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Alcohol 32 (2004) 223–241

ALCOHOL

Mouse striatal transcriptome analysis: effects of oral self-administration of alcohol

Mariko Saito[a,b], Istvan Szakall[a,1], Reka Toth[a], Krisztina M. Kovacs[a], Melinda Oros[a,2], Vidudala V. T. S. Prasad[a], Miroslav Blumenberg[c], Csaba Vadasz[a,b,*]

[a]*Laboratory of Neurobehavioral Genetics, Nathan S. Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY 10962, USA*
[b]*Department of Psychiatry, New York University Medical Center, New York, NY 10016, USA*
[c]*The Ronald O. Perelman Department of Dermatology, New York University Medical Center, New York, NY 10016, USA*

Received 6 October 2003; received in revised form 24 February 2004; accepted 28 February 2004

Abstract

Results of recent studies support the notion that substance self-administration is partially a genetically controlled component of addiction tied to habit formation and cellular modification of the striatum. Aiming to define pathways among genomic, neural, and behavioral determinants of addiction, we investigated global striatal gene expression in a paradigm of oral self-administration of alcohol by using genomically very similar alcohol-nonpreferring B6.Cb$_5$i$_7$-α3/Vad (C5A3) and alcohol-preferring B6.Ib$_5$i$_7$-β25A/Vad (I5B25A) quasi-congenic mouse strains and their progenitors, C57BL/6By (B6By) and BALB/cJ. Expression of 12,488 genes and expressed sequence tags (ESTs) was studied by using 24 high-density oligonucleotide microarrays. Transcript signal intensity differences were analyzed with *z* test after iterative median normalization across groups and Hochberg step-down Bonferroni procedure. As expected, striatal transcriptome differences were far more extensive between the independently derived progenitor strains than between the quasi-congenic strains and their background partner, B6By. However, the genes, which were differentially expressed between the quasi-congenic strains and their background partner, were not subsets of the progenitorial differences and were not located on the chromosome segments introgressed into the quasi-congenic strains from the donor BALB/cJ strain that have been so far defined. Although 25 transcripts showed significantly different expression between the progenitor strains, only two transcripts, phosphatidylserine decarboxylase and a hypothetical 21.2-kDa protein, and one transcript, molybdenum co-factor synthesis 2, showed significantly different expression between C5A3 and I5B25A, and between B6By and I5B25A, respectively. The latter three transcripts are not located on previously identified chromosome segments introgressed from the donor BALB/cJ strain, supporting the suggestion of *trans*-acting regulatory variations among strains. Exposure to alcohol did not induce statistically significant striatal gene expression changes in any of the mouse strains. In conclusion, the results support the hypothesis that in functional genomic studies the chance of detecting function-relevant genes can be increased by the comparative analysis of quasi-congenic and background strains because the number of functionally irrelevant, differentially expressed genes between genomically similar strains is reduced. Lack of statistically significant alcohol-induced changes in transcript abundance indicated that oral self-administration had subtle effects on striatal gene expression and directed attention to important implications for the experimental design of future microarray gene expression studies on complex behaviors.

Keywords: DNA microarray; Alcohol; Addiction; Quasi-congenic mouse strains; Striatum; QTL analysis; Gene expression

1. Introduction

The neurobiologic mechanisms responsible for the development of alcoholism are not well known. Alcoholism is a complex, heritable behavioral phenotype with a significant environmental component (Prescott & Kendler, 1999), which has been the subject of intensive gene-mapping efforts [for a review, see Foroud and Li (1999)]. However, no genetic polymorphism has been identified with certainty, except the well-known alcohol and aldehyde dehydrogenases that metabolize alcohol (Chen et al., 1999; Thomasson et al., 1991).

Alcohol preference in animal models is an accepted experimental paradigm for the study of certain aspects of alcoholism (McClearn & Rodgers, 1959). This paradigm of oral self-administration of alcohol has been used in numerous quantitative trait locus (QTL)–mapping experiments. Some of the mapping experiment results have been confirmed, but,

* Corresponding author. Tel.: +1-845-398-5536; fax: +1-845-398-5531.
E-mail address: vadasz@nki.rfmh.org (C. Vadasz).
[1] Present address: Medical/Regulatory Department, Bayer Hungaria Kft, Pálya str. 4-6, Budapest, Hungary H-1012.
[2] Present address: Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary H-4012.
Editor: T.R. Jerrells

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doi: 10.1016/j.alcohol.2004.02.005

as is the case with studies with human subjects, the identity of the variant alleles is not known (Belknap et al., 2001). This lack of success in finding the genes for excessive alcohol consumption accurately signals the difficulty of the problems ahead in complex trait gene mapping (Belknap et al., 2001; Nadeau & Frankel, 2000; Vadasz, 2000). It is encouraging, however, that novel combinations of special animal models, gene mapping, and comprehensive gene expression analysis may help in identifying genes for alcohol-related behaviors (Liang et al., 2003; Tabakoff et al., 2003).

The purpose of the current study was to identify genes that may be related to alcohol dependence in alcohol-preferring and alcohol-nonpreferring quasi-congenic strains of mice by investigating strain differences in gene expression as well as the effects of alcohol on striatal gene expression. The behavioral paradigm is based on oral self-administration, which shares some characteristics with the human alcohol abuse condition. Because there is a significant genetic variation in oral self-administration of alcohol among quasi-congenic strains (Vadasz et al., 2000b), as well as that both instrumental and Pavlovian learning are involved in the development of alcohol drinking habits [for a review, see Gerdeman et al. (2003)], we hypothesized that genes that influence plasticity in alcohol-related neural and behavioral functions represent a significant component of the total genetic variation in voluntary alcohol consumption. Accordingly, the striatum was chosen as a brain region of interest in which to study gene expression because it has been implicated in habit formation in substance abuse (Ito, 2002). For reducing genetic background "noise," which could arise from heterogeneous genetic background of animal models, we used quasi-congenic alcohol-nonpreferring B6.Cb$_5$i$_7$-α3/Vad (C5A3) and alcohol-preferring B6.Ib$_5$i$_7$-β25A/Vad (I5B25A) recombinant QTL introgression (RQI) mouse strains that share about 97% of their genome (Fig. 1). This strategy offers the potential to limit differential gene expression that is unrelated to alcohol preference by limiting genetic variability to only a few loci that differ between C57BL/6By (B6By) and the quasi-congenic strains, and thereby focus attention on alcohol consumption–related changes.

In the current study, we tested the hypotheses that (1) reduced complexity of genomic strain differences is reflected as reduced complexity of striatal transcriptomic strain differences and (2) self-administration of alcohol induces significant striatal transcriptome changes. To date, in only a few studies has a congenic strain strategy been combined with microarray gene expression analysis (Aitman et al., 1999; Eaves et al., 2002; Gu et al., 2002; Lichtigfeld & Gillman, 1996; Rozzo et al., 2001). In this article, we describe the first application of global gene expression profiling of quasi-congenic RQI strains in a behavioral paradigm for self-administration of alcohol.

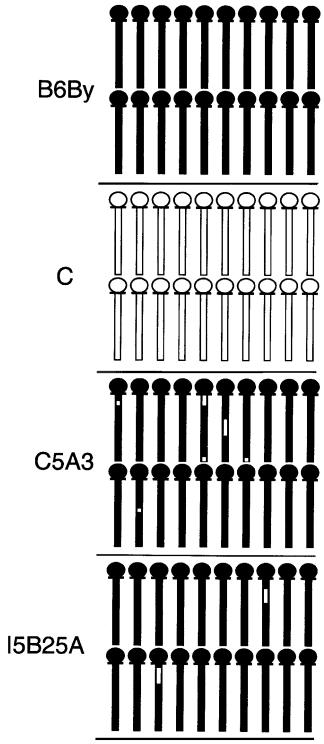

Fig. 1. Schematic illustration of the genomic architecture of quasi-congenic recombinant QTL introgression (RQI) animal models. The RQI strains were developed by small series of backcrosses to the background strain with concomitant phenotypic selection, followed by inbreeding (Abiola et al., 2003). Chromosomes of the C57BL/6By (B6By) background strain and those of the BALB/cJ (C) donor strain are shown in solid black and solid white, respectively. Genomes of alcohol-nonpreferring B6.Cb$_5$i$_7$-α3/Vad (C5A3) and alcohol-preferring B6.Ib$_5$i$_7$-β25A/Vad (I5B25A) mice are highly similar to the genome of the B6By background strain. However, they carry small chromosome segments of BALB/cJ origin (shown as small solid white blocks in the black chromosomes), which can harbor quantitative trait loci responsible for behavioral differences (unpublished observations, C. Vadasz, 2003).

2. Materials and methods

2.1. Experimental design

Four strains, two experimental conditions per strain, and three oligonucleotide microarrays per condition (24 Affymetrix GeneChip gene expression analysis arrays, Murine Genome U74v2 Set; Affymetrix, Santa Clara, CA) were analyzed. Striatal tissue samples for each microarray were pooled from two subjects. Littermate mice were chosen as pairs, and each member of the pair was assigned to either the alcohol treatment group or the control group. In the alcohol treatment group, subjects were offered alcohol and water in a two-bottle-choice, oral self-administration paradigm. Subjects in the control group were offered water in both bottles. It had been established that the progenitor and congenic strains expressed distinctly different levels of alcohol consumption in the above paradigm (Vadasz et al., 2000b). Accordingly, preplanned comparisons included only within-strain alcohol treatment versus control groups and between-strain control versus control groups comparisons. The voluntary nature of alcohol and water consumption predicted significant within-strain variation in alcohol intake and, consequently, in alcohol-induced gene expression. This source of nongenetic variation was reduced by choosing

only those pairs of littermates in which the alcohol-exposed member exhibited a consumption level of 12% alcohol similar to that of the previously established strain mean value (±20%) and the total liquid consumption was closest among the littermate pairs (Vadasz et al., 2000b).

We selected a moderately alcohol-preferring strain (B6By) as a background strain, and quasi-congenic partner strains with low alcohol preference (C5A3) and with high alcohol preference (I5B25A). Because deviations from the background strain alcohol consumption can be attributed only to genes introgressed from the donor strain, the alcohol-avoiding BALB/cJ was also included in the study. The quasi-congenic C5A3 is not highly alcohol avoiding, like the BALB/cJ is. This is a useful feature in a self-administration paradigm, because the effects of alcohol on gene expression can be studied while there is a significant genetically determined reduction in the amount of consumption.

2.2. Animals and tissue collection

Background B6By, donor BALB/cJ, and quasi-congenic C5A3 and I5B25A mice were raised and maintained in our research colony at the Animal Facility of The Nathan S. Kline Institute (Orangeburg, NY). Animals were maintained on a 12-h light/12-h dark schedule (lights on at 6:00 a.m./lights off at 6:00 p.m.) with free access to food (Purina #5008). After weaning at the age of about 5 weeks (depending on the development of the litter), littermate male mice were housed together. Construction of the quasi-congenic strains has been described (Vadasz, 1990). For the experiment on oral self-administration of alcohol, 10- to 12-week-old male mice were used and had been in the study room for at least 1 week before start of the experiment. The care and use of, as well as all procedures involving, animals were approved by The Nathan Kline Institute for Psychiatric Research Institutional Animal Care and Use Committee. All procedures were in accordance with the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research, 2001). For isolation of striatal tissue samples, 48 animals were used. Animals were euthanized by cervical dislocation, brains were hand dissected on a Peltier cold plate, and both left and right striata (caudate putamen) were collected as described (Vadasz et al., 1982). The targeted sample (head of caudate putamen) was situated in the area dorsal to the anterior commissure. The average weight of the dissected striata was 18.4 mg. For each microarray, striata from two animals were pooled.

2.3. Behavioral testing

We used a two-bottle-choice, oral self-administration paradigm with escalating alcohol concentration to measure alcohol preference (Vadasz et al., 2000a, 2000b). Briefly, the test consisted of six 3-day trials, during which male mice were housed singly and allowed to choose between alcohol solution and tap water offered in 50-ml centrifuge tubes equipped with standard stainless steel sippers. Ball bearings were not used in sipper tubes. Alcohol solution was offered in escalating concentrations: a 3% solution for trial 1 (days 1–3) was increased to 6% in trial 2 (days 3–6) and further increased to 12% for trials 3–6 (days 6–18). This arrangement provided four measures of alcohol preference at 12% [volume/volume (vol./vol.)] concentration. On the basis of the alcohol and water consumption values for the trials with 12% alcohol solution after correction for evaporation and spillage, alcohol consumption was expressed as $g*kg^{-1}*day^{-1}$. Owing to the fact that mice consume more liquid from drinking tubes closer to the food container, the position of the water and alcohol drinking tubes on the cage cover was alternated in each 3-day preference trial. The weights of the drinking tubes were measured before and after a 3-day trial by using an A&D electronic analytic balance (Bradford, MA) connected to an IBM AT computer. Data were entered automatically by using A&D COLLECT software and QUATTRO spreadsheets. Evaporation and spillage were estimated by using two control cages equipped with water and alcohol drinking tubes but without animals (Vadasz et al., 2000a).

After completion of the last consumption measurements, all drinking tubes were replaced immediately into the cages of origin, to avoid potential withdrawal effects owing to the time interval between the end of the last trial and tissue collection. For technical reasons, animals were tested and killed in two sets. Experiments with the second set started 1 day after the start of the first set. On the last day of the experiment, consumption of alcohol and water was measured, and data were processed to allow us to identify animals with alcohol consumption similar to the mean alcohol consumption of their strain of origin. The final data were available on the afternoon/evening of the last day of experiment. Accordingly, dissection was performed on the next day. In the light phase between 10:00 a.m. and 2:00 p.m. (about 30 min before animals were killed), water and alcohol drinking tubes were removed from the cage, and the cage was transported from the animal facility (within the same building) to the laboratory. Because mice are nocturnal animals with peak activities, including eating and drinking, occurring in the dark phase of the cycle, alcohol is likely to be ingested in the dark phase. The experiments were designed for the detection of long-term effects of alcohol exposure, and to ensure a uniformly low blood alcohol level (BAL) at the time animals were killed. Assuming nocturnal drinking, we estimated that, even in the case of alcohol-preferring animals with relatively high alcohol consumption ($10\ g*kg^{-1}*day^{-1}$), the BAL would be negligible by 10:00 a.m. in the light phase, when brain dissection started.

2.4. RNA preparation

The dissected striata were immediately homogenized in 0.5 ml of TRIzol reagent (Invitrogen Life Technologies, Inc.,

Rockville, MD) by using a Kontes homogenizer and a pestle with a cordless motor. The homogenate was incubated at room temperature for 5 min, frozen on dry ice, and stored at −80ºC until RNA isolation was continued. The frozen samples were thawed at room temperature, and samples from two animals in the same group were pooled. The subsequent RNA isolation with TRIzol was performed according to the manufacturer's instruction. The total RNA was further purified by using RNeasy Mini Kits (Qiagen, Valencia, CA) following the manufacturer's protocol. The quality of the total RNA was assessed by agarose gel electrophoresis and spectrophotometry. RNA gave discrete bands of 28S and 18S on agarose gel electrophoresis, and the intensity of 28S was higher than that of 18S. The A_{260}:A_{280} ratios were between 1.9 and 2.1. The yield of total RNA was 1 to 2 μg per milligram of striatum. The quality of the RNA obtained by this method was similar to that of RNA isolated from the fresh tissue (without freezing) and better than that of RNA isolated from samples frozen in liquid nitrogen and stored at −80ºC before homogenizing in TRIzol reagent.

2.5. Microarray technology

Preparation of cRNA probes was performed according to the protocol provided by Affymetrix. Briefly, 15 μg of total RNA was converted to double-stranded cDNA by using SuperScript Choice System (Invitrogen Life Technologies, Inc.) and an oligo $(dT)_{24}$ primer containing a T7 RNA polymerase promoter site (GENSET, La Jolla, CA). The resulting double-stranded cDNA was biotin-labeled by IVT (in vitro transcription) reaction by using BioArray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY) following the manufacturer's protocol, and the IVT product was purified by using RNeasy Mini Kits (Qiagen). cRNA (20 μg) was fragmented at 94ºC for 35 min in 40 μl of fragmentation buffer containing TRIS-acetate (40 mM), potassium acetate (100 mM), and magnesium acetate (30 mM). The approximate sizes of cRNAs before and after the fragmentation were checked by using agarose gel electrophoresis. Fifteen micrograms of labeled, fragmented cRNA (0.5 μg/μl) was used for hybridization to Affymetrix GeneChip expression array (Murine Genome U74v2 Set). Arrays were washed, stained with anti-biotin streptavidin-phycoerythrin–labeled antibody, and scanned by using the Agilent GeneArray Scanner system (Hewlett-Packard, Palo Alto, CA).

2.6. Quantitative real-time polymerase chain reaction

For quantitative real-time polymerase chain reaction (qPCR) experiments, 6 mice (3 in the control group and 3 in the alcohol treatment group) from each strain (B6By, BALB/cJ, C5A3, and I5B25A) were used. The experiment for oral self-administration of alcohol, the dissection of the striatum, and the total RNA isolation were performed as described above for microarray experiments except that the RNA samples from the animals were not pooled, but processed separately. Reverse transcription was performed as follows by using reagents obtained from Invitrogen Life Technologies, Inc. Reaction mixture (12 μl) containing total RNA (2 μg) and oligo (dT) primers (100 ng) was heated at 70ºC for 10 min and chilled on ice. Next, 5X First-Strand Buffer (4 μl), 0.1 M dithiothreitol [(DTT); 2 μl], and 10 mM 2′-deoxynucleoside 5′-triphosphate (dNTP) mixture (1 μl) were added, followed by incubation at 42ºC for 2 min. SuperScript II reverse transcriptase (1 μl) was mixed in, and incubation was continued at 42ºC for 60 min. The reaction was inactivated by incubation at 70ºC for 15 min. After *Escherichia coli* RNaseH [1 μl (2 units)] was added, incubation was continued at 37ºC for 20 min. The qPCR analyses were performed by using an ABI Prism 7900 sequence-detection system (Applied Biosystems, Foster City, CA). The qPCR reaction mixture consisted of cDNA from the diluted (×40) reverse transcriptase reactions (2 μl), forward and reverse primers (1 μl) with empirically determined concentrations, and 2X SYBR Green PCR Master Mix (Applied Biosystems) (12.5 μl) in a final volume of 25 μl. The qPCR was performed in triplicate for each cDNA sample. For the primers, published primer sequences were used for β-actin (Vincent et al., 2002), *Aldh1a1* [Mouse Genome Informatics (MGI) Accession ID: MGI: 1926893], and *Gnb1* (MGI Accession ID: MGI: 1205467). Primers for other genes were designed from published mouse sequences by using Primer Express software (Applied Biosystems) or Primer 3 available from the Whitehead Institute for Biomedical Research (Cambridge, MA). Intron-spanning primers were chosen whenever possible. Otherwise, total RNA samples were treated with amplification-grade deoxyribonuclease I (Invitrogen Life Technologies, Inc.) following the manufacturer's protocol. All primers were obtained from Integrated DNA Technologies (Coralville, IA). The PCR product from each primer pair gave one single peak when analyzed by dissociation curves method with the use of an ABI Prism 7900 sequence-detection system. Relative quantification of mRNA expression of each sample was calculated by a $2^{-\Delta\Delta C_T}$ method, as described by Livak and Schmittgen (2001), by using β-actin as an internal control and the sample from B6By (in the control group) as a calibrator. The results were expressed as the mean fold changes (relative to findings for the B6By control) obtained from three sets of animals. β-Actin was used as the internal standard because the result of microarray showed that the signals for β-actin were constant among the samples from different groups. The amplification efficiency of each target gene was approximately equal to that of β-actin when determined by serial dilution of template cDNA (Livak & Schmittgen, 2001).

2.7. Array data analysis

The following major steps were followed: array quantification, qualitative analysis, heuristic analysis, quantitative statistical analysis, confirmation tests, and data mining. Although working with freely behaving animals is an advantage in terms of validity of the alcoholism model, the lack of

control of the alcohol dosage represents an inherent problem because different genetic preparations with different alcohol preferences cannot be easily compared. Accordingly, alcohol-induced differential gene expression groups were not compared across strains.

2.7.1. Array quantification

To compare data from multiple arrays, the signal of each probe array was scaled to the same target intensity value. We used Affymetrix Microarray Suite version 5.0 (MAS 5.0) for data extraction, and ArrayStat version 1.0 (Imaging Research Inc., St. Catharines, Ontario, Canada) for parametric analysis.

2.7.2. Qualitative analysis

Comparisons of B6By versus BALB/cJ, B6By versus C5A3, B6By versus I5B25A, and control versus alcohol treatment in each strain were performed. The MAS 5.0 provides a statistically based Present call when a transcript is reliably detectable on the chip. If Present calls were obtained for all three chips in one group, and an Absent call was obtained for all three chips in the other group for the same transcript, the two groups were considered qualitatively different for that transcript.

2.7.3. Heuristic analysis

For multivariate heuristic analysis, a data set was used consisting of 3,427 genes and expressed sequence tags (ESTs) with Present call on all 24 chips. Data were imported into ArrayStat and normalized across eight groups centering on the median. The mean and standard deviation (S.D.) were subsequently calculated for each group. By using the mean signal intensity values for each strain, the logged (base 2) alcohol treatment group:control group ratios were calculated, and the results for B6By, C5A3, and I5B25A were subjected to multivariate analysis with self-organizing map (SOM) algorithms (J-Express Pro, MolMine AS, Bergen, Norway). The size of the SOM was 4×4 neurons. The following parameters were used: theta/momentum, 0.9/0.998; phi/momentum, 0.45/0.998; iteration limit, 4,000; nonexclusive sweep circumference, 10 (in percentage of maximum distance); neighborhood function, Gauss; distance function, Euclidean; random seed, 970767757; and lattice structure, hexagonal. Profiles of the 16 neurons were inspected in 16 thumbnail images. Each thumbnail image showed the mean expression profile of all genes lying within the sweep circumference of 10. In each thumbnail, strains are shown in the following order: B6By, C5A3, and I5B25A. Data subset represented by thumbnail #8 was chosen for complete linkage hierarchical cluster analysis with Euclidean distance measure because it showed the greatest strain differences and the smallest standard deviations for the logged ratios in the identified set of genes (see Fig. 4B).

2.7.4. Quantitative statistical analysis

The MAS 5.0 data were filtered to remove all transcript signal intensity values labeled with a statistically based Absent call. Parametric statistical comparisons of control groups were preplanned for B6By versus BALB/cJ, B6By versus C5A3, B6By versus I5B25A, C5A3 versus I5B25A, and control group versus alcohol treatment group in each strain. First, the relation between the mean and the S.D. was assessed. For removing the correlation between the mean and the S.D., log transformation (base 10) was used, and data were subsequently normalized within group by centering. The minimum sample size was set to $n = 3$ for each group. Lack of correlation between the S.D. and the mean allowed us to use the pooled–common error method for random error estimation. The program ArrayStat, in the range of ± 3.0 median absolute deviation (M.A.D.) to ± 3.5 M.A.D., established outlier-detection thresholds automatically. When an outlier was detected, the row (representing a transcript) was removed from further statistical analysis (Fig. 2). Groups were compared as independent conditions by z test. In this step, data were subjected to iterative normalization by centering to the median across groups. For correcting systematic errors across conditions, we normalized our data. We assume that most of the genes are not differentially expressed across conditions. Normalization by mean across condition may create spurious results because, if there are strongly differentially expressed genes in the different conditions, such normalization may take out part of the real effect. Iterative normalization may minimize the spurious results by attempting to base normalization on genes that are not differentially expressed. In the iterative process, first we identify which 2% of the genes exhibit the strongest differential expression. Next, this 2% is removed, and the remaining values are renormalized. These steps are repeated, removing successive 2% of the most strongly differentially expressed genes until they all appear to have been excluded from the normalization. For normalization, we used the median because strongly differentially expressed genes affect it less. In addition, the above-described iteration was applied to further minimize spurious results. Nominal alpha was set to .05. For multiple testing correction, the Hochberg step-down Bonferroni procedure was used (Hochberg, 1988).

Raw data from these studies will be made available at http://RQIgenetics.org.

2.8. Bioinformatics

Gene symbol, GenBank accession number, description, and chromosomal localization of genes from Affymetrix GeneChip expression array (Murine Genome U74v2 Set) were obtained from NCBI LocusLink (www.ncbi.nlm.nih.gov/LocusLink) and NetAffix Analysis Center (www.affymetrix.com/analysis/index.affx). The data of sequences of some of the primers used for qPCR experiments were obtained from Mouse Genome Informatics database (www.informatics.jax.org). The data obtained from Mouse Genome Server (www.ensembl.org/Mus_musculus) was used for the analysis of genome sequences of B6By mice, and Celera Discovery System (www.celeradiscoverysystem.com/index.cfm)

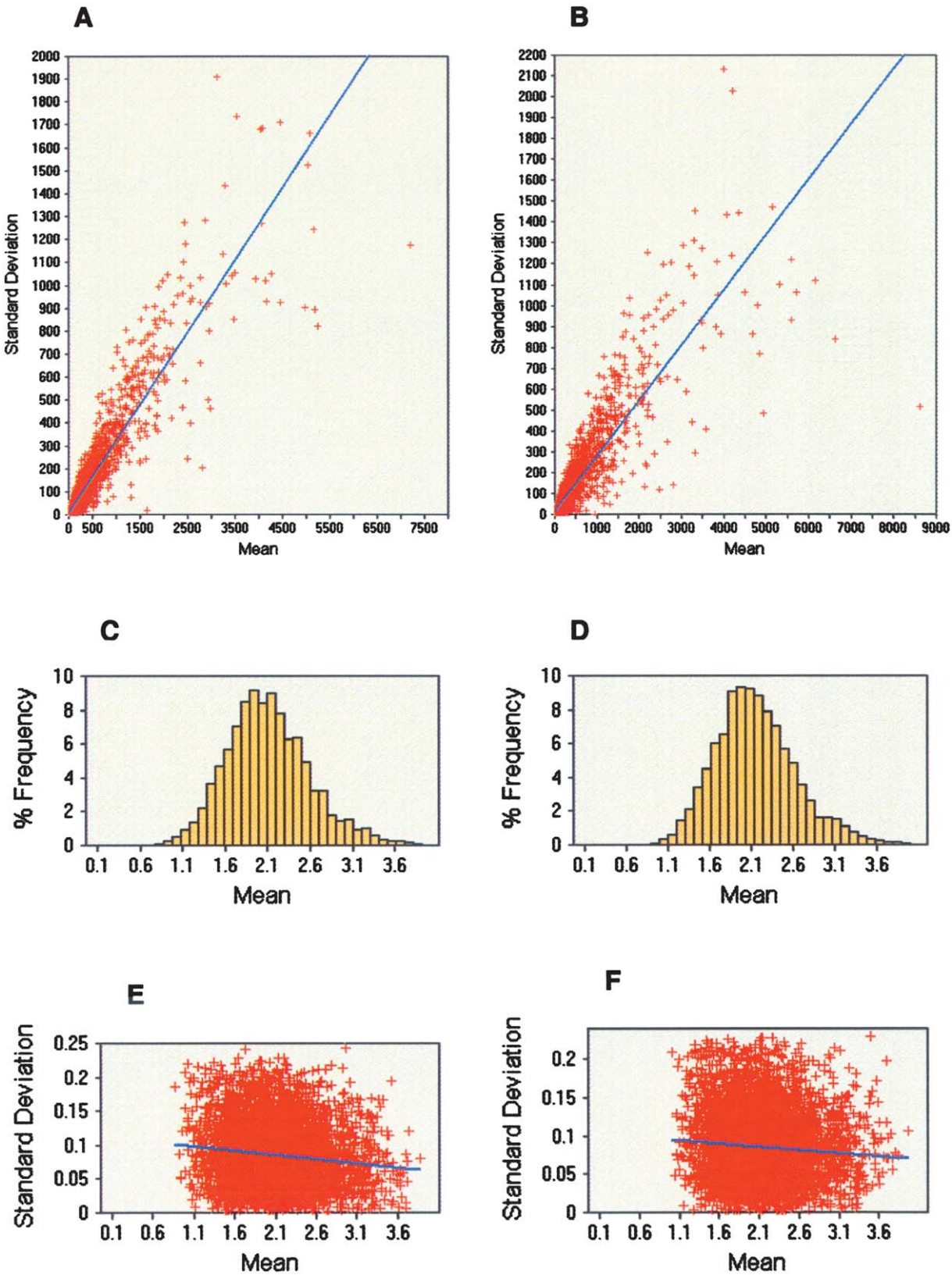

Fig. 2. Graphs of C57BL/6By (B6By) control (A, C, E) and BALB/cJ control (B, D, F) gene expression data. The charts demonstrate the distribution of data before (A, B) and after (C, D, E, F) log transformation, normalization within group, and removal of outliers. The minimum number of replicates was $n = 3$. Random error was estimated by the pooled–common error method.

was used for the analysis of genome sequences of other mouse-inbred strains. The data for gene polymorphisms were obtained from dbSNP (www.ncbi.nlm.nih.gov/SNP), Mouse Phenome Database (aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home), and Celera Discovery System. The data for the comparison of brain mRNA expression between C57BL/6J (B6) and DBA/2J (D2) were obtained from WebQTL (http://webqtl.org). Gene annotation is primarily based on the data of Rouillard et al. (2002) and the Gene Ontology Consortium data (http://dot.ped.med.umich.edu:2000/ourimage/pub/shared/JMR_pub_affyannot.html and http://cgap.nci.nih.gov/Genes/GOBrowser). The regulated genes were classified according to this table.

3. Results

3.1. Alcohol preference behavior

In this study, we used adult male mice from four strains in an alcohol preference test (18 days) offering a choice between two bottles. Each strain was divided into two groups. The alcohol treatment group had a choice between alcohol solution and water. The control group was subjected to identical conditions; however, both bottles contained water. From each group six animals were selected for gene expression studies on the basis of similarity between their individual alcohol drinking and alcohol consumption characteristic to that strain. The alcohol consumption results are shown in Fig. 3. These results are in agreement with the previously reported mean alcohol consumption values (Vadasz et al., 2000b): 0.12 (BALB/cJ), 7.82 (B6By), 2.89 (C5A3), and 11.89 (I5B25A) $g*kg^{-1}*day^{-1}$. Blood alcohol levels were not determined in this experiment. However, in a previous study BALs were measured after a 2-h two-bottle-choice preference test with 12% (vol./vol.) alcohol (Vadasz et al., 2000a). Regression analysis of these data indicated a linear relation between alcohol consumption and BAL. In the 2-h preference test, consumption at 1.0 g/kg/h corresponded to a BAL of about 50 mg/dl. In our current study, alcohol consumption was covered in a range of about 0 to 10 g/kg/day [Fig. 3; strain average for four 3-day trials with 12% (vol./vol.) alcohol]. If, for a simplified estimate, we assume that alcohol consumption was restricted to the dark period (12 h; from 6:00 p.m. until 6:00 a.m.), animals in the strain with the highest alcohol consumption consumed about 10 g/kg on the average in the course of 12 h. Thus, we may calculate an average consumption rate of 0.83 g/kg/h, and a corresponding average BAL of 41.7 mg/dl, which can be considered as a moderate value for human intoxication (Thombs et al., 2003). Because alcohol drinking is not evenly distributed, and we did not correct for the effect of alcohol metabolism, we may assume that there were periods with significantly higher and lower BALs during the dark period. In the current study, animals were killed in the light period between 10:00 a.m. and 2:00 p.m. Thus, assuming no alcohol drinking in the light period and a steady rate of alcohol metabolism of about 0.3 g/kg/h (Froehlich et al., 2001), we may conclude that the BAL was negligible at the time of removing the brains. The experimental design relied on a low estimated BAL at the time of killing the animals, because the goal of this experiment was not the detection of acute alcohol-induced changes, but detection of long-term (18-day) effects of alcohol exposure.

3.2. Categorical comparison of microarray gene expression data

The Microarray Suite 5.0 program detected 5,088 ± 322 transcripts (mean number of Present calls ± S.D. per chip in a set of 24 Affymetrix GeneChip gene expression analysis arrays). Accordingly, the mouse striatal transcriptome represents about 41% of the 12,488 probe sets of the Affymetrix GeneChip expression array (Murine Genome U74v2 Set).

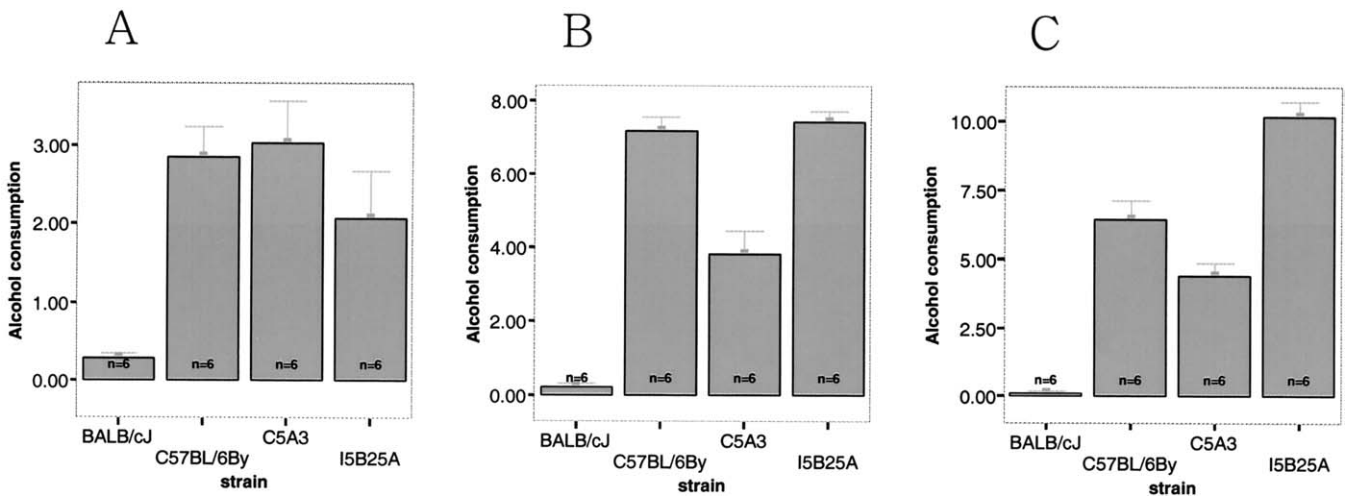

Fig. 3. Strain differences in oral self-administration of alcohol. Panels A, B, and C show consumption of 3% (trial 1), 6% (trial 2), and 12% (trials 3–6) solutions of alcohol, respectively. Bars and error bars show means and standard error of the mean (S.E.M.). Alcohol consumption on the Y axis is expressed as $g*kg^{-1}*day^{-1}$.

For a qualitative assessment of differential gene expression in two groups, transcripts were selected if they were reliably detectable (Present) on all three chips in one group and labeled as Absent on all three chips of the other group. Using the criterion of complete presence and complete absence of a transcript in two strains and looking for transcripts, which were expressed in B6By but not detectable in BALB/cJ, or in C5A3, or in I5B25A, we found 13, 3, and 9 transcripts, respectively (Table 1). In the opposite condition when the transcript was not expressed in B6By, but it was detected (Present) in the other strains, we found 8 (BALB/cJ) and 4 (C5A3) genes (Table 1). The genes differentially expressed between the quasi-congenic strains (C5A3, I5B25A) and the background B6By strain in Table 1 were not located on the previously identified chromosome segments introgressed from the donor BALB/cJ strain. A similar categorical comparison of control and alcohol treatment groups in each strain indicated no alcohol-induced gene expression changes in B6By and I5B25A. In contrast, in BALB/cJ and C5A3, 3 transcripts in each comparison showed alcohol exposure–associated changes (Table 2).

3.3. Heuristic analysis of microarray data

Filtering the 12,488 probe sets yielded 3,427 transcripts, which were Present on all 24 Affymetrix GeneChip gene expression analysis arrays. The filtering procedure removed those genes that dropped to below detectable levels from one condition to another. However, genes with consistent detectability (only Present or only Absent calls on all three chips in a condition) were subjected to categorical comparisons (see above). Signal intensities of these transcripts were subjected to multivariate analysis, applying SOM of log (base 2) transformed (alcohol treatment:control) gene expression ratios for exploring patterns in genetic differences in alcohol self-administration–induced changes in gene expression. BALB/cJ mice were not included because of their low alcohol consumption (Fig. 3). The SOM was projected on a principal components analysis plot. The two principal components extracted explained 98.1% of the total variance (Fig. 4A). After individual inspection of the profiles of each neuron (Fig. 4B), thumbnail image #8 was selected because it indicated a relatively clear pattern of strain-dependent differences in alcohol treatment:control gene expression signal intensity ratios, showing an inverse relation between gene expression and alcohol consumption across strains. Hierarchical cluster analysis of the involved transcripts separated the B6By background strain (characterized by moderate alcohol preference and no or little increase in gene expression) and the quasi-congenic C5A3 strain (characterized by low alcohol preference and little increase in gene expression) from the quasi-congenic I5B25A strain (characterized by high alcohol preference and decreased gene expression) (Fig. 5). However, parametric statistical analysis of the transcripts presented in the cluster did not support the heuristic observation: Only statistically insignificant differences were found between the alcohol treatment and control groups in the B6By, C5A3, and I5B25A strains ($P > .05$, z test).

3.4. Quantitative parametric analysis of gene expression

First, after excluding transcripts with Absent calls, as determined by the MAS 5.0 program, we assessed striatal gene expression differences between the control group of the background B6By strain and control groups of the other strains (BALB/cJ, C5A3, and I5B25A). Because in the raw data the S.D. was positively correlated with the mean, as in all other microarray data sets in this study, log (10 base) transformation was applied, which effectively removed the correlation (Fig. 2). After removing the outliers, the total sample sizes were 4,716, 4,586, 4,671, and 4,522 for B6By, BALB/cJ, C5A3, and I5B25A, respectively.

Transcript signal intensity differences between the quasi-congenic and progenitor strains were analyzed with z test after iterative median normalization across groups and Hochberg step-down Bonferroni procedure (Table 3). Between the progenitor strains, 25 statistically significant transcript differences were detected (effective α: 1.19e-005). No significant differences were found in the B6By versus C5A3 comparison when the same methods and tests were applied (effective α: 1.16e-005). Statistical comparison of the B6By and I5B25A strains yielded 1 significant transcript difference (effective α: 1.19e-005). This gene, *Mocs2* [chromosome 13, 111.6 megabase (Mb); 64 cM position], was not located on any previously identified chromosome segment introgressed from the donor BALB/cJ strain. Finally, comparison of the quasi-congenic strains C5A3 and I5B25A revealed that striatal expression of a phosphatidylserine decarboxylase transcript is higher in C5A3, whereas a transcript coding for a hypothetical 21.2-kDa protein (moderately similar to erythroid differentiation regulator) was more abundant in I5B25A (effective α: 1.51e-005).

To investigate the statistical behavior of the data, we tested the effects of the more liberal Benjamini and Hochberg False Discovery Rate (FDR) multiple testing correction. With the use of the FDR multiple testing correction, 47 significant differences were found between B6By and BALB/cJ. If the minimum number of chips per group was lowered from $n = 3$ to $n = 2$ to allow for outlier removal, and if the results were corrected with FDR multiple testing correction, the number of significant differences increased to 70. In the B6By versus C5A3 comparison, we found that keeping the minimum $n = 3$ and using the FDR multiple testing correction did not increase the number of significant differences. However, we found that allowing outlier removal and setting the minimum number of chips to $n = 2$, and using the FDR multiple testing correction, resulted in 15 statistically significant differences. Finally, comparison of B6By and I5B25A revealed that, with a minimum $n = 3$ and FDR multiple testing correction, the significant transcript

Table 1
Qualitative differences in striatal gene expression in alcohol-preferring and alcohol-nonpreferring mouse strains

GenBank accession #	Affymetrix probe	Gene/EST symbol	Strain 1 Mean ± S.E.M.	Strain 2 Mean ± S.E.M.	Description	Position Chr. #	Mb
			B6By Control	BALB/cJ Control			
Z31664	100449_g_at	*Acvrl1*	81 ± 25.5	A	activin A receptor, type II-like 1	15	101.583
L48015	100450_r_at	*Acvrl1*	226 ± 49.0	A	activin A receptor, type II-like 1	15	101.580
AI194333	103471_at	4432405K22Rik	31 ± 4.7	A	RIKEN cDNA 4432405K22 gene	10	114.841
AA673769	103934_at	*Gabt3*	74 ± 12.2	A	gamma aminobutyric acid (GABA-A) transporter-3	6	122.028
AW123271	104312_at	1110013B16Rik	89 ± 31.8	A	RIKEN cDNA 1110013B16 gene	9	106.546
AI843518•	160163_at	AA415817	95 ± 12.2	A	expressed sequence AA415817	16	13.315
AV354117	161616_f_at	2410012A13Rik	24 ± 3.3	A	RIKEN cDNA 2410012A13 gene	2	54.860
X04653	93078_at	*Ly6a*	156 ± 32	A	lymphocyte antigen 6 complex, Locus A	15	75.202
AI180784•	94312_at	AA415817	67 ± 8.1	A	expressed sequence AA415817	16	13.315
U06119	94834_at	*Ctsh*	36.4 ± 7.9	A	cathepsin H	9	90.045
L20315	99071_at	*Mpeg1*	94.5 ± 19.9	A	macrophage expressed gene 1	19	11.656
U56243	93016_at	*Ywhaq*	27 ± 7.9	A	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase, Activation protein, theta polypeptide	4	67.675
AF100956	103828_at	*Scam21*	129 ± 23.5	A	SAC2 (suppressor of actin mutations 2, homolog)-like *(S. cerevisiae)*	17	32.565
AB019600	100367_g_at	*Casp9*	A	91 ± 22.6	caspase 9	4	139.955
D13664	92593_at	*Osf2-pending*	A	22 ± 4.0	osteoblast specific factor 2 (fasciclin I-like)	3	54.561
U58974	92695_at	*Frat1*	A	44.3 ± 8.3	frequently rearranged in advanced T-cell lymphomas	19	41.417
AJ223071	93210_g_at	*Nek4*	A	12.9 ± 0.6	NIMA (never in mitosis gene a)-related expressed kinase 4	14	25.677
AW048608	96621_at	1110061L23Rik	A	151 ± 31.8	RIKEN cDNA 1110061L23 gene	7	33.784
AB026807	96979_at	*Syt10*	A	27 ± 5.2	synaptotagmin 10	15	90.113
AW214336•	97713_at	AA415817	A	224 ± 42	expressed sequence AA415817	16	13.315
U11860	98332_at	*Kcnj9*	A	98 ± 16.4	Potassium inwardly-rectifying channel, subfamily J, member 9	1	173.065
			B6By Control	C5A3 Control			
M36411	102202_s_at	*Mpv17*	33 ± 10.1	A	Mpv17 transgene, kidney disease mutant	5	29.363
AV339278	161101_r_at	*Btbd3*	51 ± 10.0	A	BTB (POZ) containing 3	2	139.955
AV292380	161421_r_at	A1504353	11 ± 3.4	A	expressed sequence A1504353	7	34.467
AW122061	104076_at	1190017O12Rik	A	23 ± 1.9	RIKEN cDNA 1190017O12Rik	16	92.963
AW124785	104410_at	*Midn*	A	101 ± 8.4	midnolin	10	79.976
AW045808	93185_at	AU021092	A	28 ± 2.9	expressed sequence AU021092	16	2.844
X66117	98863_at	*Grik2*	A	33 ± 8.1	glutamate receptor, ionotropic kainate2 (beta2)	10	49.027
			B6By Control	I5B25A Control			
AF068615	101651_at	*Cntfr*	57 ± 13.9	A	ciliary neurotrophic factor receptor	4	41.547
AA797556	103625_at	*Afg3l1*	85 ± 13.7	A	AFG3 (ATPase family gene 3)-like 1 (yeast)	8	123.731
AV292380	161421_r_at	A1504353	11 ± 3.4	A	expressed sequence A1504353	7	34.467
AV354117	161616_f_at	2410012A13Rik	24 ± 3.3	A	RIKEN cDNA 2410012A13 gene	2	54.860
AF086824	93686_s_at	*Cit*	18 ± 4.5	A	citron	5	113.242
AW045202	94209_g_at	*P-5 pending*	45 ± 10.6	A	protein disulfide isomerase related protein	12	17.397
AI838996	95061_at	*Bcas2*	13 ± 4.0	A	breast carcinoma amplified sequence 2	3	103.477
AI847282	160186_at	C030004A17Rik	29 ± 4.2	A	C030004A17 gene	9	35.138
L47335	96035_at	*Bckdha*	65 ± 22.1	A	Branched chain ketoacid dehydrogenase E1, alpha polypeptide	7	17.034

Mean and standard error of the mean (S.E.M.) represent original (untransformed) signal intensities from three Affymetrix GeneChip arrays ($n = 3$).
A indicates Absent calls for all three Affymetrix GeneChip arrays in a group.
•Differences were confirmed by quantitative real-time polymerase chain reaction.
B6By = C57BL/6By mice; C5A3 = alcohol-nonpreferring B6.Cb$_5$i$_7$-α3/Vad mice; I5B25A = alcohol-preferring B6.Ib$_5$i$_7$-β25A/Vad mice; Chr. # = chromosome number; EST = expressed sequence tag; Mb = megabase.

Table 2
Qualitative differences in striatal gene expression between alcohol treatment and control groups

GenBank accession #	Affymetrix probe	Gene/EST symbol	Control	Alcohol treatment	Description	Position	
			Mean ± S.E.M.	Mean ± S.E.M.		Chr. #	Mb
			BALB/cJ	BALB/cJ			
AA420337	101661_r_at	U	A	10.4 ± 3.1	EST	8	28.752
AV091448	161495_r_at	*Ckmt1*	35.5 ± 8.9	A	creatine kinase, mitochondrial 1, ubiquitous	2	122.994
D26089	93041_at	*Mcmd4*	A	30.1 ± 3.5	mini chromosome maintenance deficient 4 homolog (*S. cerevisiae*)	16	15.085
			C5A3	C5A3			
AW121234	101505_at	2810442O16Rik	158.2 ± 15.7	A	RIKEN cDNA 2810442O16 gene	2	154.009
AI882440	104293_at	1810045K06Rik	143.4 ± 9.7	A	RIKEN cDNA 1810045K06 gene	4	151.303
AI847033	160105_r_at	U	A	29.7 ± 3.96	EST	U	U

Mean and standard error of the mean (S.E.M.) represent original (untransformed) signal intensities from three Affymetrix GeneChip arrays ($n = 3$).
A indicates Absent calls for all three Affymetrix GeneChip arrays in a group.
C5A3 = Alcohol-nonpreferring B6.Cb$_5$i$_7$-α3/Vad mice; Chr. # = chromosome number; EST = expressed sequence tag; Mb = megabase; U = unknown.

differences increased from 1 to 3. In contrast, we found that allowing outlier removal with a minimum $n = 2$ chips per group increased the significant differences to 39 after FDR multiple testing correction.

The effects of alcohol on striatal gene expression were analyzed by methods similar to those described above, namely log (10 base) transformation, pooled-curve fit random error estimation, z test after iterative normalization across groups, and Hochberg step-down Bonferroni procedure (minimum $n = 3$ in all groups). No statistically significant alcohol drinking–induced effects were detected in B6By, BALB/cJ, C5A3, and I5B25A strains. With the use of data obtained from the most changed transcripts in the highly alcohol-preferring I5B25A strain, power analysis indicated that if we set the effective $\alpha = 1e\text{-}006$ and apply z test, we need 8 or 9 replicates to detect significant alcohol-induced changes with the power of about 0.9. Using less stringent criteria (minimum $n = 2$ per chip with outlier removal; FDR multiple testing correction), we identified significant alcohol-induced differences in gene expression (11, 7, and 17 transcripts of B6By, BALB/cJ, and I5B25A, respectively).

3.5. Confirmation with quantitative real-time polymerase chain reaction

To confirm the results of the microarray experiment, qPCR for selected genes was performed. Among 18 genes

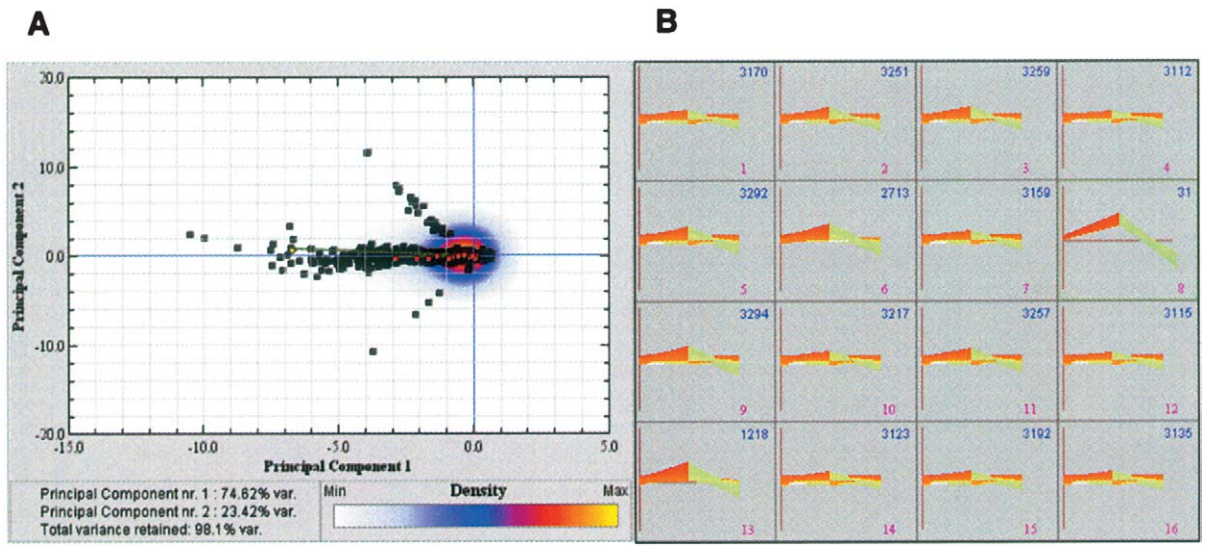

Fig. 4. Analysis of $\log_2$(alcohol treatment:control) ratios of 3,427 transcripts in alcohol-nonpreferring and alcohol-preferring quasi-congenic strains and their C57BL/6By (B6By) background strain by self-organizing map (SOM) algorithms. The left panel (A) shows the SOM projected on principal components. Each black dot represents a transcript. Density of overlapping black dots is shown by color density gradient. Neurons of SOM are indicated by red spots. The right panel (B) shows 16 thumbnail images, each representing a neuron containing the profile lying within the sweep circumference (sweep distance threshold = 10). Thumbnail #8 was selected for further analysis because it shows a consistent pattern of alcohol-induced changes in 31 transcripts. From left to right: B6By (red, increase), B6.Cb$_5$i$_7$-α3/Vad (C5A3) (red, increase), and B6.Ib$_5$i$_7$-β25A/Vad (I5B25A) (light green, decrease). BALB/cJ data were not included because of the very low alcohol consumption characteristic of this strain.

showing qualitative differences in the expression between B6By and BALB/cJ (Table 1), the expression of AA415817 gene was examined by qPCR.

The microarray result indicates that the AA415817 gene is expressed only in B6By when detected by probe sets '160163_at' and '94312_at', whereas the gene is expressed only in BALB/cJ when detected by a probe set '97713_at' (Table 1). The National Center for Biotechnology Information (NCBI) database search indicates that the sequences of the two probes ('160163_at' and '94312_at') are located in 3′ untranslated region (3′UTR) of a cDNA clone AK054329 (derived from C57BL/6J), and the sequence of '97713_at' is located in 3′UTR of a cDNA clone BC005541 (derived from CZECH II) (Fig. 6A). The probes are shown

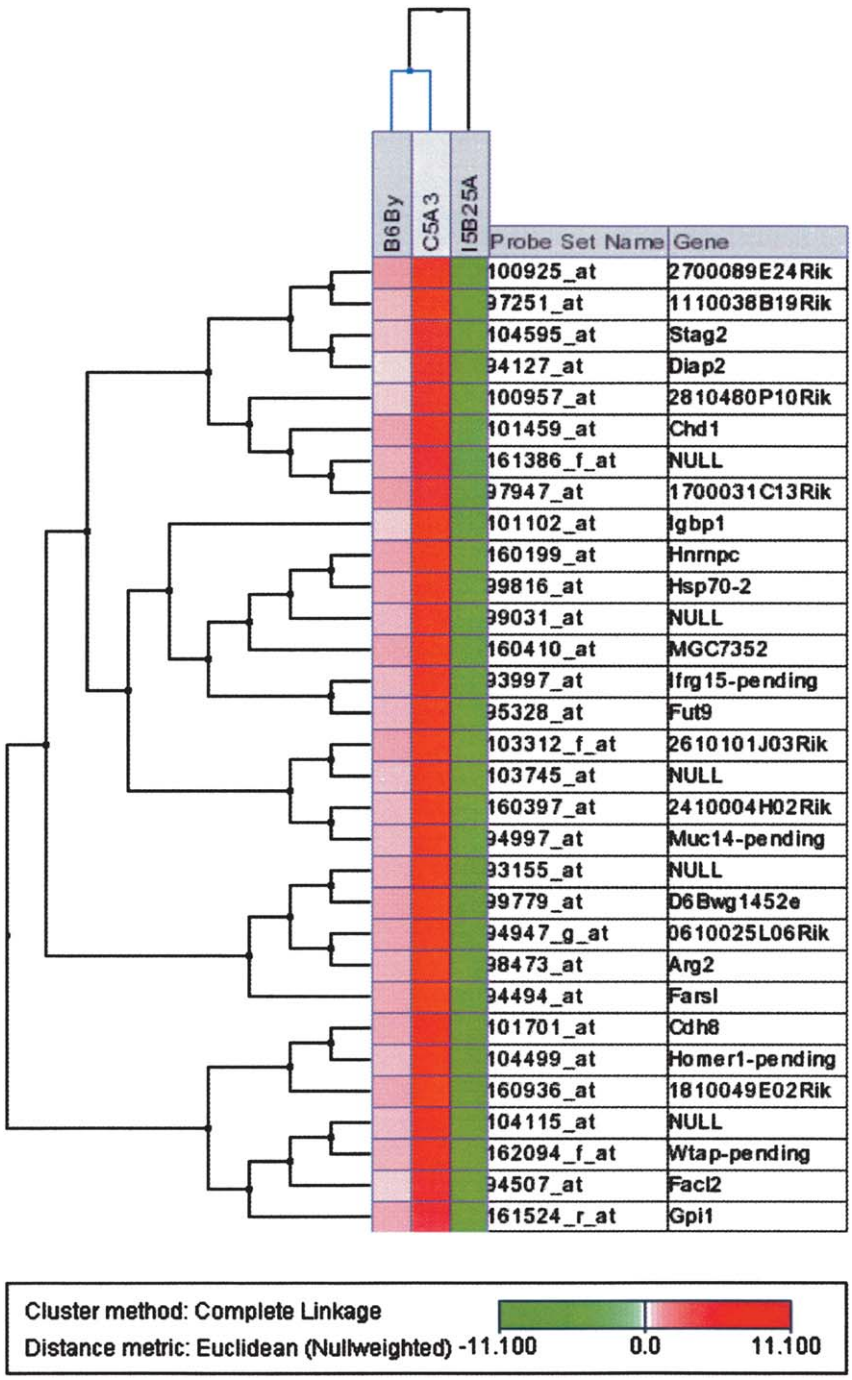

Fig. 5. Cluster analysis. A subset of data identified by self-organizing map (SOM) algorithms in thumbnail profile #8 of Fig. 4. is further analyzed by complete linkage hierarchical clustering with a Euclidean (nullweighted) distance measure. Color scale indicates alcohol-induced decrease (light to dark green) or increase (light orange to dark red) in striatal gene expression in alcohol-nonpreferring B6.Cb$_5$i$_7$-α3/Vad (C5A3), alcohol-preferring B6.Ib$_5$i$_7$-β25A/Vad (I5B25A), and moderately alcohol-preferring C57BL/6By (B6By) mouse strains. Note that in the behavioral paradigm of oral self-administration of alcohol subjects in the alcohol treatment group consumed different amounts of alcohol in a strain-dependent manner (see Fig. 3).

Table 3
Quantitative differences in striatal gene expression in alcohol-preferring and alcohol-nonpreferring mouse strains

GenBank accession #	Affymetrix probe	Gene/EST symbol	Strain 1 Mean ± S.D.	Strain 2 Mean ± S.D.	*P*	Description	Position Chr. #	Position Mb
			B6By Control	BALB/cJ Control				
Metabolism								
M74570•	100068_at	*Aldh1a1*	2.05 ± 0.10	1.48 ± 0.10	7.91E-13	aldehyde dehydrogenase family 1, subfamily A1	19	19.924
AI835461	97449_at	*Aldh7a1*	2.37 ± 0.10	1.98 ± 0.10	9.97E-07	aldehyde dehydrogenase family 7, member A1	18	56.901
X60367	104716_at	*Rbp1*	2.13 ± 0.10	1.74 ± 0.10	1.16E-06	retinol binding protein 1, cellular	9	98.418
AI841705	160482_at	*Acaa1*	2.36 ± 0.10	1.98 ± 0.10	1.92E-06	acetyl-Coenzyme A acyltransferase 1	9	119.454
Transport								
X82648	93592_at	*Apod*	2.54 ± 0.10	2.05 ± 0.10	5.10E-10	apolipoprotein D	16	31.080
U86090	160417_at	*Kif5b*	2.22 ± 0.10	1.77 ± 0.10	1.89E-08	kinesin family member 5B	18	6.183
Biosynthesis								
X80699	100729_at	*Rpl26*	2.86 ± 0.10	3.27 ± 0.10	3.37E-07	ribosomal protein L26	11	69.560
X13752	101044_at	*Alad*	1.95 ± 0.10	2.35 ± 0.10	9.52E-07	aminolevulinate, delta-, dehydratase	4	59.914
AF092050	98291_at	*B13gnt1*	1.83 ± 0.10	1.45 ± 0.10	1.71E-06	UDP-GlcNAc:betaGal beta-1,3-N-Acetylglucosaminyltransferase	11	22.917
Signal transduction and cell cycle regulation								
U29055•	94854_g_at	*Gnb1*	2.14 ± 0.10	2.66 ± 0.10	5.37E-11	guanine nucleotide binding protein, beta 1	4	152.210
U29055•	97458_at	*Gnb1*	2.44 ± 0.10	2.93 ± 0.10	9.63E-10	guanine nucleotide binding protein, beta 1	4	152.210
U29055•	94853_at	*Gnb1*	1.65 ± 0.10	2.16 ± 0.10	1.73E-10	guanine nucleotide binding protein, beta 1	4	152.143
AW046496	98149_s_at	1110033J19Rik	1.99 ± 0.10	1.57 ± 0.10	1.60E-07	RIKEN cDNA 1110033J19 gene	6	148.732
Regulation of transcription								
U33196	93740_at	*Nsep1*	3.45 ± 0.10	2.94 ± 0.10	2.79E-10	nuclease sensitive element binding protein 1	4	117.4 12
X82648	93728_at	*Tgfb1i4*	2.57 ± 0.10	2.15 ± 0.10	2.03E-07	transforming growth factor beta 1 induced transcript 4	14	66.954
Other								
AI851052	94426_at	6330575P11Rik	2.20 ± 0.10	1.63 ± 0.10	1.03E-12	RIKEN cDNA 6330575P11 gene	7	107.994
AA874329•	96156_at	1110008H02Rik	2.46 ± 0.10	1.62 ± 0.10	0	RIKEN cDNA 1110008H02 gene	1	9.964
AI504338	160975_at	U	2.02 ± 0.10	1.20 ± 0.10	0	EST	17	70.374
AB003305	101741_at	U	2.71 ± 0.10	2.01 ± 0.10	0	Psmb 5 pseudogene	11	103.503
M55424	103575_at	AI847934	1.97 ± 0.10	1.41 ± 0.10	2.53E-12	expressed sequence AI847934	14	58.752
D38613•	98782_at	U	2.65 ± 0.10	3.20 ± 0.10	9.42E-12	Mus musculus, clone IMAGE:6544937, mRNA	13	52.446
AA689927	104444_at	9430098E02Rik	1.71 ± 0.10	2.09 ± 0.10	1.41E-06	RIKEN cDNA 9430098E02 gene	8	70.418
AI595382	96535_at	U	2.23 ± 0.10	1.85 ± 0.10	2.53E-06	cDNA, RIKEN, clone:8030410G23	7	8.743
AJ001373	100989_at	*Itgb1bp1*	2.11 ± 0.10	2.58 ± 0.10	7.30E-09	integrin beta 1 binding protein 1	12	16.619
AI845165	97448_at	U	2.73 ± 0.10	2.31 ± 0.10	2.06E-07	Mus musculus, similar to phosphatidylserine decarboxylase, clone MGC:7133, mRNA	17[a]	60.943
			B6By Control	I5B25A Control				
Biosynthesis								
AW060325•	160637_at	*Mocs2*	1.54 ± 0.10	2.00 ± 0.09	4.13E-09	molybdenum cofactor synthesis 2	13	111.572
			C5A3 Control	I5B25A Control				
Other								
AI153421	96215_f_at	U	2.23 ± 0.10	2.82 ± 0.09	1.1E-12	Mus musculus mRNA for erythroid differentiation regulator, partial	U	U
AI845165•	97448_at	U	2.81 ± 0.10	2.38 ± 0.09	2.5E-07	Mus musculus, similar to phosphatidyl serine decarboxylase, clone MGC:7133 mRNA	17[a]	60.943

Mean values and standard deviation (S.D.) represent normalized, logged (base 10) signal intensity values. Sample size was $n = 3$ for all groups. Only transcripts with significantly different expression are shown (z test, nominal $\alpha = .05$; Hochberg step-down Bonferroni procedure for false-positive results).

•Differences were confirmed by quantitative real-time polymerase chain reaction.

[a]This DNA sequence was also located on chromosome 5 at 30.984 Mb and on chromosome 11 at 3.006 Mb.

B6By = C57BL/6By mice; C5A3 = alcohol-nonpreferring B6.Cb$_5$i$_7$-α3/Vad mice; I5B25A = alcohol-preferring B6.Ib$_5$i$_7$-β25A/Vad mice; Chr. # = Chromosome number; EST = expressed sequence tag; Mb = megabase; *P* = probability; U = unknown.

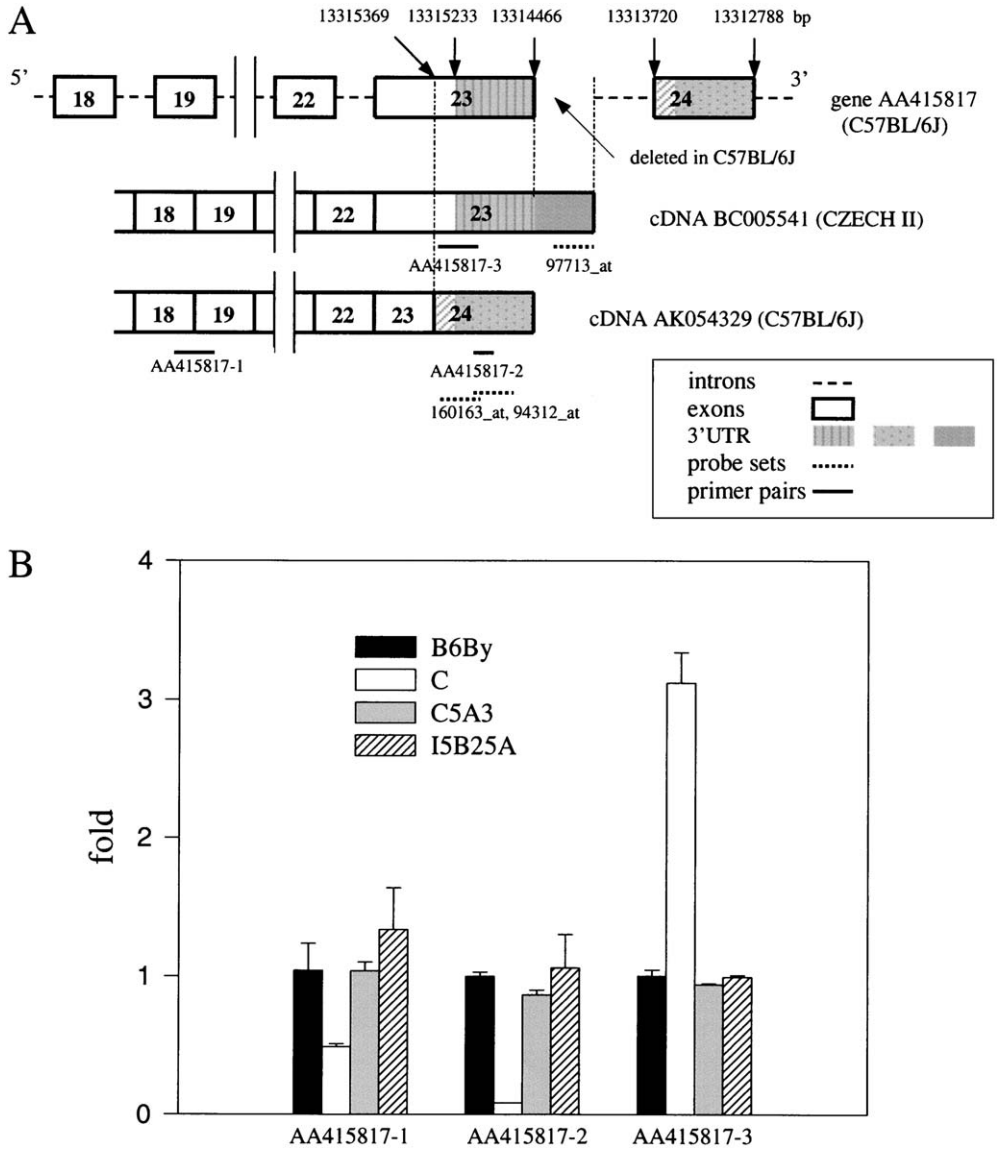

Fig. 6. Gene and cDNA structures of AA415817 and the mRNA levels measured by quantitative real-time polymerase chain reaction (qPCR). A. Schematic illustration of AA415817 gene and the cDNA variants (clones BC005541 and AK054329). AA415817 gene is located on chromosome 16 at 13.3 megabase (Mb) (www.ensembl.org/Mus_musculus). The cDNA clones BC005541 and AK054329 have mostly identical sequences except for short coding sequences near the 3′ end (illustrated with oblique lines in exon 24 in AK054329) and 3′ untranslated region (3′UTR) (illustrated as gray solid and patterned boxes) (www.ncbi.nlm.nih.gov/LocusLink). The same pattern in the boxes indicates identical sequences. Part of sequence of 3′UTR of BC005541 (shown as a solid gray box) was not listed in the public database (Ensembl) of B6 strain but was found in the consensus genome sequence derived from A/J, DBA/2J, 129X1/SvJ, and 129S1/SvImJ mice (Celera database; http://www.celeradiscoverysystem.com/index.cfm), suggesting the deletion of this sequence in C57BL/6By (B6By) mice. The position of the deletion is at 13314466 bp (www.ensembl.org/Mus_musculus), as indicated. Affymetrix probe sets (‘97713_at’, ‘160163_at’, and ‘94312_at’) are shown as dotted lines under each cDNA. B. Quantitative real-time polymerase chain reaction of variants of AA415817. Primer pairs, AA415817-1, AA415817-2, and AA415817-3, were designed by using Primer Express software. The locations of the primer pairs are shown under each cDNA as solid lines in Fig. 6A. Quantitative real-time polymerase chain reaction was performed as described in Materials and Methods, and relative quantification of mRNA expression of each sample was calculated by a $2^{-\Delta\Delta CT}$ method by using β-actin as an internal control and the sample from B6By (in the control group) as a calibrator. The results were expressed as fold changes of means, relative to B6By control. Error bars represent ± standard error of the mean (S.E.M.) obtained from three animals.

as dotted lines under the schematic view of each cDNA in Fig. 6A. Although these transcripts are similar and derived from AA415817 gene, their 3′UTR and a part of the coding region of exon 23 sequences are different. The schematic view of AA415817 gene in Fig. 6A was constructed from the public database of B6By mouse genome sequence (www.ensembl.org/Mus_musculus). It indicates that the sequence of a part of 3′UTR of BC005541 (shown as a

gray box) was not found in the gene derived from B6By mice. However, this sequence was found in the consensus genome sequence derived from A/J, DBA/2J, 129X1/SvJ, and 129S1/SvImJ in Celera database. It is likely that alternate splicing and alternate polyadenylation (that gives the clone AK054329) occurs in B6By strain because of the deletion of the DNA sequence.

Fig. 6B shows the results of qPCR performed with the use of three primer sets (AA415817-1, AA415817-2, and AA415817-3). The location of primer sets is shown in Fig. 6A as solid lines under each cDNA. The results seem to indicate that the expression of AK054329 (measured by using AA415817-2 primers) is more abundant in B6By, and the expression of BC005541 (measured by using AA415817-3) is more abundant in BALB/cJ, confirming the microarray results. Expression measured by using AA415817-1 reflected a much smaller difference between B6By and BALB/cJ, which barely reached the accepted level of significance ($P = .048$). Results of the qPCR studies show that signal levels detected by hybridization in microarray experiments do not always reflect the levels of transcript abundance, because the signal levels may be different owing to sequence variations of the transcripts caused by single nucleotide polymorphisms (SNPs), deletion, alternate splicing, alternate polyadenylation, and so forth. Affymetrix probes are often located in 3′UTR and may only hybridize to a particular variant of transcript. Therefore, confirmation by qPCR, with the use of primers located in coding regions, may be important.

From the list of genes that show strain differences assessed by Hochberg step-down Bonferroni procedure (Table 3), the expression of six randomly chosen genes (D38613, *Gnb1*, 1110008H02Rik, *Aldh1a1*, AI845165, and *Mocs2*) was compared among strains by qPCR. The results are shown in Fig. 7. The expression of D38613 and *Gnb1* was higher in BALB/cJ, and the expression of 1110008H02Rik, *Aldh1a1*, and AI845165 was lower in BALB/cJ, compared with findings for other strains, confirming the microarray result. Also, the expression of *Mocs2* was higher in I5B25A. Thus, the microarray results of all six genes selected from Table 3 are confirmed by qPCR. However, the qPCR result also shows that the expression of AI845165 was lower in I5B25A than in B6By. The microarray result of this gene indicated a twofold difference between B6By and I5B25A (B6By/I5B25A, 2.14 ± 0.6), although the significance level did not reach the criteria used in the genes listed in Table 3. Because the qPCR experiment confirmed the difference between B6By and I5B25A in the expression of AI845165, and for this difference the P value (3.68E-05) in the microarray experiment was higher than that for any other gene listed in Table 3, it is likely that the results of Table 3 would be confirmed by qPCR.

The qualitative changes observed in expression of genes *Mcmd4*, 2810442O16Rik, and 1810045K06Rik (Table 2) owing to alcohol treatment could not be confirmed with qPCR, supporting the suggestion that the selection of differentially expressed genes with the use of the Absent and Present calls is associated with a risk of type I error.

Using less stringent criteria (minimum $n = 2$ per chip with outlier removal; FDR multiple testing correction), we identified significant alcohol-induced differences in gene expression (11, 7, and 17 transcripts of B6By, BALB/cJ, and I5B25A, respectively). However, when qPCR was performed for 5 representative transcripts (*Nsf*, *Syt11*, *Copg1*, *Plp*, and *Kif5a*) the differences could be confirmed neither in B6By nor in I5B25A strains.

3.6. Expression of aldehyde dehydrogenase and alcohol dehydrogenase genes

It is noticeable that two aldehyde dehydrogenase (ALDH) genes, *Aldh1a1* and *Aldh7a1*, are among the 25 transcripts showing statistically significant differences in the expression between B6By and BALB/cJ strains (Table 3). Table 4 presents the microarray results of ALDHs and alcohol dehydrogenases (ADHs). In addition to *Aldh1a1* and 7a1, *Aldh2*, 3a2, and 9a1 were detected in our microarray experiments. The expression levels of *Aldh2* did not differ between B6By and BALB/cJ. Among ADH families, only *Adh5* (Adh III family) was detected, and the expression levels were similar in these strains.

4. Discussion

Results of the current study revealed significant natural genetic variation in striatal expression of several genes. As expected, the number of differentially expressed genes was considerably higher between genomically different alcohol-avoiding and alcohol-preferring strains than in genomically similar alcohol-nonpreferring and alcohol-preferring quasi-congenic strains. Unexpectedly, oral self-administration of alcohol did not induce striatal transcriptome changes in moderately, or even in highly, alcohol-preferring strains, supporting the suggestion that the applied methods require improvements in sensitivity and experimental design. Also, we cannot exclude the possibility that there were small or no biologically significant differences between alcohol-exposed and control animals.

4.1. Genes expressed differentially between B6By and BALB/cJ

As shown in Tables 1 and 3, there are considerable numbers of genes expressed differentially between B6By and BALB/cJ as expected from their phenotypic differences. Some of these genes may be related to strain differences in alcohol preference (Fig. 3). In human beings, it is well known that the polymorphisms in mitochondrial class 2 aldehyde dehydrogenase (ALDH2) influence the risk of alcohol dependence (Dick & Foroud, 2003). Although the mechanism of alcohol metabolism in the brain is not well known,

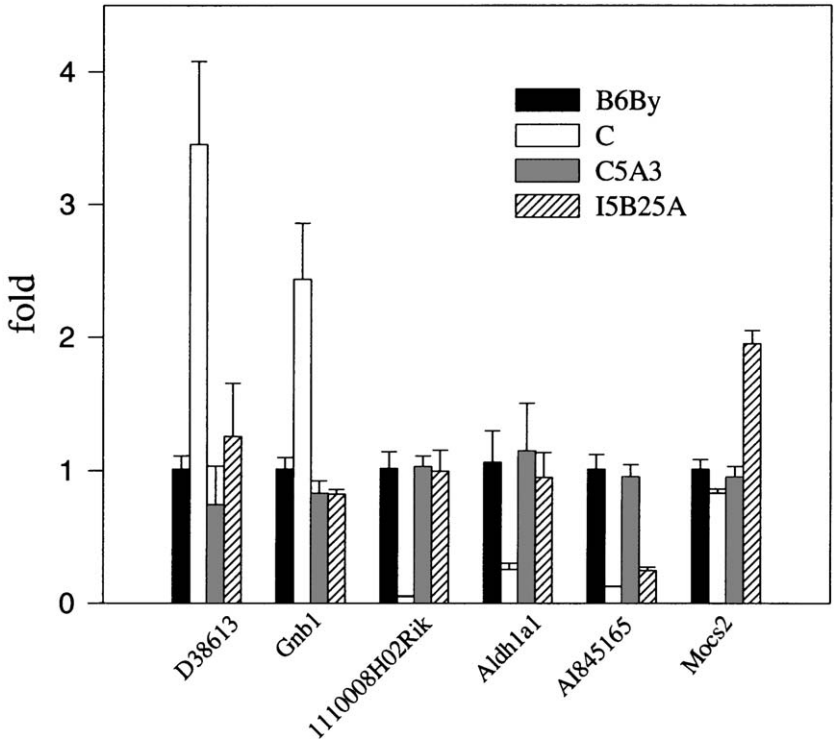

Fig. 7. Confirmation of microarray results by quantitative real-time polymerase chain reaction (qPCR). The expression of six genes listed in Table 3 was measured by qPCR to confirm the microarray results. The relative quantification of mRNA expression of each sample was calculated by a $2^{-\Delta\Delta CT}$ method by using β-actin as an internal control and the sample from C57BL/6By (B6By) mice (in the control group) as a calibrator. Genes on the X axis are represented either by their gene symbols (*Gnb1*, 1110008H02Rik, *Aldh1a1*, *Mocs2*) or by their GenBank accession number (D38613 and AI845165). The results are expressed as fold changes of means, relative to B6By control. Error bars represent ± standard error of the mean (S.E.M.) obtained from three sets of animals. C = BALB/cJ donor strain; C5A3 = alcohol-nonpreferring B6.Cb_5i_7-α3/Vad mice; I5B25A = alcohol-preferring B6.Ib_5i_7-β25A/Vad mice.

the observed differences in ALDH gene expression may affect aldehyde levels in the brain. Among ALDH families detected by our microarray experiments (*Aldh1a1*, *2*, *3a2*, *7a1*, *9a1*), the expression levels of *Aldh1a1* and *7a1* are different between B6By and BALB/cJ (Table 4). It has been reported that mRNA expression of *Aldh7a1* in the brain of alcohol-avoiding D2 mice is lower than that of B6 mice, although the expression levels of *Aldh1a1* are similar in both strains (http://webqtl.org). Kharchenko (2000) has reported that *Aldh1* and *Aldh2* were found in brain cytosol fractions of alcohol-preferring rats, whereas only *Aldh2* was found in alcohol-avoiding rats. It is possible that *Aldh1a1* and *Aldh7a1* influence aldehyde metabolism in the brain, leading to differences in alcohol preference between the strains. The difference in the expression of *Aldh7a1* between B6 and D2 seems to be due to the polymorphisms of the gene itself because the gene shows polymorphisms, including mis-sense mutation between these strains (Celera Discovery System), and because QTL for gene expression resides in the locus of the gene itself (http://webqtl.org). It remains to be solved whether the differential expression between B6By and BALB/cJ is also due to the gene polymorphisms.

Confirming findings in a recent report (Galter et al., 2003), among ADH families only *Adh5* (Adh III family) was detected in our microarray experiments. Catalases and cytochrome P450 2E1 (CYP2E1), implicated in the alcohol metabolism in the brain (Hunt, 1996; Lieber, 1997; Riveros-Rosas et al., 1997), were undetected. It has been shown that *Aldh1a1* relates to the metabolism of retinol (Molotkov & Duester, 2003). In this context, it is interesting that the BALB/cJ strain shows lower expression, not only in *Aldh1a1* but also in retinol-binding protein (Table 3). It is possible that some of the genes in the retinoic acid–mediated signaling pathway show differential expression between B6By and BALB/cJ. Two genes, activin A receptor (Table 1) and transforming growth factor (TGF) beta 1 induced transcript 4 (Table 3), are related to the TGF signaling pathway, which cross-talks with the retinoic acid pathway (Nugent et al., 2001). Interactions between TGF beta 1 and ethanol in primary cultures of cortical neurons have been demonstrated (Miller & Luo, 2002). Guanine nucleotide binding protein beta 1 (*Gnb1*), with higher expression in alcohol-avoiding mice—BALB/cJ (Table 3), 129SvEv (Sandberg et al., 2000), and DBA/2J (http://webqtl.org)—than in B6By mice, has been noted as a gene located near to a QTL for alcohol preference (Bachmanov et al., 1997; Tarantino et al., 1998). Belknap and Atkins (2001) compared eight independent studies on QTLs for murine alcohol preference drinking behavior, and the replicability of the QTLs was confirmed

Table 4
Expression of aldehyde dehydrogenase and alcohol dehydrogenase genes in B6By and BALB/cJ mouse strains

			B6By Control	BALB/cJ Control	Position	
GenBank accession #	Affymetrix probe	Gene symbol	Mean ± S.D.	Mean ± S.D.	Chr. #	Mb
Aldehyde dehydrogenase						
M74570	100068_at	*Aldh1a1*	2.05 ± 0.10	1.48* ± 0.10	19	19.924
AV364015	161956_at	*Aldh1a1*	A	A	19	19.883
X99273	101707_at	*Aldh1a2*	A	A	9	71.545
AW050387	98732_at	*Aldh1a3*	A	A	7	54.516
U96401	94778_at	*Aldh1a7*	A	n/a	19	19.974
AI647943	96057_at	*Aldh2*	2.16 ± 0.10	2.07 ± 0.10	5	118.897
U07235	96058_at	*Aldh2*	2.23 ± 0.10	2.19 ± 0.10	5	118.896
AV329607	161997_f_at	*Aldh2*	n/a	1.45 ± 0.10	5	118.896
AF033034	99548_at	*Aldh3a1*	A	n/a	11	61.740
AV089850	162331_f_at	*Aldh3a1*	A	n/a	11	61.732
U14390	99559_at	*Aldh3a2*	1.89 ± 0.10	1.78 ± 0.10	11	61.768
AV276715	161401_f_at	*Aldh3a2*	1.28 ± 0.10	n/a	11	61.768
AV362606	161783_at	*Aldh7a1*	n/a	n/a	18	56.895
AI835461	97449_at	*Aldh7a1*	2.37 ± 0.10	1.98* ± 0.10	18	56.901
AA986258	97450_s_at	*Aldh7a1*	2.49 ± 0.10	2.22* ± 0.10	18	56.901
AW120804	96243_f_at	*Aldh9a1*	1.59 ± 0.10	1.55 ± 0.10	1	168.023
Alcohol dehydrogenase						
M22679	94906_at	*Adh1 (I)*	A	A	3	138.924
AI046345	103983_at	*Adh4 (II)*	A	A	3	139.061
AJ245750	103982_s_at	*Adh4 (II)*	A	A	3	139.061
U48971	95765_at	*Adh5 (III)*	2.32 ± 0.10	2.3 ± 0.10	3	139.088
M84147	98625_s_at	*Adh5 (III)*	2.07 ± 0.10	2.09 ± 0.10	3	139.080
U20257	93695_at	*Adh7 (IV)*	A	A	3	138.865

Mean and standard deviation (S.D.) values represent normalized, logged (base 10) signal intensity values. Sample size was $n = 3$ for all groups.
A indicates Absent calls for all three Affymetrix GeneChip arrays in a group. n/a indicates inconsistency in the calls among three chips of a group.
*Group mean values are statistically different from group mean values of B6By control after Hochberg step-down Bonferroni procedure ($P < .05$).
B6By = C57BL/6By mice; Chr. # = chromosome number; Mb = megabase.

in chromosomes 2 (proximal to mid), 3 (mid to distal), 4 (distal), and 9 (proximal to mid). In addition to *Gnb1* located on distal chromosome 4, *Nsep1* (distal chromosome 4) and *Rbp1* (mid chromosome 9), listed in Table 3, seem to be located in the replicated QTLs.

4.2. Genes expressed differentially between B6By and RQI strains

The RQI strains (C5A3 and I5B25A) contain about 3% BALB/c genes on B6By genetic background (Vadasz et al., 2000b). In agreement with the genetic architecture of the strains (Fig. 1), the expression patterns of these strains were similar to the pattern observed in B6By. One may assume that the differences in expression between B6By and the two quasi-congenic strains would be proportionately smaller (hypothesis #1) and would be a subset of the differences seen between B6By and BALB/cJ (hypothesis #2). Our results on quantitative comparisons fully supported hypothesis #1, and the qualitative comparison provided tentative support. In a comparison of quantitative gene expression data in B6By and BALB/cJ, we found 25 differences, whereas only one difference was found between B6By and the quasi-congenic strains (GenBank accession number AW060325; Table 3), which corresponds well with the <5% genomic difference between background and quasi-congenic strains.

In the qualitative comparison of Present and Absent calls of gene expression in B6By and BALB/cJ, we found 21 differences (Table 1). The number of differences between B6By and C5A3 (7) and between B6By and I5B25A (9) was somewhat larger than expected. Results of our qPCR confirmation studies support the suggestion that the reliability of signal intensity data near detection level is low. In qualitative strain comparisons, when a transcript Absent call was obtained in one of the strains and Present was obtained for the other, the original untransformed signal intensity values were often low in the Present calls, indicating a higher probability of false-positive results. However, further confirmation tests are required to show whether chance occurrence of differences in qualitative comparisons might have contributed to the deviation from the expected proportions.

Our study results did not support hypothesis #2. Of the total 17 differences between the background and the quasi-congenic strains, only 1 (GenBank accession number AV354117) was present in the total 46 differences between B6By and BALB/cJ. This observation is consistent with the idea that the relation between gene polymorphism and gene expression is not necessarily direct. Gene expression should be considered a complex quantitative trait, which occasionally may reflect gene polymorphism directly. Because of the complexity of the gene expression phenotype (including

genetic and epigenetic interactions and environmental variation), we cannot expect emergence of clear subsets of progenitorial differential gene expressions in quasi-congenic strains. Variation in differential gene sets between strains can result in a complex cascade of overlapping effects in these quantitative phenotypes obscuring the expected patterns. In addition to the genetic factors, methodologic issues, such as sensitivity and reproducibility, can also contribute to the difficulties.

Comparison of the genetic architecture and gene expression data allows us to put forward hypotheses about possible causes of gene expression changes. For instance, we detected that *Mocs2* expression in I5B25A is twice as high as that of either B6By or BALB/cJ (Table 3). This pattern indicates that genes derived from B6By and BALB/cJ interact and determine the expression levels of *Mocs2* or there is a specific new mutation in the I5B25A strain. From the qPCR study, we also found that the expression levels of a gene (A1845165, similar to phosphatidylserine decarboxylase) are lower in both BALB/cJ and I5B25A, although results of our ongoing QTL studies did not indicate DNA segments introgressed from the donor BALB/cJ near the gene (A1845165) locus in I5B25A. These results support the notion of *trans*-acting regulatory variation, which was indicated for several genes whose expression levels were different between B6By and RQI strains and did not map to the differential chromosome intervals of the quasi-congenic strains (Table 1).

Although in some studies on non-behavioral phenotypes a combination of congenic lines and microarray gene expression analysis has been successfully used to identify disease susceptibility loci (Aitman et al., 1999; Rozzo et al., 2001), results of other congenic studies seem to indicate that the phenotypic effects of QTLs (residing on introgressed chromosome segments) could not be explained by global gene expression changes (Eaves et al., 2002). In this respect, our study results are similar to those of Eaves et al. (2002), indicating that the combination of quasi-congenic strains and gene expression studies can yield complex results. Because gene expression is influenced not only by its own polymorphism (*cis*-regulatory variation) but also by *trans*-acting regulatory variation induced by transcription factors (e.g., siRNA), which may reside on QTLs, it may be important to study the genes differentially expressed between congenic and background strains, even though the genes themselves are not located on QTLs. It is also possible that transcript levels of a certain polymorphic gene in a QTL may be similar between congenic and background strains, although the transcripts have different DNA sequences, and different protein activities may arise from the variant DNA sequences, leading to different phenotypes or to different *trans*-acting effects on other genes.

4.3. Changes in striatal gene expression related to oral self-administration of alcohol

Oral self-administration of alcohol in the two-bottle, free-choice paradigm requires numerous repetitions of the appetitive (substance-seeking) and consummatory (licking-drinking) phases of alcohol intake. We hypothesized that (1) in genetic models of excessive alcohol preference, alcohol-seeking and alcohol-consuming habits will develop parallel with alcohol-induced changes of the striatum in advanced stages of addiction, and (2) genes that influence plasticity in alcohol-related neural and behavioral functions represent a significant component of the total genetic variation in voluntary alcohol consumption. These hypotheses are based on theories about substance abuse, which emphasize that addiction usurps the function of the phylogenetically developed brain reward mechanism that serves food procurement and sexual reproduction (Wise & Bozarth, 1987). We selected the striatal transcriptome for study because the mesolimbic circuitry plays a prominent role in reinforcement, and during repeated exposures to reinforcing substances there is a shift of activity from the ventral striatum to the dorsal striatum, which coincides with the development of automatic behavioral patterns and habit learning (Berke & Hyman, 2000; Everitt & Wolf, 2002; Gerdeman et al., 2003; Hyman & Malenka, 2001; Koob & Le Moal, 2001). In the current study, we did not detect statistically significant alcohol-induced quantitative gene expression changes. Lack of such changes prevented analysis of the effects of genetic variability on plasticity and habit formation in the development of addiction.

Alcohol has been reported to induce differential gene expression in vitro (de la Monte et al., 2000; Luo & Miller, 1998; Thibault et al., 2000); in alcoholics (Mayfield et al., 2002; Rajgopal & Vemuri, 2001; Schafer et al., 2001); and in various species, including rats (Rimondini et al., 2002; Saito et al., 2002) and mice (Daniels & Buck, 2002; Murphy et al., 2002; Rimondini et al., 2002). The different results from these studies may have arisen for a variety of reasons. First, we used brain tissue from experimental subjects, which exercised oral self-administration of alcohol on the basis of free choice between water and alcohol solution. This behavioral paradigm is presumably less stressful than paradigms with experimenter-administered drugs, and it may affect finely regulated processes, such as habit learning and synaptic plasticity, whose changes require more sensitive experimental design/methods of detection. Second, about two thirds of the mouse genome was not tested for differential expression because those genes were not represented on the Affymetrix GeneChip gene expression analysis array we used. Third, the tissue sampling method may not be sensitive enough. If there are spatiotemporal changes in striatal activity during the development of alcohol dependence, a more refined tissue sampling design may be necessary. Fourth, the rigorous statistical methods applied may be associated with a risk of type II error. Also, power calculation supported the suggestion that the sample size used ($n = 3$) was too small to detect the effects of alcohol on gene expression, because the required sample size was $n = 8$ (at power = 0.9 and corrected effective α = 1e-005).

As to qualitative differences in striatal gene expression between alcohol treatment and control groups, altogether six genes showed differences in Present/Absent status in BALB/cJ and C5A3 strains (Table 2). Because BALB/cJ mice avoided alcohol at all concentrations, consumption of alcohol and temporary elevations of BAL during the six trials could not significantly affect gene expression in BALB/cJ. The detected qualitative differences between the BALB/cJ control and alcohol treatment groups may reflect olfactory effects, although the raw signal intensities were low for the Present calls, thus questioning the reliability of the differences. It seems that alcohol may suppress expression of 2810442O16Rik and 1810045K06Rik, whose raw signal intensities in the control group were in a range that could be confirmed by qPCR in other cases (see Table 1). However, additional experiments are needed with independent methods to confirm the results presented in Table 2.

We demonstrated that genomically similar genetic preparations such as quasi-congenic strains exhibit considerably fewer differentially expressed genes than exhibited by unrelated progenitor strains. Therefore, the use of such preparations can significantly increase the probability of finding genes relevant to the phenotype in which the preparations differ. The observation that the differentially expressed genes in the background versus quasi-congenic strain comparisons were not located on chromosome segments introgressed from the donor strain supports the idea of a greater level of complexity of the pathways from DNA polymorphism to behavior at the initial step of transcription control than previously appreciated.

In conclusion, we identified several genes differentially expressed between genetic models of alcoholism. Some of these genes may be related to the predisposition to alcohol preference.

Acknowledgments

Research described in this article was supported in part by National Institutes of Health NIAAA grant RO1 AA11031, USAMRAA grant DAMD17-00-1-0578, and Philip Morris USA Inc.

References

Abiola, O., Angel, J. M., Avner, P., Bachmanov, A. A., Belknap, J. K., Bennett, B., Blankenhorn, E. P., Blizard, D. A., Bolivar, V., Brockmann, G. A., Buck, K. J., Bureau, J. F., Casley, W. L., Chesler, E. J., Cheverud, J. M., Churchill, G. A., Cook, M., Crabbe, J. C., Crusio, W. E., Darvasi, A., de Haan, G., Dermant, P., Doerge, R. W., Elliot, R. W., Farber, C. R., Flaherty, L., Flint, J., Gershenfeld, H., Gibson, J. P., Gu, J., Gu, W., Himmelbauer, H., Hitzemann, R., Hsu, H. C., Hunter, K., Iraqi, F. F., Jansen, R. C., Johnson, T. E., Jones, B. C., Kempermann, G., Lammert, F., Lu, L., Manly, K. F., Matthews, D. B., Medrano, J. F., Mehrabian, M., Mittlemann, G., Mock, B. A., Mogil, J. S., Montagutelli, X., Morahan, G., Mountz, J. D., Nagase, H., Nowakowski, R. S., O'Hara, B. F., Osadchuk, A. V., Paigen, B., Palmer, A. A., Peirce, J. L., Pomp, D., Rosemann, M., Rosen, G. D., Schalkwyk, L. C., Seltzer, Z., Settle, S., Shimomura, K., Shou, S., Sikela, J. M., Siracusa, L. D., Spearow, J. L., Teuscher, C., Threadgill, D. W., Toth, L. A., Toye, A. A., Vadasz, C., Van Zant, G., Wakeland, E., Williams, R. W., Zhang, H. G., & Zou, F.; Complex Trait Consortium. (2003). The nature and identification of quantitative trait loci: a community's view. *Nat Rev Genet 4*, 911–916.

Aitman, T. J., Glazier, A. M., Wallace, C. A., Cooper, L. D., Norsworthy, P. J., Wahid, F. N., Al-Majali, K. M., Trembling, P. M., Mann, C. J., Shoulders, C. C., Graf, D., St. Lezin, E., Kurtz, T. W., Kren, V., Pravenec, M., Ibrahimi, A., Abumrad, N. A., Stanton, L. W., & Scott, J. (1999). Identification of *Cd36 (Fat)* as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet 21*, 76–83.

Bachmanov, A. A., Reed, D. R., Ninomiya, Y., Inoue, M., Tordoff, M. G., Price, R. A., & Beauchamp, G. K. (1997). Sucrose consumption in mice: major influence of two genetic loci affecting peripheral sensory responses. *Mamm Genome 8*, 545–548.

Belknap, J. K., & Atkins, A. L. (2001). The replicability of QTLs for murine alcohol preference drinking behavior across eight independent studies. *Mamm Genome 12*, 893–899.

Belknap, J. K., Hitzemann, R., Crabbe, J. C., Phillips, T. J., Buck, K. J., & Williams, R. W. (2001). QTL analysis and genomewide mutagenesis in mice: complementary genetic approaches to the dissection of complex traits. *Behav Genet 31*, 5–15.

Berke, J. D., & Hyman, S. E. (2000). Addiction, dopamine, and the molecular mechanisms of memory. *Neuron 25*, 515–532.

Chen, Y. C., Lu, R. B., Peng, G. S., Wang, M. F., Wang, H. K., Ko, H. C., Chang, Y. C., Lu, J. J., Li, T. K., & Yin, S. J. (1999). Alcohol metabolism and cardiovascular response In an alcoholic patient homozygous for the *ALDH2*2* variant gene allele. *Alcohol Clin Exp Res 23*, 1853–1860.

Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research. (2001). *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*. Washington, DC: The National Academies Press.

Daniels, G. M., & Buck, K. J. (2002). Expression profiling identifies strain-specific changes associated with ethanol withdrawal in mice. *Genes Brain Behav 1*, 35–45.

de la Monte, S. M., Ganju, N., Banerjee, K., Brown, N. V., Luong, T., & Wands, J. R. (2000). Partial rescue of ethanol-induced neuronal apoptosis by growth factor activation of phosphoinositol-3-kinase. *Alcohol Clin Exp Res 24*, 716–726.

Dick, D. M., & Foroud, T. (2003). Candidate genes for alcohol dependence: a review of genetic evidence from human studies. *Alcohol Clin Exp Res 27*, 868–879.

Eaves, I. A., Wicker, L. S., Ghandour, G., Lyons, P. A., Peterson, L. B., Todd, J. A., & Glynne, R. J. (2002). Combining mouse congenic strains and microarray gene expression analyses to study a complex trait: the NOD model of type 1 diabetes. *Genome Res 12*, 232–243.

Everitt, B. J., & Wolf, M. E. (2002). Psychomotor stimulant addiction: a neural systems perspective. *J Neurosci 22*, 3312–3320.

Foroud, T., & Li, T.-K. (1999). Genetics of alcoholism: a review of recent studies in human and animal models. *Am J Addict 8*, 261–278.

Froehlich, J. C., Stewart, R. B., Li, T.-K., Mosemiller, A. K., McCullough, D. E., Ho, M. C., & Kisner, J. M. (2001). Induction of steady-state blood alcohol levels: application to the study of within-session alcohol tolerance in rats. *Alcohol Clin Exp Res 25*, 370–376.

Galter, D., Carmine, A., Buervenich, S., Duester, G., & Olson, L. (2003). Distribution of class I, III and IV alcohol dehydrogenase mRNAs in the adult rat, mouse and human brain. *Eur J Biochem 270*, 1316–1326.

Gerdeman, G. L., Partridge, J. G., Lupica, C. R., & Lovinger, D. M. (2003). It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci 26*, 184–192.

Gu, W., Li, X., Lau, W.K.-H., Edderkaoui, B., Donahae, L. R., Rosen, C. J., Beamer, W. G., Shultz, K. L., Srivastava, A., Mohan, S., & Baylink, D. J. (2002). Gene expression between a congenic strain that

contains a quantitative trait locus of high bone density from CAST/EiJ and its wild-type strain C57BL/6J. *Funct Integr Genomics 1*, 375–386.

Hochberg, Y. (1988). A sharper Bonferroni procedure for multiple tests of significance. *Biometrika 75*, 800–803.

Hunt, W. A. (1996). Role of acetaldehyde in the actions of ethanol on the brain—a review. *Alcohol 13*, 147–151.

Hyman, S. E., & Malenka, R. C. (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat Rev Neurosci 2*, 695–703.

Ito, C. (2002). Analysis of overall gene expression induced by amphetamine and phencyclidine: novel targets for the treatment of drug psychosis and schizophrenia. *Curr Pharm Des 8*, 147–153.

Kharchenko, N. K. (2000). [Role of opiate system in mechanisms of formation of alcohol-dependence]. *Fiziol Zh 46*, 9–13.

Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology 24*, 97–129.

Liang, T., Spence, J., Liu, L., Strother, W. N., Chang, H. W., Ellison, J. A., Lumeng, L., Li, T.-K., Foroud, T., & Carr, L. G. (2003). α-Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats. *Proc Natl Acad Sci U S A 100*, 4690–4695.

Lichtigfeld, F. J., & Gillman, M. A. (1996). Role of dopamine mesolimbic system in opioid action of psychotropic analgesic nitrous oxide in alcohol and drug withdrawal. *Clin Neuropharmacol 19*, 246–251.

Lieber, C. S. (1997). Ethanol metabolism, cirrhosis and alcoholism. *Clin Chim Acta 257*, 59–84.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods 25*, 402–408.

Luo, J., & Miller, M. W. (1998). Growth factor–mediated neural proliferation: target of ethanol toxicity. *Brain Res Brain Res Rev 27*, 157–167.

Mayfield, R. D., Lewohl, J. M., Dodd, P. R., Herlihy, A., Liu, J., & Harris, R. A. (2002). Patterns of gene expression are altered in the frontal and motor cortices of human alcoholics. *J Neurochem 81*, 802–813.

McClearn, G. E., & Rodgers, D. A. (1959). Differences in alcohol preference among inbred strains of mice. *Q J Stud Alcohol 20*, 691–695.

Miller, M. W., & Luo, J. (2002). Effects of ethanol and transforming growth factor β (TGFβ) on neuronal proliferation and nCAM expression. *Alcohol Clin Exp Res 26*, 1281–1285.

Molotkov, A., & Duester, G. (2003). Genetic evidence that retinaldehyde dehydrogenase *Raldh1 (Aldh1a1)* functions downstream of alcohol dehydrogenase *Adh1* in metabolism of retinol to retinoic acid. *J Biol Chem 278*, 36085–36090.

Murphy, B. C., Chiu, T., Harrison, M., Uddin, R. K., & Singh, S. M. (2002). Examination of ethanol responsive liver and brain specific gene expression, in the mouse strains with variable ethanol preferences, using cDNA expression arrays. *Biochem Genet 40*, 395–410.

Nadeau, J. H., & Frankel, W. N. (2000). The roads from phenotypic variation to gene discovery: mutagenesis versus QTLs. *Nat Genet 25*, 381–384.

Nugent, P., Kusek, J. C., Pisano, M. M., & Greene, R. M. (2001). Convergence of cAMP, TGF-beta and retinoic acid signaling pathways in cells of the embryonic palate. *Life Sci 69*, 2091–2102.

Prescott, C. A., & Kendler, K. S. (1999). Age at first drink and risk for alcoholism: a noncausal association. *Alcohol Clin Exp Res 23*, 101–107.

Rajgopal, Y., & Vemuri, M. C. (2001). Ethanol induced changes in cyclin-dependent kinase-5 activity and its activators, P35, P67 (Munc-18) in rat brain. *Neurosci Lett 308*, 173–176.

Rimondini, R., Arlinde, C., Sommer, W., & Heilig, M. (2002). Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J 16*, 27–35.

Riveros-Rosas, H., Julian-Sanchez, A., & Pina, E. (1997). Enzymology of ethanol and acetaldehyde metabolism in mammals. *Arch Med Res 28*, 453–471.

Rouillard, J.-M., Herbert, C. J., & Zuker, M. (2002). OligoArray: genome-scale oligonucleotide design for microarrays. *Bioinformatics 18*, 486–487.

Rozzo, S. J., Allard, J. D., Choubey, D., Vyse, T. J., Izui, S., Peltz, G., & Kotzin, B. L. (2001). Evidence for an interferon-inducible gene, *Ifi202*, in the susceptibility to systemic lupus. *Immunity 15*, 435–443.

Saito, M., Smiley, J., Toth, R., & Vadasz, C. (2002). Microarray analysis of gene expression in rat hippocampus after chronic ethanol treatment. *Neurochem Res 27*, 1221–1229.

Sandberg, R., Yasuda, R., Pankratz, D. G., Carter, T. A., Del Rio, J. A., Wodicka, L., Mayford, M., Lockhart, D. J., & Barlow, C. (2000). Regional and strain-specific gene expression mapping in the adult mouse brain. *Proc Natl Acad Sci U S A 97*, 11038–11043.

Schafer, G. L., Crabbe, J. C., & Wiren, K. M. (2001). Ethanol-regulated gene expression of neuroendocrine specific protein in mice: brain region and genotype specificity. *Brain Res 897*, 139–149.

Tabakoff, B., Bhave, S. V., & Hoffman, P. L. (2003). Selective breeding, quantitative trait locus analysis, and gene arrays identify candidate genes for complex drug-related behaviors. *J Neurosci 23*, 4491–4498.

Tarantino, L. M., McClearn, G. E., Rodriguez, L. A., & Plomin, R. (1998). Confirmation of quantitative trait loci for alcohol preference in mice. *Alcohol Clin Exp Res 22*, 1099–1105.

Thibault, C., Lai, C., Wilke, N., Duong, B., Olive, M. F., Rahman, S., Dong, H., Hodge, C. W., Lockhart, D. J., & Miles, M. F. (2000). Expression profiling of neural cells reveals specific patterns of ethanol-responsive gene expression. *Mol Pharmacol 58*, 1593–1600.

Thomasson, H. R., Edenberg, H. J., Crabb, D. W., Mai, X. L., Jerome, R. E., Li, T.-K., Wang, S. P., Lin, Y. T., Lu, R. B., & Yin, S. J. (1991). Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet 48*, 677–681.

Thombs, D. L., Olds, R. S., & Snyder, B. M. (2003). Field assessment of BAC data to study late-night college drinking. *J Stud Alcohol 64*, 322–330.

Vadasz, C. (1990). Development of congenic recombinant inbred neurological animal model lines. *Mouse Genome 88*, 16–18.

Vadasz, C. (2000). Analysis of complex traits: mutagenesis versus QTLs. *Nat Genet 26*, 395.

Vadasz, C., Baker, H., Joh, T. H., Lajtha, A., & Reis, D. J. (1982). The inheritance and genetic correlation of tyrosine hydroxylase activities in the substantia nigra and corpus striatum in the C x B recombinant inbred mouse strains. *Brain Res 234*, 1–9.

Vadasz, C., Saito, M., Balla, A., Kiraly, I., Vadasz, C. II, Gyetvai, B., Mikics, E., Pierson, D., Brown, D., & Nelson, J. C. (2000a). Mapping of quantitative trait loci for ethanol preference in quasi-congenic strains. *Alcohol 20*, 161–171.

Vadasz, C., Saito, M., Gyetvai, B., Mikics, E., & Vadasz, C. II. (2000b). Scanning of five chromosomes for alcohol consumption loci. *Alcohol 22*, 25–34.

Vincent, V. A. M., DeVoss, J. J., Ryan, H. S., & Murphy, G. M. Jr. (2002). Analysis of neuronal gene expression with laser capture microdissection. *J Neurosci Res 69*, 578–586.

Wise, R. A., & Bozarth, M. A. (1987). A psychomotor stimulant theory of addiction. *Psychol Rev 94*, 469–492.