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Transcription Profiling Reveals Mitochondrial, Ubiquitin and Signaling Systems Abnormalities in Postmortem Brains From Subjects With a History of Alcohol Abuse or Dependence

Boris P. Sokolov,[1]* Lixin Jiang,[2] Niraj S. Trivedi,[2] and Christopher Aston[2]
[1]Molecular Neuropsychiatry Branch, National Institute on Drug Abuse, National Institutes of Health, Baltimore, Maryland
[2]Neuroscience Discovery Research, Wyeth Research, Princeton, New Jersey

Alcohol abuse is a common human disorder with high rate of comorbidity with other psychiatric disorders. To identify candidate mechanisms for alcohol abuse, the expression of 12,626 genes was measured in postmortem temporal cortex from 11 subjects with a history of alcohol abuse or dependence, with or without other psychiatric diagnoses and compared pairwise with the expression in 11 nonalcoholic subjects matched for the other psychiatric diagnoses and demographics. Genes were defined to have altered expression in alcohol abuse if: 1) the gene showed decreased expression in at least 10 of 11 subjects with alcohol abuse, or showed increased expression in at least 10 of 11 subjects with this diagnosis compared to matched non-abusers ($P < 0.007$, χ^2test); or 2) the difference in the mean abuser/non-abuser ratio for the gene from value of 1.0 was significant at $P < 0.05$ (one sample t-test). In subjects with a history of alcohol abuse or dependence, 163 genes were changed significantly. The most abundant and consistent changes were in gene families encoding mitochondrial proteins, the ubiquitin system, and signal transduction. These alterations indicate disturbances in energy metabolism and multiple signaling mechanisms in the temporal cortex of subjects with a history of alcohol abuse or dependence. We hypothesize that these mechanisms may be related to alcohol abuse traits or long-term effects of alcohol.

Key words: microarray; substance abuse; alcoholism; psychiatric disorders

Alcohol abuse is a common human disorder that has a tremendous medical and economic impact on society. Molecular mechanisms associated with alcohol abuse may manifest themselves through changes in gene expression. Microarray technology enables expression of many genes to be determined simultaneously, making it possible to examine the complex interplay of multiple genetic and environmental factors that may contribute to complex disorders. Expression profiling of postmortem brain tissue using microarrays has been applied to examine the molecular basis of schizophrenia (Mirnics et al., 2000; Hakak et al., 2001; Vawter et al., 2001; Hemby et al., 2002), bipolar disorder (Bezchlibnyk et al., 2001), and to identify changes associated with alcoholism (Lewohl et al., 2000; Mayfield et al., 2002). We used microarrays to identify genes whose expression was changed in subjects with the diagnosis of alcohol abuse or dependence.

Current evidence suggests that susceptibility to alcoholism involves a complex interaction of a number of genetic and environmental factors (Enoch and Goldman, 1999; Thome et al., 2000). Importantly, subjects with alcoholism frequently have concurrent psychiatric diagnoses such as bipolar disorder, major depressive disorder, schizophrenia, anxiety disorder, or panic disorder (Guze, 1990; Chignon and Lepine, 1993; Kendler et al., 1993; Merikangas et al., 1994; Dawson and Grant, 1998; Nurnberger et al., 2001). This necessitates examining mechanisms of alcoholism in both subjects without other psychiatric disorders and subjects with comorbid psychiatric disorders. Importantly, the high comorbidity of alcoholism and other psychiatric disorders suggests that alcohol abuse/dependence may share some common underlying mechanisms with other psychiatric disorders. This may complicate identification of alcohol abuse-specific mechanisms using molecular analysis of brains from subjects with alcohol abuse compared to nonalcoholic subjects.

*Correspondence to: Dr. Boris P. Sokolov, Molecular Neuropsychiatry Branch, National Institute on Drug Abuse, NIH, DHHS, 5500 Nathan Shock Dr., Baltimore, MD 21224.
E-mail: BSokolov@intra.nida.nih.gov

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Indeed, alterations identified in subjects with alcohol abuse may potentially represent both alcohol abuse-specific mechanisms and mechanisms shared with other psychiatric disorders. In addition, there might be a risk of confounding undiagnosed or pre-clinical psychiatric conditions.

Examining molecular alterations across subjects with a history of alcohol abuse with or without other psychiatric diagnoses, compared to nonalcoholic subjects matched for psychiatric diagnosis and other demographics, may be a useful approach to identify candidate mechanisms specific for alcohol abuse. It is plausible to suggest that alterations conserved across pairs of alcoholic/nonalcoholic subjects with different psychiatric diagnoses may be relatively specific for alcohol abuse. The logic behind this assumption is that comparison of abusers without psychiatric comorbidities versus non-abusers without psychiatric comorbidities is expected to reveal alcohol abuse-specific alterations and, in addition, alterations shared with other psychiatric disorders. By contrast, comparison of alcohol abusers who have schizophrenia versus non-abusers who have schizophrenia is expected to reveal alterations specific for alcohol abuse but not to reveal alterations that are shared by alcohol abuse and schizophrenia. Similarly, comparing alcohol abusers who have bipolar disorder versus non-abusers who have bipolar disorder will exclude alterations related to mechanisms shared by alcohol abuse and bipolar disorder. Further, comparison of alcohol abusers who have major depressive disorder versus non-abusers who have major depressive disorder will exclude alterations shared with alcoholism abuse and major depressive disorder. Thus, a search for alterations that are present simultaneously in matched pairs of non-psychiatric alcohol abusers/non-abusers and in matched pairs of alcohol abusers/non-abusers who have schizophrenia, bipolar disorder or major depressive disorder may help to identify alterations specific for alcohol abuse and reduce the chance of identifying alterations shared by alcohol abuse and other psychiatric disorders. Another practical reason for examining alcohol abusers with and without psychiatric comorbidities is that it is very difficult to obtain a sufficient number of postmortem brains from individuals with alcohol abuse and control individuals who would have undergone a comprehensive psychiatric assessment that would exclude other psychiatric disorders. This is important, because examining samples without comprehensive psychiatric assessment may lead to confounds due to undiagnosed concurrent diseases. By contrast, matched pairs of abusers and non-abusers with or without psychiatric comorbidities who have undergone an intensive psychiatric evaluation were available to study from the Stanley Foundation Brain Bank.

We used microarrays to examine changes across subjects with the diagnosis of alcohol abuse/dependence who had no other psychiatric diagnoses as well as across subjects with alcohol abuse/dependence who had diagnoses of schizophrenia, bipolar disorder, or major depressive disorder. We chose to focus on alterations that were present in alcohol abusers with or without other concurrent psychiatric diagnoses, as an approach to exclude changes shared by alcohol abuse and other psychiatric disorders. Furthermore, we chose to focus on alterations that were present in both: 1) subjects with alcohol abuse/dependence at time of death, and 2) subjects who had a history of alcohol abuse/dependence but were abstinent for a long time before death. The rationale for using this approach was that it may help to reduce the findings related to immediate short-term effects of alcohol use and increase the probability of identifying alterations related to an alcohol abuse trait or to irreversible consequences of alcohol abuse.

MATERIALS AND METHODS

Eleven subjects with the diagnosis of past or current alcohol abuse or dependence, with or without other psychiatric diagnoses (schizophrenia, bipolar disorder, or major depressive disorder) and eleven closely matched control subjects were selected from the Stanley Foundation Brain Bank. Demographics for the cases studied are shown in Table I. Cases were diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) as described in detail previously (Torrey et al., 2000). Of the cases, five with a history of alcohol abuse were abstinent for up to 30 years before death and six other cases displayed alcohol abuse or dependence at time near death (Table I). Control cases were nondrinkers or social drinkers with no evidence of a history of alcohol abuse or dependence. Data from 11 subjects with alcohol abuse/dependence was compared to 11 nonalcoholic subjects. Subjects with alcohol abuse/dependence were paired with nonalcoholic subjects. Pairs were matched primarily by concurrent psychiatric disease, then by age, gender, history of neuroleptic treatment, then by direct measures of overall RNA preservation and cRNA quality (i.e., S28:S18 RNA ratio determined by gel electrophoresis, 5′:M ratio for β-actin, and 5′:3′ ratio for GAPDH, determined from Affymetrix chip hybridization analysis), indirect indicators of overall RNA preservation (postmortem interval delay [PMI] and brain pH), and then by the side of the brain, if possible (Table I). Of the 11 pairs, seven pairs were matched by side of brain; however, the other four pairs were not matched: two pairs had left/right (L/R) and two pairs had right/left (R/L). There were two pairs without suicide; one pair with suicide in both alcoholic and nonalcoholic cases; four pairs with suicide in the alcoholic but not in the nonalcoholic case; and four pairs with suicide in the nonalcoholic but not in the alcoholic case. Thus, suicide and side of the brain were balanced overall across the pairs, thereby downplaying potential contribution of these factors to the expression data.

The temporal cortex (middle temporal gyrus corresponding to Brodmann's area 21) was examined because it has been implicated in regulation of mental and emotional states, and has been suggested to play roles in disturbances of thought, behavior, and altered consciousness (Barta et al., 1997; Pearlson, 1997; Levitan et al., 1999), which may be involved in alcoholism.

The brain tissue was collected by medical examiners trained to process the brain tissue in a standardized manner. One-half of the cerebrum was cut into 1.5-cm thick coronal slices and frozen in a mixture of isopentane and dry ice (see

TABLE I. Demographics for Cases Examined

Case	Age (years)	Neuroleptic at death	Gender	GAPDH 5′-3′ ratio	β-actin 5′-M ratio	PMI (hr)	pH	Side of brain	Cause of death[a]	Duration of psych disorder (years)	Alcohol abuse/dependence (DSM-IV)
No other psychiatric diagnosis											
49C53	52	N	M	1.02	0.74	28	6.5	R	CP	—	Abuse past, abstinent 8 yr
85C26	52	N	M	0.97	0.41	8	6.5	R	CP	—	No
124C55	53	N	M	0.80	0.68	28	6.2	R	CP	—	Abuse past, abstinent 30 yr
165C6	58	N	M	0.88	0.48	27	6.0	R	CP	—	No
Subjects with major depressive disorder											
104D13	51	N	M	0.6	0.34	26	6.3	L	S	50	Abuse past, abstinent 14 yr
92D18	52	N	M	0.93	0.5	12	6.5	L	CP	46	No
171D7	43	N	M	1.06	0.49	43	5.9	R	CP	30	Dependence, other drug use
138D33	33	N	M	0.85	0.39	42	nd	R	S	32	No
38D51	53	N	F	0.97	0.67	40	6.3	L	AI	11	Dependence
16D20	32	N	F	0.64	0.27	47	6.0	R	S	32	No, some cocaine use
135D34	39	N	M	0.51	0.21	23	6.0	R	S	17	Abuse, amphetamine abuse
101D37	42	N	M	0.60	0.29	25	6.3	L	CP	39	No
Subjects with bipolar disorder											
72B11	57	Y	M	0.78	0.35	19	6.2	R	CP	30	Abuse past, abstinent 25 yrs
83B2	48	Y[b]	M	1.08	0.64	13	6.1	L	S	27	No, some marijuana use in months prior death
33B50	25	Y	F	0.75	0.73	24	6.4	L	S	19	Dependence, other drug use
47B8	37	Y	F	0.94	0.6	29	6.5	R	S	14	No
75B32	34	Y	M	0.65	0.09	23	6.3	L	S	19	Abuse
60B16	30	Y	M	0.58	0.18	31	6.1	L	CP	22	No
Subjects with schizophrenia											
93S27	25	Y	M	0.63	0.16	32	6.6	R	S	20	Abuse past; marijuana use
30S40	30	Y	M	0.42	0.07	32	5.8	R	CP	13	No
66S43	32	Y	M	0.96	0.69	19	6.1	R	AI	27	Abuse, amphetamine abuse
81S1	31	Y	M	0.61	0.39	14	5.8	R	S	18	No

[a]CP, cardiopulmonary; S, suicide; AI, acute alcohol intoxication.
[b]History of neuroleptic treatment, but was untreated for over 20 years.

Torrey et al., 2000). Frozen tissue was stored at −70°C. Gray matter was used for the analysis.

RNA was purified from 500 mg of tissue by Trizol reagent, treated with DNase to remove genomic DNA and then purified additionally using phenol/chloroform followed by ethanol precipitation as described (Sokolov, 1998). RNA integrity was evaluated by agarose gel electrophoresis and by measuring the 5′- to 3′-end ratio for GAPDH and 5′- to M ratio for β-actin mRNAs in the array data.

Transcriptional profiling was carried out using Affymetrix HgU95A GeneChip oligonucleotide arrays (Affymetrix, Santa Clara, CA) and 5 μg of total RNA. Each array contained 12,626 probe sets corresponding to full-length genes and clustered ESTs. Sample labeling, hybridization, and scanning were carried out according to Affymetrix recommendations. The samples were coded to blind the investigators. The codes were broken after the arrays were scanned and data were analyzed using Affymetrix GeneChip version 4.0 software. The target intensity was set to 1,500 to normalize expression levels across all samples. "Average difference" (level of expression) and "absent/present" calls were generated for each gene. Subsequent analyses used Microsoft Excel, Microsoft Access, SPPS 10.5 and S-plus statistical programs.

Array analysis of one sample (C26) was repeated three times. Array analysis of four other samples (D20, B50, S27, and S43) was repeated twice. The average correlation coefficient in these re-hybridized experiments was >0.99, demonstrating consistency of the arrays. The average correlation coefficient between different subjects was lower (≈0.94). Mean values for repeated experiments were used in comparing different subjects.

Ratios were generated for expression of each gene in each matched abuser/non-abuser pair. We defined genes with candidate changes in alcohol abuse/dependence as genes for which: 1) the gene showed decreased expression in at least 10 of 11 subjects with alcohol abuse/dependence or showed increased expression in at least 10 of 11 subjects with alcohol abuse/dependence compared to matched nonalcoholics (significance of $P < 0.007$, $\chi^2 = 7.364$); 2) the difference in the mean abuser/non-abuser ratio for the gene from a value of 1.0 was significant at $P < 0.05$ (one sample *t*-test after natural log transformation of ratios to reduce skewness); 3) hybridization was sufficiently robust that the expression of the gene was called "present" by Affymetrix criteria in at least one of the samples; and 4) there were no negative mean average difference values in any of the groups (because these represented low-level expression and were therefore of low confidence). Fold change was not used as a

filtering criterion because even subtle differences in the expression levels may be of potential biological significance. Functional grouping of genes was based on annotation from LocusLink, OMIM, sequence homology analysis, and literature searches.

Real-time reverse-transcription polymerase chain reaction (RT-PCR) was carried out as follows: template cDNA was synthesized using Superscript II reverse transcriptase and random-hexamers (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. TaqMan oligonucleotide probes were designed according to recommendations of the manufacturer (Applied Biosystems, Foster City, CA). Amplicons were typically 70 bp in length. The reporter dye was FAM and the quencher dye was TAMRA. PCR was carried out using the Platinum qPCR Supermix-UDG kit (Invitrogen). Each gene was profiled separately, but the same pool of cDNA was used for all reactions.

RESULTS

Of the 12,626 genes assayed by the arrays, 7,597 (60%) reached Affymetrix criteria for being "expressed: in at least one sample, and 163 genes satisfied criteria for "changed in alcohol abuse/dependence" as described above, with 102 genes decreased and 61 genes increased (Table II). Of these, 21 genes showed changed expression in all 11 subjects with a history of alcohol abuse/dependence compared to matched nonalcoholics (Table II). Real-time RT-PCR was used to confirm the changes in gene expression detected by microarray analysis. The expression of six genes (*ME2*, *HHARI*, *STX12*, *HPF7*, *PAHX*, and *SeP*) representing major functional gene families demonstrating changes in alcohol abuse (see below) was measured by RT-PCR in 10 abuser/non-abuser pairs and confirmed to have mean fold changes in expression levels directionally similar to those determined by microarray analysis (data not shown).

The most remarkable decrease was in the expression of 11 nuclear genes encoding mitochondrial proteins (Table II). Two of these genes were decreased in all 11 subjects with a history of alcohol abuse/dependence, whereas nine genes were decreased in 10 of 11 subjects with a history of alcohol abuse/dependence. This uniform decrease in expression of multiple genes for mitochondrial proteins may indicate an overall decline in mitochondrial function in the brains of subjects with alcohol abuse/dependence.

Six genes involved in cell adhesion were upregulated in the brains of subjects with a history of alcohol abuse/dependence. These included cadherins 12 and α9, a non-classic-type cadherin CELSR1, extracellular matrix protein 2, and a cell adhesion molecule, APC, that binds β-catenin (Table II).

Alterations in expression of genes encoding key components of the ubiquitin-proteosome system were present in subjects with a history of alcohol abuse/dependence (Table II). Two genes (*HHARI* and *CDC34*) for enzymes that conjugate ubiquitin with target proteins (Pickart, 2001a,b) were decreased in each of the 11 subjects with a history of alcohol abuse/dependence. Two other ubiquitination-related genes, *ANCR* and *P27*, were decreased in 10 of 11 subjects with a history of alcohol abuse/dependence. *ANCR* is an ubiquitin ligase (E3), which selects the targets for ubiquitination (Hershko and Ciechanover, 1998). *P27* is a component of the 26S proteosome, an ATP-dependent protease that selectively degrades ubiquitinated proteins. These coordinated deficits in ubiquitin-related genes indicate a deficit in ubiquitination-mediated intracellular proteolysis in the brains of subjects with a history of alcohol abuse/dependence. Further disturbances in the ubiquitin-mediated proteolysis in alcohol abuse/dependence may be caused by increased expression of the ubiquitin protease *Upnh* (Table II), which removes ubiquitin from ubiquitin-conjugated proteins. In addition to genes mediating ubiquitin-mediated proteolysis, there was a decrease in expression of *Calpain1*, a large subunit of calcium-dependent neutral protease (Table II).

Genes encoding phospholipases C and D1 were upregulated in subjects with a history of alcohol abuse/dependence (Table II). Besides these, several other phosphatases, protein kinases, GTP-binding proteins, and ion channels displayed altered expression in subjects with a history of alcohol abuse/dependence, indicating widespread abnormalities in signal transduction. Of interest was the coordinated decrease in expression of two catalytic subunits (calcineurin A1 and Aγ) of calmodulin-regulated protein phosphatase 3, which is involved in a wide range of biologic activities, acting as a Ca^{2+}-dependent modifier of phosphorylation status (Hashimoto et al., 1988).

Downregulation was found for 21 genes involved in regulation of gene expression or replication and 9 genes were upregulated in subjects with a history of alcohol abuse/dependence (Table II). Decreased expression of *ATRX*, a DNA-dependent ATPase and helicase causing X-linked mental retardation, was revealed by two independent probe sets targeted to two different regions of its transcript, which added confidence to the results.

Among other interesting changes was decreased expression of selenoprotein P, which is implicated in oxidant defense in the extracellular space and in the transport of selenium. The expression of the microsomal cytochrome p450IIA4 gene was increased in subjects with a history of alcohol abuse/dependence. Alcohol is a known modulator of the hepatic cytochrome P450 (CYP)-dependent microsomal monooxygenase system, which is involved in oxidizing alcohol (Niemela et al., 1999). All 11 subjects with a history of alcohol abuse/dependence displayed increased expression of Bcl10, a novel member of the caspase recruitment domain/membrane-associated guanylate kinase family of proteins that regulate apoptosis and NF-κB signaling pathways.

DISCUSSION

Analysis of alcohol abuse/dependence-related changes across subjects with or without other psychiatric diagnoses has both advantages and limitations. The advantage in this approach is to potentially decrease the risk of confounding undiagnosed or pre-clinical psychiatric con-

TABLE II. Genes with Altered Expression in Subjects With a History of Alcohol Abuse/Dependence[†]

Description	Gene name	GenBank accession number	Map	*P**	Fold change
Mitochondrial					
NAD(P)+ dependent malic enzyme, mitochondrial	**ME2**	**M55905**	**6p25-p24**	**0.0009**	**0.65**
dynamin-related protein, maintenance of mitochondrial DNA	**OPA1**	**AB011139**	**3q28-qter**	**0.00008**	**0.73**
NADH-ubiquinone oxidoreductase B12 subunut		AA203354		0.005	0.70
Similar to 10 kD HSP, mitochondrial		AI912041		0.003	0.72
Cytochrome c oxidase precursor	COX5B	M19961	2cen-q13	0.029	0.84
HS1 binding protein, localized to the mitochondrial membrane	HAX1	U68566	1	0.046	0.88
Translocase of outer mitochondrial membrane homologue A	TOMM70A	AB018262	3	0.019	0.83
Acyl-coa dehydrogenase, mitochondrial protein	ACADSB	U12778	10q25-q26	0.008	0.79
ATP synthase, H+ transporting, mitochondrial F1F0, subunit γ		AA917672		0.023	0.74
Function in the assembly of complex III of the respiratory chain	BCS1	AF038195	2q33	0.013	0.72
Low molecular mass ubiquinone-binding protein (9.5kd)	QP-C	AI540957	5	0.014	0.86
Cytochrome c oxidase subunit via polypeptide 2	COX6A2	F27891		0.018	1.32
Ubiquitin-proteosome/proteolysis					
Ubiquitin-conjugating enzyme (E2), putative RING finger protein	**HHAR1**	**AJ009771**	**15q24**	**0.00029**	**0.60**
Ubiquitin conjugating enzyme (E2), a putative G2 checkpoint gene	**CDC34**	**L22005**	**19p13.3**	**0.0027**	**0.66**
Ubiquitin protein ligase E3A, Angelman syndrome, mental retardation	ANCR	U84404	15q11-q13	0.002	0.21
Proteasome subunit p27	P27	AB003177	12q24.31-32	0.049	0.81
Targets beta-catenin for ubiquitin degradation	SIAH1	U76247	16q12	0.001	0.81
Ubiquitination factor E4B, function unknown	UBE4B	AB014584	1p36.3	0.0169	1.94
Ubiquitin protease, removes ubiquitin from ubiquitinated proteins	Unph	U20657	3p21.3	0.016	2.08
Calpain 1, large subunit, calcium dependent neutral protease	**CAPN1**	**X04366**	**11q13**	**0.0003**	**0.60**
Cell adhesion					
Extracellular matrix protein	**ECM2**	**AB011792**	**9q22.3**	**0.0005**	**1.75**
Nonclassic-type cadherin, contact-mediated communication	CELSR1	AL031588	22q13.3	0.0036	1.29
Cadherin 12, brain specific, neuronal development, synaptogenesis	CDH12	L34057	5p13-p14	0.0004	1.68
Protocadherin alpha 9	PCDHA9	AB002343	5q31	0.037	2.62
Beta-2 integrin alphad subunit (ITGAD)	ITGAD	U40279		0.049	1.31
Cell adhesion, binds beta-catenin, adenomatous polyposis coli	APC	M74088	5q21-q22	0.01187	4.80
Signal transduction					
TEK tyrosine kinase, endothelial, morphogenesis of veins	**TEK**	**L06139**	**9p21**	**0.0067**	**0.77**
Calcitonin receptor activity modifying protein 1	**RAMP1**	**AJ001014**	**2q36-q37.1**	**0.0028**	**0.63**
21-Glutamic acid-rich protein, SH3 domain binding	**21-GARP**	**X93498**	**21q22.3**	**0.0048**	**0.63**
Calcineurin A1, calmodulin-dep protein phosphatase 3 catalytic subunit α	CALNAB	M29550	4q21-q24	0.001	0.77
Calcineurin A, calmodulin-dep protein phosphatase catalytic subunit γ	CALNA3	S46622	8	0.008	0.65
Sac domain-containing inositol phosphatase 2	KIAA0966	AB023183	10	0.033	0.71
Casein kinase I alpha isoform	CSNKIA1	L37042	13q13	0.0025	0.72
Testis-specific kinase 1	TESK1	D50863	9p13	0.002	0.76
Similar to RAS-like protein TC21	TC21	A1365215	11	0.015	0.73
Ras-related GTP-binding protein homologue enriched in brain	Rheb	D78132	7q36	0.015	0.85
Similar to GTP-binding protein		AI057115		0.024	0.68
Ras association (RalGDS/AF-6) domain family 2	KIAA0168	D79990	20	0.0079	0.81
Insulin receptor precursor	INSR	X02160	19p13.2-3	0.019	0.64
Multiple membrane spanning receptor (TRC8), patched related protein	RCA1	AF064801	8q24	0.031	0.81
Similarity to tyrosine-phosphorylated protein DOK1	DKFZ	AL050069	20	0.005	0.76
Transient receptor potential channel	TRP1	X89066	3q22-q24	0.0058	0.69
K+ channel beta subunit	KCNAB1	L39833	3q26.1	0.011	0.80
Syntaxin 12	STX12	AL035306	1p34.1-1p35	0.0098	0.77
Subunit of putative vesicle coat adaptor complex AP-3	ADTD	AF002163	19p13.3	0.0004	0.75
Protein tyrosine kinase, vascular endothelial growth factor receptor-2	**VEGFR2**	**AF035121**	**4q11-q12**	**0.0072**	**1.78**
Phospholipase C, gamma 2 (phosphatidylinositol-specific)	PLC1	M37238	20q12-q13.1	0.0046	1.46
Glycosylphosphatidylinositol specific phospholipase D1	PIGPLD1	L11702	6p22.3-p22.2	0.046	1.80

Table II continues on next page

TABLE II. Genes with Altered Expression in Subjects With a History of Alcohol Abuse/Dependence† (continued)

Description	Gene name	GenBank accession number	Map	P*	Fold change
Protein tyrosine phosphatase, receptor type E	PTPRE	X54134		0.007	1.48
Membrane-associated kinase, activates Cdc2 kinase	Myt1	AF014118		0.0059	1.46
Regulatory partner for cdk5 kinase	CDK5P35	X80343	17	0.0012	2.52
Serine/threonine kinase 9		X89059		0.033	1.90
Ligand for the flt3/flk-2 tyrosine kinase receptor	FLT3LG	U03858	19q13.3	0.0054	2.34
SH3-domain binding protein 1	SH3BP1	Z83844	22	0.010	1.71
IgG Fc fragment receptor precursor	IgG FcRII.	M31932		0.003	2.09
Estrogen receptor 1	ESR	X03635	6q25.1	0.017	1.31
CD40 antigen, nerve growth factor receptor-related	CD40	X60592	20q12-q13.2	0.023	1.41
Peropsin, G protein-coupled receptor, light detection	RRH	AF012270	4q	0.016	1.95
Hepatoma-derived growth factor	HMGIL2	D16431	Xq25	0.036	1.54
Transcription/translation/replication					
Transcription factor-like 1, nuclear protein with DNA-binding ability	YL1	D43642	1q21	0.0021	0.80
Sim to RNA-binding protein Hlark, embryonic development	LARK	U89505	11q13	0.00001	0.74
DNA-dependent ATPase and helicase, X-linked mental retardation	**ATRX**	**U72936**	**Xq13.3**	**0.0026**	**0.67**
DNA-dependent ATPase and helicase	ATRX	U72936	Xq13.3	0.004	0.80
RNA helicase A, nuclear DNA helicase II, DEAD/H box polypeptide 9	LKP	L13848	1q25	0.0089	0.69
ATP-dependent RNA helicase #46, DEAD/H box polypeptide 15	DBP1	AB001636	4p15.3	0.0053	0.78
DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 16	N36997	0.0038	0.84		
Cysteine and glycine-rich protein 2; two zinc-finger LIM domains	CSRP2	U57646	12q21.1	0.012	0.74
Cysteine and glycine-rich protein 1, two zinc-finger LIM domains	CSRP1	M33146	1q32	0.015	0.77
Gene activation, antagonizing chromatin-mediated transcriptional repress	BAF53	AF041474	3	0.039	0.80
Transcription factor, forkhead box GIB	FKH2	X74142	14q12-q13	0.0021	0.82
General transcription factor IIA, 2 (12 kD subunit)	TFILA	U14193	15	0.0027	0.73
Heat shock transcription factor 2	HSF2	M65217	6	0.033	0.84
PC4, developmental regulator; nerve growth factor-inducible protein	PC4	Y10313	7q22-q31	0.0033	0.69
TG-interacting factor (TALE family homeobox), holoprosencephaly-4	HPE4	X89750	18p11.3	0.0072	0.64
FBRNP, heterogeneous nuclear protein, helix destabilizing protein	D10S102	S63912	10	0.0043	0.68
C protein, heterogeneous nuclear ribonucleoprotein C (C1/C2)	HNRNPC	M16342	14	0.0023	0.75
Eukaryotic translation elongation factor 1 beta 2	EEF1B2	X60489	2	0.0075	0.65
Matrix associated, regulator of chromatin, subfamily a, member 5	HSNF2H	AB010882	4q31.1-q31.2	0.0017	0.69
Binds subunit 5 of RNA polymerase II, negatively regulates RNA Pol II	NNX3	AB006572	19q12	0.0002	0.79
Speckle-type POZ protein	SPOP	AJ000644	17	0.020	0.85
Associates with DNA polymerase al/primase	CDK2AP1	AF006484	12q24.31	0.032	0.85
RNA polymerase I transcription factor RRN3	**A-270G1.2**	**AF001549**	**16p12**	**0.0001**	**1.58**
Zinc finger protein 261, X-linked mental retardation candidate gene	ZNF261	X95808	Xq13.1	0.0051	3.73
Nuclear factor I/B	NFI-B3	U70862	9p24.1	0.020	1.82
Zinc finger protein 91 (HPF7, HTF10), Kruppel related	HPF7	L11672	19p13.1-p12	0.0093	2.13
Zinc finger protein 91 (HPF7, HTF10), Kruppel related	HPF7	L11672	19p13.1-p12	NS	1.99
Helix-loop-helix-PAS transcription factor family, primarily in brain	MOP4	U77970	2p11.2-q13	0.011	3.02
HB9 homeobox gene, Currarino syndrome	HOXHB9	U07664	7q36	0.0014	2.28
Homcobox gene, hemopoietic progenitor homeobox	HPX42B	AF068006	10q26	0.0019	1.21
RNA polymerase II elongation factor ELL2	ELL2	U88629	5	0.015	2.78
Splicing factor, arginine/serine-rich 8	SWAP	U08377	12	0.1025	1.23
Others					
ATPase type IV, phospholipid transporting	**ATPASEP**	**AJ006268**	**18q23**	**0.0023**	**0.71**
3-Hydroxy-3-methylglutaryl-coenzyme A reductase, cholesterol path	**HMGCR**	**M11058**	**5q13.3-q14**	**0.0013**	**0.71**
Astrotactin, glial-guided neuronal migration	ASTN	AB006627	1q25.2	0.0034	0.73
DPD; dihydrouracil dehydrogenase	DPD	U20938	Ip22	0.045	0.80
Uracil-DNA glycosylase 2		X52486	5	0.072	0.81
N-myristoyltransferase 1	NMT	AF043324	17	0.007	0.77
Guanidinoacetate N-methyltransferase, GAMT deficiency		AC005329	19p13.3	0.010	0.79
Phytanoyl-coa hydroxylase (Refsum disease, a neurologic disorder	PAHX	AF023462	10pter-p11.2	0.0017	0.71

Table II continues on next page

TABLE II. Genes with Altered Expression in Subjects With a History of Alcohol Abuse/Dependence[†] (continued)

Description	Gene name	GenBank accession number	Map	*P**	Fold change
1,4-Alpha-glucan branching enzyme	HGBE	L07956	3p21	0.002	0.70
Protein disulfide isomerase.	ERp57	Z49835	15q15	0.0048	0.73
ADP-ribosylation factor-binding protein GGA3, γ adaptin	GGA3	D63876	17	0.0083	0.76
Selenoprotein P, oxidant defense, transport of selenium	SeP	Z11793	5q31	0.0035	0.60
Calcium-binding protein, chaperone, secretion of proteins from the ER	Calnexin	L10284	5q35	0.0031	0.83
Lysosomal membrane protein	LAMPB	U36336	Xq24	0.049	0.81
Tubulin-specific chaperone, beta-tubulin cofactor A	TBCA	AF038952	5	0.0017	0.76
Myosin class I, myh-1c	myh-1	AJ001381		0.023	0.73
β2 Subunit of a complex associated with non-clathrin coated vesicles	COPB2	X70476	3q23	0.020	0.84
Dystroglycan 1, linkage between subsarcolemmal cytoskeleton and EMC	DAG A3a	L19711	3p21	0.026	0.77
Phosphoprotein that encircles the lipid storage droplet in adipocytes	perilipin	AB005293	15q26	0.024	0.77
Clathrin-like protein, adaptor-related protein complex 3, mu 2	CLA20	D38293	8p11.2	0.036	0.87
MTCP1 gene, mature T-cell proliferation 1	MTCP1	Z24459	Xq28	0.0067	0.72
Similar to rat Hras-revertant gene 107 protein	orf	X92814	11	0.0052	0.66
UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter	KIAA0260	D87449	1	0.033	0.80
Apoptosis-related protein	TFAR15	AF022385	3	0.015	0.81
Caspase recruitment domain/memb-associated guanylate kinase	**CARD10**	**L049851**	**22Q13.1**	**0.0046**	**1.41**
Cyclophilin F, peptidylprolyl isomerase F	CYP3	M80254	10q22-q23	0.019	1.70
Phosphoribosylpyrophosphate synthetase subunit III	PRPS3	M57423		0.0023	1.48
Cytochrome P450IIA4, subfamily IIA	CYP2A7	M33317	19q13.2	0.0089	1.70
Peroxisomal long-chain acyl-coA thioesterase	PTE2	L40401	14q24.3	0.010	1.31
Unconventional myosin, deafness, neurosensory	DFNA11	U39226	11q13.5	0.023	1.57
Dentatorubro-pallidoluysian atrophy, neurodegenerative disorder	DRPLA	D31840	12p13.31	0.0021	1.62
Monocarboxylate transporter, solute carrier family 16, member 5	MCT5	U59299		0.0099	1.17
Testis amiloride-sensitive cation channel 3, isoform b	TNAC1	AB010575	7q35	0.010	2.07
UDP-N-acetylglucosamine transporter		AB021981	1p21	0.022	2.08
Similar to ganglioside GM2 activator precursor	GM2A	AA224768	5q31.3-q33.1	0.010	1.44
Alpha adducin, cytoskeleton proteins	ADD1	L07261	4p16.3	0.032	5.90
Kappa light chain constant region		X96754		0.0021	1.50
Mucin	MUCCF	M57417		0.0081	2.81
Unknown function/EST					
HBV associated factor	XAP4	AA160708	20	0.010	0.80
Similar to yeast Upf3, variant A		N36842		0.0024	0.80
Homolog of rat kinase D-interacting substance of 220 kD		W27233		0.0017	0.74
Hypothetical protein FLJ11191		AF038179		0.013	0.80
Homo sapiens clone 24626		AF052141		0.0027	0.80
cDNA DKFZp586C1019		AL049397		0.012	0.80
Clone 23620 mRNA sequence		AF052107		0.0056	0.72
Erythrocyte membrane protein band 4.1-like 3	DAL-1 4.1B	AB023204	18	0.0067	0.84
KIAA0560 gene product	KIAA0560	AB011132	15	0.0003	0.80
KIAA0455 gene product	KIAA0455	AB007924	1	0.017	0.83
KIAA0623 gene product	KIAA0623	AB014523	17	0.011	0.80
Clone IMAGE:2338438 3′, mRNA sequence		AI692348		0.0012	0.71
cDNA DKFZp586N1720 (from clone DKFZp586N1720)		AL049442		0.0038	0.71
KIAA0133 gene-related protein	KIAA0133	D50923	1	0.029	0.71
Leptin receptor gene-related protein	HSOBRGRP	AW026535		0.032	0.77
Activity-dependent neuroprotective protein	KIAA0784	AB018327	20q13.1	0.0064	0.72
KIAA1116 protein	KIAA1116	AB029039	6	0.0042	0.76
KIAA0648 protein	KIAA0648	AB014548	4	0.020	0.72
Similar to S. Pombe dim1+	DIM1	AF023612	18	0.014	0.80
Predicted osteoblast protein	GS3786	D87120	7	0.038	0.76
DAZ associated protein 2	KIAA0058	D31767	2q33-q34	0.011	0.79
Hypothetical protein, hypothetical protein LOC57158	dJ1183121.1	AL035447	20	0.0077	0.74
Transmembrane trafficking protein	TMP21	L40397	14q24.3	0.030	0.81
HSPC035 protein	LOC51669	W26659	8	0.017	0.80
Meningioma expressed antigen 5 (hyaluronidase)	KIAA0679	AB014579	10q24.1-3	0.010	0.66

Table II continues on next page

TABLE II. Genes with Altered Expression in Subjects With a History of Alcohol Abuse/Dependence[†] (continued)

Description	Gene name	GenBank accession number	Map	P*	Fold change
cDNA clone IMAGE:305046 3′, mRNA sequence		N92548		0.002	0.63
KIAA0844 protein	KIAA0844	AB020651	10	0.013	0.75
HMBA-inducible	HEXIM1	AB021179	17	0.005	0.59
Acidic 82 kD protein mRNA		U15552	1	0.010	0.84
Hepatitis B virus x-interacting protein (9.6 kD)	XIP	AF029890	1	0.020	0.73
DKFZP5641052 protein		AL080063	1	0.0041	0.78
Hypothetical protein FLJ10120		**H16917**		**0.0005**	**2.17**
Clone B18 unknown mRNA		**AF052497**		**0.0006**	**1.75**
Clone 23821 mRNA sequence		**AF038194**		**0.0008**	**1.27**
cDNA DKFZp58611518 (from clone DKFZp58611518)		AL049378		0.044	1.30
Retina cDNA randomly primed sublibrary cDNA		W26326		0.048	1.79
KIAA0514 gene product	KIAA0514	AB011086		0.012	1.24
KIAA0416 protein	KIAA0416	AB007876	5	0.0026	1.56
KIAA0484 protein	KIAA0484	AB007953		0.007	1.30
Chromosome 11 open reading frame 11	KIAA0659	AB014559	11	0.031	2.08
Chromosome 1 open reading frame 1	PO42	U88965	1p36.3	0.0033	1.82
Hypothetical protein MGC5350		W25866		0.0097	1.54
KIAA0751 gene product	KIAA0751	AB018294	8	0.0046	1.36
PAI-1 mRNA-binding protein	LOC51624	AL080119	3	0.0004	2.24
Cytochrome c-like antigen		S80864	22	0.048	1.44
V-myc avian myelocytomatosis viral oncogene homolog	MYC	M13929	8q24	0.0070	1.69

[†]Bold indicates changes in 11 of 11 subjects with a history of alcohol abuse or dependence; normal font indicates changes in 10 of 11 subjects with a history of alcohol abuse or dependence ($P < 0.007$, $\chi^2 = 7.364$). Fold change indicates mean fold change for 11 abuser/nonabuser pairs. Boxed text represents duplicate probes for *ATRX* and *HPF7,* homologous genes, or genes with similar function. NS, not significant.
*P-value for significance from the one sample t-test.

ditions. Furthermore, this approach is more likely to identify abnormalities that are relatively specific for alcohol abuse/dependence, instead of those that may be common to alcohol abuse/dependence and other psychiatric disorders, which is a potential risk when studying nonpsychotic alcohol abusers only. One disadvantage is that this approach may decrease the power of analysis and does not reveal changes that may be shared by alcohol abuse/dependence and other psychiatric disorders.

Oligonucleotide microarray analysis identified 163 genes that showed abnormal expression in subjects with a history of alcohol abuse/dependence. These genes were changed in subjects who had no other psychiatric disorders as well as in those subjects who had diagnoses of schizophrenia, bipolar disorder, or major depressive disorder. Furthermore, these genes were changed in subjects who were alcohol abusers or dependent at time of death as well as in those subjects who had a history of alcohol abuse, but were abstinent for a long time (up to 20 years) before death. Thus, these may be candidate genes for alcohol abuse/dependence-associated alterations in the brain.

Altered expression in subjects with a history of alcohol abuse/dependence was identified for groups of genes that carry out key cellular functions. The decreased expression of 11 nuclear genes encoding mitochondrial proteins in the brains of alcoholics is remarkable in its consistency. Previous studies have shown structural alterations in mitochondria in animals fed with alcohol (Wahid et al., 1980; Tavares and Paula-Barbosa, 1983; Morvai and Ungvary, 1987; Thayer and Rottenberg, 1992; Chen et al., 1997; Cunningham and Bailey, 2001). A coordinated decrease in multiple genes for mitochondrial proteins indicates that altered gene regulation may underlie structural abnormalities in mitochondria. Furthermore, it suggests significant mitochondrial abnormalities in the brains of subjects with alcohol abuse/dependence. The brain is an extremely energy-dependent and sensitive organ, therefore mitochondrial dysfunction in alcoholism might cause profound changes in brain function.

Another important system that displays alterations in the brains of subjects with a history of alcohol abuse/dependence, consistent with previous findings in experimental animals, is the ubiquitin-proteosome system for intracellular proteolysis. Inhibition of the ubiquitin-proteosome pathway has been reported previously in the liver of rats fed chronically with ethanol (Gouillon et al., 1999; French et al., 2001). Ubiquitin, ubiquitin conjugates, and proteosomal isopeptidase activity were inhibited, although the mechanism of inhibition was not understood. These findings in the liver of rats are consistent with our findings in the brains of subjects with a history of alcohol abuse/dependence. Coordinated and internally consistent alterations were revealed for genes involved in three major steps of ubiquitin-mediated proteolysis, i.e.; selecting the targets for ubiquitination (decreased expression of *ANCR*), conjugation of ubiquitin with target proteins (decreased expression of *HHARI* and *CDC34*), and selective degradation of ubiquinated proteins (de-

creased expression of *P27*). Because proteolysis is an energy-dependent process, decreases in ubiquitin-proteosome function may be exacerbated further by the compromised mitochondrial function in alcohol abuse/dependence. Disturbances in ubiquitin-mediated proteolysis, and decreased expression of a calcium-dependent protease *Calpain1* are consistent with a significant inhibition of cerebral protein breakdown reported in mice receiving ethanol (Toth and Lajtha, 1984).

One consequence of a deficit in the ubiquitin system may be a disturbance in removal of misfolded or unwanted proteins. Within the nervous system, accumulation of unwanted proteins as a result of defective ubiquitin-dependent proteolysis may contribute to aggregation events, which underlie the pathogenesis of several major human neurodegenerative and neurobehavioral diseases, including Alzheimer's disease (Lam et al., 2000; Layfield et al., 2001) and Angelman syndrome (Malzac et al., 1998; Bank et al., 2000; Lossie et al., 2001). Importantly, ubiquitination-dependent mechanisms regulate synaptic growth and function (DiAntonio et al., 2001), which may be related directly to altered brain functions in alcoholism. The ubiquitin system is now recognized as an important signaling mechanism whose signaling functions are remarkably diverse and involved in regulation of many different processes, including, but not limited to, gene regulation, intracellular trafficking, growth, and development (Pickart, 2001a).

Other signaling mechanisms also may be altered in alcohol abuse/dependence. Calpains (Ca^{2+}-dependent proteases) participate in signaling via Ca^{2+}-associated signal transduction pathways (Zhang et al., 1996). Reduced expression of Calpain 1 may therefore indicate a disturbance of this function in alcohol abuse/dependence. Phospholipases C and D1 were upregulated in the brains of subjects with a history of alcohol abuse/dependence. Ethanol has been shown to interact with membrane-associated signal transduction mechanisms, which rely on the reaction of phospholipases with their phospholipid substrates in the membrane. In several cell and membrane preparations, alcohol activates the polyphosphoinositide-specific phospholipase C and triggers the complete battery of intracellular signaling responses that are characteristic of hormones acting through this pathway. These include the formation of inositol-1,4,5-trisphosphate, the release of Ca^{2+} from intracellular storage sites, with the consequent activation of cytosolic Ca^{2+}-dependent enzymes, and the formation of diacylglycerol, leading to activation of protein kinase C. Further interaction of ethanol with the intracellular second messenger system is mediated through a hormone-sensitive phospholipase D (Hoek and Rubin, 1990; Hoek et al., 1992), which is also decreased in alcoholics. Membrane-associated signal transduction may additionally be impaired due to decreased expression of *ATPASEP*, a gene encoding phospholipid transporting ATPase type IV (Table II). Besides these genes, a number of other phosphatases, protein kinases, GTP-binding proteins, and ion channels displayed altered expression in alcohol abuse/dependence, indicating widespread abnormalities in signal transduction. Of interest was a coordinated decrease in expression in subjects with alcohol abuse/dependence of two catalytic subunits (calcineurin A1 and Aγ) of calmodulin-regulated protein phosphatase 3, involved in a wide range of biologic activities, acting as a Ca^{2+}-dependent modifier of phosphorylation status (Hashimoto et al., 1988).

There was a consistent increase in expression of six cell adhesion-related genes in alcohol abuse/dependence, including β-2 integrin αD (*ITGAD*), cadherins 12 and α9 (*CDH12* and *PCDHA9*), a non-classic-type cadherin *CELSR1*, a cell adhesion molecule binding to β-catenin (*ITGAD*), and an extracellular matrix protein 2 (*EMC2*). These findings are consistent with increased expression of the β1, α1, and α5 integrin subunits in perivenous hepatocytes from ethanol-fed rats (Tuma et al., 1999; Schaffert et al., 2001). Cell adhesion molecules play well-recognized roles in building and maintaining synaptic structures (Benson et al., 2000), which may suggest disturbances of synaptic neuroplasticity in alcohol abuse/dependence.

The increased expression of the microsomal cytochrome p450IIA4 gene was also of interest. This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. Ethanol is a known modulator of the hepatic cytochrome P450 (CYP)-dependent microsomal monooxygenase system (Niemela et al., 1999). Alcohol is oxidized to acetaldehyde by alcohol dehydrogenase (*ADH*) and cytochrome P-450, and then to acetate by aldehyde dehydrogenase (*ALDH*). The cytochrome p450 2D6 pharmacogenetic polymorphism has been implicated in substance abuse (Sellers et al., 1997).

The significance of changes in other candidate genes in alcohol abuse/dependence remains to be explored in future studies, including such interesting genes as *PAHX* (phytanoyl-CoA hydrolase) and *ATRX* (a DNA-dependent ATPase and helicase. *PAHX* is a gene causing Refsum disease, an autosomal recessive neurologic disorder (Mihalik et al., 1997; Wierzbicki et al., 2000). Mutations of the *ATRX* gene give rise to a severe, X-linked form of syndromal mental retardation (ATR-X syndrome) (Picketts et al., 1996). In addition to DNA helicase *ATRX*, three RNA helicases (*LKP*, *DBP1* and DEAD/H box polypeptide 16) were downregulated in alcohol abuse/dependence. RNA helicases of the DEAD box proteins are associated with all processes involving RNA molecules, including transcription, editing, splicing, ribosome biogenesis, RNA export, translation, RNA turnover, and organelle gene expression (Linder et al., 2001).

Clinical and epidemiological studies have consistently revealed an association between alcoholism and other psychiatric disorders, particularly mood disorders and schizophrenia; however, the evidence regarding the nature of these associations is unclear. There are several

lines of evidence for some shared genetic and environmental sources of comorbidity, as well as evidence for significant disease-specific risk factors (Kendler et al., 1993, 1995; Maier et al., 1994; Maier and Merikangas, 1996; Prescott et al., 2000; Nurnberger et al., 2001; Preisig et al., 2001). The identification of multiple candidate genes uniformly changed in subjects with past or present alcohol abuse/dependence and with or without other different psychiatric diagnoses supports the idea of the existence of risk factors that are specific for alcohol abuse/dependence.

Two recent microarray studies of frontal and motor cortices from alcoholics, defined on the basis of high level of alcohol intake (Lewohl et al., 2000; Mayfield et al., 2002), reported a number of changes, which are largely different from changes identified in our study of temporal cortex from subjects with psychiatric diagnosis of alcohol abuse or dependence. These differences may provide initial evidence for existence of molecular abnormalities specific for alcohol abuse/dependence. It should be noted, however, that besides differences in subject selection criteria, some technical differences may contribute to differences between findings in subjects with high alcohol intake (Lewohl et al., 2000; Mayfield et al., 2002) and individuals with alcohol abuse/dependence in the present study. These include the use of different brain regions, the analysis of pooled samples (Lewohl et al., 2000) versus the analysis of individual samples, as well as differences in the criteria for "changed" (replication in two pools [Lewohl et al., 2000] or in 6 of 11 hybridization experiments [Mayfield et al., 2002] vs. statistical significance in both χ^2 and in one sample *t*-test). Of note also, is that no psychiatric assessments were reported in the study of alcoholics defined based on high alcohol intake (Lewohl et al., 2000), providing an opportunity for possible concurrent psychiatric conditions among the subjects examined. This may be an important factor considering marked similarities in changes of myelination-related genes reported in these two studies of alcoholics (Lewohl et al., 2000; Mayfield et al., 2002) compared to those reported in a recent study of elderly patients with chronic schizophrenia (Hakak et al., 2001). Importantly, the study of elderly patients with chronic schizophrenia (Hakak et al., 2001) specifically excluded any cases with a history of alcohol abuse. Furthermore, our preliminary microarray analysis of patients with schizophrenia from the Stanley Consortium confirmed changes in myelination-related genes in schizophrenia regardless of the presence or absence of a history of alcohol abuse (Aston C, Jiang L, Sokolov BP, submitted). In addition, changes in myelination-related genes were found in patients with mood disorders (Aston C, Jiang L, Sokolov BP, in preparation). Therefore, it is plausible to suggest that disturbances in myelination may be common for both alcohol abuse and other psychiatric disorders. These common disturbances in myelination may underlie, in part, high rate of comorbidity of alcohol abuse and other psychiatric disorders. Such common abnormalities may apparently be excluded by identification changes, which are preserved in alcoholic/nonalcoholic pairs with different psychiatric diagnoses. Examining molecular alterations across subjects with alcohol abuse, with or without other psychiatric diagnoses compared to nonalcoholic subjects matched for psychiatric diagnosis may therefore be a useful alternative to identify candidate mechanisms relatively specific for alcohol abuse.

Some of the changes revealed in the current study may represent the consequences of alcohol abuse rather than preexisting risk factors. The convergence of mitochondrial, ubiquitin-proteosome, phospholipase C, cell adhesion, and cytochrome P450 (CYP) systems in the brains of subjects with the diagnosis of alcohol dependence/abuse and other findings in alcohol-treated animals support such a possibility. It is possible, however, that some changes found in the brains of alcoholics may represent both the predisposing abnormalities and the consequences of alcohol use, in a scenario when alcohol use aggravates preexisting abnormalities, leading to evolution of "being at risk," to clinical alcohol abuse/dependence. Further gene expression and proteomic studies in human and experimental animals fed with ethanol, together with genetic studies, will clarify this issue. Importantly, 6 of 11 cases with a history of alcohol abuse/dependence studied here were abstinent for a long period (up to 30 years) before death. Still, these former alcohol abusers shared multiple abnormalities with subjects who had been alcohol abusers at time of death. Separate analysis of the subgroups of "current" and "past" alcohol abusers revealed similar fold changes in both subgroups (data not shown). This is consistent with the idea that long-lasting and possibly irreversible damage to some basic brain functions may occur as a result of alcohol abuse and that there might be inherited risk factors for alcohol abuse. Note that selection criteria for "changed" genes in this study were designed specifically to select genes that were altered in both past and present alcohol abusers. The functions altered in subjects with alcohol abuse may include, but are not limited to, abnormal energy metabolism, proteolysis, and multiple widespread abnormalities in signaling mechanisms. If confirmed, some of these abnormalities may represent valuable targets for treatment of this disorder. The current study provides novel and potentially important evidence on the molecular mechanisms associated with alcohol abuse; however, the results should be treated with caution. Among the limitations are the small number and heterogeneity of the cases studied and practical difficulties in selecting comparison pairs that are completely matched for all demographics. In addition, because several cases were polydrug abusers, it remains to be elucidated whether changes revealed here are related to alcohol abuse per se or to overall substance abuse traits. Co-occurrence of different forms of substance abuse is common, suggesting that they may share some underlying mechanisms (Enoch and Goldman, 1999). Furthermore, it remains to be elucidated whether changes revealed in the current study are present in other cases of alcohol abuse or dependence. Future studies using larger numbers of well-characterized cases

without psychiatric comorbidities, as well as cases with other concurrent psychiatric diagnoses, are necessary to validate these findings.

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