medium supplemented with 15% FBS, 2 mM glutamine, 50 U/mL penicillin, and 50 µg/mL streptomycin at 37 °C. For each treatment (cell line and ethanol concentration), 5 identical parallel flasks were seeded. At a given time, cells in 1 flask were counted twice, and the average number was used to calculate a growth curve and doubling time for each individual.

**Microarray analysis of LCLs**

For the microarray experiment, 2 × 10⁶ LCLs from each of 21 alcoholics and 21 non-alcoholics were seeded in 10 mL of RPMI1640 medium supplemented with 15% FBS, 2 mM glutamine, 50 U/mL penicillin, and 50 µg/mL streptomycin. Cultures were maintained in tightly capped flasks to minimize evaporation. Alcoholics were defined as meeting DSM-IV criteria for alcohol dependence (American Psychiatric Association, 1994) at age 18 years or younger. Non-alcoholics were defined as having been taken at least 1 drink of alcohol and not meeting any of 4 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 define alcoholism (Feighner et al., 1972); none was dependent on any illicit drug. Each phenotypic group (alcoholic or non-alcoholic) contained 12 males and 9 females. Growth of ethanol-treated and untreated cells was parallel by 22 h even up to 100 mM ethanol; we chose 75 mM to be within this range and to offer a good possibility of discerning effects. Cells were cultured in the absence or presence of 75 mM ethanol for 24 h, at which time cells were harvested and lysed with buffer RLT, supplied in the Qiagen RNeasy kit, and RNA extractions were conducted per the manufacturer’s protocol.

Reverse transcription and labeling used the Affymetrix 3’ IVT labeling kit and protocols (GeneChip® Expression Analysis Technical Manual, Affymetrix, Santa Clara, CA). Samples were labeled in groups balanced by sex and phenotype to the extent possible; pairs of treated and untreated samples from the same individual were labeled and hybridized at the same time. Samples were hybridized to Affymetrix HG U133 Plus 2 GeneChips® for 17 h, then washed and stained using the standard Affymetrix protocols. GeneChips® were scanned using an Affymetrix Model 3000 scanner controlled by GCOS software (Affymetrix, Santa Clara, CA). MAASS signals and detection calls were generated by GCOS. Data are available from NCBI GEO, Accession number GSE52553.

To avoid analyzing genes that were not expressed, only probe sets that were called “present” in at least 33% of the arrays in at least 1 experimental group (phenotype, treatment, sex) were selected for analysis (McClintick & Edenberg, 2006). Using these criteria, 31,528 of the 54,675 probe sets on the GeneChips were retained for analysis. The MASS data were imported into Partek Genomics Suite (Partek Inc., St. Louis, Mo.). Because we expected cell lines from different individuals to differ, analysis was done using a general linear method with repeated measures for 0 and 75 mM ethanol; the main effects factors were ethanol treatment, phenotype (alcoholic vs. non-alcoholic), sex, and labeling batch. Addition of the 3 interaction terms (sex*treatment, sex*phenotype, and phenotype*treatment) to the model did not improve the results; none of the interaction terms reached significance after correcting for multiple testing. Therefore, we present the data from the simpler model with main effects only. The p values for each factor tested were imported into R to compute false discovery rate (FDR) using the Storey q-value package (Storey & Tibshirani, 2003). Partek Genomics Suite was used for hierarchical clustering of the arrays using Euclidean distance and average linkage.

Genes that were differentially expressed either by alcohol treatment or by phenotype were analyzed using Ingenuity Pathway Analysis (Ingenuity® Systems, spring 2013 release). Duplicate probe sets were eliminated by selecting the entry with the best p value. Parameters were set to use the Ingenuity knowledge base as the reference set. Due to the large number of genes that were differentially expressed after ethanol treatment, we limited the analysis to those genes with FDR < 0.05 and minimum absolute fold change ≥1.2; for phenotype, FDR was set at ≤0.36 with no minimum fold change. We used the canonical pathway analysis to identify modified pathways and the upstream regulator analysis to identify putative factors responsible for the changes in expression. The upstream regulator analysis looks for transcription factors, cytokines, hormones, vitamins, and other signaling molecules that may be responsible for a portion of the differential expression. IPA uses its knowledge base of causal effects and the list of differentially expressed genes to predict whether a particular regulator could be activated. The activation z-score sign (±) indicates whether the upstream ‘factor’ is activated or less active in either the LCLs treated with ethanol or from alcoholics.

**Measurement of gene expression by real time PCR**

Two micrograms of total RNA (from the same RNA used for microarrays) was reverse-transcribed using the TaqMan Reverse Transcription Reagent kit (Applied Biosystems, Foster City, CA). An aliquot of the cDNA was amplified for 40 cycles on a GeneAmp 7900HT Sequence Detection System with gene-specific primers designed using the Primer Express software (Applied Biosystems). Sybr Green was used for signal detection. All analyses were carried out in triplicate, and no-template controls and dissociation curves were used to ensure specific amplification. For each primer pair, serial dilutions of a control cDNA were used to determine standard
curves, and curves with $R^2 > 0.98$ were then used to determine the mRNA levels in individual samples. The expression levels were calculated as a ratio of the mRNA level for a given gene relative to the mRNA level for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same cDNA.

**Microarray analysis of brain tissues**

Samples from 9 different regions of the brains of each of 4 individuals (2 male and 2 female; an alcoholic and a control of each sex) were obtained from the NIAAA-supported brain bank at the Tissue Resource Center located in the Neuropathology Unit of the Department of Pathology, University of Sydney, Australia. We extracted total RNA from each of the 9 regions of each individual brain: prefrontal cortex, cerebral cortex, thalamus, visual cortex, hippocampus, amygdala, caudate nucleus, putamen, and cerebellum. RNA was extracted using Trizol (Invitrogen), with a higher tolerance, withdrawal, craving, and maximum number of drinks DSM-IV symptom count, initial sensitivity to alcohol, alcohol-related phenotypes: age of onset of DSM-IV alcohol dependence, 2013; Xuei et al., 2006; Zlojutro et al., 2011; Zuo, Gelernter, et al., 2011; Lind et al., 2010; Treutlein et al., 2009; Wang et al., 2007, 2006; Mayfield et al., 2002; McClintick et al., 2013; Sokolov, Jiang, Trivedi, & Aston, 2003; Zhou, Yuan, Mash, & Goldman, 2011).

**Results**

**Effects of ethanol treatment on cell growth**

To select ethanol concentrations that would not be toxic over the 24-h course of the experiment, the response of 3 LCLs to increasing concentrations of ethanol up to 100 mM were examined. The 3 LCLs differed in their rates of doubling in the absence of ethanol (22, 28, and 35 h). Ethanol prolonged the lag phase before LCLs began logarithmic growth, but in the period from 22 to 70 h after ethanol was added, LCLs treated with 0, 50, 75, or 100 mM ethanol were in log phase. A plot of log10 (cell number) vs. time during this period fit a linear regression with $R^2 > 0.98$ for all LCLs with all concentrations of ethanol. The average doubling time in the absence of ethanol was 27.4 h, and it was 27.7 h in 75 mM ethanol (Supplementary Fig. 1). Thus at the time studied, the cells were growing exponentially. Based upon these data, we chose to examine gene expression with and without 24 h exposure to 75 mM ethanol.

**Effects of ethanol on gene expression**

For a global picture of differential gene expression, we used hierarchical clustering of the arrays. The differences between individuals were greater than the differences due to either ethanol treatment or phenotype: the ethanol-treated and untreated samples from each person invariably clustered together, whether using all 31,522 probe sets expressed or the 5000 most variable probe sets (those with the largest coefficient of variation; data not shown). Although between-person effects were large, the paired design in which ethanol-treated and untreated LCLs from each of 42 individuals were used as repeated measures allowed us to detect the widespread effects of ethanol on gene expression, even when differences were small; each individual cell line acted as its own control, reducing the noise due to inter-individual differences.

Ethanol treatment significantly affected the expression of 11,734 probe sets (37% of the expressed probe sets), representing 7183 unique, named genes, at a stringent Storey FDR < 5% (nominal $p$ value $< 0.039$). Most of the expression differences, however, were small (Fig. 1). There were 1393 named genes with absolute fold changes $>1.2$, of which 165 had an absolute fold change $>1.4$.

**Cross comparison with GWAS and human gene expression results**

We compared the LCL results with results from 14 recent genome-wide association studies (GWAS) for alcohol dependence or related phenotypes (Bierut et al., 2010; Edenberg et al., 2010; Foroud et al., 2007; Gelernter et al., 2014; Hack et al., 2011; Johnson, Drgn, Walther, & Uhl, 2011; Kapoor et al., 2013; Kendler et al., 2011; Lind et al., 2010; Treutlein et al., 2009; Wang et al., 2013; Xuei et al., 2006; Zlojutro et al., 2011; Zuo, Gelernter, et al., 2012). These studies used alcohol dependence and/or 1 or more related phenotypes: age of onset of DSM-IV alcohol dependence, DSM-IV symptom count, initial sensitivity to alcohol, alcohol tolerance, withdrawal, craving, and maximum number of drinks within a 24-h period (maxdrinks). Gene symbols were matched to gene names reported by the various groups, which frequently represented genes within a given distance from the SNP.

We also compared the LCL results to a list of genes identified as differentially expressed by 1 or more of 11 post-mortem gene expression studies in humans (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.,
Twenty-three histone genes were all decreased, more than half with absolute fold changes larger than 1.5-fold. A large number of heat shock proteins were affected by ethanol treatment. A list of differentially expressed genes with fold changes $\geq 1.1$ can be found in Supplementary Table 1.

There were 567 probe sets, representing 478 unique named genes, that differed in expression between cell lines derived from alcoholics and cell lines from non-alcoholics (at an FDR $\leq 36\%$, nominal $p$ value $\leq 0.0076$; Fig. 2). Sixty-four percent of the genes that differed by phenotype were also affected by ethanol treatment (305 genes); compared to 51% of named genes being affected by ethanol. Supplementary Table 2 lists the genes differentially expressed between alcoholics and controls.

Not unexpectedly, sex had a significant effect on gene expression: 122 probe sets, associated with 58 unique named genes, were expressed differently in males than in females, FDR $\leq 0.05$ (nominal $p$ value $\leq 2 \times 10^{-4}$). This list includes genes such as XIST and EIF1AX, which are not detectably expressed in males, and EIF1AY, DDX3Y, and NLCN4Y, which are not detectably expressed in females. Of these 58 loci, 48 mapped to either the X or Y chromosome.

Pathway analysis

The 1393 genes affected by ethanol treatment with an absolute fold change $\geq 1.2$ were used for pathway analysis. Forty-one pathways were significantly affected by ethanol treatment (Table 1). Among these were several inflammatory pathways, including IL-6 signaling, dendritic cell maturation, CD40 signaling, IL-10, and IL-9 signaling. TNFR2 (tumor necrosis factor receptor 2) signaling showed mostly increased expression. Four NFkB-related genes (NFKB2, NFKBIA, NFKBIE, and IKKBE), all with increased expression, including the NFkB pathway itself. The results from the upstream regulator analysis, shown in Supplementary Table 3, reinforce these findings. NFkB was identified as the most significantly activated upstream regulator. TNF signaling also appears activated; TNF$\alpha$, which has increased expression, is found in 17 of the pathways. Also affected were 45 cytokines, including IL6 and IL1$\beta$. All were activated except 3, 2 of which, IL10 and IL1RN, have known anti-inflammatory effects. Other harbingers of inflammation were seen: activation of interferons and Toll-like receptors.

The pathways that differed between cells from alcoholics and controls included phospholipase C signaling, G beta gamma signaling, RAN signaling, signaling by Rho family GTPases, androgen signaling, hypoxia signaling in the cardiovascular system, RhoGDI signaling, netrin signaling, tec kinase signaling, paxillin signaling, telomerase signaling, and ephrin B signaling (Table 2). RAC1, GNG2, GNA11, and RHOT2, with decreased expression in alcoholics, were common to several pathways. GNA13, SOS2, PKRKE, and RHQO2, with increased expression, were also common to multiple pathways. The upstream regulator analysis of the phenotype differences (Supplementary Table 4) shows increased signaling due to retinoic acid, vitamin D, TP53, and APP. The growth factors IGF1 (insulin-like growth factor 1) and EGFR (epidermal growth factor receptor), along with transcription factors MYC and MAX, are less active in the alcoholics.

Protein ubiquitination pathway and hypoxia signaling in the cardiovascular system were the only 2 pathways in common for treatment and phenotype. The only affected gene common to these 2 pathways is UBE2Q, a ubiquitin-conjugating enzyme, which was decreased in alcoholics and because of treatment by ethanol.

Comparison to brain expression

We detected 20,165 unique genes expressed in at least 1 brain region. Ninety-nine percent of the genes expressed in the LCLs that could be mapped to the Gene 1.0 ST arrays were expressed in at least 1 of the 9 brain regions (Supplementary Tables 1 & 2).

Confirmation by qRT-PCR

qRT-PCR was used to confirm microarray results. Genes that were previously identified by animal or human studies or related to stress or inflammatory response were selected for testing. Of the 22 genes selected for qRT-PCR based on different expression after treatment with ethanol, 20 were confirmed with a $p$ value $< 0.05$, and 1 (FOXP1) had a similar fold and direction but with $p = 0.09$ (Supplementary Table 5, Sections A & B). SRSF11, which was not confirmed, was measured on the array by 2 non-overlapping probe sets with different results, reflecting different splice variants; the differentially expressed variant contained a longer 3' UTR that was not captured by the qRT-PCR. The 11 genes selected based on differential expression between alcoholics and controls (8 overlapped with the set affected by ethanol) were confirmed with $p < 0.05$ (Supplementary Table 5, Sections B & C).

Discussion

Analyzing the effects of a 24-h exposure to ethanol on lymphoblastoid cell lines (LCLs) under identical culture conditions allowed us to focus on the direct effects of ethanol on gene expression in a single cell type without complications of organismal environmental variables such as hormonal and nutritional status or different distributions of cell types. The differences in gene expression among individuals were large, but since each individual cell line was its own control, the effects of ethanol could be isolated and measured. Ethanol at 75 mM altered the expression of 37% of the probe sets expressed in LCLs, representing 31% of the unique named genes, which is remarkable, but most changes were small in magnitude (Fig. 1). This concentration, corresponding to a blood level of 0.345 mg%, is within the range seen after heavy drinking by alcoholics (Adachi et al., 1991; Lindblad & Olsson, 1976). Almost all of these genes were also expressed in brain. Given that one cannot sample brain from living subjects, LCLs offer a well-controlled, living cell alternative that can be examined for genes affected by...
ethanol, and can help in prioritizing findings from genetic studies and biomarker studies of expression in the more complex mixture of blood cells.

Gene expression affected by ethanol

Ethanol activated many pathways related to inflammation (Table 1, Supplementary Tables 1 & 3). The Nfkb and Tnf pathways are central to inflammatory responses and alcoholic liver disease (Roh & Seki, 2013; Wang, Gao, Zakhari, & Nagy, 2012). These pathways showed strong increases in expression of many genes, including Tnfa, 15 Tnf receptors or Tnf-associated genes, and 5 Nfkb related genes (Nfkb1, Nfkb2, Nfkb1a or Nfkb1b, Ikbke). It is notable that Nfkb1 was found to be associated with risk for alcoholism (Edenberg et al., 2010). Seventy-seven genes downstream of Nfkb and 151 downstream of Tnfa were affected, as were
numerous genes downstream of the activated cytokines and more than 120 downstream of the interferons. The Toll-like receptors are also activated by ethanol. TNXP (thioredoxin interacting protein; 1.5-fold higher in LCL from alcoholics) is also increased 10% by ethanol treatment. TNXP, which functionally links ER stress to the inflammasome and activation of NFκB, was found to be 1.7-fold higher in the hippocampus of alcoholics (McClintick et al., 2013). Recently, neuroinflammation has been linked to alcoholism and may play a role in the addiction process (Crews et al., 2011; Mayfield et al., 2013). It has been hypothesized that lipopolysaccharides (LPS) introduced into circulation from the gut may be responsible for the inflammatory response in the brain barrier. Others have shown that a robust inflammatory response to ethanol does not require lipopolysaccharides from the gut—liver axis, and that a direct effect of ethanol on Toll-like receptor 4 can initiate neuroinflammation (Fernandez-Lizarbe, Montesinos, & Guerri, 2013). Our data show that a 24-h exposure to ethanol was sufficient to initiate this inflammatory response in LCLs without exposure to LPS.

Among the LCL genes differentially expressed upon exposure to ethanol, 1043 were differentially expressed in brain in 1 or more of 11 post-mortem gene expression studies, 58 of which also differed between alcoholics and controls (Supplementary Table 1). Most GWAS findings are in the non-protein coding portion of the genome, and are thought to influence gene expression. Trait-associated SNPs are more likely to be expression quantitative trait loci (Nicolae et al., 2010). We therefore examined the overlap between genes whose expression in LCLs was altered by ethanol and genes reported in GWAS studies. Two hundred eighty-four were identified by at least 1 GWAS (Supplementary Table 1, GWAS references therein), including 8 that also differed between alcoholics and controls (Supplementary Tables 1 & 2). Among the 284 genes, 12 were reported by 2 GWAS, including 2 genes associated with alcohol dependence (Kendler et al., 2011) and alcohol dependence symptom count (Wang et al., 2013); it was also associated with smoking (Vink et al., 2009) and ADHD (Kondapalli et al., 2013). SLC9A9 expression was also altered in the frontal cortex of alcoholics (Liu et al., 2006; Wang et al., 2013). SLC1A1 (high affinity glutamate transporter) is associated with alcohol dependence (Edenberg et al., 2010; Kendler et al., 2011); it was also associated with obsessive-compulsive disorder (Wendland et al., 2009) and schizophrenia (Horiiuchi et al., 2012). Three SNPs in or near SLC1A1 are correlated with gene expression levels in LCLs (Wendland et al., 2009), and are associated with increased expression in post-mortem prefrontal cortex (Horiiuchi et al., 2012). AWK3 (ankyrin 3, node of Ranvier) is associated with alcoholism (Kendler et al., 2011) and alcohol plus illegal substance dependence (Johnson et al., 2013), and also with posttraumatic stress disorder and externalizing behavior (Logue et al., 2013), bipolar disorder especially associated with stress (Leussis et al., 2013), and autism susceptibility (Bi et al., 2012). EPHB1 (ephrin receptor B1) is associated with alcoholism (Edenberg et al., 2010; Kendler et al., 2011) and also shown to differ in expression in the frontal cortex of alcoholics (Liu et al., 2007). SH3BP5, which was also differentially expressed in
alcoholics compared to controls, was identified in 2 GWAS related to alcohol dependence (Bierut et al., 2010; Johnson et al., 2011) and has been replicated recently in alcohol and nicotine co-dependence (Zuo, Zhang, et al., 2012).

Gene expression in alcoholics vs. controls

Genes that differ between alcoholics and controls were harder to detect, given the relatively high level of expression heterogeneity observed among all subjects. Such differences could reflect genetic variation between subjects including gene expression differences and gene product variations that contribute to risk, effects of repeated exposure to ethanol in the subject from whom the cells were derived, or gene × environment interactions. Most of the pathways that exhibited expression differences between LCLs from alcoholics and controls are signaling pathways, including ones associated with brain functions (Table 2). PRKCE is known to affect ethanol consumption (Olive, Mehmert, Messing, & Hodge, 2000). Thirteen genes differentially expressed in the alcoholics were associated with alcoholism in at least 1 of 14 GWAS (Supplementary Table 2; references therein). ZNF415 (Zinc finger 415, a transcriptional regulator) had the largest fold difference between alcoholics and controls (1.9-fold increase) and was previously identified by post-mortem expression (Sokolov et al., 2003) and GWAS (Kendler et al., 2011).

We did not detect significant interaction between alcoholic status and ethanol exposure. After correction of the interaction term for multiple testing, only 1 probe set for an unknown transcript had an FDR <0.95. This may be an issue of power, given the relatively small number of genes detected as differentially expressed between the alcoholics and controls. There was substantial heterogeneity between LCL from different subjects, which reduces power to detect differences between alcoholics and controls but did not greatly interfere with detection of the effects of ethanol because of our paired design.

We have identified genes and pathways that differ in expression between alcoholics and controls, and genes that are affected by ethanol treatment. In a complex disease such as alcoholism, both pre-existing genetic risk factors that might influence gene expression, and expression differences that result from heavy drinking, can contribute to the disease. LCLs are an accessible tissue model, and 99% of the genes differentially expressed in LCLs treated with ethanol that could be mapped to the Gene 1.0 ST array are also expressed in at least 1 part of the brain. Many were also identified in studies of post-mortem brain. These data can be used to prioritize genes reported by GWAS at sub-genome-wide levels.

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Appendix. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.alcohol.2014.07.004.

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