

Candidate genes, pathways and mechanisms for alcoholism: an expanded convergent functional genomics approach

ZA Rodd¹, BA Bertsch^{1,2},
WN Strother¹, H Le-Niculescu^{1,2},
Y Balaraman^{1,2}, E Hayden¹,
RE Jerome³, L Lumeng⁴,
Jl Nurnberger Jr¹, HJ Edenberg³,
WJ McBride¹ and
AB Niculescu^{1,2}

¹Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN, USA; ²Department of Psychiatry, Laboratory of Neurophenomics, Indiana University School of Medicine, Indianapolis, IN, USA; ³Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA and ⁴Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Correspondence:

Professor A Niculescu, Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202, USA.
E-mail: anicules@iupui.edu

Received 5 June 2006; revised 27 July 2006;
accepted 7 August 2006; published online
10 October 2006

We describe a comprehensive translational approach for identifying candidate genes for alcoholism. The approach relies on the cross-matching of animal model brain gene expression data with human genetic linkage data, as well as human tissue data and biological roles data, an approach termed convergent functional genomics. An analysis of three animal model paradigms, based on inbred alcohol-preferring (iP) and alcohol-non-preferring (iNP) rats, and their response to treatments with alcohol, was used. A comprehensive analysis of microarray gene expression data from five key brain regions (frontal cortex, amygdala, caudate-putamen, nucleus accumbens and hippocampus) was carried out. The Bayesian-like integration of multiple independent lines of evidence, each by itself lacking sufficient discriminatory power, led to the identification of high probability candidate genes, pathways and mechanisms for alcoholism. These data reveal that alcohol has pleiotropic effects on multiple systems, which may explain the diverse neuropsychiatric and medical pathology in alcoholism. Some of the pathways identified suggest avenues for pharmacotherapy of alcoholism with existing agents, such as angiotensin-converting enzyme (ACE) inhibitors. Experiments we carried out in alcohol-preferring rats with an ACE inhibitor show a marked modulation of alcohol intake. Other pathways are new potential targets for drug development. The emergent overall picture is that physical and physiological robustness may permit alcohol-preferring individuals to withstand the aversive effects of alcohol. In conjunction with a higher reactivity to its rewarding effects, they may be able to ingest enough of this nonspecific drug for a strong hedonic and addictive effect to occur.

The Pharmacogenomics Journal (2007) 7, 222–256; doi:10.1038/sj.tpj.6500420; published online 10 October 2006

Keywords: alcoholism; genetics; convergent functional genomics; brain; microarrays; pharmacotherapy

Introduction

Alcoholism is a prevalent problem in the United States and across the world. During the course of their lives, approximately 10–13% of Americans are affected by alcohol dependence.¹ Alcohol dependence is a chronic relapsing disorder characterized by the inability to self-control drinking, tolerance to the effects of alcohol, characteristic withdrawal symptoms and negative personal as well as social consequences.^{2–5} Family, twin and adoption studies have shown that genetic factors contribute 40–70% of the risk for the development of

alcoholism⁶⁻⁹ and exposure to alcohol has been shown to change gene expression patterns in the brains of both experimental animals and humans.^{10,11} Alcoholism is likely a polygenic, non-Mendelian disorder with variable penetrance and complex clinical phenotypes. As a result, with a few notable exceptions,¹²⁻²⁰ human genetic studies so far have had limited success in definitively identifying individual genes involved in the pathophysiology of alcoholism.

Convergent functional genomics (CFG)²¹⁻²⁴ is an approach that aims to unravel the genetics of complex neuropsychiatric disorders, and has been applied with some success to bipolar and related disorders.²⁵ CFG integrates gene expression data from a relevant animal model with human genetic linkage data, as well as human tissue gene expression data (post-mortem brain, lymphocytes) and biological roles data, as a Bayesian way of cross-validating findings, reducing uncertainty and coming up with a short list of high probability candidate genes. Those genes can subsequently be followed up in a prioritized manner and studied on an individual basis, in mouse transgenic models and human candidate gene association studies, for definitive confirmation of their involvement in disease pathophysiology. In this study, we have applied our expanded CFG approach^{24,25} (Figure 1) to identify candidate genes for alcoholism and related disorders.

Internal lines of evidence

The experimental approaches used to produce the animal model data for CFG analysis were carried out in two rat lines selectively bred for divergent alcohol preference; inbred alcohol-preferring (iP) vs inbred alcohol-non-preferring (iNP) rats. Five brain regions were chosen for gene expression studies in these rat lines: the frontal cortex (FC), amygdala (AMY), caudate-putamen (CP), nucleus accumbens (NAC) and hippocampus (HIP). Animal studies, human imaging and post-mortem analyses have previously provided evidence that these regions are implicated in alcoholism.²⁶⁻²⁸

Data for the analysis came from studies of three experimental paradigms. Paradigm 1²⁹ examined basal level of gene expression in the brains of the alcohol-naïve iP and iNP lines of rats. This basal comparison was performed to determine innate differences between these two lines with a marked divergence in the willingness to consume alcohol. It is hypothesized that the innate differences in gene expression between the iP and iNP would involve some of the genes associated with an increased susceptibility for alcohol dependence. The second paradigm examined the effects of chronic 24-h free-choice alcohol consumption on gene expression in iP rats compared with alcohol-naïve iP rats.¹⁰ This paradigm looked for gene expression changes in the brain associated with the direct influence of peripherally self-administered alcohol in the genetically susceptible rats. In the third paradigm,³⁰ iP rats were allowed to directly self-infuse alcohol directly into the posterior ventral tegmental area (VTA), the originating area of the mesolimbic dopamine system. The advantage of this latter procedure is that it isolates the neurocircuitry involved in alcohol reinforce-

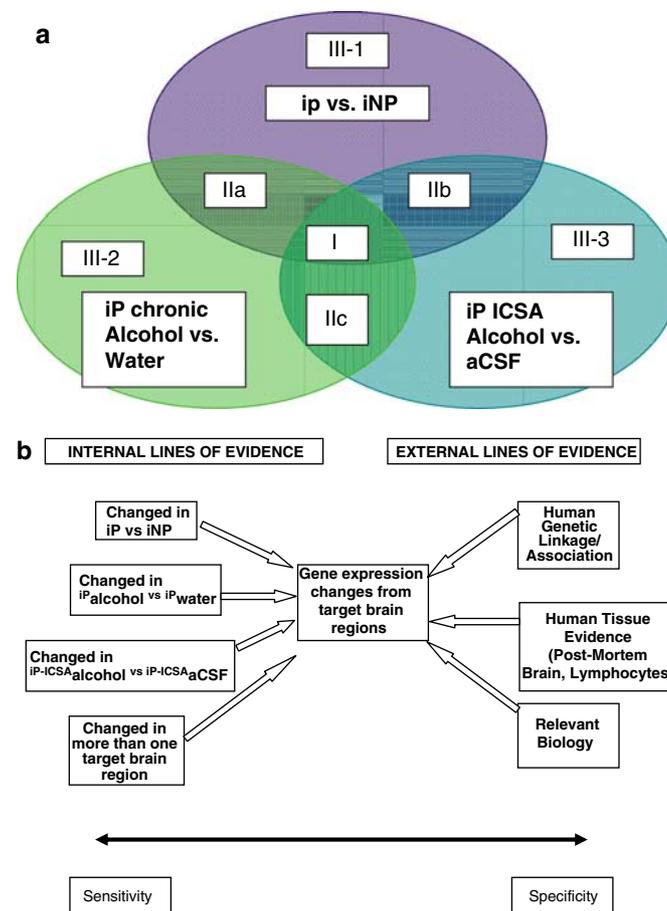


Figure 1 Design of experiments and data analysis. (a) Venn diagram depicting genes changed in the various paradigms used and their classification into Categories I–III. (b) CFG: Multiple converging independent internal and external lines of evidence for Bayesian cross-validation of findings.

ment, and eliminates the peripheral effects of alcohol. Following the establishment of alcohol self-administration into the posterior VTA, gene expression levels in target brain areas were measured and compared with P rats that received artificial cerebral spinal fluid (aCSF) infusions into the posterior VTA.

Recognizing that not all the genes that show alterations in expression in the individual paradigms are necessarily associated with alcoholism, we propose that genes that show changes in expression in more than one paradigm are more likely to be central to the pathophysiology of alcoholism and are higher probability candidate genes. We labeled those genes that had expression changes in all three paradigms in the same brain region, Category I genes. Category II genes showed changes in two paradigms in the same brain region (Category IIa genes were those that changed both in Paradigms 1 and 2, Category IIb genes changed both in Paradigms 1 and 3 and Category IIc genes were those that changed in both Paradigms 2 and 3). Category III genes changed in only one paradigm (Category III-1, III-2 and III-3

genes were from Paradigms 1, 2 and 3, respectively) (Figure 1 – Venn diagram). We also reasoned that genes that had expression changes in more than one of the brain regions have a higher probability of being positive findings compared to genes that changed in a single region, at the very least for reproducibility reasons, as the assaying of different brain regions are essentially independent experiments.

Our approach of looking at the overlaps of three very different alcohol-related paradigms also has the virtue of factoring out, at least in part, gene expression changes that are potentially artifactual, related to the inbreeding process or to the experimental manipulations involved in each particular paradigm, and not to alcohol. This is a very important point, as inbred strains are vulnerable to the accumulation of a variety of aberrations at later generations.^{31,32} For these reasons, we are not focusing in this report on genes that are changed exclusively in a single paradigm of the three studied (Category III genes).

External lines of evidence

We used three external cross-validators in our expanded CFG analysis.²⁵ First, each gene was assessed to see if it mapped to a linkage locus that had been reported to be associated with alcoholism. Our criterion was mapping within 10 centimorgans (cM) of a marker that has shown significant evidence of linkage²¹ to alcoholism, with a lod score >2 in at least one published study. We also looked more broadly at cross-matching with linkage data from other neuropsychiatric disorders (bipolar disorder, schizophrenia), based on the rationale that their clinical comorbidity with alcoholism may be due, at least in part, to genetic overlap.³³ Second, we searched to see if there was any human tissue data (post-mortem brain, lymphocytes, fibroblasts) showing expression changes of the gene in patients who had had alcoholism or, more broadly, other neuropsychiatric disorders (bipolar disorder, schizophrenia, major depression, anxiety, other substance dependence disorders, dementia, suicide). Third, we looked at the known biological functions associated with the gene and asked if it had any relevance to the pathophysiology of alcoholism and/or other neuropsychiatric disorders. Including disorders other than alcoholism in our external lines of evidence arguably dilutes the specificity of our approach. We nevertheless decided to include them as a way of increasing sensitivity, based on the emerging clinical, neurobiological and genetic evidence of substantial overlap between these disorders and alcoholism,^{18,33–37} and the likelihood that published alcohol-related data sets to date are non-exhaustive. To address the issue of specificity, we decided to weight differentially the significance of the association with alcoholism with a score of 1, and with any other neuropsychiatric disorder with a lesser score of 0.5.

Scoring the lines of evidence

Each gene in our data set was studied using four internal and three external lines of evidence (Figure 2). For each paradigm, an empirical score of 1 was given for its

contributory line of evidence (gene expression change) in the same brain region. As such, a Category I gene would receive 3 points, a Category II genes 2 points and a Category III gene 1 point. If a gene showed expression changes in multiple brain regions, 0.5 point was given for each paradigm in which it showed up as changed in other brain regions also. A maximum score of 4.5 can thus be generated by the four internal lines of evidence. For the external lines of evidence, a gene that mapped within 10cM of a marker for alcoholism was given 1 point, and within 10cM of a marker for another neuropsychiatric disorder, 0.5 point. Genes that had a biological function relevant to alcoholism received 1 point, and if their biological function was relevant to the pathophysiology of another neuropsychiatric disorder (bipolar disorder, schizophrenia, major depression, anxiety, other substance dependence disorders, dementia, suicide), 0.5 point. Last but not least, a gene that had published evidence of expression changes in human tissue data (post-mortem brains, lymphocytes, fibroblasts) from patients with a history of alcoholism was given 1 point, and for tissue data from another neuropsychiatric disorder, 0.5 point. The maximum score a gene could receive in any external line of evidence was set at 1.5 points. A maximum score of 4.5 can be generated by the external lines of evidence, like for the internal lines of evidence. Thus, totaling all the internal and external lines of evidence gives a maximum possible score of 9 points, with the internal evidence and the external evidence weighted equally.

It has not escaped our attention that different ways of scoring the independent lines of evidence could be used, which might give somewhat different results in terms of the prioritization of the top candidate genes, if not in terms of the actual content of the list *per se*. However, our simple weighted scoring is arguably a reasonable compromise between specificity and sensitivity, between focus and broadness.

Results

Number of genes

A total of 3246 unique genes were changed with a $P < 0.05$ in the three paradigms we used (Figure 1a) (iP vs INP, chronic alcohol in iP, intracranial self-administration (ICSA) alcohol in iP), and in the five brain regions examined (FC, AMY, CP, NAC, HIP). In Paradigms 1 and 2, the highest number of genes changed was in the CP, followed by the AMY. In Paradigm 3, NAC had the highest number of gene changes, followed by AMY.

By using our internal convergence analysis (Figure 1b), we narrowed the field to just four Category I genes (changed in all three paradigms – three independent lines of evidence) and 166 Category II genes (changed in at least two paradigms – two independent lines of evidence). The four genes changed in common in all three rat model experimental paradigms, two in the AMY (CD81, nucleoporin-like 1 (NUPL1)) and two in the CP (phosphatidylethanolamine-binding protein (PBP) and aldehyde dehydrogenase family

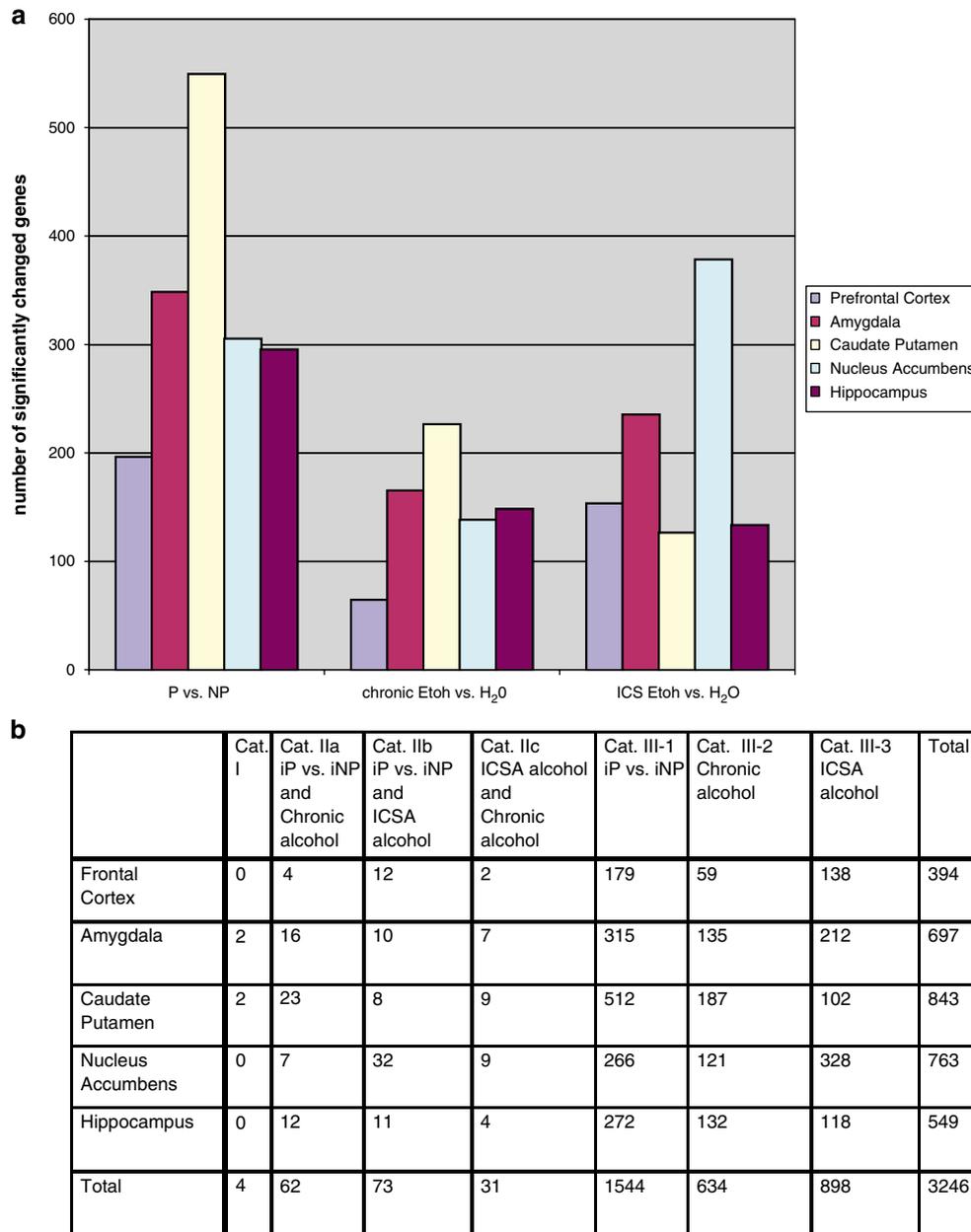


Figure 2 Number of genes changed. (a) Brain-region-specific changes in gene expression. (b) Number of genes changed in the different Categories (I–III).

6, member A1 (ALDH6A1)) (Table 1). These regions are likely associated with the anxiolytic, respectively, the locomotor effects of alcohol.

Top findings

The genes in Categories I are shown in Table 1, and Category II genes in Tables 2–4. Our internal convergence analysis also included determining whether a gene is changed in multiple brain regions, which adds another internal line of evidence. The maximum score that a gene can get based on internal lines of evidence is 4.5. By then applying our

external convergence analysis to all the genes in the data set, a maximum total score of 9 can be generated. Figure 3a summarizes in a pyramid format the assigned empirical probability scores based on the multiple internal and external lines of evidence, showing the 56 genes we identified that had a total score of 4 or greater. While the CP has the highest number of overall gene expression changes (Figure 2b), it is interesting to note that the region with the highest average score per gene in the pyramid is the AMY, followed by the HIP, FC, NAC and lastly, CP (Figure 3b). This may speak to the more specific effects of alcohol on

Table 1 Category I genes (changed in all three paradigms)

Genebank accession number	Symbol – description	<i>P</i> vs NP fold-change/ <i>P</i> -value	Chronic EtOH fold-change/ <i>P</i> -value	ICS EtOH fold-change/ <i>P</i> -value	Multiple brain regions		Human genetic linkage/ association	Relevant biology	Human tissue (post-mortem brains)	Scoring of lines of evidence
<i>AMY</i>										
AI103957	CD81 – cell surface protein (CD81 antigen)	1.11/0.01304	1.16/0.02013	–1.53/0.01399	NAC-2 –1.07/0.01952	NAC-3 –1.47/0.0162	11p15.5 EtOH ⁶⁵ BP ⁶⁶ SZ ¹⁶⁷	Cocaine ^{62–64}		6.0/9.0
AF000899	NUPL1 – nucleoporin-like 1	–1.25/0.04643	1.22/0.02919	1.19/0.04192	PFC- 21.16/0.0215	PFC-3 –1.26/0.03678	13q12.13 EtOH ¹¹⁹ BP ¹⁶⁸ SZ ^{45,169}			5.5/9.0
<i>CP</i>										
X75253	PBP – phosphatidylethanolamine-binding protein	–1.14/0.02503	1.08/0.01012	–1.12/0.02565			12q24.23 EtOH ⁵⁶ BP ¹⁷⁰	AZ ¹⁷¹		5.0/9.0
M93401	ALDH6A1 – aldehyde dehydrogenase family 6, member A1	–1.63/0.00713	–1.17/0.01745	1.28/0.01221			14q24.23 EtOH ¹¹⁰ Other psychiatric disorder ¹⁷²			4.5/9.0

Abbreviations: AMY = amygdala; BP = bipolar disorder; CP = caudate putamen; EtOH = alcohol/alcoholism; FC = frontal cortex; ICS = intracranial self-administration; NAC = nucleus accumbens; NP = inbred alcohol-non-preferring rats; P = inbred alcohol-preferring rats; PFC = prefrontal cortex; SZ = schizophrenia.

Fold-changes and *P*-values were calculated using Affymetrix Microarray Analysis Suite version 5.0 analysis software. All *P*-values were <0.05.

Table 2 Top Category IIa genes (changed in both Paradigms 1 and 2)

Genebank accession number	Symbol – description	Paradigm 1 iP vs iNP fold-change/ P-value	Paradigm 2 chronic alcohol fold-change/ P-value	Multiple brain regions		Human genetic linkage/association	Relevant biology	Human tissue (post-mortem brains)	Scoring of lines of evidence
FC									
AA943892	AGT – angiotensinogen precursor	1.37/0.02455	-1.26/0.03856	CP-1 -14.25/0.00019	NAC-1 1.52/0.0333	1q42.2 BP ^{105,174} SZ ^{57,106}	EtOH ¹⁰¹	Downregulated in frontal lobe of EtOH ¹⁷⁵	5.5/9.0
AA892775	LYZ – lysozyme	1.67/0.00658	1.25/0.03833	HIP-3 1.30/0.01423 AMY-1 1.54/0.00678 NAC-1 2.05/0.00039	CP-1 -5.12/0.0063 HIP-3 1.69/0.04432	12q15	EtOH ¹⁷⁶ Morphine ¹⁷⁷	EtOH ¹⁷⁵	5.5/9.0
AA875127	CDC2L5 – cholinesterase-related cell division controller	-1.45/0.02797	1.16/0.0307	NAC-1 -1.45/0.02797	NAC-2 1.16/0.0307	7p14.1 SZ ¹¹¹		AZ ¹⁷⁸	4.0/9.0
AMY									
X15906	FN1 – fibronectin 1	-1.43/0.0007	1.17/0.03532	PFC-1 -1.36/0.03277 NAC-1 -1.27/0.03304	CP-2 -1.20/0.01435 HIP-1 -1.69/0.00002	2q35 EtOH ^{56,179}	EtOH ^{51,54} SZ ⁵⁸	EtOH ⁵⁹	6.5/9.0
X75207	CCND1 – cyclin d1	-1.24/0.04365	1.17/0.01999			11q13.3 BP ¹¹⁷ SZ ¹¹⁸	EtOH ¹⁸⁰ Morphine ¹⁸¹	EtOH ⁵⁹ and upregulated in HIP of AZ ¹⁸²	5.5/9.0
M18331	PRKCE – protein kinase C, epsilon	-1.48/0.04593	1.08/0.04006	NAC-2 1.13/0.02729		2p21 SZ ⁸⁶	EtOH ^{97,183} Lithium ¹⁰⁰	AZ ¹⁸⁴	5.0/9.0
U14192	VDP – general vesicular transport factor p115 (transstosis-associated protein)	1.18/0.04646	1.28/0.0281	PFC-1 1.23/0.01816	NAC-3 -1.36/0.03632	4q21.1 EtOH ^{110,73} SZ ⁵⁷			4.5/9.0
X92097	RNP24 – coated vesicle membrane protein	1.47/0.00145	1.10/0.02935	PFC-1 1.57/0.00001	NAC-1 1.35/0.00398	12q24.31 EtOH ⁵⁶ BP ¹⁷¹			4.5/9.0
M17525	GNAS – GNAS complex locus (adenylate cyclase-stimulating G alpha protein)	-1.17/0.02556	-1.15/0.02417	HIP-1 1.55/0.0047	PFC-3 1.16/0.01044	20q13.32 EtOH ¹¹⁹	EtOH ¹⁴⁴ Anxiety ¹⁴⁵		4.5/9.0
AA891935	CAPNS1 – calpain, small subunit 1	1.42/0.00215	1.18/0.03113			19q13.12	EtOH ¹⁰⁹ AZ ¹⁸⁵	Upregulated in frontal lobe of EtOH ¹⁷⁵	4.5/9.0
M64780	AGRN – agrin protein	1.09/0.04878	1.13/0.01067	CP-1 -1.14/0.04135	NAC-1 -1.22/0.00057	1p36.33	EtOH ^{186,107,187}	AZ ¹⁸⁸	4.0/9.0
CP									
U88958	NRN1 – neuritin 1	-1.56/0.0226	-1.30/0.0033	AMY-1 1.17/0.00763	NAC-3 1.29/0.01865	6p25.1 EtOH ⁹⁶ SZ ^{45,189}	SZ ¹⁹⁰	BP ¹⁹¹	5.5/9.0
U73142	MAPK14 – mitogen-activated protein kinase 14	-2.16/0.00308	1.28/0.03672			6p21.31 EtOH ¹¹⁰ SZ ^{111,45}	EtOH ¹⁰⁹	Upregulated in PFC and NAC in EtOH ²⁷	5.5/9.0

Table 2 Continued

Genebank accession number	Symbol – description	Paradigm 1 iP vs iNP fold-change/ P-value	Paradigm 2 chronic alcohol fold-change/ P-value	Multiple brain regions	Human genetic linkage/association	Relevant biology	Human tissue (post-mortem brains)	Scoring of lines of evidence	
D37951	HIVEP2 – human immuno-deficiency virus type 1 enhancer-binding protein 2	-1.90/0.04284	-1.16/0.01406	NAC-2 1.38/0.01524	HIP-1 -1.11/0.01516	Psychosis ¹⁹² 6q24.2 EtOH ⁵⁶ SZ ¹⁹³		4.5/9.0	
X84039	LUM – lumican precursor	-2.65/0.02745	-1.33/0.00489	NAC-1 1.38/0.03949		12q21.33 EtOH ⁵⁶ BP ¹⁷¹		4.0/9.0	
AA944156	BTG2 – B-cell translocation gene 2	1.55/0.00913	-1.35/0.00596	AMY-2 -1.35/0.00596		1q32.1 EtOH ¹⁹⁴ SZ ^{195,57}		4.0/9.0	
AA849769	FSTL1 – follistatin-like 1	-2.37/0.03495	1.20/0.04728	AMY-1 -1.30/0.02779	AMY-3 -1.62/0.03143	3q13.33 BP ⁸⁵	Upregulated in PFC pf BP ¹⁹⁶	4.0/9.0	
L42810	CAMKK1 – Ca ²⁺ /calmodulin-dependent protein kinase 1, alpha	-1.86/0.00376	-1.12/0.02406	PFC-1 1.15/0.04194		17p13.2 EtOH ⁵⁶	Neuronal survival ¹⁹⁷	4.0/9.0	
<i>NAC</i>									
AA799726	KIAA1737 – KIAA1737 protein	-1.60/0.00806	1.22/0.02364	AMY-1 -1.21/0.00318	HIP-1 -1.24/0.0121	14q24.3 EtOH ⁵⁶	EtOH ¹⁰⁷	4.5/9.0	
AA875127	CDC2L5 – cholinesterase-related cell division controller	-1.34/0.01056	1.28/0.00624	PFC-1 -1.45/0.02797	PFC-2 1.16/0.0307	7p14.1 SZ ¹¹¹	AZ ¹⁷⁸	4.0/9.0	
<i>HIP</i>									
M58040	TFRC – transferrin receptor mRNA	1.36/0.00154	1.22/0.03867	CP-1 -6.42/0.01677		3q29 EtOH ⁸⁴ BP ^{85,87} SZ ^{87,86}	EtOH ^{83,186,107} Mania ¹⁹⁸	EtOH ⁸⁸	6.5/9.0
AF044581	STX12 – syntaxin 12-binding protein	1.20/0.01049	-1.18/0.0486	NAC-3 -1.36/0.01294		1p35.3 BP ¹⁶⁹ SZ ⁴⁵	EtOH ¹⁹⁹	Changed in temporal lobe of EtOH ²⁰⁰	5.0/9.0
AI176658	HSPB1 – heat-shock 27 kDa protein 1	1.38/0.02837	1.65/0.0255	PFC-1 1.29/0.01916 CP-1 -2.82/0.03322	PFC-3 -1.40/0.02212 NAC-1 1.37/0.01799	7q11.23	EtOH ²⁰¹	BP ¹¹² AZ ¹¹³	4.5/9.0

Abbreviations: AMY = amygdala; AZ = Alzheimer's disease; BP = bipolar disorder; CP = caudate-putamen; EtOH = alcohol/alcoholism; FC = frontal cortex; HIP = hippocampus; NAC = nucleus accumbens; P = inbred alcohol-preferring rats; NP = inbred alcohol-non-preferring rats; SZ = schizophrenia.

Fold-changes and *P*-values were calculated using Affymetrix Microarray Analysis Suite version 5.0 analysis software. All *P*-values were <0.05. Numbers with each brain region in the multiple brain regions column represent the paradigm in which the gene expression was changed. Genes with a line of evidence score of 4 or higher are shown.

Table 3 Top Category IIb Genes (changed in both Paradigms 1 and 3)

Genebank accession number	Gene symbol – description	Paradigm 1 iP vs iNP fold-change/ P-value	Paradigm 3 ICSA EtOH fold-change/ P-value	Multiple brain regions		Human genetic linkage/association	Relevant biology	Human tissue (post-mortem brains)	Scoring of lines of evidence
FC									
AA998683	HSPB1 – heat-shock 27 kDa protein 1	1.29/0.01916	–1.40/0.02212	CP-1 –2.82/0.03322 HIP-1 1.38/0.02837	NAC-1 1.37/0.01799 HIP-2 1.65/0.00255	7q11.23	EtOH ²⁰⁹	BP ¹¹² AZ ¹¹³	4.5/9.0
U48288	AKAP11 – A kinase (PRKA) anchor protein 11	4.77/0.00097	–1.42/0.02557	AMY-1 4.72/0 CP-1 –8.86/0.000139	NAC-1 9.46/0 HIP-1 5.14/0	13q14.11 EtOH ⁵⁶ BP ⁸⁵ SZ ²¹¹			4.0/9.0
AMY									
AI030286	BDNF – brain-derived neurotrophic factor	1.31/0.01665	1.83/0.01878			11p14.1 EtOH ¹³³ BP ^{134–138} SZ ¹³⁹	EtOH ^{130,131} Depression ¹³²	Downregulated in PFC of SZ ¹⁴² Lymphoblasts ^{213,214} and downregulated in HIP of BP ¹⁴⁰ Downregulated in PFC and HIP in suicide ¹⁴¹ Downregulated in HIP of AZ ¹⁴³	5.5/9.0
D90035	PPP3CA – protein phosphatase 3, catalytic subunit, alpha isoform (calcineurin A alpha)	1.23/0.02995	1.26/0.01712	PFC-3 1.29/0.00282 CP-3 1.17/ 0.01792	NAC-1 –1.11/0.03117	4q24 EtOH ^{84,215}	BP ²¹⁶		4.5/9.0
AA893454	SLC41A2 – solute carrier family 41, member 2	1.23/0.00431	1.22/0.0476	PFC-3 1.31/0.04405		12q23.3 EtOH ⁵⁶ BP ⁸⁵			4.0/9.0
AA849769	FSTL1 – follistatin-like 1	–1.30/0.02779	–1.62/0.03143	CP-1 –2.37/0.03459	CP-2 1.20/0.04728	3q13.33 BP ⁸⁵		Upregulated in frontal lobe of BP ¹⁹⁹	4.0/9.0
CP									
AI145494	SYN2 – synapsin 2	–3.13/0.00399	1.29/0.03921	PFC-3 –1.32/0.03653 AMY-3 1.83/0.04186 NAC-2 1.16/0.02601	NAC-3 –1.25/0.0220 HIP-1 1.25/0.0301	3p25.2 SZ ^{57,125}	Neurotransmitter release ¹²⁴	EtOH ^{59,128} Downregulated in HIP of SZ and BP ^{126,127}	6.0/9.0
AI009801	MIF – macrophage migration inhibitory factor	–1.85/0.00537	1.16/0.01057			22q11.23 BP ²¹⁹	EtOH ²²⁰ AZ ²²¹	Downregulated in HIP of BP ²¹⁷	4.5/9.0
AA892547	C2orf25 – chromosome 2 open-reading frame 25	–9.13/0.00001	1.34/0.01608	PFC-1 1.27/0.00795		2q23.2 EtOH ¹¹⁰ BP ⁸⁵			4.0/9.0
AB005143	UCP2 – uncoupling protein 2	–1.85/0.04981	1.16/0.04772			11q13.4 BP ¹¹⁷ SZ ¹¹⁸	EtOH ^{222,223} Methamphetamine ²²⁴		4.0/9.0
L07736	CPT1A – carnitine palmitoyltransferase 1, liver	–2.55/0.02672	–1.25/0.038	AMY-2 1.14/0.04671		11q13.3 BP ¹¹⁷ SZ ¹¹⁸	EtOH ²²⁵		4.0/9.0

Table 3 Continued

Genebank accession number	Gene symbol – description	Paradigm 1 iP vs iNP fold-change/ P-value	Paradigm 3 ICSA EtOH fold-change/ P-value	Multiple brain regions	Human genetic linkage/association	Relevant biology	Human tissue (post-mortem brains)	Scoring of lines of evidence	
NAC									
X63722	VCAM1 – vascular cell adhesion molecule 1	1.31/0.00394	-1.49/0.00421	CP-1 -2.67/0.00433	1p21.2 EtOH ⁷¹⁻⁷⁴ BP ²²⁶ SZ ⁷⁵	EtOH ⁶⁸ Cocaine ⁶⁹	Upregulated in anterior cingulate of BP ²²⁷ Upregulated in PFC of depression thomas ⁷⁶	6.0/9.0	
AI009191	FYN – fyn proto-oncogene	-1.14/0.01724	-1.41/0.00865	AMY-3 -1.07/0.01831	6q21 EtOH ²²⁸ BP ²²⁹ SZ ⁴⁵	EtOH ²³⁰	Upregulated in PFC of SZ ²³¹ Increased in leukocytes of BP ¹²⁶	5.5/9.0	
L29232	IGF1R – insulin-like growth factor 1 receptor	-1.24/0.01173	-1.28/0.04214	CP-2 1.40/0.00989	15q26.3 BP ⁸⁵	EtOH ²³²	EtOH ^{59,88} AZ ²³³	5.5/9.0	
AA946313	SPARC – secreted protein, acidic, cysteine-rich (osteonectin)	1.19/0.00509	1.25/0.04174		5q33.1 BP ¹⁷⁰ SZ ^{234,86,45} Psychosis ²³⁵	EtOH ²³⁶ Morphine ²³⁷	BP ¹¹²	4.5/9.0	
AA800693	PDXK – pyridoxal (pyridoxine, vitamin B6) kinase	-1.17/0.0257	-1.38/0.02938		21q22.3 EtOH ¹¹⁹ BP ²³⁸	EtOH ²³⁹		4.5/9.0	
AI230748	TPT1 – tumor protein, translationally controlled 1	1.34/0.00084	-1.15/0.00313	PFC-1 -1.37/0.04084 AMY-1 -1.25/0.0012	HIP-1 -1.33/0.00183	13q14.13 EtOH ⁵⁶ BP ⁸⁵ SZ ²¹¹		4.0/9.0	
AI179012	ACTB – actin, beta	-1.11/0.03067	-1.18/0.00409	AMY-3 1.27/0.02027		7p22.1 EtOH ⁵⁶	AZ ²⁴⁰	4.0/9.0	
AA957930	MAPT – microtubule-associated protein tau	-1.16/0.03966	1.17/0.0152			17q21.31 AZ ²⁴¹	Upregulated in PFC and NAC in EtOH ²⁷ Downregulated in anterior cingulate cortex of BP and SZ ²⁴² AZ ²⁴³	4.0/9.0	
AA800175	PIN1 – protein (peptidyl-prolyl <i>cis/trans</i> isomerase) NIMA-interacting 1	-1.35/0.00177	1.33/0.00646	PFC-1 -1.81/0.02123 AMY-1 -1.26/0.00945	CP-1 2.01/0.00347 HIP-1 -1.30/0.00004	19p13.2 EtOH ¹⁰⁷ AZ ²⁴⁴		4.0/9.0	
HIP									
AF001898	ALDH1A1 – aldehyde dehydrogenase family 1, member A1	1.22/0.02931	1.39/0.00103	PFC-1 1.34/0.00211		9q21.12 EtOH ^{119,120,121,245} BP ¹⁷³	Downregulated in ventral tegmentum of SZ ¹²²	5.5/9.0	
J03190	ALAS1 – aminolevulinic acid synthase 1	1.20/0.04986	1.37/0.03944	AMY-1 1.32/0.048	CP-1 -1.68/0.03238	3p21.2 EtOH ⁷⁴ SZ ¹⁷³	EtOH ²⁴⁹ Other ⁹¹	5.5/9.0	
AB012759	PREP – prolyl endopeptidase	-1.41/0.03113	-1.53/0.04888			6q21 BP ²²⁹	EtOH ²⁵⁰ Psychosis ²⁵⁰	AZ ²⁵¹	4.5/9.0
D10874	ATP6VOC – ATPase, H+ transporting, lysosomal (vacuolar proton pump) 16 kDa, V0 subunit c	-1.08/0.04141	1.11/0.04032	AMY-2 -1.08/0.01948		16p13.3 EtOH ^{212,110} BP ²²⁶	BP ¹⁹⁴	4.5/9.0	

Abbreviations: AMY = amygdala; AZ = Alzheimer's disease; BP = bipolar disorder; CP = caudate-putamen; EtOH = alcohol/alcoholism; FC = frontal cortex; HIP = hippocampus; ICS = intracranial self-administration; NAC = nucleus accumbens; P = inbred alcohol-preferring rats; NP = inbred alcohol-non-preferring rats; SZ = schizophrenia.

Fold-changes and *P*-values were calculated using Affymetrix Microarray Analysis Suite version 5.0 analysis software. All *P*-values were <0.05. Numbers with each brain region in the multiple brain regions column represent the paradigm in which the gene expression was changed. Genes with a line of evidence score of 4 or higher are shown.

Table 4 Top Category IIc genes (changed in both Paradigms 2 and 3)

Genebank accession number	Gene symbol – description	Paradigm 2 chronic alcohol fold-change/ P-value	Paradigm 3 ICS alcohol fold-change/ P-value	Multiple brain regions		Human genetic linkage/ association	Relevant biology	Human tissue (post-mortem brains)	Scoring of lines of evidence
<i>FC</i> AF000899	NUPL1 -nucleoporin-like 1	1.16/0.0215	-1.26/0.03678	AMY-1 -1.25/0.04643 AMY-3 1.19/0.04192	AMY-2 1.22/0.02919	13q12.13 EtOH ¹¹⁹ BP ¹⁶⁸ SZ ^{45,169}			5.5/9.0 (Category I)
<i>CP</i> AA944177	HMGB1 – high-mobility group box 1	-1.23/0.03433	1.63/0.04859	HIP-1 1.19/0.00319	HIP-3 1.28/0.03744	13q12.3 EtOH ⁵⁶	AZ ²⁵⁴		4.5/9.0
M18567	GOT2 – glutamate oxaloacetate transaminase 2, mitochondrial (aspartate aminotransferase 2)	-1.25/0.02632	-1.22/0.0052			16q21 EtOH ²⁵⁵	EtOH ²⁵⁶		4.0/9.0
<i>NAC</i> AI103957	CD81 – cell surface protein (CD81 antigen)	-1.07/0.01952	-1.47/0.0162	AMY-1 1.11/0.01304	AMY-2 1.16/0.02013	11p15.5 EtOH ⁶⁵ BP ⁶⁶ SZ ¹⁶⁷	Cocaine ^{62–64}		5.5/9.0
M27925	SYN2 – synapsin 2	1.16/0.02601	-1.25/0.02205	AMY-3 1.53/0.01399 PFC-3 -1.32/0.03653 CP-1 -3.13/0.00399 HIP-1 1.25/0.0301	AMY-3 1.83/0.04186 CP-3 1.29/0.03921	3p25.2 SZ ^{57,125}	Neurotransmitter release ¹²⁴	EtOH ^{59,128} Downregulated in HIP of SZ and BP ^{126,127}	5.5/9.0
<i>HIP</i> M64986	HMGB1 – high-mobility group box 1	1.19/0.00319	1.28/0.03744	CP-2 -1.23/0.03433	CP-3 1.63/0.04859	13q12.3 EtOH ⁵⁶	AZ ²⁵⁴		4.5/9.0
X68283	RPL29 – ribosomal protein L29	-1.09/0.0358	1.11/0.02863	CP-1 -1.46/0.02565		3p21.2 EtOH ⁷⁴		Downregulated in frontal lobe in EtOH ¹⁷⁴	4.5/9.0

Abbreviations: AMY = amygdala; AZ = Alzheimer's disease; BP = bipolar disorder; CP = caudate-putamen; EtOH = alcohol/alcoholism; FC = frontal cortex; HIP = hippocampus; ICS = intracranial self-administration; NAC = nucleus accumbens; P = inbred alcohol-preferring rats; NP = inbred alcohol-non-preferring rats; SZ = schizophrenia.

Fold-changes and *P*-values were calculated using Affymetrix Microarray Analysis Suite version 5.0 analysis software. All *P*-values were <0.05. Numbers with each brain region in the multiple brain regions column represent the paradigm in which the gene expression was changed. Genes with a line of evidence score of 4 or higher are shown.

anxiety (AMY), followed less specifically by its effects on memory formation (HIP), cognition (FC), hedonia (NAC) and lastly, locomotion (CP).

At the top of our pyramid, we have 20 genes that show a score of greater than 4.5. This is of interest because this is the maximum score obtainable from internal lines of evidence alone, without any external confirmatory evidence. Six of these genes are from the AMY – fibronectin 1 (FN1) located at 2q35, CD81 located at 11p15.5, brain-derived neurotrophic factor (BDNF) located at 11p14.1, cyclin D1 (CCND1) located at 11q13.3, NUPL1 located at 13q12.13 and protein kinase C epsilon (PRKCE) located at 2p21; four from the HIP – transferrin receptor (TFRC) located on 3q29, aldehyde dehydrogenase family 1, member A1 (ALDH1A1) on 9q21.2, 5-aminolevulinic acid synthase 1 (ALAS1) located on 3p21.2 and syntaxin 12-binding protein (STX12) at 1p35.3; four from the CP – synapsin II (SYN2) located at 3p25.2, mitogen-activated protein kinase 14 (MAPK14) at 6p21.31, neuritin 1 (NRN1) located at 6p25.1 and PBP located at 12q24.23; three from the NAC – vascular cell adhesion molecule 1 (VCAM1) located at 1p21.2, *fyn* oncogene (FYN) located at 6q21 and IGF1R located at 15q26.3; two from the FC – angiotensinogen (AGT) located at 1q42.2 and lysozyme (LYZ) located at 12q15; and one in multiple regions – tyrosine hydroxylase (TH) located at 11p15.5.

Fibronectin 1

Notably, 14 of these top 20 genes are known to interact in a network with FN1 at its core (Figure 4a): VCAM1³⁸ and CD81³⁹ (cell adhesion and signaling), TFRC⁴⁰ and ALAS1⁴¹ (iron-heme metabolism), AGT⁴² and PRKCE⁴³ (cardiovascular regulation), insulin-like growth factor 1 receptor (IGF1R),⁴⁴ BDNF,⁴⁵ CCND1,⁴⁶ FYN⁴⁷ and MAPK14⁴⁸ (cell proliferation and differentiation), and SYN2⁴⁹ and NRN1⁵⁰ (synaptic transmission and neurite outgrowth). Moreover, several other genes in our data set, with a score of 4.5 or less, are part of FN1 pathways and interactions (neuronal cell adhesion molecule (NRCAM), PTK2B, RHOB, BCAR1). For a more complete view of all the known direct interactions between our top candidate genes, we have conducted a Pathways Assist analysis, as depicted in Figure 4b. This analysis also identified MAPK14 and AGT as key nodes in the interaction network. These genes will be discussed in more detail below.

As a caveat, it should be noted that most of the above inter-relationships were inferred from work in tissues other than the brain. However, it is reasonable to assume that similar inter-relationships might be functional in glial or neuronal populations. Other investigators have previously implicated a majority of the above-named genes, individually or as part of functional groups, in various biological and genetic contexts germane to the pathophysiology of alcoholism and related disorders, as discussed below. Our results, identifying these genes as top candidate genes, are thus a strong validation of the heuristic value and internal consistency of the approach we have used. Moreover, they outline networks of potentially co-acting genes, and support

an important role for FN1 pathway in alcoholism and related disorders.

FN1 has previously been implicated in the myocardial fibrosis induced by chronic alcohol,⁵¹ acute lung injury susceptibility induced by alcohol⁴⁶ as well as in cirrhosis of the liver.^{52,53} FN1 expression was also changed, in tissue culture, in glial cells treated with alcohol.⁵⁴ This suggests that perhaps a fibrosis-type process may underlie detrimental changes in the brains of alcoholics – a cirrhosis of sorts in the brain. Our data show that alcohol-preferring rats (iP), which are better able to withstand the intoxicating effects of alcohol, have lower baseline level of FN1 than alcohol-non-preferring (iNP) rats, in multiple brain regions (Table 2). iP rats are less susceptible to the motor-impairing effects of alcohol than iNP rats.⁵⁵ Additionally in iP rats, chronic alcohol consumption alters the sensitivity of the posterior VTA (lower concentrations of alcohol are self-administered) and increases the tolerance to the reinforcing properties of higher concentrations of alcohol (higher concentrations of alcohol are self-administered in chronically drinking iP rats than alcohol-naïve iP rats).^{10,30} Therefore, there appears to be an innate ability to withstand the intoxicating effects of alcohol in iP rats, and perhaps a genetic predisposition to develop further tolerance to the effects of alcohol. FN1 maps to a locus on 2q35 identified in a genome-wide screen for alcohol susceptibility genes.⁵⁶ That chromosomal location was also identified as linked to a cognitive trait component (visual working memory) in schizophrenia.⁵⁷ Interestingly, FN1 content was reported to be altered in fibroblasts from schizophrenia patients.⁵⁸ Moreover, FN1 gene expression was reported to be altered in post-mortem brains of human alcoholics.⁵⁹

Cell adhesion signaling and alcoholism

Besides FN1, other molecules involved in cell adhesion signaling were among our top candidate genes.

CD81, a member of the tetraspanin superfamily of proteins, has been linked to a number of biologic functions, including cellular proliferation, differentiation, activation and degranulation. Mice lacking CD81 have been shown to have increased brain size and glial cell number.⁶⁰ CD81 seems to be important for neuronally induced glial cell proliferation arrest.⁶¹ CD81 is increased in the NAC by cocaine administration, and has been shown to mediate some of the behavioral activating effects of cocaine.^{62–64} Our data show that CD81 is a Category I gene, changed in expression in the AMY in all three alcohol-related experimental paradigms we studied. While there seems to be an increase in the expression of CD81 in alcohol-preferring rats at baseline, of note there is a strong decrease in its expression in the NAC following ICSA administration of alcohol in the VTA (Table 1), opposite to the effects of systemic administration of cocaine. This elevation of CD81 in the NAC of iP rats vs iNP rats, similar to the effects of cocaine in wild-type animals, suggests they may have a higher hedonic activity at baseline. Alternately, the level of CD81 in iP rats may have to do with the ability of the alcohol-preferring rats to withstand the aversive (sedative

and intoxicating) effects of alcohol, and its decreased expression following alcohol administration may be a manifestation of those effects. The effects of alcohol in decreasing CD81 may be particularly relevant to brain development and glial proliferation during fetal alcohol syndrome. CD81 maps to a locus on 11p15.5 that has been linked to alcohol dependence by a genome-wide study in an American Indian population.⁶⁵ That locus also shows evidence for linkage to bipolar disorder⁶⁶ and schizophrenia.⁶⁷

VCAM1, involved in endothelial adhesion and migration of leukocytes, has previously been shown to be deficient in central nervous system (CNS) injury response in adult rats who had been exposed to alcohol *in utero*.⁶⁸ This suggests that neuroimmunomodulatory responses to tissue injury might be altered in alcoholics. VCAM1 has also been shown to be upregulated by another drug of abuse, cocaine,⁶⁹ probably through a noradrenergic mechanism.⁷⁰ Our data show that alcohol-preferring rats have higher baseline level of VCAM1 than alcohol-non-preferring rats in the NAC, and those levels are decreased by ICSA administration of alcohol directly in the VTA (Table 3), opposite to the effects of systemic administration of cocaine. VCAM1 maps to a locus on 1p21.2 that shows linkage not only to alcoholism, in multiple studies,^{71–74} but also to bipolar disorder⁶⁷ and schizophrenia.⁷⁵ Last but not least, VCAM1 was reported to be upregulated in human post-mortem brains of patients with depression.⁷⁶

Taken together, these data on cell adhesion molecules, and the apparent opposite effects of cocaine and alcohol on them, support the possibility of them having a role more in withstanding the aversive effects of alcohol rather than in hedonic effects. These data also have interesting implications for the effect of alcohol on brain infrastructure, and the neurobiological and clinical overlap with bipolar disorder and schizophrenia. Moreover, at baseline the iP rats have a molecular profile that resembles the effects of cocaine administration in wild-type rats, and which translates phenotypically in increased locomotor behavior.⁷⁷ iP rats may also be particularly susceptible to the effects of cocaine and other stimulant drugs,⁷⁸ which mimics the human condition, where alcoholics are often polysubstance abusers.⁷⁹ Specifically, P rats self-administer cocaine directly into the accumbens shell at a greater than twofold lower concentration compared to Wistar rats.³⁰ In addition, P rats develop sensitization to cocaine-induced locomotor stimulation more readily than Wistar and iNP rats.⁷⁷

An opposite example to this paradigm is provided by another candidate gene in our data set, NRCAM, which has a role in cell adhesion and a role in the response to drug of abuse. NRCAM knockout mice display reduced opiate- and stimulant-conditioned place preferences.⁸⁰ NRCAM is not higher at baseline in iP rats than in iNP rats, but is increased by alcohol treatment in the CP, in our data set, in two independent paradigms (Table 4). It maps to a locus on 7q31.1 implicated in alcohol dependence in the context of major depression.¹⁴ Given the similarity of response of NRCAM to very different drugs (opiates, stimulants, alco-

hol), its role may have to do with the common hedonic effects of various drugs of abuse.

Iron–heme metabolism and alcoholism

Among our top candidate genes, we have two genes involved in iron-heme metabolism, TFRC and ALAS1. Alcoholism is associated with an increased risk for cancers. Accumulation of iron and associated oxidative stress may contribute to this risk.⁸¹ Carbohydrate-deficient transferrin has been established clinically as a biomarker of alcohol abuse, providing a reliable estimate of long-term alcohol intake.⁸² TFRC expression is upregulated in hepatocytes by habitual alcohol drinking, and is implicated in hepatic iron overload in alcoholic liver disease.⁸³ Our data show that alcohol-preferring rats have a higher baseline level of TFRC than alcohol-non-preferring rats in the HIP, and that those levels are increased by the administration of alcohol (Table 2). TFRC maps to a locus on 3q29 that has been implicated in alcoholism,⁸⁴ as well as in bipolar disorder and schizophrenia.^{85–87} Moreover, TFRC gene expression was reported to be altered in post-mortem brains of human alcoholics.⁸⁸ ALAS1 is involved in the coordinated upregulation of apoprotein and heme synthesis in response to exogenous and endogenous signals controlling heme levels.⁸⁹ ALAS1 is upregulated in the liver and in peripheral blood cells in response to alcohol.⁹⁰ Interestingly, the circadian clock and heme biosynthesis are reciprocally regulated through ALAS1.⁹¹ Our data show that alcohol-preferring rats have a higher baseline level of ALAS1 than alcohol-non-preferring rats in the HIP and that those levels are increased by administration of alcohol (Table 3). It would be of interest to study whether ALAS1 levels may mediate some of the circadian rhythm abnormalities associated with alcoholism.⁹² ALAS1 maps to a locus on 3p21.2 that has been implicated in alcoholism,⁷⁴ as well as schizophrenia.⁶⁷

The importation of iron into cells by TFRC and heme synthesis by ALAS1 may serve, in moderation, a useful trophic function, supporting cellular proliferation and activity in multiple tissues, including the HIP, not only hematopoietic tissue. The higher levels of TFRC and ALAS1 in baseline iP rats are consistent with the emerging picture of them being more robust and active. Similarly, selection for high alcohol preference in other rat lines have produced rats that have a greater survival rate, lower rates of kidney disease, benign tumors and cardiovascular disease than rats selected for low alcohol preference.⁹³ Additionally, a lifetime of alcohol consumption slightly increased the lifespan of Alko, Alcohol (AA) rats.⁹³ Thus, the increase in TFRC and ALAS1 in response to alcohol may be, at least initially, a tonic, and might underlie some of the health benefits in humans of intermittent use of low to moderated doses of alcohol.⁹⁴

Cardiovascular regulation and alcoholism

Among our top candidate genes (Figure 3), we have two genes that are known to be involved in cardiovascular regulation, PRKCE and AGT. Excessive alcohol consumption has been associated with cardiovascular disorders, including

cardiomyopathy, hypertension, coronary artery disease and stroke. However, recent evidence suggests that moderate alcohol intake may provide a measure of cardioprotection, especially against coronary disease and ischemia–reperfusion injury.⁹⁴

PRKCE is a calcium-independent, phospholipid-dependent, serine- and threonine-specific enzyme involved in signal transduction in a wide variety of tissues. PKC has previously been implicated by cell culture studies of the effects of alcohol.^{95,96} Mice lacking PRKCE consume less alcohol and show greater acute sensitivity to alcohol than do wild-type mice.⁹⁷ Moderate alcohol consumption induces cardiac protection by activating PRKCE.^{58,98} PRKCE was also identified as a candidate gene for alcoholism by a recent mouse model gene expression meta-analysis.⁹⁹ Our data show that alcohol-preferring rats have a lower baseline level of PRKCE than alcohol-non-preferring rats in the AMY. Additionally, our data show that those levels are increased by chronic alcohol (Table 2). The lower levels of PRKCE in the alcohol-preferring rats, whereas puzzling in view of the above-mentioned mouse literature relating to intake, may have less to do with alcohol consumption regulation and more to do with greater regio-specific sensitivity to alcohol in the AMY of these rats, perhaps in terms of fear reduction. This is consistent with the emerging picture of baseline iP rats being more sensitive to the desirable effects of alcohol than iNP rats. The increase of PRKCE by alcohol may be a phenomenon of tolerance, and is consistent with potential favorable cardiovascular benefits of moderate alcohol intake, like in the case of AGT. PRKCE maps to a locus on 2p21 that has been linked to schizophrenia,⁸⁶ as well as has been shown to be regulated by the mood-stabilizing drug lithium,¹⁰⁰ which may provide some mechanistic explanation for clinical comorbidities, and supports the use of lithium for the treatment of human alcoholism, especially when comorbid bipolar mood disorder exists.

AGT is a precursor of angiotensin II, a key enzyme in the renin–angiotensin system (RAS), involved in blood pressure regulation and other processes. Pharmacological and genetic manipulations of the RAS have been found to alter the voluntary consumption of alcohol.^{101–103} Specifically, transgenic rats that express an antisense RNA against AGT and consequently have reduced AGT and Ang II levels exclusively in the central nervous system consumed markedly less alcohol in comparison to their wild-type controls. Moreover, spirapril, an inhibitor of the angiotensin-converting enzyme (ACE), which passes the blood–brain barrier, did not influence alcohol consumption in the transgenic rats, but it significantly reduced alcohol intake in wild-type rats.¹⁰² Consistent with this, our data show that alcohol-preferring rats have higher baseline levels of AGT than alcohol-non-preferring rats in the prefrontal cortex (PFC). Additionally, our data show that those levels are decreased by chronic alcohol (Table 2). Last but not least, a Pathways Assist analysis of the known direct interactions between our top candidate genes identified AGT as a key node in the interaction network, as depicted in Figure 4b. The higher levels of AGT may have a tonic effect on blood pressure in

these alcohol-preferring animals, and their capability to respond to the environment.¹⁰⁴ This is consistent with the emerging picture of baseline iP rats being more robust and active. The lowering of AGT by alcohol is consistent with potential favorable cardiovascular benefits of moderate

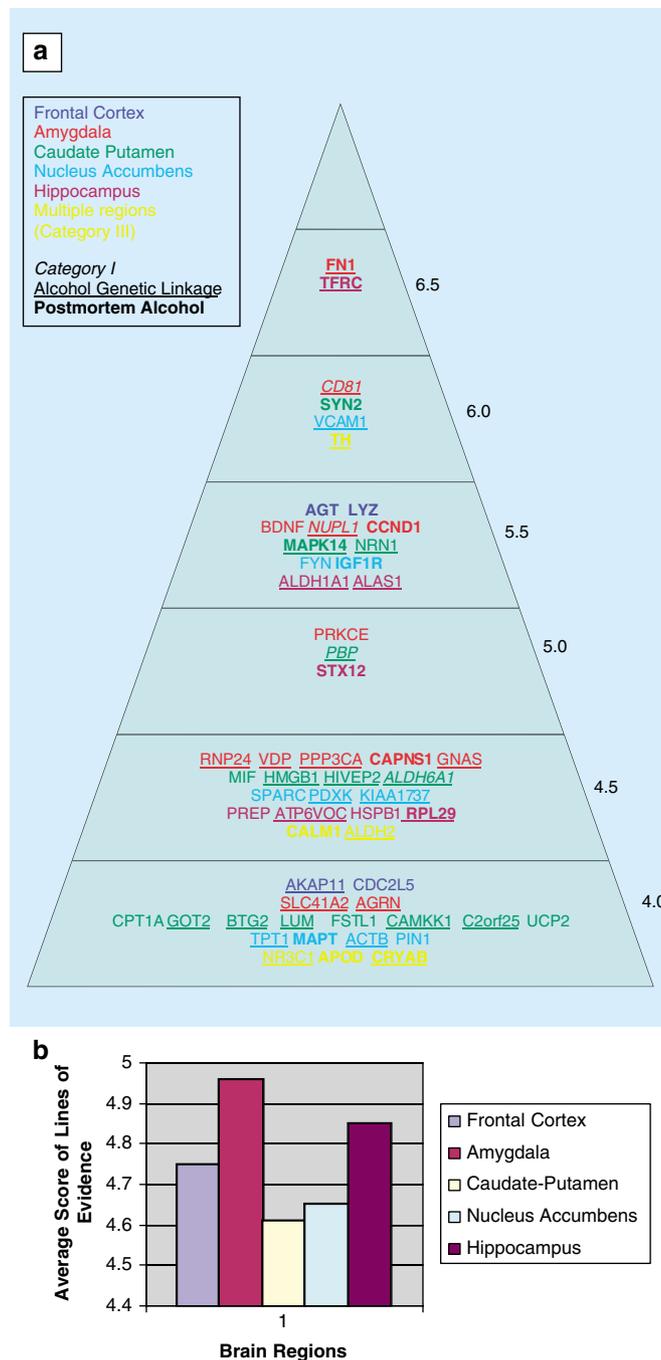


Figure 3 Top candidate genes. (a) Pyramid generated by the tabulation of independent converging lines of evidence. For full description of gene symbols see Tables 1–5. (b) Comparison of different brain regions in terms of average score per candidate gene in the pyramid. (c) Clustering of top candidate genes in iP vs iNP rats, and the reciprocal effects of ICSA alcohol.

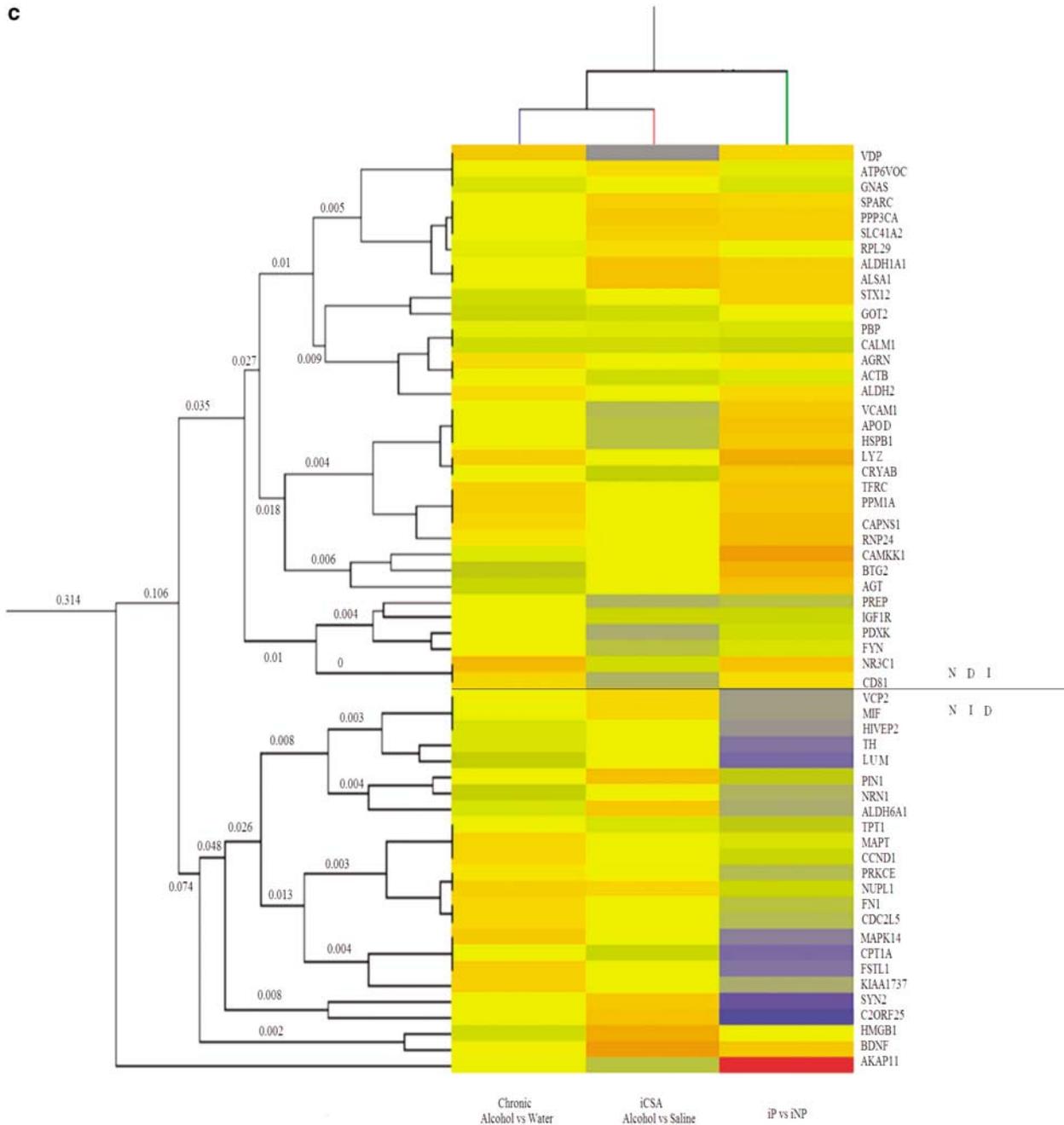


Figure 3 (Continued)

alcohol intake. AGT maps to a locus on 1q42.2 that has been linked to bipolar disorder,^{67,105} as well as schizophrenia.^{57,106} Last but not least, AGT gene expression was reported to be downregulated in the FC in post-mortem brains of human alcoholics.¹⁰⁷

ACE inhibition effectively reduced alcohol drinking in animals with elevated RAS activity and not at all in animals with suppressed RAS activity, indicating that initial levels of RAS activity may determine the speed and ability of ACE inhibition to attenuate alcohol intake.¹⁰³ The alcohol-preferring rats, which have higher levels of AGT than

alcohol-non-preferring rats, should be susceptible to ACE inhibitors in terms of reducing their alcohol intake behavior. To test this hypothesis, we treated alcohol-preferring rats with the ACE inhibitor lisinopril, and measured alcohol intake.

Effects of daily administration of lisinopril on the acquisition of ethanol and saccharin consumption

Daily administration of lisinopril suppressed the acquisition of ethanol (EtOH) drinking during the initial 7 days of exposure in alcohol-preferring rats (Figure 5; top panel). A

repeated-measure analysis of variance (ANOVA) revealed that across the 10 initial days of EtOH exposure, there was a significant effect of drug treatment ($F_{3,27} = 5.9$; $P = 0.003$), a significant effect of day ($F_{6,22} = 2.6$; $P = 0.04$) and a nonsignificant day \times treatment interaction ($F_{18,72} = 0.98$; $P = 0.50$). One-way ANOVAs were conducted on the initial ten 24-h periods following injection. The results indicated that there were significant differences between the groups during injection days 3–7 and the first post-injection day ($F_{3,27} > 3.5$; P -values < 0.28). Overall, *post hoc* comparisons (Tukey's *b*) indicated that receiving 3 and 10 mg/kg consistently consumed less EtOH than saline-treated rats, and rats administered 1 mg/kg consumed an intermediate amount of EtOH.

Additionally, administration of lisinopril in these rats did not significantly reduce concurrent water intake (Figure 5; top panel inset). A repeated measure performed on water intake levels conducted on the 2 days before lisinopril testing and the 7 days of lisinopril administration revealed that there was a significant effect of drug treatment ($F_{3,27} = 4.4$; $P = 0.012$), a significant effect of day ($F_{8,20} = 6.9$; $P < 0.0001$) and a significant day \times treatment interaction ($F_{24,66} = 2.4$; $P = 0.002$). Rats administered saline or 1 mg/kg lisinopril reduced water intake when EtOH was made available (indicating the preference for EtOH that the P rat was selected), whereas the rats administered 3 or 10 mg/kg lisinopril maintained their water intake levels. Administration of lisinopril did not alter body weight (Figure 1; bottom panel inset). Comparable analysis revealed a significant effect of day ($P < 0.0001$), which indicated that all rats put on weight, but no effect of drug treatment or a day \times treatment interaction (P -values > 0.33).

Peripheral administration of lisinopril did not alter saccharin consumption in the alcohol-preferring rats (Figure 5; bottom panel). There were no significant effects of drug treatment ($P = 0.45$) or a drug treatment \times day interaction ($P = 0.23$). In general, alcohol-preferring rats consumed a large quantity of saccharin (> 80 g/day), and this was not altered by lisinopril. Repeatedly, there was no alteration in body weight or water intake (water intake was greatly reduced in all groups with the introduction of saccharin) following lisinopril (P -values > 0.56).

Cellular stress response and alcoholism

MAPK14 responds to activation by environmental stress, proinflammatory cytokines and lipopolysaccharide by phosphorylating a number of transcription factors, such as ELK1 and ATF2, and several downstream kinases, such as MAPKAPK2 and MAPKAPK5. It plays a critical role in the production of some cytokines, for example, interleukin-6. MAPK14 was also identified as a differentially expressed gene in carefully carried out microarray studies and analyses in alcohol-preferring AA (alko, alcohol) rats vs alcohol-avoiding ANA (alko, non-alcohol) rats.^{108,109} Our data show that alcohol-preferring iP rats have a lower baseline level of MAPK14 than alcohol-non-preferring iNP rats in the CP. Additionally, our data show that those levels are

increased by chronic alcohol (Table 2). Last but not least, a Pathways Assist analysis of the known direct interactions between our top candidate genes identified MAPK14 as a key node in the interaction network, as depicted in Figure 4b. The lower levels of MAPK14 in alcohol-preferring rats may permit them to be associated with lower responsiveness to alcohol-induced cellular stress changes, and thus presumably to the aversive effects of alcohol. As mentioned previously, iP rats are less susceptible to the motor-impairing effects of alcohol than iNP rats.⁵⁵ MAPK14 maps to a region on 6p21.31 that has been implicated in human linkage studies of alcoholism,¹¹⁰ as well as schizophrenia.^{111,45} Moreover, MAPK14 has been shown to be upregulated in the PFC and NAC in post-mortem human brains from alcoholics.²⁷

Heat-shock 27 kDa protein 1 (HSPB1) is downstream of MAPK14 in the MAPK pathway, and is involved in stress resistance and actin organization. Mutations in HSPB1 are the cause of Charcot–Marie–Tooth disease type 2 and of distal hereditary motor neuropathy. Our data show that alcohol-preferring rats have a higher baseline level of HSPB1 than alcohol-non-preferring rats in the HIP. Additionally, our data show that those levels are increased by chronic alcohol (Table 2). The higher levels of HSPB1

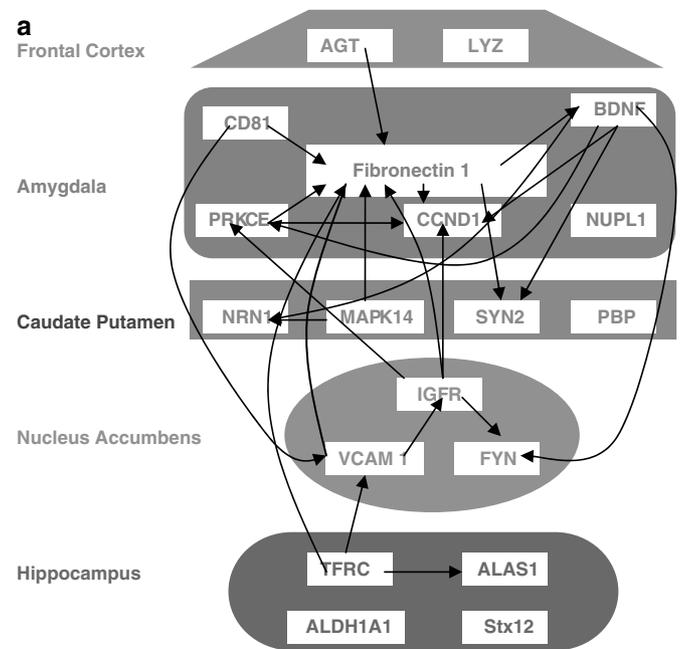


Figure 4 Candidate genes, pathways and mechanisms. (a) Top candidate genes and their relationships with FN1. All the genes from Figure 3a with a score of greater than 4.5 are illustrated. Genes are depicted in the brain region where they were reproducibly changed. For genes that showed changes in multiple brain regions, the gene is depicted in the brain region in which the gene showed the most lines of evidence. (b) Pathway Assist analysis of the interactions among top candidate genes. (c) GO analysis-derived model of biological processes and mechanisms. Numbered categories refer to GO analysis categories from Table 6a (bold) and 6b (italic).

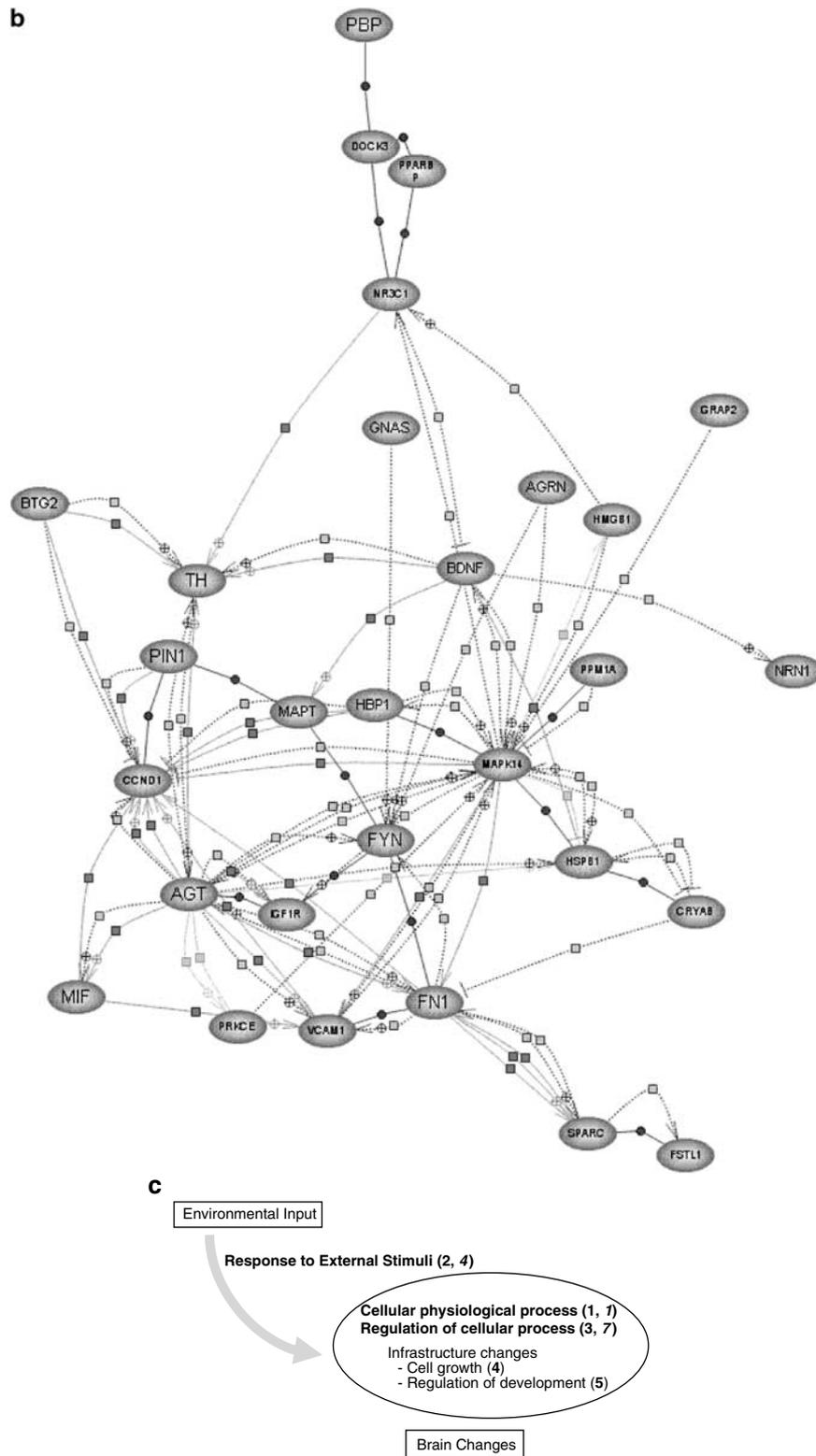


Figure 4 (Continued)

in alcohol-preferring rats may permit them to be more resilient to alcohol-induced cellular stress changes, and thus presumably to the aversive effects of alcohol. The increase in HSPB1 with chronic alcohol may be part of a hormesis-like

neuroadaptation mechanism. HSPB1 levels have also been shown to be increased in post-mortem human brains from bipolar disorder and schizophrenia,¹¹² and as part of the reactive gliosis in Alzheimer disease.¹¹³

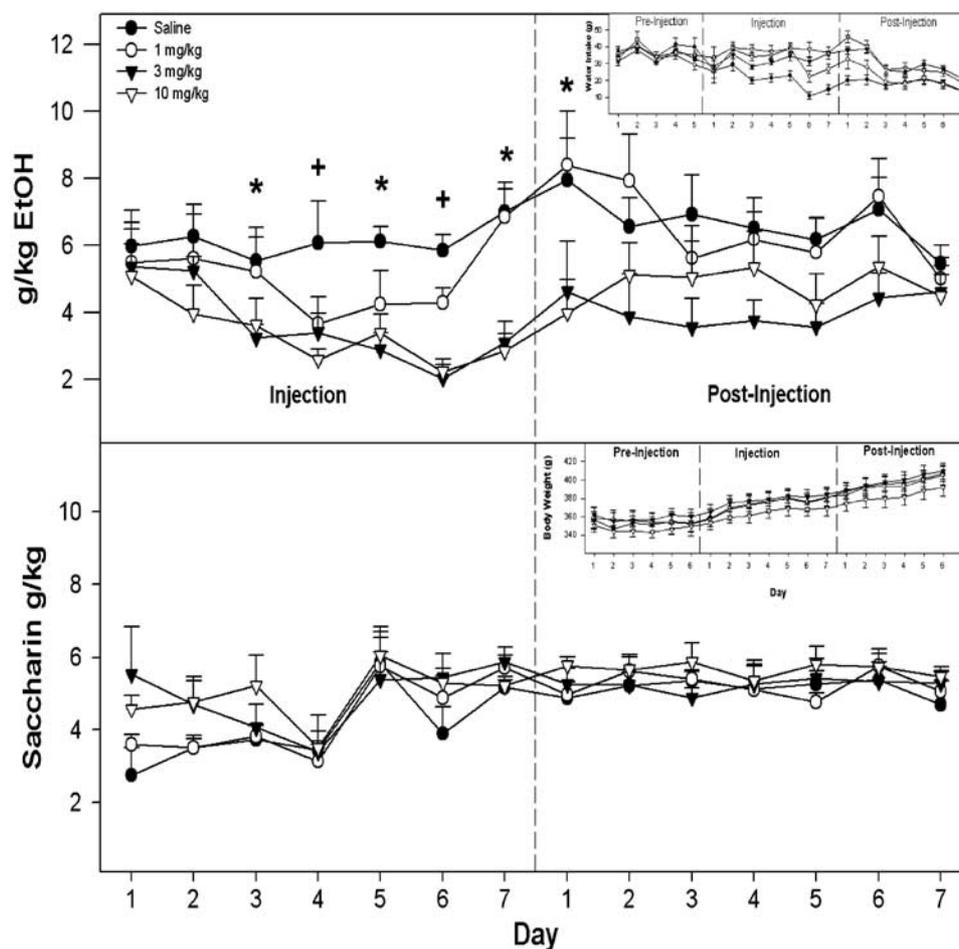


Figure 5 ACE inhibitor treatment experiments. Effects of single daily i.p. administrations of lisinopril (0, 1, 3, or 10 mg/kg) on the acquisition of 24-h free choice EtOH intake (15% v/v; top panel), concurrent water intake (top panel insert), saccharin intake (0.025%; bottom panel), and body weight in adult, male P rats ($n=6-8$ /group). Lisinopril only affected the EtOH intake (top panel). Asterisks represent that rats administered 3 or 10 mg/kg lisinopril consumed significantly less EtOH than rats administered saline or 1 mg/kg lisinopril. Pound symbols indicate that rats administered 1, 3, or 10 mg/kg lisinopril consumed less EtOH than rats treated with saline.

Cell proliferation and alcoholism

Among our top candidate genes, we have at least three genes involved in cell proliferation: CCND1, FYN and IGF1R. Alcoholism is associated with an increased risk for cancers. Cyclin D1, FYN and IGF1R have key roles in promoting cell proliferation, and higher levels of them are observed in various tumors.¹¹⁴⁻¹¹⁶ Our data show that alcohol-preferring rats have lower baseline levels of cyclin D1 than alcohol-non-preferring rats in the AMY, and these levels are increased by the chronic administration of alcohol (Table 2). Cyclin D1 maps to a locus on 11q13.3 that has been linked to bipolar disorder¹¹⁷ and schizophrenia.¹¹⁸ Last but not least, cyclin D1 gene expression was reported to be altered in post-mortem brains of human alcoholics.⁵⁹ Our data also show that alcohol-preferring rats have lower baseline levels of FYN and IGF1R than alcohol-non-preferring rats in the NAC, and that these levels are decreased by the ICSA of alcohol in the VTA (Table 3). The lower baseline levels of cyclin D1, FYN and IGF1R in iP rats suggest

that they may be less susceptible to overreact with cell proliferation and develop cancers, whether due to alcohol or other environmental factors. This is consistent with a picture of physiological resilience in these alcohol-preferring animals.

Alcohol metabolism

Among our top candidate genes, we have three genes involved directly or indirectly in alcohol metabolism: ALDH1A1, aldehyde dehydrogenase 2 (ALDH2) on 12q24.1 and ALDH6A1 on 14q24.23. ALDH1A1 is an important enzyme in the metabolism of acetaldehyde and the synthesis of retinoic acid. It maps to a region on chromosome 9q21.12 that has been linked to alcoholism^{107,119-121} and bipolar disorders.⁶⁷ Moreover, a recent study has shown association between ALDH1 promoter polymorphisms and alcohol-related phenotypes in southwest California Indians.¹²⁰ Moreover, there is evidence of decreases in ALDH1 levels in post-mortem brains of persons with

schizophrenia.¹²² Our data show that alcohol-preferring rats have higher baseline level of ALDH1A1 than alcohol-non-preferring rats in the HIP, as well as the FC. Additionally, our data show that those levels are increased in the HIP in the ICSA alcohol administration paradigm also (Table 3). ALDH1 may serve an important function in metabolizing the by-products of alcohol, and thus limiting its aversive effects. This is consistent with the iP rats being more resilient to the aversive effects of alcohol, and thus being able to ingest higher amounts. Similarly, ALDH2 alleles have

been associated with inability to ingest large amounts of alcohol and facial flushing in response to alcohol.¹²³ Our data show that alcohol-preferring rats have higher baseline level of ALDH2 than alcohol-non-preferring rats in the NAC. Additionally, our data show that those levels are increased in the AMY in the ICSA alcohol administration paradigm (Table 5). Last but not least, ALDH6A1, which maps to a region on 14q24.23 implicated in alcoholism,¹¹⁰ was changed in the CP in all the three paradigms included in our analysis (Table 1).

Table 5 Top Category III candidate genes (changed in multiple single paradigms)

Genebank accession number	Symbol – description	Brain regions	Human genetic linkage/ association	Relevant biology	Human tissue (post-mortem brains)	Scoring of lines of evidence
A1104389	TH – tyrosine hydroxylase	CPIII-1 –2.49/0.03415 HIPIII-2 –1.17/0.0406	11p15.5 EtOH ^{65,242–244} BP ⁶⁶ SZ ¹⁶⁸	EtOH ²⁴⁵ Suicide ²⁴⁶	Downregulated in frontal lobes of EtOH ⁸⁸ Upregulated in locus coeruleus of major depression ^{247,248} Downregulated in coeruleus of suicides ¹⁴⁸	6.0/9.0
AA892470	CALM1 – calmodulin 1	CPIII-1 –1.28/0.01296 AMYIII-3 –1.22/0.01605	FCIII-2 –1.20/0.04837	14q32.11 EtOH ²⁴⁹ Morphine ²⁵⁰	Upregulated in frontal lobe of EtOH ¹⁷⁵	4.5/9.0
A1172017	ALDH2 – aldehyde dehydrogenase 2	AMYIII-2 1.13/0.03773	NACIII-1 1.19/0.03525	12q24.2 EtOH ⁵⁶ BP ⁸⁵	EtOH ^{251,252} Anxiety-Depression ²⁵³	4.5/9.0
M14053	NR3C1 – nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor gene)	AMYIII-1 1.37/0.018 FCIII-2 1.47/0.0497	HIPIII-3 –1.22/0.04664	5q31.3 EtOH ¹⁹⁴ SZ ⁸⁶	Stress ²⁵⁴	4.0/9.0
X55572	APOD – apolipoprotein D	FCIII-1 1.45/0.01115 AMYIII-3 –1.71/0.02982	NACIII-1 1.234/0.00642 CPIII-3- 1.15/0.01755	3q26.2-qter Antipsychotics ²⁵⁵	Downregulated in frontal lobes of EtOH ^{125,175} Upregulated in AMY and thalamus of SZ and upregulated in parietal ctx and cingulated ctx of BP ⁷⁶	4.0/9.0
X60351	CRYAB – crystallin, alpha B	FCIII-1 1.19/0.04609 CPIII-3 –1.29/0.04608	AMYIII- 11.36/0.00567	11q23.1 EtOH ¹⁹⁴	EtOH ⁵⁹	4.0/9.0

Abbreviations: AMY = amygdala; AZ = Alzheimer's disease; BP = bipolar disorder; CP = caudate-putamen; EtOH = alcohol/alcoholism; FC = frontal cortex; HIP = hippocampus; NAC = nucleus accumbens; SZ = schizophrenia.

Fold-changes and *P*-values were calculated using Affymetrix Microarray Analysis Suite version 5.0 analysis software. All *P*-values were <0.05. Numbers with each brain region in that column represent the paradigm in which the gene expression was changed.

Neuropsychiatric disorders genes

Among our top candidate genes, we have several genes that have been implicated directly or indirectly in major neuropsychiatric disorders, such as schizophrenia (SYN2 on 3p25.2 and APOD on 3q26.2-qter), bipolar disorder (BDNF on 11p14.1), anxiety (guanine nucleotide-binding protein G(s), alpha subunit (GNAS) on 20q13.32 and NR3C1 on 5q31.3) or all three (TH on 11p15.5), as well as neurite outgrowth (NRN1 on 6p25.1) and myelin formation (PBP on 12q24.23). These data may explain why alcohol abuse can lead to psychotic, mood and anxiety symptoms, and have permanent deleterious effects on brain infrastructure.

SYN2, is a neuronal phosphoprotein that coats synaptic vesicles, binds to the cytoskeleton and functions in the regulation of neurotransmitter release.¹²⁴ SYN2 is decreased in the CP of iP vs iNP rats, and expression is increased by the ICSA administration of alcohol (Table 3). SYN2 maps to a locus on chromosome 3p25.2 that has been implicated in schizophrenia.^{57,125} There is evidence of downregulation in post-mortem brain tissue of persons with schizophrenia and bipolar illness,^{126,127} as well as in persons with alcohol dependence.^{59,128} Our findings of lower SYN2 in iP rats, and its increase by alcohol administration, suggest that iP rats, and by extension human alcoholics, may be using alcohol to improve a baseline cognitive deficit.

BDNF promotes the survival of neuronal populations in the CNS. BDNF has been implicated in substance abuse,¹²⁹ alcoholism^{130,131} and depression.¹³² In our data sets, we see BDNF increased in the AMY in both the iP rats vs iNP rats paradigm, and in the ICSA administration of alcohol paradigm (Table 3). These findings are intriguing, and are consistent with the physiological robustness of the iP rats, and perhaps a positive effect of the stress of exposure to alcohol (hormesis). BDNF maps to a locus on chromosome 11p14.1 that has been implicated in human genetic studies of alcoholism,¹³³ bipolar affective disorder^{134–138} and schizophrenia.¹³⁹ There is post-mortem human brain evidence regarding its downregulation in bipolar,¹⁴⁰ suicide,¹⁴¹ schizophrenia¹⁴² and Alzheimer disease.¹⁴³ Our findings of increased BDNF are intriguing. They contrast with the decrease in human post-mortem data, and are consistent with the physiological robustness of the iP rats, perhaps positive effects of the stress of exposure to alcohol (hormesis) and elevated mood.

GNAS is involved in hormonal regulation of adenylate cyclase. It activates the cyclase in response to beta-adrenergic stimuli. GNAS has been implicated in both alcoholism¹⁴⁴ and anxiety.¹⁴⁵ In our data sets, GNAS is decreased in the AMY in both the iP rats vs iNP rats paradigm, and in the chronic alcohol administration paradigm (Table 2). GNAS maps to a locus on chromosome 20q13.32 that has been implicated in low level of response to alcohol.¹¹⁹ Our findings of lower GNAS in iP rats in the AMY suggest a decreased reactivity to fearful stimuli. The decrease of GNAS induced by chronic alcohol may underlie some of the anxiolytic effects of alcohol.

TH, mainly expressed in the brain and adrenal glands, is the rate-limiting enzyme in the biosynthesis of catechola-

mines. TH has been suggested to be implicated in both bipolar disorder and alcoholism by earlier human genetic studies,^{146,147} although the evidence published since has been contradictory. In our data sets, we see TH decreased in the CP of iP rats vs iNP rats, and also decreased in the HIP by chronic alcohol (Table 5). TH maps to a locus on chromosome 11p15.5 that has been implicated in human genetic studies of alcoholism, bipolar disorder and schizophrenia. There is biological evidence of its involvement in alcohol abuse, anxiety and suicidality, including changes in post-mortem human brains (decreased in the FC of alcoholics⁸⁸ and in the locus coeruleus of suicide victims¹⁴⁸). Our findings of lower TH in iP rats, and its decrease by alcohol administration, may fit together into a picture of impulsivity and poor behavioral control.

Taken together, the data for SYN2, BDNF, GNAS and TH point to a picture of decreased cognition, physiological robustness, elevated mood, decreased anxiety, low response to alcohol and impulsivity in the male iP rats, and by extrapolation, alcohol-preferring men. This is not dissimilar to type B alcoholism,¹⁴⁹ or indeed anti-social personality disorder, and hyperthymic temperament,¹⁵⁰ as we go in our translational comparison from DSM-IV axis I (psychiatric disorders), to axis II (personality disorders), to normal population variants (temperaments).

GeneSpring clustering analysis of top candidate genes (Figure 3c)

Unsupervised hierarchical clustering of the top candidate genes in the pyramid using GeneSpring revealed, first, that the two alcohol treatment paradigms (Paradigms 2 and 3) cluster more closely together than with the baseline iP vs iNP strain comparison (Paradigm 1). This is reassuring, as it indicates a distinguishable effect of alcohol administration in two very different paradigms. Second, the analysis revealed two major clusters of genes that show a reciprocal relationship of increase (I) and decrease (D) in Paradigm 1 (iP vs iNP) and Paradigm 3 (ICSA alcohol), with no overall marked effect (N) in the chronic alcohol treatment (Paradigm 2). The lower cluster, containing genes that are predominantly lower in iP rats, involves genes that may have to do with mediating the aversive effects of alcohol (FN1, MAPK14, PRKCE, ALDH6A1), and the upper cluster, containing genes that are predominantly higher in iP rats, involves genes that may have to do with physiological resilience and rewarding effects of alcohol (VCAM1, CD81, TFRC, LYZ, ALDH1A1, AGT, CAMKK1 (Ca²⁺/calmodulin-dependent protein kinase 1, alpha), GNAS). This is consistent with the emerging overall picture of physical and physiological robustness, tolerance and increased reward in the alcohol-preferring male rats, and by extension, male alcoholics, somewhat evocative of the 'Irishman stereotype'.^{151–153}

GeneOntology analysis of results

GeneOntology (GO) analysis of the complete data set, Categories I and II (Table 6a), revealed that the highest probability genes were genes having to do with cellular processes (1, 3), infrastructure (4, 5) and response to external

Table 6 Biological processes classification obtained from GO: (a) analysis of the complete data set of Category I and II genes and (b) analysis of the top candidate genes based on lines-of-evidence score

(a)
Go analysis – biological processes

	Categories			
	I	II A	II B	II C
	Number of genes			
1. Cellular physiological process	1	12	10	10
2. Response to external stimuli	1	2	4	1
3. Regulation of cellular process	1	1	1	3
4. Cell growth	1	1	2	2
5. Regulation of development	1	1	2	2
6. Metabolism		11	18	5
7. Cell communication		8	8	4
8. Morphogenesis		2	5	3
9. Regulation of physiological process		4	1	3
10. Organismal physiological process		3	3	3
11. Secretion		1	1	3
12. Cell differentiation		1	1	1
13. Chemosensory behavior		1		
14. Reproduction		1	1	
15. Pigmentation			1	
16. Cell death				1
17. Membrane fusion		1		
18. Extracellular structure organization and biogenesis			1	
19. Regulation of enzyme activity		1		
20. Homeostasis		1		

(b)
Go analysis – biological processes

	Number of lines of evidence						
	7	6.5	6	5.5	5	4.5	4
	Number of genes						
1. Cellular physiological process		2	3	8	2	6	11
2. Metabolism		2	2	8	1	10	9
3. Cell communication		1	2	4	1	3	2
4. Response to stimulus		1	2	3		4	2
5. Localization		1	2		1	5	4
6. Regulation of physiological process			3	2	1	3	3
7. Regulation of cellular process			1	4	1	3	3
8. Morphogenesis			1	5		1	2
9. Organ development			1	5		1	2
10. Organismal physiological process			3	1		1	1
11. Regulation of biological process			1	2			3
12. Cell death				2		1	1
13. Secretion					1	2	
14. Regulation of development			1	1			
15. Cell differentiation				2			
16. Homeostasis		1				1	
17. Regulation of enzyme activity			1				1
18. Viral infectious cycle			1				
19. Interaction between organism			1				
20. Growth			1				
21. Extracellular structure organization and biogenesis				1			
22. Feeding behavior				1			
23. Sex determination				1			
24. Membrane fusion						1	
25. Aging						1	
26. Locomotory behavior							1
27. Reproduction							1
28. Tissue regeneration							1
29. Embryonic development							1

GO = GeneOntology.

stimuli (2). This is consistent with a model of alcohol abuse disorders that might be speculated to involve a reaction to external stimuli in the form of modified cellular functions, and infrastructure changes/tissue re-modeling (Figure 4b). Of note, a GO analysis of only the top candidate genes revealed a similar distribution of cellular functions (1, 7) and response to stimulus (4) (Table 6b and Figure 4b).

Our approach described so far is to generate data in an appropriate discovery paradigm and let the data coalesce into possible mechanistic interpretations. An opposite, hypothesis-driven approach for mining our data sets is to ask whether genes related to known biological mechanisms of interest (Table 7), linkage loci (Table 1S – see Supplementary material) or post-mortem findings (Table 8) are present in them – spanning the spectrum from the more sensitive (biological) to the more specific (post-mortem) external corroborative lines of evidence.

Biological roles

An interrogation of our complete data set of reproducibly changed genes, Categories I–III, for classification into functional groups that have been previously implicated or hypothesized to have relevance to the pathophysiology of alcoholism and related disorders, yielded genes related to neurotransmission (GABA, dopamine, acetylcholine, adenosine), cellular mechanisms (cell adhesion signaling, cellular stress response, clock genes, transporters, synaptic function) and physiological functions (alcohol metabolism, lipid metabolism, iron metabolism, growth factors) (Table 7).

Cross-validation with human linkage loci

An interrogation of our data set for genes that map to the linkage loci for alcoholism, as well as loci for bipolar disorder and schizophrenia, yielded a series of candidate genes at those loci (Table 1S – see Supplementary material), which may help prioritize future candidate gene research for each of these loci.

Cross-validation of human post-mortem findings

Last but not least, an interrogation of our data set with genes that have previously been reported in the literature to be altered in post-mortem brains from subjects with alcoholism, as well as post-mortem brains from subjects with other neuropsychiatric disorders (bipolar, schizophrenia, dementia, depression, suicide), confirmed in our data set some of those earlier findings (Table 8). This cross-validation, on the one hand, reinforces the validity of our approach and, on the other hand, reduces the likelihood that those particular post-mortem findings are methodological or gene–environment interactions artifacts of work with post-mortem human tissue. Moreover, it illustrates at a genetic and neurobiological mechanism level the overlap among major neuropsychiatric disorders.¹⁵⁴

Discussion

With the goal of helping break the genetic code of alcoholism, we have used a comprehensive translational

approach for identifying high-probability candidate genes, pathways and mechanisms for alcoholism, by integrating in a Bayesian fashion multiple independent lines of evidence. The analysis revealed that alcohol has pleiotropic effects on multiple systems, with a consequent wide anatomical and physiological impact, which may explain the diverse neuropsychiatric and medical pathology in alcoholism.

Limitations and confounds

Inbred rat lines, such as those used to generate the data analyzed in this paper, are prone to accumulating genetic defects that may be reflected in gene expression levels. While we have no way of knowing if some of the genes we captured in our screen are the result of such random accumulated defects rather than being involved in the preference or response to alcohol, it is to be noted that we have used three very different animal model paradigms in our analysis (alcohol preference selection, response to chronic oral alcohol and response to the ICSA of alcohol in the VTA), focusing on genes that are changed in common among these different paradigms rather than genes that are unique to one paradigm or another. It is to be noted that some of these gene changes have also been reported in post-mortem brains of alcoholic patients or patients with other neuropsychiatric disorders (Table 8). Moreover, we have candidate genes in our data set that are involved in pathways known from previous work to be associated with alcohol response (such as GABA genes), or with alcohol metabolism (such as aldehyde dehydrogenase genes) (Table 7). More work, in transgenic mouse models, for example, should nevertheless be pursued to establish unambiguously the roles of our top candidate gene findings in the neurobiology of response to alcohol.

Different combinations of alcohol animal models, treatments and comparisons others than the ones we have used could be integrated in a comprehensive approach such as the one we have described. They could conceivably lead to different results, which would be of interest and quite useful, as it is unlikely that we are capturing with the models that we have used the full spectrum of gene expression changes and mechanisms involved in alcoholism. However, if those other models indeed mimic and modulate the same core phenomenology, the Venn diagram of overlaps between different experimental paradigms will be of high utility and interest in terms of identifying the key molecular players involved in the effects, as opposed to those involved in the (very different) artifacts of the different individual experimental models. Along these lines, microarray studies in microdissected brain regions of alcohol-preferring AA (alko, alcohol) rats vs alcohol-avoiding ANA (alko, non-alcohol) rats^{108,109} have identified some of the same genes identified in our data set: MAPK14, microtubule-associated protein tau (MAPT), KCNA4 (potassium channel voltage-gated, shaker-related subfamily, member 4), GABRD (gamma-aminobutyric acid receptor, delta), ADORA2A (adenosine A2a receptor), THRA and RB1. Of note, an elegant meta-analysis of mouse alcoholism models was reported recently.⁹⁹ We do not see a significant overlap

Table 7 Candidate genes and biological roles

<i>Accession number</i>	<i>Gene symbol – description</i>	<i>Brain region/ category/paradigm</i>
<i>Neurotransmission</i>		
GABA		
NM_080587	GABRA4 – gamma-aminobutyric acid (GABA) A receptor, alpha 4	CPIII-1
NM_012956	GABRB1 – gamma-aminobutyric acid receptor, beta-1	CPIII-1 HIPIII-1
M35162	GABRD – gamma-aminobutyric acid receptor, delta	CPIII-1 HIPIII-2
L08497	GABRG2 – gamma-aminobutyric acid receptor, gamma-2	CPIII-1
X51922	GABRA5 – gamma-aminobutyric acid (GABA) A receptor, alpha 5	PFCIII-3
L08490	GABRA1 – gamma-aminobutyric acid (GABA) A receptor, alpha 1	AMYIII-3
Serotonin		
J03481	QDPR – quinoid dihydropteridine reductase	NACIIb
Dopamine		
J03481	QDPR – quinoid dihydropteridine reductase	NACIIb
L19998	SULT1A1 – sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	PFCIIb
AI008131	AMD1 – s-adenosylmethionine decarboxylase	CPIIc
Cholinergic		
M16407	CHRM3 – cholinergic receptor, muscarinic 3	AMYIII-1 HIPIII-3
L31619	CHRNA7 – cholinergic receptor, nicotinic, alpha polypeptide 7	CPIII-1
M16409	CHRM4 – cholinergic receptor, muscarinic, 4	CPIII-2
AI764103	ACHE – acetylcholinesterase	CPIII-2
Adenosine		
L08102	ADORA2A – adenosine A2a receptor	NACIII-1
<i>Cellular mechanisms</i>		
Cell adhesion		
X15906	FN1 – fibronectin 1	AMYIIa
X63722	VCAM1 – vascular cell adhesion molecule 1	NACIIb
U81037	NRCAM – neuronal cell adhesion molecule	CPIIc
AI103957	CD81 – cell surface protein (CD81 antigen)	AMY I; NACIIc
Cellular stress response		
U73142	MAPK14 – mitogen-activated protein kinase 14	CPIIa
AI176658	HSPB1 – heat-shock 27 kDa protein 1	HIPIIaPFCIIb
Ion channels		
M18331	PRKCE – protein kinase c, epsilon	AMYIIa
M32867	KCNA4 – potassium channel voltage-gated, shaker-related subfamily, member 4	NACIIb
Circadian clock genes		
J03179	DBP – D-box binding protein	PFC III-1
Transporters		
AF000899	NUPL1 – nucleoporin-like 1	PFCIIc
Synaptic function		
AF000423	SYT11 – synaptotagmin 11	AMYIIc
M27925	SYN2 – synapsin 2	NACIIc CPIIb
M24104	VAMP1 – vesicle-associated membrane protein 1 (synaptobrevin 1)	HIPIII-2
M64780	AGRN – agrin	AMYIIa
<i>Physiological functions</i>		
Alcohol metabolism		
af001898	ALDH1A1 – aldehyde dehydrogenase 1 family, member A1	HIPIIb
AI172017	ALDH2 – acetaldehyde dehydrogenase 2	NACIII-1
AI172017	ALDH2 – acetaldehyde dehydrogenase 2	NACIII-1
Growth factors		
D14839	FGF9 – fibroblast growth factor 9	PFCIII-1
AF030286	BDNF – brain-derived neurotrophic factor	AMYIIb
Lipid metabolism		
AI237731	LPL – lipoprotein lipase	CPIII-1

Table 7 Continued

Accession number	Gene symbol – description	Brain region/ category/paradigm
Iron metabolism		HIPIII-1 NACIII-3
M58040	TFRC – transferrin receptor	HIPIIa
D38380	TF – transferrin	PFCIII-1
AI169802	FTH1 – ferritin, heavy polypeptide 1	PFCIII-1
D50436	FDX – ferredoxin 1	CPIIa

between our results and the top results of that analysis, with the exception of PRKCE, CRYAB and NICN1. It has to be pointed, however, that the design of their study was different than ours and had two major potential limitations: (1) the use of whole-brain gene expression data, which could confound signal from specific brain regions; (2) the cross-matching of their results to congenic mouse strain data instead of human genetic and post-mortem data, which arguably reduces the specificity and relevance of their findings for the human condition purported to be modeled.

Our experimental approach for detecting gene expression changes relies on a single methodology, Affymetrix Gene-Chip microarrays. It is possible, indeed likely, that at least some of the gene expression changes detected from a single biological experiment, with a one-time assay with this technology, are technical or biological artifacts. Cognizant of that, we have designed our analysis to minimize the likelihood of having false positives, even at the expense of having false negatives. Our approach, as described above, based on the same genes being changed across different paradigms, ensures some measure of biological and technical reproducibility, as the different paradigm animal experiments and microarray assays are carried out at different times. Moreover, it is weighted toward genes that are being changed in multiple paradigms in the same brain region, and less weighted toward genes that are changed in different brain regions in different paradigms. Finally, the external convergences, with human genetic linkage data, post-mortem brain data and biological roles data, all serve to create a hierarchy in our findings that puts at the top genes with a reduced likelihood of type I error.

Our animal model data was generated from inbred male rats, and we may arguably miss gender-related differences in the underlying neurobiology of alcoholism. As such, our speculative translational conclusions below should be viewed with this major caveat in mind, and might be more germane to type II (or type B) alcoholism, which has a male preponderance, rather than to type I (or type A) alcoholism.

We have performed our convergent analysis at an individual gene level, rather than a pathway level. As such, we may be missing many things. Arguably, pathways are more conserved than individual genes.²⁴ It may be worthwhile, with the emerging availability of more sophisticated bioinformatics approaches, to re-examine our data sets by

doing convergence at a pathway rather than individual gene level. Another higher order analysis that needs to be carried out is that of epistasis, based on the possibility that ‘genes that change together (may) work together’.^{21,23} Candidate genes need to be further validated by studying the phenotype of transgenic mice in which the gene of interest is ablated (knockout, small interfering RNA), or overexpressed. More definitive proof will consist of demonstrating association of polymorphisms in the gene with the illness in human candidate gene association studies. The ultimate proof, of course, would be evidence that those polymorphisms have functional significance.²⁴

Conclusions and future directions

The current data analysis includes examination of innate neurological differences in gene expression in two rats lines selected for divergent propensity to consume alcohol (Paradigm 1). The genetic difference between these rat lines should reflect, in part, the components that facilitate the acquisition and maintenance of alcohol consumption. Theoretically, iP rats should have genetic differences from iNP rats that correspond with an increase in the rewarding properties of alcohol, an increase in the tolerance to the negative consequences of consuming copious amounts of alcohol and perhaps genes that increase the propensity to consume fluids and/or substances. However, a single gene could possibly mediate all three proposed components. Examination of the genetic alterations produced by chronic alcohol consumption in iP rats (paradigm 2) should have provided a list of genes that are induced by alcohol consumption that regulate alcohol reward/tolerance and fluid consumption. Correspondence of genes between the chronic alcohol consumption (Paradigm 2) and innate differences studies (Paradigm 1) should indicate the genes that are involved in both the propensity to consume alcohol and maintenance of alcohol consumption. However, genes differentially expressed following chronic alcohol consumption but not observed during the innate analysis may be solely inducible genes that mediate the same biological processes. Changes in gene expression following ICASA of alcohol into the posterior VTA (Paradigm 3) should primarily be the result of the reinforcing actions of alcohol in this region. Therefore, convergent genetic alterations in

Table 8 Candidate genes and human post-mortem brain data

Genes from our data set (Categories I–III) with evidence of human post-mortem brain changes *Brain region/category/paradigms (change)*

Alcohol

STX12 – syntaxin 12	HIP-IIa (I/D)
CAPNS1 – calpain, small subunit 1	AMY-IIa (I/I)
AGT – angiotensinogen	PFC-IIa (I/D)
RPL29 – ribosomal protein L29	HIP-IIc (D/I)
SYT11 – synaptotagmin 11	AMY-IIc (I/D)
UGCG – UDP-glucose:ceramide glycosyltransferase	PFC-IIc (I/D)
IGF1R – insulin-like growth factor 1 receptor	NAC-IIb (D/D)
FN1 – fibronectin 1	AMY-IIa (D/I)
CCND1 – cyclin D1	AMY-IIa (D/I)
TFRC – transferrin receptor	HIP-IIa (I/I)
LYZ – lysozyme	PFC-IIa (I/I)
COPB2 – coatamer protein complex, subunit beta 2 (beta prime)	CP-IIa (D/I)
MAPK14 – mitogen-activated protein kinase 14	CP-IIa (I/D)
MAPT – microtubule-associated protein tau	NAC-IIb (D/I)
SYN2 – synapsin 2	CP-IIb (D/I); NAC-IIc (I/D)
CALM1 – calmodulin 1	CP-III-1 (D); PFC-III-2 (D); AMY-III-3 (D)
TH – tyrosine hydroxylase	CP-III-1 (D); PFC-III-2 (I); HIP-III-2 (D)
CRYAB – crystallin, alpha B	PFC-III-1 (D); AM-YIII-1 (I); CP-III-3 (D)
APOD – apolipoprotein D	PF-CIII-1 (I); AMY-III-1 (D);NAC-III-1 (I); CP-III-3 (D)

Bipolar

BDNF – brain-derived neurotrophic factor	AMY-IIb (I/I)
ATP5C1 – ATP synthase, H ⁺ transporting, mitochondrial F1 complex, gamma polypeptide 1	AMY-IIb (I/D)
DNM1L – dynamin 1-like	AMY-IIb (D/I)
ATRN – attractin	NAC-IIb (I/D)
MIF – macrophage migration inhibitory factor	CP-IIb (D/I)
DNAJB1 – DNAJ (HSP40) homolog subfamily B, member 1	NAC-IIc (I/D)
QDPR – quinoid dihydropteridine reductase	NAC-IIb (I/D)
NRN1 – neuritin	CP-IIa (D/D)
SGNE1 – secretory granule neuroendocrine protein 1	PFC-IIb (I/I)
ATP6V0C – ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump) 16 kDa	HIP-IIb (D/I)
SPARC – secreted acidic cystein-rich glycoprotein (osteonectin)	NAC-IIb (I/I)
MAPT – microtubule-associated protein tau	NAC-IIb (D/I)
FYN – fyn proto-oncogene	NAC-IIb (D/D)
VCAM1 – vascular cell adhesion molecule 1	NAC-IIb (I/D)
FSTL1 – follistatin-like 1	CP-IIa (D/I); AMY-IIb (D/D)
HSPB1 – heat-shock 27 kDa protein 1	HIP-IIa (I/I); PFC-IIb (I/D)
SYN2 – synapsin 2	NAC-IIc (D/I); NAC-IIc (I/D)
CNTN1 – contactin 1	CP-III-1 (D); AMY-III-3 (I); NAC-III-3 (I)
ATP5O – ATP synthase, H ⁺ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein)	AMY-III-1 (D); CP-III-1 (D); HIP-III-2 (D)
APOD – apolipoprotein D	PFC-III-1 (I); AMY-III-3 (D); NAC-III-1 (I);CP-III-3 (D)

Schizophrenia

FYN – fyn proto-oncogene	NAC-IIb (D/D)
BDNF – brain-derived neurotrophic factor	AMY-IIb (I/I)
ALDH1A1 – aldehyde dehydrogenase family 1, member A1	HIP-IIb (I/I)
MAPT – microtubule-associated protein tau	NAC-IIb (D/I)
SYN2 – synapsin 2	NAC-IIc (D/I); NACIIc (I/D)
PSMA1 – proteasome (prosome, macropain) subunit, alpha type 1	AMY-III-1 (I); CPIII-1 (D); NACIII-3 (D)
APOD – apolipoprotein D	PFC-III-1 (I); NAC-1 (I); AMYIII-3 (D); CPIII-3 (D)

Dementia

PREP – prolyl endopeptidase	HIP-IIb (D/D)
NEFL – neurofilament, light polypeptide 68 kDa	CP-IIa (D/D)
PIK4CB – phosphatidylinositol 4-kinase	NAC-IIb (I/I)

Table 8 *Continued*

Genes from our data set (Categories I–III) with evidence of human post-mortem brain changes *Brain region/category/paradigms (change)*

AGRN – agrin	AMY-IIa (I/I)
PRKCE – protein kinase C, epsilon	AMY-IIa (D/I)
CDC2L5 – cell division cycle 2-like 5 (cholinesterase-related cell division controller)	PFC-IIa (D/I)
LRPAP1 – low-density lipoprotein receptor-related protein-associated protein 1	NAC-IIb (I/I)
IGF1R – insulin-like growth factor 1 receptor	NAC-IIb (D/D)
MAPT – microtubule-associated protein tau	NAC-IIb (D/I)
ACTB – actin, beta	NA-IIb (D/D)
BDNF – brain-derived neurotrophic factor	AMY-IIb (I/I)
ATP5C1 – ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	AMY-IIb (I/D)
HSPB1 – heat-shock 27 kDa protein 1	HIP-IIa (I/I); PFC-IIb (I/D)
Depression	
VCAM1 – vascular cell adhesion molecule 1	NAC-IIb (I/D)
TH – tyrosine hydroxylase	CP-III-1 (D); PFC-III-2 (I); HIP-III-2 (D)
Suicide	
BDNF – brain-derived neurotrophic factor	AMY-IIb (I/I)
TH – tyrosine hydroxylase	CP-III-1 (D); PFC-III-2 (I); HIP-III-2 (D)

Candidate genes in our data set that have also been reported in human post-mortem brain studies of alcoholism and other neuropsychiatric disorders.

all three paradigms should primarily reflect genes involved in mediating alcohol effects.

The analysis revealed at the top of the list genes related to cell adhesion and signaling (FN1 on 2q35, VCAM1 on 1p21.2 and CD81 on 11p15.5), catecholamine biosynthesis (TH on 11p15.5), iron–heme metabolism (TFRC (transferin receptor mRNA) on 3q29 and ALAS1 on 3p21.2), cardiovascular regulation (AGT on 1q42.2 and PRKCE on 2p21), cellular stress response (MAPK14 on 6p21.31), cell proliferation and differentiation (cyclin D1 on 11q13.3, FYN on 6q21, IGFR1 on 15q26.3), nuclear pore function (NUPL1 on 13q12.13), anti-bacterial protection (LYZ on 12q15), Golgi/endoplasmic reticulum function (STX12 on 1p35.3) and alcohol metabolism (ALDH1 on 9q21.12, ALDH6A1 on 14q24.23 and ALDH2 on 12q24.1). Nine of these 17 genes map to genetic linkage loci for alcoholism, and eight out of these 17 genes have published evidence of human post-mortem brain changes in alcoholism. These data reveal that alcohol has pleiotropic effects on multiple systems, with a consequent wide anatomical and physiological impact, which explains the diverse medical and neuropsychiatric pathology in alcoholism. More specifically for the latter, among the top candidate genes we have genes related to schizophrenia (SYN2 on 3p25.2 and APOD on 3q26.2-qter), bipolar disorders (BDNF on 11p14.1), anxiety (GNAS on 20q13.32 and NR3C1 on 5q31.3) or all three (TH on 11p15.5), as well as neurite outgrowth (NRN1 on 6p25.1) and myelin formation (PBP on 12q24.23). Moreover, 20 of the 24 genes mentioned above map to genetic linkage loci previously implicated in bipolar disorder and/or schizo-

phrenia, and seven out of these 24 genes have published evidence of human post-mortem brain changes in bipolar and/or schizophrenia. These data may explain why alcohol abuse can lead to mood, psychotic and anxiety symptoms, and have permanent deleterious effects on brain infrastructure. In addition, other candidate genes (involved in response to external stimuli, cellular physiological processes, metabolism and infrastructure), pathways and mechanisms of likely importance in pathophysiology were identified from the complete data sets.

The results presented in this paper have a series of direct implications. First, in terms of pharmacotherapy and drug development, some of the candidate genes in our data sets encode for proteins that are modulated by existing pharmacological agents (Table 9), which may suggest future avenues for rational polypharmacy using existing agents. Such existing, non-addictive drugs include, for example, ACE inhibitors for the AGT pathway, and mood-stabilizing drugs (lithium for PRKCE, lamotrigine and zonisamide for SCN1A, olanzapine for GABRA1 (gamma-aminobutyric acid receptor, alpha 1)). Moreover, our data sets of the effects of alcohol on gene expression in different key brain regions (Tables 1–5), as well as the pathways and mechanisms identified, may be of use as a source of new targets for drug development. We have pursued one of these leads, modulating the AGT pathway with the ACE inhibitor lisinopril, and showed significant reduction in alcohol consumption in iP rats (Figure 5). Given the high comorbidity of alcoholism and hypertension, the original medical condition for which ACE inhibitors have been approved and marketed, human

Table 9 Top candidate genes in our data sets that encode targets of existing pharmacological agents (Ingenuity analysis)

Genebank accession number	Symbol – description	Brain region (Paradigm) fold-change/ P-value		Family	Drug
AF001898	ALDH1A1 – aldehyde dehydrogenase family 1, member A1	PFC(1) 1.34/0.00211 HIP(1) 1.22/0.02931	CP(2) 1.21/0.028 HIP(3) 1.39/0.00103	Enzyme	Disulfiram
D90035	PPP3CA – protein phosphatase 3, catalytic subunit, alpha isoform	PFC(3) 1.29/0.00282 AMY(3) 1.26/0.01712 NAC(1) –1.11/0.0311	AMY(1) 1.23/0.02995 CP(3) 1.17/0.01792	Phosphatase	Cyclosporine, Fk506
M22253	SCN1A – sodium channel, voltage-gated, type 1, alpha polypeptide	PFC(1) 1.46/0.01392 NAC(1) 1.78/0.00133 CP(3) 1.17/0.0254	AMY(1) 1.69/0.03608 CP(2) –1.25/0.00874 HIP(1) 1.44/0.01154	Ion channel	Lamotrigine, lidocaine, phenytoin, prilocaine, procaine, ropivacaine, zonisamide
M14053	NR3C1 – nuclear receptor subfamily 3, group C, member 1	PFC(2) 1.47/0.0497 HIP(1) –1.22/0.04664	AMY(1) 1.37/0.018	Ligand-dependent nuclear receptor	Beclomethasone dipropionate, betamethasone, dexamethasone/tobramycin, fluticasone, fluticasone/salmeterol, methylprednisolone, mometasone furoate, prednisolone, prednisone, triamcinolone acetonide
M16407	CHRM3 – cholinergic receptor, muscarinic 3	AMY(1) 1.24/0.0131	HIP(3) –1.18/0.03711	G-protein coupled receptor	Ipratropium, olanzapine, tolterodine
A1172017	ALDH2 – aldehyde dehydrogenase 2	AMY(2) 1.13/0.03773	NAC(1) 1.19/0.03525	Enzyme	Disulfiram
M31174	THRA – thyroid hormone receptor alpha	AMY(3) 1.23/0.04192 HIP(3) 1.27/0.01358	CP(1) 1.63/0.01932	Ligand-dependent nuclear receptor	Amiodarone, thyroxine
L08490	GABRA1 – gamma-aminobutyric acid receptor, subunit beta 1	AMY(3) 1.40/0.02694		Ion channel	Clonazepam, diazepam, floricet, lorazepam, muscimol, olanzapine, sevoflurane, temazepam, zaleplon, zolpidem
A1237731	LPL – lipoprotein lipase	CP(1) –1.58/0.04801 HIP(1) 1.32/0.02446	NAC(3) –1.81/ 0.02148	Enzyme	Gemfibrozil, nicotinic acid
NM_012956	GABRB1 – gamma-aminobutyric acid receptor, subunit beta 1	CP(1) –2.65/0.02562	HIP(1) 1.57/0.00735	Ion channel	Clonazepam, diazepam, floricet, lorazepam, muscimol, olanzapine, sevoflurane, temazepam, zaleplon, zolpidem
M35162	GABRD – gamma-aminobutyric acid A receptor, delta	CP(1) –2.11/0.0258 HIP(2) –1.14/0.01673	CP(1) –1.72/0.03088	Ion channel	Clonazepam, diazepam, floricet, lorazepam, muscimol, olanzapine, sevoflurane, temazepam, zaleplon, zolpidem

clinical trials in alcohol-abusing subjects with comorbid hypertension are warranted, and satisfy the Hippocratic principle of *primum non nocere* (first do no harm).

Second is the uncovered relationship between brain genes involved in alcohol response and cell adhesion signaling processes. The research literature has long contained references to cell adhesion molecules, not only in relationship to alcohol but also for other drugs of abuse. It seems possible that nature, given the limited repertoire of genes and proteins, has recruited more primitive mechanisms related to cell adhesion signaling for higher functions such as neuropsychiatric processes involved in addiction. The utility of regulating cell adhesion processes in response to alcohol abuse is of speculative evolutionary interest, and of pragmatic clinical importance. Speculatively, the emergent overall picture is that physical and physiological robustness may permit alcohol-preferring individuals to withstand the aversive effects of alcohol, which is essentially an organic solvent. In conjunction with a higher reactivity to its rewarding effects, they may be able to ingest enough of this nonspecific drug for a strong hedonic and addictive effect to occur. Pragmatically, it underscores the profound psychophysiological impact of alcohol abuse, the potential prenatal teratogenicity and postnatal cumulative end-organ damage, and points to the importance of prevention and abstinence in the treatment of alcoholism.

Third, the model that emerges out of the GO analysis of our data is that of environmental input leading to changes in cellular function and infrastructure changes (Figure 4b). The simplicity of the model should not eclipse the important fact that it is the result of empirical coalescence of data in a non-hypothesis-driven, discovery-type approach. Moreover, the implications for understanding the pathophysiology and treatment of alcohol disorders are profound. One needs to modulate environmental input, internal cellular functions and infrastructure changes in the treatment of these disorders. It is a place where both pharmacology and cognitive-behavioral psychotherapeutic interventions can and should go hand in hand.

Fourth, the identification of candidate genes for alcoholism that have also been implicated in other neuropsychiatric disorders, such as schizophrenia, bipolar disorder and anxiety may explain the comorbidity between alcohol abuse, psychosis and affective disorders. Whether the initial entry point into pathology is an underlying neuropsychiatric disorder with subsequent alcohol abuse, or whether alcohol abuse leads to neuropsychiatric symptoms, the genes and pathways identified may provide an opportunity to understand, and ultimately treat, these relatively refractory dual-diagnosis disorder patients.

In conclusion, we propose that our comprehensive translational CFG analysis of microarray data derived from various experimental paradigms in an established rodent model of alcoholism has produced novel candidate genes, pathways and mechanisms that could be of important heuristic value in the understanding of the biological basis of alcoholism, and the development of pharmacotherapies for the treatment of alcoholism. It arguably generates a

series of immediate leads for both future research and clinical practice.

Materials and methods

Alcohol-naïve iP and iNP rats

Most rodents do not voluntarily consume alcohol in sufficient quantities to produce pharmacologically meaningful blood alcohol levels. Through selective breeding, however, it has been possible to produce rats with either high (alcohol-preferring or P) or low (alcohol-non-preferring or NP) alcohol-drinking characteristics.^{155–157} The P line of rats has been well characterized and meets the criteria for an animal model of alcoholism. The P line of rat prefers an EtOH solution over water at least at 2:1 ratio, whereas the NP rats show a preference ratio of less than 0.5:1. These rat lines also demonstrate distinct physiological and behavioral phenotypes in response to alcohol.^{157,158} Alcohol-naïve, male iP and iNP rats, 90–100 days old, were housed and killed as described previously.²⁹

Chronic alcohol vs water in iP rats

Experimentally naïve, male iP rats ($n=6$ per group; iP 5c strain) from the 34th and 35th inbred generations, 4–6 months of age at the start of the experiment, were used as subjects. Food and water were available *ad libitum* throughout the experiments. Rats were given continuous free-choice access in the home cage to 15% (v/v) EtOH and water (alcohol group) or water only. Alcohol intake was relatively constant throughout the experiment (ranging from 5.8 to 6.4 g/kg/day). Samples were collected after the 10th week of alcohol access.

Intracranial self-administration of alcohol into the posterior VTA in iP rats

Our third paradigm focuses on the neurocircuitry involved in alcohol reinforcement, and its gene expression response to alcohol.³⁰ The ICSA technique has been employed to identify specific brain regions involved in the initiation of response-contingent behaviors for the delivery of a reinforcer.^{159,160} Studies utilizing the ICSA procedure have successfully isolated discrete brain regions where opioids,^{161,162} amphetamine¹⁶³ and cocaine¹⁶⁴ may produce their rewarding effects. Recently, we have reported that alcohol is self-administered into the posterior, but not anterior, VTA of Wistar and P rats,^{30,165} that P rats are more sensitive to the reinforcing properties of alcohol in the posterior VTA than Wistar rats³⁰ and that previous experience with oral alcohol consumption can significantly alter alcohol self-administration into the posterior VTA in P rats.³⁰ Additionally, microinjection of alcohol into the posterior VTA increases the amount of extracellular dopamine in the NAC shell.¹⁶⁶

Experimentally naïve, male iP rats were used. Rats were randomly assigned to one of two groups ($n=4-5$ /group). A vehicle group received infusions of aCSF for eight sessions, each 4 h in duration, occurring every other day. The other group received infusions of 150 mg % alcohol for eight

sessions. Immediately after the 8th session, rats were killed by decapitation.³⁰

ACE inhibitor (lisinopril) experiments

Experimentally naïve, male alcohol-preferring P rats from the 60th and 61st selected generations, 4–6 months of age at the start of the experiment, were used as subjects. Rats were individually housed in hanging metal cages and allowed to acclimate to room and housing conditions for 6 days before any experimental manipulations. The room was maintained on a reverse 12L/12D hour cycle with lights on at 2100 hours. Before the acclimation period, all rats were handled at least seven times. During handling, the napes of the rats' necks were gently pinched to habituate the animal to the injection procedure. Food and water were available *ad libitum* throughout the experiments.

Groups were matched for water intake and body weight during the acclimation period. Thirty minutes before the introduction of EtOH (15% (v/v)), separate groups of P rats received intraperitoneal (i.p.) injections of 0, 1, 3 or 10 mg/kg lisinopril ($n=7-8$ /group). For 7 consecutive days, rats received the same treatment at approximately 1200 hours. Rats were then allowed 20 days of continuous access to 15% EtOH. In the saccharin experiment, rats were treated identical to the EtOH acquisition study, except that 0.025% saccharin was concurrently available with water. Similarly, rats received 7 daily administration of lisinopril (0, 1, 3 or 10 mg/kg; $n=5-6$ /group) followed by 20 days of continuous access to saccharin. The left–right positions of the two bottles were randomly switched each day. Fluid intake was recorded to the nearest 0.01 g by weighing the water and 15% EtOH bottles before and after each 24-h period. Body weights were also recorded every day for the duration of the injection phase. Fluid intake measures were converted into g EtOH/kg body weight (g/kg) and amount of water consumed (ml/day).

Data analysis consisted of a group \times day mixed ANOVA with repeated measures on 'day'. Dependent variables were 24-h EtOH (g/kg), water intake, body weight, and EtOH preference.

Microarray analysis

Tissues were dissected, and RNA was extracted and analyzed using standard Affymetrix GeneChip protocols, as described previously.²⁹ Each GeneChip was scanned and analyzed using Affymetrix Microarray Analysis Suite version 5.0 (Affymetrix: Affymetrix Microarray Suite User's Guide, version 5.0, Santa Clara, CA, USA; Affymetrix; 2001). Each sample was scaled to a target intensity of 1000 using the 'all probeset' scaling option; this option scales the trimmed mean target intensity to the specified value. For each probeset, absolute analysis generates a signal value (expression level), a detection call of 'absent', 'present' or 'marginal' and a *P*-value associated with the detection call. To reduce false positives from genes that were not reliably detected, the data were pre-filtered by only analyzing genes that were called 'present' in at least half of the arrays in at least one of the groups. This is a conservative way to remove from

further analyses most genes that were not reliably detected in either group, while retaining those detected in at least one of the groups.¹⁶⁷ This filtering reduces the number of comparisons and of false positives, because genes not reliably detected exhibit high levels of noise.¹⁶⁷

For analyses, we compared the \log_2 of the level of gene expression (signal) using Welch's approximate *t*-test, assuming unequal variance. This is a conservative test that we selected based upon our evaluation of the variances in many different microarray experiments. The log-transformed signal data more closely approximated a normal distribution than did the signal data. The transformation did not greatly affect the analyses. Nearly all of the genes that significantly differed would have been identified by analyses of untransformed data. We truncated the significance level, so that any *P*-value below 0.000005 is listed as 0.000000.

Gene identification

The identities of transcripts were established using NetAFFX (Affymetrix, Santa Clara, CA, USA), and confirmed by cross-checking the target mRNA sequences that had been used for probe design in the Affymetrix RGU34A GeneChip with the GenBank database. Where possible, identities of expressed sequence tags (ESTs) were established by BLAST searches of the nucleotide database. A National Center for Biotechnology Information (Bethesda, MD, USA) BLAST analysis of the accession number of each probeset was carried out to identify each gene name. BLAST analysis identified the closest known rat gene existing in the database (the highest known rat gene at the top of the BLAST list of homologs), which then could be used to search the GeneCards database (Weizmann Institute, Rehovot, Israel) to identify the human homolog. Probesets that did not have a known gene were labeled 'EST' and their accession numbers kept as identifiers.

Genetic linkage convergence

To designate convergence for a particular gene, the gene had to map within 10 cM of a microsatellite marker for which at least one published study showed evidence for linkage to alcoholism, or another neuropsychiatric disorder. The University of Southampton's sequence-based integrated map of the human genome (The Genetic Epidemiological Group, Human Genetics Division, University of Southampton, UK: http://cedar.genetics.soton.ac.uk/public_html/) was used to obtain cM locations for both genes and markers. The sex-averaged cM value was calculated and used to determine convergence to a particular marker. For markers that were not present in the Southampton database, the Marshfield database (Center for Medical Genetics, Marshfield, WI, USA: <http://research.marshfieldclinic.org/genetics>) was used to evaluate linkage convergence. Further information on specific gene function and biology was taken from the Online Mendelian Inheritance of Man database (<http://ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>).

Biological and tissue (post-mortem brain, lymphocytes) convergence

Information about our candidate genes was obtained using GeneCards, as well as database searches using PubMed (<http://ncbi.nlm.nih.gov/PubMed>) and various combinations of keywords (gene name, alcohol, alcoholism, bipolar, schizophrenia, depression, suicide, dementia, opiates, cocaine, marijuana, hallucinogens, amphetamines, benzodiazepines, human, brain, post-mortem, lymphocytes, fibroblasts). Genes were deemed to have biological convergence if their known biological function was relevant to the pathophysiology of alcoholism and/or related disorders in human or animal models. Tissue convergence was deemed to occur for a gene if there were published reports of human post-mortem brain data (or, rarely, lymphocytes and other tissue data) showing changes in expression of that gene in tissue from patients with alcoholism and/or another neuropsychiatric disorder.

GeneSpring analysis

GeneSpring version 7.2 was used (Agilent Technologies, Palo Alto, CA, USA). Unsupervised two-way hierarchical clustering of gene expression fold-change data of the top candidate genes (from Figure 3a) was carried out.

GO analysis

The NetAffx Gene Ontology Mining Tool (Affymetrix, Santa Clara, CA, USA) was employed to categorize the genes in our data sets into functional categories, using the Biological Process ontology branch.

Pathway assist analysis

The Pathway Assist Version 3.0 Tool (Stratagene, San Diego, CA, USA) was employed to analyze the direct interactions (expression, regulation, transport, binding) of the top candidate genes resulting from our CFG analysis.

Ingenuity analysis

Ingenuity Pathway Analysis 3.0 (Ingenuity Systems, Redwood City, CA, USA) was employed to identify genes in our data sets that are the target of existing drugs.

Acknowledgments

We would like to acknowledge our debt of gratitude to the efforts and results of the Collaborative Genetics of Alcoholism (COGA) consortium, and to the pioneering efforts to develop inbred alcohol preferring and non-preferring rat lines by T-K Li, L Lumeng and collaborators at Indiana University. This work was supported by funds from INGEN (Indiana Genomics Initiative at Indiana University) to ABN. This work was also supported by the Indiana Alcohol Research Center through P60AA007611 and the Integrative Neuroscience Initiative in Alcoholism through U01AA013518 (HJE, ZAR, WJM). Microarray data were generated in the Center for Medical Genomics at Indiana University School of Medicine, which is supported in part by INGEN (HJE). ABN would also like to thank Marc Schuckit for excellent clinical mentorship and introduction to the alcohol research field.

Duality of Interest

The authors declare that they have no competing financial interests.

References

- Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL *et al*. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *JAMA* 1990; **264**: 2511–2518.
- Muthen BO, Grant B, Hasin D. The dimensionality of alcohol abuse and dependence: factor analysis of DSM-III-R and proposed DSM-IV criteria in the 1988 National Health Interview Survey. *Addiction* 1993; **88**: 1079–1090.
- Lennox RD, Steele PD, Zarkin GA, Bray JW. The differential effects of alcohol consumption and dependence on adverse alcohol-related consequences: implications for the workforce. *Drug Alcohol Depend* 1998; **50**: 211–220.
- Koob GF. Alcoholism: allostasis and beyond. *Alcohol Clin Exp Res* 2003; **27**: 232–243.
- Schuckit MA, Smith TL, Danko GP, Kramer J, Godinez J, Bucholz KK *et al*. Prospective evaluation of the four DSM-IV criteria for alcohol abuse in a large population. *Am J Psychiatry* 2005; **162**: 350–360.
- Hicks BM, Krueger RF, Iacono WG, McGue M, Patrick CJ. Family transmission and heritability of externalizing disorders: a twin-family study. *Arch Gen Psychiatry* 2004; **61**: 922–928.
- Tyndale RF. Genetics of alcohol and tobacco use in humans. *Ann Med* 2003; **35**: 94–121.
- Walters GD. The heritability of alcohol abuse and dependence: a meta-analysis of behavior genetic research. *Am J Drug Alcohol Abuse* 2002; **28**: 557–584.
- Oroszi G, Goldman D. Alcoholism: genes and mechanisms. *Pharmacogenomics* 2004; **5**: 1037–1048.
- McBride WJ, Kerns RT, Rodd ZA, Strother WN, Edenberg HJ, Hashimoto JG *et al*. Alcohol effects on central nervous system gene expression in genetic animal models. *Alcohol Clin Exp Res* 2005; **29**: 167–175.
- Worst TJ, Vrana KE. Alcohol and gene expression in the central nervous system. *Alcohol Alcohol* 2005; **40**: 63–75.
- Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO *et al*. Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* 2004; **74**: 705–714.
- Jones KA, Porjesz B, Almasy L, Bierut L, Goate A, Wang JC *et al*. Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. *Int J Psychophysiol* 2004; **53**: 75–90.
- Wang JC, Hinrichs AL, Stock H, Budde J, Allen R, Bertelsen S *et al*. Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. *Hum Mol Genet* 2004; **13**: 1903–1911.
- Covault J, Gelernter J, Hesselbrock V, Nellissery M, Kranzler HR. Allelic and haplotypic association of GABRA2 with alcohol dependence. *Am J Med Genet B* 2004; **129**: 104–109.
- Lappalainen J, Krupitsky E, Remizov M, Pchelina S, Taraskina A, Zvartau E *et al*. Association between alcoholism and gamma-amino butyric acid alpha2 receptor subtype in a Russian population. *Alcohol Clin Exp Res* 2005; **29**: 493–498.
- Pierucci-Lagha A, Covault J, Feinn R, Nellissery M, Hernandez-Avila C, Oncken C *et al*. GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. *Neuropsychopharmacology* 2005; **30**: 1193–1203.
- Belfer I, Hipp H, McKnight C, Evans C, Buzas B, Bollettino A *et al*. Association of galanin haplotypes with alcoholism and anxiety in two ethnically distinct populations. *Mol Psychiatry* 2006; **11**: 301–311.
- Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcohol Clin Exp Res* 2005; **29**: 8–16.

- 20 Hinrichs AL, Wang JC, Bufe B, Kwon JM, Budde J, Allen R et al. Functional variant in a bitter-taste receptor (hTAS2R16) influences risk of alcohol dependence. *Am J Hum Genet* 2006; **78**: 103–111.
- 21 Niculescu III AB, Segal DS, Kuczynski R, Barrett T, Hauger RL, Kelsoe JR. Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. *Physiol Genomics* 2000; **4**: 83–91.
- 22 Niculescu III AB, Kelsoe JR. Convergent functional genomics: application to bipolar disorder. *Ann Med* 2001; **33**: 263–271.
- 23 Kelsoe JR, Niculescu III AB. Finding genes for bipolar disorder in the functional genomics era: from convergent functional genomics to phenomics and back. *CNS Spectr* 2002; **7**: 215–226.
- 24 Bertsch B, Ogden CA, Sidhu K, Le-Niculescu H, Kuczynski R, Niculescu AB. Convergent functional genomics: a Bayesian candidate gene identification approach for complex disorders. *Methods* 2005; **37**: 274–279.
- 25 Ogden CA, Rich ME, Schork NJ, Paulus MP, Geyer MA, Lohr JB et al. Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. *Mol Psychiatry* 2004; **9**: 1007–1029.
- 26 Smith DG, Learn JE, McBride WJ, Lumeng L, Li TK, Murphy JM. Alcohol-naive alcohol-preferring (P) rats exhibit higher local cerebral glucose utilization than alcohol-nonpreferring (NP) and Wistar rats. *Alcohol Clin Exp Res* 2001; **25**: 1309–1316.
- 27 Flatscher-Bader T, van der Brug M, Hwang JW, Gochee PA, Matsumoto I, Niwa S et al. Alcohol-responsive genes in the frontal cortex and nucleus accumbens of human alcoholics. *J Neurochem* 2005; **93**: 359–370.
- 28 Oscar-Berman M, Marinkovic K. Alcoholism and the brain: an overview. *Alcohol Res Health* 2003; **27**: 125–133.
- 29 Edenberg HJ, Strother WN, McClintick JN, Tian H, Stephens M, Jerome RE et al. Gene expression in the hippocampus of inbred alcohol-preferring and -nonpreferring rats. *Genes Brain Behav* 2005; **4**: 20–30.
- 30 Rodd ZA, Bell RL, Melendez RI, Kuc KA, Lumeng L, Li TK et al. Comparison of intracranial self-administration of ethanol within the posterior ventral tegmental area between alcohol-preferring and Wistar rats. *Alcohol Clin Exp Res* 2004; **28**: 1212–1219.
- 31 Sladka M, Kren V, Klir P. Spontaneous acute lymphoblastic leukemia in Sprague-Dawley rats. II. Cytogenetic analysis of nine individual leukemias. *Neoplasma* 1988; **35**: 379–388.
- 32 Hamta A, Adamovic T, Heloua K, Levan G. Cytogenetic aberrations in spontaneous endometrial adenocarcinomas in the BDII rat model as revealed by chromosome banding and comparative genome hybridization. *Cancer Genet Cytogenet* 2005; **159**: 123–128.
- 33 Nurnberger Jr JJ, Wiegand R, Bucholz K, O'Connor S, Meyer ET, Reich T et al. A family study of alcohol dependence: coaggregation of multiple disorders in relatives of alcohol-dependent probands. *Arch Gen Psychiatry* 2004; **61**: 1246–1256.
- 34 Schuckit MA. Genetic and clinical implications of alcoholism and affective disorder. *Am J Psychiatry* 1986; **143**: 140–147.
- 35 Maher BS, Marazita ML, Zubenko WN, Kaplan BB, Zubenko GS. Genetic segregation analysis of alcohol and other substance-use disorders in families with recurrent, early-onset major depression. *Am J Drug Alcohol Abuse* 2002; **28**: 711–731.
- 36 Margolese HC, Malchy L, Negrete JC, Tempier R, Gill K. Drug and alcohol use among patients with schizophrenia and related psychoses: levels and consequences. *Schizophr Res* 2004; **67**: 157–166.
- 37 Brown ES. Bipolar disorder and substance abuse. *Psychiatr Clin N Am* 2005; **28**: 415–425.
- 38 Lobb RR, Abraham WM, Burkly LC, Gill A, Ma W, Knight JA et al. Pathophysiological role of alpha 4 integrins in the lung. *Ann NY Acad Sci* 1996; **796**: 113–123.
- 39 Feigelson SW, Grabovsky V, Shamri R, Levy S, Alon R. The CD81 tetraspanin facilitates instantaneous leukocyte VLA-4 adhesion strengthening to vascular cell adhesion molecule 1 (VCAM-1) under shear flow. *J Biol Chem* 2003; **278**: 51203–51212.
- 40 Coppolino M, Migliorini M, Argraves WS, Dedhar S. Identification of a novel form of the alpha 3 integrin subunit: covalent association with transferrin receptor. *Biochem J* 1995; **306**(Part 1): 129–134.
- 41 Ponka P. Cell biology of heme. *Am J Med Sci* 1999; **318**: 241–256.
- 42 Tamura K, Chen YE, Chen Q, Nyui N, Horiuchi M, Takasaki I et al. Expression of renin-angiotensin system and extracellular matrix genes in cardiovascular cells and its regulation through AT1 receptor. *Mol Cell Biochem* 2000; **212**: 203–209.
- 43 Traub O, Monia BP, Dean NM, Berk BC. PKC-epsilon is required for mechano-sensitive activation of ERK1/2 in endothelial cells. *J Biol Chem* 1997; **272**: 31251–31257.
- 44 Meyer A, van Golen CM, Kim B, van Golen KL, Feldman EL. Integrin expression regulates neuroblastoma attachment and migration. *Neoplasia* 2004; **6**: 332–342.
- 45 Padovan CS, Jahn K, Birnbaum T, Reich P, Sostak P, Strupp M et al. Expression of neuronal markers in differentiated marrow stromal cells and CD133+ stem-like cells. *Cell Transplant* 2003; **12**: 839–848.
- 46 Han S, Sidell N, Roman J. Fibronectin stimulates human lung carcinoma cell proliferation by suppressing p21 gene expression via signals involving Erk and Rho kinase. *Cancer Lett* 2005; **219**: 71–81.
- 47 Shima T, Nada S, Okada M. Transmembrane phosphoprotein Cbp senses cell adhesion signaling mediated by Src family kinase in lipid rafts. *Proc Natl Acad Sci USA* 2003; **100**: 14897–14902.
- 48 Wang L, Kwak JH, Kim SI, He Y, Choi ME. Transforming growth factor-beta1 stimulates vascular endothelial growth factor 164 via mitogen-activated protein kinase 3-p38alpha and p38delta mitogen-activated protein kinase-dependent pathway in murine mesangial cells. *J Biol Chem* 2004; **279**: 33213–33219.
- 49 Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS. Synapsins as mediators of BDNF-enhanced neurotransmitter release. *Nat Neurosci* 2000; **3**: 323–329.
- 50 Naeve GS, Ramakrishnan M, Kramer R, Hevroni D, Citri Y, Theill LE. Neuritin: a gene induced by neural activity and neurotrophins that promotes neurogenesis. *Proc Natl Acad Sci USA* 1997; **94**: 2648–2653.
- 51 Vendemiale G, Grattagliano I, Altomare E, Serviddio G, Portincasa P, Prigallo F et al. Mitochondrial oxidative damage and myocardial fibrosis in rats chronically intoxicated with moderate doses of ethanol. *Toxicol Lett* 2001; **123**: 209–216.
- 52 Savolainen V, Perola M, Lalu K, Penttila A, Virtanen I, Karhunen PJ. Early perivascular fibrogenesis – precirrhotic lesions among moderate alcohol consumers and chronic alcoholics. *J Hepatol* 1995; **23**: 524–531.
- 53 Stenback F, Wasenius VM, Sotaniemi EA. Interstitial collagen in alcoholic human liver. *Histol Histopathol* 1987; **2**: 401–411.
- 54 Ren LQ, Garrett DK, Syapin M, Syapin PJ. Differential fibronectin expression in activated C6 glial cells treated with ethanol. *Mol Pharmacol* 2000; **58**: 1303–1309.
- 55 Bell RL, Stewart RB, Woods II JE, Lumeng L, Li TK, Murphy JM et al. Responsivity and development of tolerance to the motor impairing effects of moderate doses of ethanol in alcohol-preferring (P) and -nonpreferring (NP) rat lines. *Alcohol Clin Exp Res* 2001; **25**: 644–650.
- 56 Hill SY, Shen S, Zezza N, Hoffman EK, Perlin M, Allan W. A genome wide search for alcoholism susceptibility genes. *Am J Med Genet B* 2004; **128**: 102–113.
- 57 Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T et al. Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. *Hum Mol Genet* 2004; **13**: 1693–1702.
- 58 Miyamae M, Rodriguez MM, Camacho SA, Diamond I, Mochly-Rosen D, Figueredo VM. Activation of epsilon protein kinase C correlates with a cardioprotective effect of regular ethanol consumption. *Proc Natl Acad Sci USA* 1998; **95**: 8262–8267.
- 59 Mayfield RD, Lewohl JM, Dodd PR, Herlihy A, Liu J, Harris RA. Patterns of gene expression are altered in the frontal and motor cortices of human alcoholics. *J Neurochem* 2002; **81**: 802–813.
- 60 Geisert Jr EE, Williams RW, Geisert GR, Fan L, Asbury AM, Maecker HT et al. Increased brain size and glial cell number in CD81-null mice. *J Comp Neurol* 2002; **453**: 22–32.
- 61 Kelic S, Levy S, Suarez C, Weinstein DE. CD81 regulates neuron-induced astrocyte cell-cycle exit. *Mol Cell Neurosci* 2001; **17**: 551–560.
- 62 Bahi A, Boyer F, Kafri T, Dreyer JL. CD81-induced behavioural changes during chronic cocaine administration: *in vivo* gene delivery with regulatable lentivirus. *Eur J Neurosci* 2004; **19**: 1621–1633.
- 63 Michna L, Brenz Verca MS, Widmer DA, Chen S, Lee J, Rogove J et al. Altered sensitivity of CD81-deficient mice to neurobehavioral effects of cocaine. *Brain Res Mol Brain Res* 2001; **90**: 68–74.

- 64 Michna L, Brenz-Verca M, Dreyer JL, Wagner GC. Methods to examine molecular changes and behavioral effects of drug administration. *Brain Res Brain Res Protocol* 2002; **9**: 181–196.
- 65 Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E et al. Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am J Med Genet* 1998; **81**: 216–221.
- 66 Zandi PP, Willour VL, Huo Y, Chellis J, Potash JB, MacKinnon DF et al. Genome scan of a second wave of NIMH genetics initiative bipolar pedigrees: chromosomes 2, 11, 13, 14, and X. *Am J Med Genet B* 2003; **119**: 69–76.
- 67 Segurado R, Detera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger Jr JI et al. Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: bipolar disorder. *Am J Hum Genet* 2003; **73**: 49–62.
- 68 DeVito WJ, Stone S. Prenatal exposure to ethanol alters the neuroimmune response to a central nervous system wound in the adult rat. *Alcohol* 2001; **25**: 39–47.
- 69 Gan X, Zhang L, Berger O, Stins MF, Way D, Taub DD et al. Cocaine enhances brain endothelial adhesion molecules and leukocyte migration. *Clin Immunol* 1999; **91**: 68–76.
- 70 Gruber-Olipitz M, Stevenson R, Olipitz W, Wagner E, Gesslbauer B, Kungl A et al. Transcriptional pattern analysis of adrenergic immunoregulation in mice. Twelve hours norepinephrine treatment alters the expression of a set of genes involved in monocyte activation and leukocyte trafficking. *J Neuroimmunol* 2004; **155**: 136–142.
- 71 Lappalainen J, Kranzler HR, Petrakis I, Somberg LK, Page G, Krystal JH et al. Confirmation and fine mapping of the chromosome 1 alcohol dependence risk locus. *Mol Psychiatry* 2004; **9**: 312–319.
- 72 Nurnberger Jr JI, Foroud T, Flury L, Su J, Meyer ET, Hu K et al. Evidence for a locus on chromosome 1 that influences vulnerability to alcoholism and affective disorder. *Am J Psychiatry* 2001; **158**: 718–724.
- 73 Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P et al. Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet* 1998; **81**: 207–215.
- 74 Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL et al. Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcohol Clin Exp Res* 2000; **24**: 933–945.
- 75 Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21–q22. *Science* 2000; **288**: 678–682.
- 76 Thomas AJ, Perry R, Kalaria RN, Oakley A, McMeekin W, O'Brien JT. Neuropathological evidence for ischemia in the white matter of the dorsolateral prefrontal cortex in late-life depression. *Int J Geriatr Psychiatry* 2003; **18**: 7–13.
- 77 McKinzie DL, Nowak KL, Murphy JM, Li TK, Lumeng L, McBride WJ. Development of alcohol drinking behavior in rat lines selectively bred for divergent alcohol preference. *Alcohol Clin Exp Res* 1998; **22**: 1584–1590.
- 78 Rodd ZA, Bell RL, Sable HJ, Murphy JM, McBride WJ. Recent advances in animal models of alcohol craving and relapse. *Pharmacol Biochem Behav* 2004; **79**: 439–450.
- 79 Galaif ER, Newcomb MD. Predictors of polydrug use among four ethnic groups: a 12-year longitudinal study. *Addict Behav* 1999; **24**: 607–631.
- 80 Ishiguro H, Liu QR, Gong JP, Hall FS, Ujike H, Morales M et al. NrCAM in addiction vulnerability: positional cloning, drug-regulation, haplotype-specific expression, and altered drug reward in knockout mice. *Neuropsychopharmacology* 2006; **31**: 572–584.
- 81 Ho L, Guo Y, Spielman L, Petrescu O, Haroutunian V, Purohit D et al. Altered expression of a-type but not b-type synapsin isoform in the brain of patients at high risk for Alzheimer's disease assessed by DNA microarray technique. *Neurosci Lett* 2001; **298**: 191–194.
- 82 Golka K, Wiese A. Carbohydrate-deficient transferrin (CDT) – a biomarker for long-term alcohol consumption. *J Toxicol Environ Health B* 2004; **7**: 319–337.
- 83 Suzuki Y, Saito H, Suzuki M, Hosoki Y, Sakurai S, Fujimoto Y et al. Up-regulation of transferrin receptor expression in hepatocytes by habitual alcohol drinking is implicated in hepatic iron overload in alcoholic liver disease. *Alcohol Clin Exp Res* 2002; **26**: 265–315.
- 84 Dick DM, Nurnberger Jr J, Edenberg HJ, Goate A, Crowe R, Rice J et al. Suggestive linkage on chromosome 1 for a quantitative alcohol-related phenotype. *Alcohol Clin Exp Res* 2002; **26**: 1453–1460.
- 85 Maziade M, Roy MA, Chagnon YC, Cliche D, Fournier JP, Montgrain N et al. Shared and specific susceptibility loci for schizophrenia and bipolar disorder: a dense genome scan in Eastern Quebec families. *Mol Psychiatry* 2005; **10**: 486–499.
- 86 Devlin B, Bacanu SA, Roeder K, Reimherr F, Wender P, Galke B et al. Genome-wide multipoint linkage analyses of multiplex schizophrenia pedigrees from the oceanic nation of Palau. *Mol Psychiatry* 2002; **7**: 689–694.
- 87 Schosser A, Fuchs K, Leisch F, Bailer U, Meszaros K, Lenzinger E et al. Possible linkage of schizophrenia and bipolar affective disorder to chromosome 3q29; a follow-up. *J Psychiatr Res* 2004; **38**: 357–364.
- 88 Liu J, Lewohl JM, Dodd PR, Randall PK, Harris RA, Mayfield RD. Gene expression profiling of individual cases reveals consistent transcriptional changes in alcoholic human brain. *J Neurochem* 2004; **90**: 1050–1058.
- 89 Podvynec M, Handschin C, Looser R, Meyer UA. Identification of the xenosensors regulating human 5-aminolevulinate synthase. *Proc Natl Acad Sci USA* 2004; **101**: 9127–9132.
- 90 Teschke R, Gorys-Konemann C, Daldrup T, Goerz G. Influence of chronic alcohol consumption on hepatic heme and porphyrin metabolism. *Biochem Pharmacol* 1987; **36**: 1133–1138.
- 91 Kaasik K, Lee CC. Reciprocal regulation of haem biosynthesis and the circadian clock in mammals. *Nature* 2004; **430**: 467–471.
- 92 Irwin MR, Rinetti G. Disordered sleep, nocturnal cytokines, and immunity: interactions between alcohol dependence and African-American ethnicity. *Alcohol* 2004; **32**: 53–61.
- 93 Sarviharju M, Riikonen J, Jaatinen P, Sinclair D, Hervonen A, Kiiianmaa K. Survival of AA and ANA rats during lifelong ethanol exposure. *Alcohol Clin Exp Res* 2004; **28**: 93–97.
- 94 Lucas DL, Brown RA, Wassef M, Giles TD. Alcohol and the cardiovascular system research challenges and opportunities. *J Am Coll Cardiol* 2005; **45**: 1916–1924.
- 95 Coe IR, Yao L, Diamond I, Gordon AS. The role of protein kinase C in cellular tolerance to ethanol. *J Biol Chem* 1996; **271**: 29468–29472.
- 96 Gordon AS, Yao L, Jiang Z, Fishburn CS, Fuchs S, Diamond I. Ethanol acts synergistically with a D2 dopamine agonist to cause translocation of protein kinase C. *Mol Pharmacol* 2001; **59**: 153–160.
- 97 Choi DS, Wang D, Dadgar J, Chang WS, Messing RO. Conditional rescue of protein kinase C epsilon regulates ethanol preference and hypnotic sensitivity in adult mice. *J Neurosci* 2002; **22**: 9905–9911.
- 98 Zhou HZ, Karlner JS, Gray MO. Moderate alcohol consumption induces sustained cardiac protection by activating PKC-epsilon and Akt. *Am J Physiol Heart Circ Physiol* 2002; **283**: H165–H174.
- 99 Mulligan MK, Ponomarev I, Hitzemann RJ, Belknap JK, Tabakoff B, Harris RA et al. Toward understanding the genetics of alcohol drinking through transcriptome meta-analysis. *Proc Natl Acad Sci USA* 2006; **103**: 6368–6373.
- 100 Li X, Jope RS. Selective inhibition of the expression of signal transduction proteins by lithium in nerve growth factor-differentiated PC12 cells. *J Neurochem* 1995; **65**: 2500–2508.
- 101 Maul B, Siems WE, Hoehe MR, Grecksch G, Bader M, Walther T. Alcohol consumption is controlled by angiotensin II. *FASEB J* 2001; **15**: 1640–1642.
- 102 Maul B, Krause W, Pankow K, Becker M, Gembardt F, Alenina N et al. Central angiotensin II controls alcohol consumption via its AT1 receptor. *FASEB J* 2005; **19**: 1474–1481.
- 103 Lingham T, Perlanski E, Grupp LA. Angiotensin converting enzyme inhibitors reduce alcohol consumption: some possible mechanisms and important conditions for its therapeutic use. *Alcohol Clin Exp Res* 1990; **14**: 92–99.
- 104 Sun Z, Cade R, Zhang Z, Alouidor J, Van H. Angiotensinogen gene knockout delays and attenuates cold-induced hypertension. *Hypertension* 2003; **41**: 322–327.
- 105 Meira-Lima IV, Pereira AC, Mota GF, Krieger JE, Vallada H. Angiotensinogen and angiotensin converting enzyme gene polymorphisms and the risk of bipolar affective disorder in humans. *Neurosci Lett* 2000; **293**: 103–106.
- 106 Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R et al. Chromosome 1 loci in Finnish schizophrenia families. *Hum Mol Genet* 2001; **10**: 1611–1617.

- 107 Hoffman PL, Miles M, Edenberg HJ, Sommer W, Tabakoff B, Wehner JM *et al*. Gene expression in brain: a window on ethanol dependence, neuroadaptation, and preference. *Alcohol Clin Exp Res* 2003; **27**: 155–168.
- 108 Arlinde C, Sommer W, Bjork K, Reimers M, Hyytia P, Kiianmaa K *et al*. A cluster of differentially expressed signal transduction genes identified by microarray analysis in a rat genetic model of alcoholism. *Pharmacogenomics J* 2004; **4**: 208–218.
- 109 Sommer W, Arlinde C, Heilig M. The search for candidate genes of alcoholism: evidence from expression profiling studies. *Addict Biol* 2005; **10**: 71–79.
- 110 Wyszynski DF, Panhuysen CI, Ma Q, Yip AG, Wilcox M, Erlich P *et al*. Genome-wide screen for heavy alcohol consumption. *BMC Genet* 2003; **4**(Suppl 1): S106.
- 111 Fallin MD, Lasseter VK, Wolyniec PS, McGrath JA, Nestadt G, Valle D *et al*. Genomewide linkage scan for schizophrenia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 10q22. *Am J Hum Genet* 2003; **73**: 601–611.
- 112 Iwamoto K, Kakiuchi C, Bundo M, Ikeda K, Kato T. Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Mol Psychiatry* 2004; **9**: 406–416.
- 113 Renkawek K, Bosman CJ, de Jong WW. Expression of small heat-shock protein hsp 27 in reactive gliosis in Alzheimer disease and other types of dementia. *Acta Neuropathol (Berlin)* 1994; **87**: 511–519.
- 114 Myo K, Uzawa N, Miyamoto R, Sonoda I, Yuki Y, Amagasa T. Cyclin D1 gene numerical aberration is a predictive marker for occult cervical lymph node metastasis in TNM Stage I and II squamous cell carcinoma of the oral cavity. *Cancer* 2005; **104**: 2709–2716.
- 115 Granot-Attas S, Elson A. Protein tyrosine phosphatase epsilon activates Yes and Fyn in Neu-induced mammary tumor cells. *Exp Cell Res* 2004; **294**: 236–243.
- 116 Jiang Y, Wang L, Gong W, Wei D, Le X, Yao J *et al*. A high expression level of insulin-like growth factor I receptor is associated with increased expression of transcription factor Sp1 and regional lymph node metastasis of human gastric cancer. *Clin Exp Metast* 2004; **21**: 755–764.
- 117 Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G *et al*. A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci USA* 1999; **96**: 5604–5609.
- 118 Yamada K, Iwayama-Shigeno Y, Yoshida Y, Toyota T, Itokawa M, Hattori E *et al*. Family-based association study of schizophrenia with 444 markers and analysis of a new susceptibility locus mapped to 11q13.3. *Am J Med Genet B* 2004; **127**: 11–19.
- 119 Schuckit MA, Edenberg HJ, Kalmijn J, Flury L, Smith TL, Reich T *et al*. A genome-wide search for genes that relate to a low level of response to alcohol. *Alcohol Clin Exp Res* 2001; **25**: 323–329.
- 120 Ehlers CL, Spence JP, Wall TL, Gilder DA, Carr LG. Association of ALDH1 promoter polymorphisms with alcohol-related phenotypes in southwest California Indians. *Alcohol Clin Exp Res* 2004; **28**: 1481–1486.
- 121 Spence JP, Liang T, Eriksson CJ, Taylor RE, Wall TL, Ehlers CL *et al*. Evaluation of aldehyde dehydrogenase 1 promoter polymorphisms identified in human populations. *Alcohol Clin Exp Res* 2003; **27**: 1389–1394.
- 122 Galter D, Buervenich S, Carmine A, Anvret M, Olson L. ALDH1 mRNA: presence in human dopamine neurons and decreases in substantia nigra in Parkinson's disease and in the ventral tegmental area in schizophrenia. *Neurobiol Dis* 2003; **14**: 637–647.
- 123 Kim JS, Kim YJ, Kim TY, Song JY, Cho YH, Park YC *et al*. Association of ALDH2 polymorphism with sensitivity to acetaldehyde-induced micronuclei and facial flushing after alcohol intake. *Toxicology* 2005; **210**: 169–174.
- 124 Samigullin D, Bill CA, Coleman WL, Bykhovskaia M. Regulation of transmitter release by synapsin II in mouse motor terminals. *J Physiol* 2004; **561**: 149–158.
- 125 Chen Q, He G, Wang XY, Chen QY, Liu XM, Gu ZZ *et al*. Positive association between synapsin II and schizophrenia. *Biol Psychiatry* 2004; **56**: 177–181.
- 126 Mirmics K, Middleton FA, Marquez A, Lewis DA, Levitt P. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron* 2000; **28**: 53–67.
- 127 Vawter MP, Thatcher L, Usen N, Hyde TM, Kleinman JE, Freed WJ. Reduction of synapsin in the hippocampus of patients with bipolar disorder and schizophrenia. *Mol Psychiatry* 2002; **7**: 571–578.
- 128 Grebb JA, Greengard P. An analysis of synapsin II, a neuronal phosphoprotein, in postmortem brain tissue from alcoholic and neuropsychiatrically ill adults and medically ill children and young adults. *Arch Gen Psychiatry* 1990; **47**: 1149–1156.
- 129 Zhang H, Ozbay F, Lappalainen J, Kranzler HR, van Dyck CH, Charney DS *et al*. Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. *Am J Med Genet B* 2006; **141**: 387–393.
- 130 McGough NN, He DY, Logrip ML, Jeanblanc J, Phamluong K, Luong K *et al*. RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. *J Neurosci* 2004; **24**: 10542–10552.
- 131 MacLennan AJ, Lee N, Walker DW. Chronic ethanol administration decreases brain-derived neurotrophic factor gene expression in the rat hippocampus. *Neurosci Lett* 1995; **197**: 105–108.
- 132 Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res* 2002; **109**: 143–148.
- 133 Matsushita S, Kimura M, Miyakawa T, Yoshino A, Murayama M, Masaki T *et al*. Association study of brain-derived neurotrophic factor gene polymorphism and alcoholism. *Alcohol Clin Exp Res* 2004; **28**: 1609–1612.
- 134 Hashimoto K, Shimizu E, Iyo M. Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Res Brain Res Rev* 2004; **45**: 104–114.
- 135 Strauss J, Barr CL, George CJ, Devlin B, Vetro A, Kiss E *et al*. Brain-derived neurotrophic factor variants are associated with childhood-onset mood disorder: confirmation in a Hungarian sample. *Mol Psychiatry* 2005; **10**: 861–867.
- 136 Strauss J, Barr CL, George CJ, King N, Shaikh S, Devlin B *et al*. Association study of brain-derived neurotrophic factor in adults with a history of childhood onset mood disorder. *Am J Med Genet B* 2004; **131**: 16–19.
- 137 Muglia P, Vicente AM, Verga M, King N, Macciardi F, Kennedy JL. Association between the BDNF gene and schizophrenia. *Mol Psychiatry* 2003; **8**: 146–147.
- 138 Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G *et al*. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neurotrophic factor. *Mol Psychiatry* 2002; **7**: 579–593.
- 139 Mundo E, Tharmalingham S, Neves-Pereira M, Dalton EJ, Macciardi F, Parikh SV *et al*. Evidence that the N-methyl-D-aspartate subunit 1 receptor gene (GRIN1) confers susceptibility to bipolar disorder. *Mol Psychiatry* 2003; **8**: 241–245.
- 140 Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF. Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem findings from the Stanley Neuropathology Consortium. *Mol Psychiatry* 2004; **9**: 609–620, 544.
- 141 Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch Gen Psychiatry* 2003; **60**: 804–815.
- 142 Webster MJ, O'Grady J, Kleinman JE, Weickert CS. Glial fibrillary acidic protein mRNA levels in the cingulate cortex of individuals with depression, bipolar disorder and schizophrenia. *Neuroscience* 2005; **133**: 453–461.
- 143 Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ. Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *J Neurosci Res* 2002; **70**: 462–473.
- 144 Yang X, Oswald L, Wand G. The cyclic AMP/protein kinase A signal transduction pathway modulates tolerance to sedative and hypothermic effects of ethanol. *Alcohol Clin Exp Res* 2003; **27**: 1220–1225.
- 145 Plagge A, Isles AR, Gordon E, Humby T, Dean W, Gritsch S *et al*. Imprinted Nesp55 influences behavioral reactivity to novel environments. *Mol Cell Biol* 2005; **25**: 3019–3026.

- 146 Lobos EA, Todd RD. Cladistic analysis of disease association with tyrosine hydroxylase: application to manic-depressive disease and alcoholism. *Am J Med Genet* 1997; **74**: 289–295.
- 147 Todd RD, Lobos EA, Parsian A, Simpson S, DePaulo JR. Manic-depressive illness and tyrosine hydroxylase markers. Bipolar Disorder Working Group. *Lancet* 1996; **347**: 1634.
- 148 Biegan A, Fieldust S. Reduced tyrosine hydroxylase immunoreactivity in locus coeruleus of suicide victims. *Synapse* 1992; **10**: 79–82.
- 149 Farren CK, Dinan TG. Alcoholism and typology: findings in an Irish private hospital population. *J Stud Alcohol* 1996; **57**: 249–252.
- 150 Akiskal HS. Toward a definition of generalized anxiety disorder as an anxious temperament type. *Acta Psychiatr Scand Suppl* 1998; **393**: 66–73.
- 151 Muhlin GL. Ethnic differences in alcohol misuse: a striking reaffirmation. *J Stud Alcohol* 1985; **46**: 172–173.
- 152 Rosenwaike I, Hempstead K. Differential mortality by ethnicity: foreign-born Irish, Italians and Jews in New York City, 1979–81. *Soc Sci Med* 1989; **29**: 885–889.
- 153 Greenslade L, Pearson M, Madden M. A good man's fault: alcohol and Irish people at home and abroad. *Alcohol Alcohol* 1995; **30**: 407–417.
- 154 Niculescu AB, Lulow LL, Ogden CA, Le-Niculescu H, Salomon DR, Schork NJ *et al*. PhenoChipping of psychotic disorders: a novel approach for deconstructing and quantitating psychiatric phenotypes. *Am J Med Genet B* 2006; **141**: 653–662.
- 155 Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ *et al*. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav Genet* 2002; **32**: 363–388.
- 156 Stewart RB, Li TK. The neurobiology of alcoholism in genetically selected rat models. *Alcohol Health Res World* 1997; **21**: 169–176.
- 157 McBride WJ, Li TK. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol* 1998; **12**: 339–369.
- 158 Li TK, Lumeng L, McBride WJ, Murphy JM. Rodent lines selected for factors affecting alcohol consumption. *Alcohol Alcohol* 1987 (Suppl 1): 91–96.
- 159 Bozarth MA, Gerber GJ, Wise RA. Intracranial self-stimulation as a technique to study the reward properties of drugs of abuse. *Pharmacol Biochem Behav* 1980; **13**(Suppl 1): 245–247.
- 160 Goeders NE, Smith JE. Intracranial self-administration methodologies. *Neurosci Biobehav Rev* 1987; **11**: 319–329.
- 161 Bozarth MA, Wise RA. Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life Sci* 1981; **28**: 551–555.
- 162 Devine DP, Wise RA. Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J Neurosci* 1994; **14**: 1978–1984.
- 163 Hoebel BC, Monaco AP, Hernandez L, Aulisi EF, Stanley BG, Lenard L. Self-injection of amphetamine directly into the brain. *Psychopharmacology (Berlin)* 1983; **81**: 158–163.
- 164 Goeders NE, Smith JE. Reinforcing properties of cocaine in the medial prefrontal cortex: primary action on presynaptic dopaminergic terminals. *Pharmacol Biochem Behav* 1986; **25**: 191–199.
- 165 Rodd-Henricks ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ. Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. *Psychopharmacology (Berlin)* 2000; **149**: 217–224.
- 166 Gonzales RA, Job MO, Doyon WM. The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. *Pharmacol Ther* 2004; **103**: 121–146.
- 167 McClintick JN, Jerome RE, Nicholson CR, Crabb DW, Edenberg HJ. Reproducibility of oligonucleotide arrays using small samples. *BMC Genomics* 2003; **4**: 4.
- 168 Gurling HM, Kalsi G, Brynjolfsson J, Sigmundsson T, Sherrington R, Mankoo BS *et al*. Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21–22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3–24 and 20q12.1–11.23. *Am J Hum Genet* 2001; **68**: 661–673.
- 169 Cichon S, Schumacher J, Muller DJ, Hurter M, Windemuth C, Strauch K *et al*. A genome screen for genes predisposing to bipolar affective disorder detects a new susceptibility locus on 8q. *Hum Mol Genet* 2001; **10**: 2933–2944.
- 170 Lindholm E, Aberg K, Ekholm B, Pettersson U, Adolfsson R, Jazin EE. Reconstruction of ancestral haplotypes in a 12-generation schizophrenia pedigree. *Psychiatr Genet* 2004; **14**: 1–8.
- 171 Morissette J, Villeneuve A, Bordeleau L, Rochette D, Laberge C, Gagne B *et al*. Genome-wide search for linkage of bipolar affective disorders in a very large pedigree derived from a homogeneous population in Quebec points to a locus of major effect on chromosome 12q23–q24. *Am J Med Genet* 1999; **88**: 567–587.
- 172 George AJ, Holsinger RM, McLean CA, Tan SS, Scott HS, Cardamone T *et al*. Decreased phosphatidylethanolamine binding protein expression correlates with Abeta accumulation in the Tg2576 mouse model of Alzheimer's disease. *Neurobiol Aging* 2006; **27**: 614–623.
- 173 Vasiliou V, Pappa A. Polymorphisms of human aldehyde dehydrogenases. Consequences for drug metabolism and disease. *Pharmacology* 2000; **61**: 192–198.
- 174 Macgregor S, Visscher PM, Knott SA, Thomson P, Porteous DJ, Millar JK *et al*. A genome scan and follow-up study identify a bipolar disorder susceptibility locus on chromosome 1q42. *Mol Psychiatry* 2004; **9**: 1083–1090.
- 175 Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, Harris RA. Gene expression in human alcoholism: microarray analysis of frontal cortex. *Alcohol Clin Exp Res* 2000; **24**: 1873–1882.
- 176 Brecher AS, Riley C, Basista MH. Acetaldehyde-modified lysozyme function: its potential implication in the promotion of infection in alcoholics. *Alcohol* 1995; **12**: 169–172.
- 177 Rodriguez Parkitna JM, Bilecki W, Mierzejewski P, Stefanski R, Ligeza A, Bargiela A *et al*. Effects of morphine on gene expression in the rat amygdala. *J Neurochem* 2004; **91**: 38–48.
- 178 Lee KY, Clark AW, Rosales JL, Chapman K, Fung T, Johnston RN. Elevated neuronal Cdc2-like kinase activity in the Alzheimer disease brain. *Neurosci Res* 1999; **34**: 21–29.
- 179 Paunio T, Shen S, Zezza N, Perola M, Perlin M, Allan W. Search for alcoholism susceptibility genes. *Hum Mol Genet* 2004; **13**: 102–113.
- 180 Yang SQ, Lin HZ, Yin M, Albrecht JH, Diehl AM. Effects of chronic ethanol consumption on cytokine regulation of liver regeneration. *Am J Physiol* 1998; **275**: G696–G704.
- 181 Gupta K, Kshirsagar S, Chang L, Schwartz R, Law PY, Yee D *et al*. Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. *Cancer Res* 2002; **62**: 4491–4498.
- 182 Smith MZ, Nagy Z, Esiri MM. Cell cycle-related protein expression in vascular dementia and Alzheimer's disease. *Neurosci Lett* 1999; **271**: 45–48.
- 183 Olive MF, Mehmert KK, Messing RO, Hodge CW. Reduced operant ethanol self-administration and *in vivo* mesolimbic dopamine responses to ethanol in PKCepsilon-deficient mice. *Eur J Neurosci* 2000; **12**: 4131–4140.
- 184 Matsushima H, Shimohama S, Chachin M, Taniguchi T, Kimura J. Ca²⁺-dependent and Ca²⁺-independent protein kinase C changes in the brain of patients with Alzheimer's disease. *J Neurochem* 1996; **67**: 317–323.
- 185 Huang Y, Wang KK. The calpain family and human disease. *Trends Mol Med* 2001; **7**: 355–362.
- 186 Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J* 2002; **16**: 27–35.
- 187 Rivier C, Lee S. Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. *Brain Res* 1996; **726**: 1–10.
- 188 Peck JA, Stopa EG. Agrin and microvascular damage in Alzheimer's disease. *Med Health R I* 2002; **85**: 202–206.
- 189 Maziade M, Bissonnette L, Rouillard E, Martinez M, Turgeon M, Charron L *et al*. 6p24–22 region and major psychoses in the Eastern Quebec population. Le Groupe IREP. *Am J Med Genet* 1997; **74**: 311–318.
- 190 Moises HW, Zoega T, Gottesman II. The glial growth factors deficiency and synaptic destabilization hypothesis of schizophrenia. *BMC Psychiatry* 2002; **2**: 8.
- 191 Jurata LW, Bukhman YV, Charles V, Capriglione F, Bullard J, Lemire AL *et al*. Comparison of microarray-based mRNA profiling technologies for identification of psychiatric disease and drug signatures. *J Neurosci Methods* 2004; **138**: 173–188.

- 192 Kohn Y, Danilovich E, Filon D, Oppenheim A, Karni O, Kanyas K *et al*. Linkage disequilibrium in the DTNBP1 (dysbindin) gene region and on chromosome 1p36 among psychotic patients from a genetic isolate in Israel: findings from identity by descent haplotype sharing analysis. *Am J Med Genet B* 2004; **128**: 65–70.
- 193 Lerer B, Segman RH, Hamdan A, Kanyas K, Karni O, Kohn Y *et al*. Genome scan of Arab Israeli families maps a schizophrenia susceptibility gene to chromosome 6q23 and supports a locus at chromosome 10q24. *Mol Psychiatry* 2003; **8**: 488–498.
- 194 Sun F, Cheng R, Flanders WD, Yang Q, Khoury MJ. Whole genome association studies for genes affecting alcohol dependence. *Genet Epidemiol* 1999; **17**(Suppl 1): S337–S342.
- 195 Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V, Arajärvi R *et al*. A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. *Am J Hum Genet* 1999; **65**: 1114–1124.
- 196 Bezchlibnyk YB, Wang JF, McQueen GM, Young LT. Gene expression differences in bipolar disorder revealed by cDNA array analysis of postmortem frontal cortex. *J Neurochem* 2001; **79**: 826–834.
- 197 Schumacher AM, Schavocky JP, Valentza AV, Mirzoeva S, Watterson DM. A calmodulin-regulated protein kinase linked to neuron survival is a substrate for the calmodulin-regulated death-associated protein kinase. *Biochemistry* 2004; **43**: 8116–8124.
- 198 Tsai SY, Lee HC, Chen CC, Lee CH. Plasma levels of soluble transferrin receptors and Clara cell protein (CC16) during bipolar mania and subsequent remission. *J Psychiatr Res* 2003; **37**: 229–235.
- 199 Treadwell JA, Pagniello KB, Singh SM. Genetic segregation of brain gene expression identifies retinaldehyde binding protein 1 and syntaxin 12 as potential contributors to ethanol preference in mice. *Behav Genet* 2004; **34**: 425–439.
- 200 Sokolov BP, Jiang L, Trivedi NS, Aston C. Transcription profiling reveals mitochondrial, ubiquitin and signaling systems abnormalities in postmortem brains from subjects with a history of alcohol abuse or dependence. *J Neurosci Res* 2003; **72**: 756–767.
- 201 Hayase T, Yamamoto Y, Yamamoto K, Muso E, Shiota K. Stressor-like effects of cocaine on heat shock protein and stress-activated protein kinase expression in the rat hippocampus: interaction with ethanol and anti-toxicity drugs. *Leg Med (Tokyo)* 2003; **5**(Suppl 1): S87–S90.
- 202 Badenhop RF, Moses MJ, Scimone A, Mitchell PB, Ewen-White KR, Rosso A *et al*. A genome screen of 13 bipolar affective disorder pedigrees provides evidence for susceptibility loci on chromosome 3 as well as chromosomes 9, 13 and 19. *Mol Psychiatry* 2002; **7**: 851–859.
- 203 Karege F, Schwald M, Papadimitriou P, Lachausse C, Cisse M. The cAMP-dependent protein kinase A and brain-derived neurotrophic factor expression in lymphoblast cells of bipolar affective disorder. *J Affect Disord* 2004; **79**: 187–192.
- 204 Karege F, Schwald M, El Kouaissi R. Drug-induced decrease of protein kinase A activity reveals alteration in BDNF expression of bipolar affective disorder. *Neuropsychopharmacology* 2004; **29**: 805–812.
- 205 Williams JT, Begleiter H, Porjesz B, Edenberg HJ, Foroud T, Reich T *et al*. Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. II. Alcoholism and event-related potentials. *Am J Hum Genet* 1999; **65**: 1148–1160.
- 206 Hasegawa H, Osada K, Misonoo A, Morinobu S, Yamamoto H, Miyamoto E *et al*. Chronic carbamazepine treatment increases myristoylated alanine-rich C kinase substrate phosphorylation in the rat cerebral cortex via down-regulation of calcineurin A alpha. *Brain Res* 2003; **994**: 19–26.
- 207 Kelsoe JR, Spence MA, Loetscher E, Foguet M, Sadovnick AD, Remick RA *et al*. A genome survey indicates a possible susceptibility locus for bipolar disorder on chromosome 22. *Proc Natl Acad Sci USA* 2001; **98**: 585–590.
- 208 Nanji AA, Lau GK, Tipoe GL, Yuen ST, Chen YX, Thomas P *et al*. Macrophage migration inhibitory factor expression in male and female ethanol-fed rats. *J Interferon Cytokine Res* 2001; **21**: 1055–1062.
- 209 Oyama R, Yamamoto H, Titani K. Glutamine synthetase, hemoglobin alpha-chain, and macrophage migration inhibitory factor binding to amyloid beta-protein: their identification in rat brain by a novel affinity chromatography and in Alzheimer's disease brain by immunoprecipitation. *Biochim Biophys Acta* 2000; **1479**: 91–102.
- 210 Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry* 2004; **61**: 300–308.
- 211 Carmiel-Haggai M, Cederbaum AI, Nieto N. Binge ethanol exposure increases liver injury in obese rats. *Gastroenterology* 2003; **125**: 1818–1833.
- 212 Horvath B, Spies C, Horvath G, Kox WJ, Miyamoto S, Barry S *et al*. Uncoupling protein 2 (UCP2) lowers alcohol sensitivity and pain threshold. *Biochem Pharmacol* 2002; **64**: 369–374.
- 213 Sriram K, Benkovic SA, Miller DB, O'Callaghan JP. Obesity exacerbates chemically induced neurodegeneration. *Neuroscience* 2002; **115**: 1335–1346.
- 214 Guzman M, Castro J. Alterations in the regulatory properties of hepatic fatty acid oxidation and carnitine palmitoyltransferase I activity after ethanol feeding and withdrawal. *Alcohol Clin Exp Res* 1990; **14**: 472–477.
- 215 Ewald H, Flint T, Kruse TA, Mors O. A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24.3 and suggestive linkage to chromosomes 1p22–21, 4p16, 6q14–22, 10q26 and 16p13.3. *Mol Psychiatry* 2002; **7**: 734–744.
- 216 Thomas AJ, Davis S, Ferrier IN, Kalalaria RN, O'Brien JT. Elevation of cell adhesion molecule immunoreactivity in the anterior cingulate cortex in bipolar disorder. *Biol Psychiatry* 2004; **55**: 652–655.
- 217 Schumann G, Rujescu D, Kissling C, Soyka M, Dahmen N, Preuss UW *et al*. Analysis of genetic variations of protein tyrosine kinase fyn and their association with alcohol dependence in two independent cohorts. *Biol Psychiatry* 2003; **54**: 1422–1426.
- 218 Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL *et al*. Genomewide linkage analyses of bipolar disorder: a new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. *Am J Hum Genet* 2003; **73**: 107–114.
- 219 Miyakawa T, Yagi T, Kitazawa H, Yasuda M, Kawai N, Tsuboi K *et al*. Fyn-kinase as a determinant of ethanol sensitivity: relation to NMDA-receptor function. *Science* 1997; **278**: 698–701.
- 220 Ohnuma T, Kato H, Arai H, McKenna PJ, Emson PC. Expression of Fyn, a non-receptor tyrosine kinase in prefrontal cortex from patients with schizophrenia and its correlation with clinical onset. *Brain Res Mol Brain Res* 2003; **112**: 90–94.
- 221 Pecherskaya A, Rubin E, Solem M. Alterations in insulin-like growth factor-I signaling in cardiomyocytes from chronic alcohol-exposed rats. *Alcohol Clin Exp Res* 2002; **26**: 995–1002.
- 222 Jafferli S, Dumont Y, Sotty F, Robitaille Y, Quirion R, Kar S. Insulin-like growth factor-I and its receptor in the frontal cortex, hippocampus, and cerebellum of normal human and Alzheimer disease brains. *Synapse* 2000; **38**: 450–459.
- 223 Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JA *et al*. Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. *Hum Mol Genet* 2001; **10**: 3037–3048.
- 224 Sklar P, Pato MT, Kirby A, Petryshen TL, Medeiros H, Carvalho C *et al*. Genome-wide scan in Portuguese Island families identifies 5q31–5q35 as a susceptibility locus for schizophrenia and psychosis. *Mol Psychiatry* 2004; **9**: 213–218.
- 225 Turner RT, Evans GL, Zhang M, Sibonga JD. Effects of parathyroid hormone on bone formation in a rat model for chronic alcohol abuse. *Alcohol Clin Exp Res* 2001; **25**: 667–671.
- 226 Ikemoto M, Takita M, Imamura T, Inoue K. Increased sensitivity to the stimulant effects of morphine conferred by anti-adhesive glycoprotein SPARC in amygdala. *Nat Med* 2000; **6**: 910–915.
- 227 Straub RE, Lehner T, Luo Y, Loth JE, Shao W, Sharpe L *et al*. A possible vulnerability locus for bipolar affective disorder on chromosome 21q22.3. *Nat Genet* 1994; **8**: 291–296.
- 228 Solomon LR, Hillman RS. Vitamin B6 metabolism in anaemic and alcoholic man. *Br J Haematol* 1979; **41**: 343–356.
- 229 Butterfield DA, Castegna A, Lauderback CM, Drake J. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. *Neurobiol Aging* 2002; **23**: 655–664.
- 230 Blennow K. CSF biomarkers for mild cognitive impairment. *J Intern Med* 2004; **256**: 224–234.

- 231 Bouras C, Kovari E, Hof PR, Riederer BM, Giannakopoulos P. Anterior cingulate cortex pathology in schizophrenia and bipolar disorder. *Acta Neuropathol (Berlin)* 2001; **102**: 373–379.
- 232 Miller BE, Whetsell Jr WO, Chong JK, Ghanbari HA. Distribution of Alzheimer's disease-associated protein (ADAP) in 10 human brains. *J Neuropsychiatry Clin Neurosci* 1992; **4**: 303–307.
- 233 Lu KP. Pinning down cell signaling, cancer and Alzheimer's disease. *Trends Biochem Sci* 2004; **29**: 200–209.
- 234 Ma JZ, Zhang D, Dupont RT, Dockter M, Elston RC, Li MD. Mapping susceptibility loci for alcohol consumption using number of grams of alcohol consumed per day as a phenotype measure. *BMC Genet* 2003; **4**(Suppl 1): S104.
- 235 Sieg I, Doss MO, Kandels H, Schneider J. Effect of alcohol on delta-aminolevulinic acid dehydratase and porphyrin metabolism in man. *Clin Chim Acta* 1991; **202**: 211–218.
- 236 Maes M, Lin A, Bonaccorso S, Vandoolaeghe E, Song C, Goossens F *et al*. Lower activity of serum peptidases in abstinent alcohol-dependent patients. *Alcohol* 1999; **17**: 1–6.
- 237 Aoyagi T, Wada T, Nagai M, Kojima F, Harada S, Takeuchi T *et al*. Increased gamma-aminobutyrate aminotransferase activity in brain of patients with Alzheimer's disease. *Chem Pharm Bull (Tokyo)* 1990; **38**: 1748–1749.
- 238 Foroud T, Bucholz KK, Edenberg HJ, Goate A, Neuman RJ, Porjesz B *et al*. Linkage of an alcoholism-related severity phenotype to chromosome 16. *Alcohol Clin Exp Res* 1998; **22**: 2035–2042.
- 239 Takata K, Kitamura Y, Kakimura J, Shibagaki K, Tsuchiya D, Taniguchi T *et al*. Role of high mobility group protein-1 (HMG1) in amyloid-beta homeostasis. *Biochem Biophys Res Commun* 2003; **301**: 699–703.
- 240 Sheffield LJ, Knauert MP, Pakstis AJ, Zhao H, Kidd KK. Analyses of the COGA data set in one ethnic group with examinations of alternative definitions of alcoholism. *Genet Epidemiol* 1999; **17**(Suppl 1): S319–S324.
- 241 Nalpas B, Vassault A, Charpin S, Lacour B, Berthelot P. Serum mitochondrial aspartate aminotransferase as a marker of chronic alcoholism: diagnostic value and interpretation in a liver unit. *Hepatology* 1986; **6**: 608–614.
- 242 Dahmen N, Volp M, Singer P, Hiemke C, Szegedi A. Tyrosine hydroxylase Val-81-Met polymorphism associated with early-onset alcoholism. *Psychiatr Genet* 2005; **15**: 13–16.
- 243 Sander T, Harms H, Rommelspacher H, Hoehe M, Schmidt LG. Possible allelic association of a tyrosine hydroxylase polymorphism with vulnerability to alcohol-withdrawal delirium. *Psychiatr Genet* 1998; **8**: 13–17.
- 244 Massat I, Souery D, Del-Favero J, Oruc L, Nothen MM, Blackwood D *et al*. Excess of allele1 for alpha3 subunit GABA receptor gene (GABRA3) in bipolar patients: a multicentric association study. *Mol Psychiatry* 2002; **7**: 201–207.
- 245 Patterson-Buckendahl P, Blakley G, Kubovcakova L, Krizanova O, Pohorecky LA, Kvetnansky R. Alcohol alters rat adrenomedullary function and stress response. *Ann NY Acad Sci* 2004; **1018**: 173–182.
- 246 Bassetti CL, Hersberger M, Baumann CR. CSF prostaglandin D synthase is reduced in excessive daytime sleepiness. *J Neurol* 2006 April 5 (Epub ahead of print).
- 247 Zhu Y, Fillenwarth MJ, Crabb D, Lumeng L, Lin RC. Identification of the 37-kd rat liver protein that forms an acetaldehyde adduct *in vivo* as delta 4-3-ketosteroid 5 beta-reductase. *Hepatology* 1996; **23**: 115–122.
- 248 Ordway GA, Smith KS, Haycock JW. Elevated tyrosine hydroxylase in the locus coeruleus of suicide victims. *J Neurochem* 1994; **62**: 680–685.
- 249 Javors MA, Houston JP, Bowden CL. EGTA-extractable calmodulin in platelet membrane is lower in alcoholics than in controls. *Alcohol* 1985; **2**: 433–436.
- 250 Ye XF, Lu Y, Zhang P, Liang JH. Calmodulin inhibitor trifluoperazine attenuates the development and expression of morphine-induced conditioned place preference in rats. *Eur J Pharmacol* 2004; **486**: 265–271.
- 251 Choi IG, Kee BS, Son HG, Ham BJ, Yang BH, Kim SH *et al*. Genetic polymorphisms of alcohol and aldehyde dehydrogenase, dopamine and serotonin transporters in familial and non-familial alcoholism. *Eur Neuropsychopharmacol* 2005; **3**: 4.
- 252 Chai YG, Oh DY, Chung EK, Kim GS, Kim L, Lee YS *et al*. Alcohol and aldehyde dehydrogenase polymorphisms in men with type I and Type II alcoholism. *Am J Psychiatry* 2005; **162**: 1003–1005.
- 253 Huang SY, Lin WW, Ko HC, Lee JF, Wang TJ, Chou YH *et al*. Possible interaction of alcohol dehydrogenase and aldehyde dehydrogenase genes with the dopamine D2 receptor gene in anxiety-depressive alcohol dependence. *Alcohol Clin Exp Res* 2004; **28**: 374–384.
- 254 Tritos N, Kitraki E, Philippidis H, Stylianopoulou F. Neurotransmitter modulation of glucocorticoid receptor mRNA levels in the rat hippocampus. *Neuroendocrinology* 1999; **69**: 324–330.
- 255 Khan MM, Parikh VV, Mahalik SP. Antipsychotic drugs differentially modulate apolipoprotein D in rat brain. *J Neurochem* 2003; **86**: 1089–1100.

Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (<http://www.nature.com/tpj>)