

Gene expression profiling of individual cases reveals consistent transcriptional changes in alcoholic human brain

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Abstract

Chronic alcohol exposure induces lasting behavioral changes, tolerance, and dependence. This results, at least partially, from neural adaptations at a cellular level. Previous genome-wide gene expression studies using pooled human brain samples showed that alcohol abuse causes widespread changes in the pattern of gene expression in the frontal and motor cortices of human brain. Because these studies used pooled samples, they could not determine variability between different individuals. In the present study, we profiled gene expression levels of 14 postmortem human brains (seven controls and seven alcoholic cases) using cDNA microarrays (46 448 clones per array). Both frontal cortex and motor

cortex brain regions were studied. The list of genes differentially expressed confirms and extends previous studies of alcohol responsive genes. Genes identified as differentially expressed in two brain regions fell generally into similar functional groups, including metabolism, immune response, cell survival, cell communication, signal transduction and energy production. Importantly, hierarchical clustering of differentially expressed genes accurately distinguished between control and alcoholic cases, particularly in the frontal cortex.

Keywords: alcoholism, frontal cortex, gene expression, individual, motor cortex.

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Chronic alcohol intake leads to long-lasting behavioral adaptations, tolerance, and dependence on the drug. These properties of alcohol likely result from changes in gene expression which may underlie neural adaptations observed at a cellular level (Nestler 2000). A number of alcohol responsive genes have been identified using methods such as differential display, northern blotting, and competitive polymerase chain reaction (Lewohl *et al.* 1997). More recently, large scale gene expression profiling has also been used to identify candidate alcohol responsive genes. For example, oligo-DNA arrays were used to identify alcohol-induced changes in gene expression in human SH-SY5Y neuroblastoma cells (Thibault *et al.* 2000). Alcohol responsive genes have been identified in rat strains selectively bred for alcohol preference (P/NP rats and AA/ANA) as well as inbred mouse strains which differ in a number of alcohol-related phenotypes (C57BL/6 and DBA/2) (Daniels and Buck 2002; Hoffman *et al.* 2003; Tabakoff *et al.* 2003). In addition, families of functionally related genes have been identified using microarrays to profile human postmortem brain tissue from alcoholics (Lewohl *et al.* 2000; Mayfield

et al. 2002) and psychiatric patients with a history of alcohol abuse (Sokolov *et al.* 2003).

Studies using postmortem human brain have shown that chronic alcohol consumption can result in significant brain damage in the frontal cortex (Kril *et al.* 1997). This brain region has major connections with the mesolimbic reward pathway, suggesting its potential role in the development of alcoholism (Matsumoto *et al.* 2001). Neuropathological studies suggest that the effects of chronic alcohol abuse may be greater in frontal cortex than other cortical regions; however, in motor cortex, basal dendritic arborization of layer III pyramidal neurons were affected significantly in both frontal and motor cortices of alcoholics (Harper and

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Abbreviations used: DAVID, database for annotation, visualization and integrated discovery; GO, gene ontology; LAD, longhorn array database; UHRR, universal human reference RNA.

Corbett 1990). In addition, chronic alcohol consumption can cause motor dysfunction even after long-term abstinence from alcohol (Welch *et al.* 1997; Sullivan *et al.* 2000; Sullivan *et al.* 2002) indicating that motor cortex may also be an important target of chronic alcohol exposure.

Our previous studies investigated the effects of long-term alcohol abuse on gene expression using pooled RNA samples from postmortem brains of alcoholics compared to matched controls (Lewohl *et al.* 2000; Mayfield *et al.* 2002). The most striking finding of both studies was that myelin-related genes were down-regulated significantly in alcoholics. In addition, families of genes involved in protein trafficking as well as calcium, cAMP, and thyroid signaling pathways were altered in both frontal and motor cortices of alcoholics compared to controls. In these studies RNA samples were pooled to reduce individual variability; however, such procedures mask individual variation in gene expression. In the present study, mRNA expression profiles were examined in the frontal and motor cortices from 14 individual cases (seven alcoholics and seven controls) using cDNA microarrays spotted with 46 448 human clones, representing 31 271 unique UniGene cluster IDs.

Methods

Case selection and total RNA preparation

Human brain tissues were obtained from the Brisbane Node of the National Health and Medical Research Council (NHMRC) Brain Bank and the Tissue Resource Centre at the University of Sydney, Australia. A total of 14 cases including seven alcoholics and seven controls were studied (Table 1). Alcoholics were defined by NHMRC/World Health Organization criteria (80 g per day), instead of criteria established by the American Psychiatric Association (DSM-IV). The alcoholics in this study often consumed more than 200 g of ethanol per day for over 20 years, exceeding the criteria established by the NHMRC. Cases were age matched as closely as possible and were not significantly different between two groups (Student's *t*-test, $p > 0.1$). Total RNA was extracted from both the frontal and motor cortices of each case using a modified guanidine isothiocyanate extraction method (Chomczynski and Sacchi 1987). The quality of the RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA). All RNA samples had two distinct ribosomal bands with minimal degradation, indicating RNA of high quality. This is

consistent with a previous study that RNA in postmortem human brain is stable for up to four days after death (Schramm *et al.* 1999). Critical factors and potential caveats for the use of autopsy material have been reviewed elsewhere (Hynd *et al.* 2003).

Array fabrication

The cDNA microarray slides were prepared at the microarray core facility center at the University of Texas at Austin (Austin, TX, USA). A set of 46 448 sequence-verified IMAGE cDNA clones (ResGen, Carlsbad, CA, USA), representing 31 271 non-redundant UniGene clusters, were PCR-amplified and spotted onto poly L-lysine coated microscope glass slides. The arrays were printed and postprocessed using succinic anhydride as described except that the UV cross-link step was skipped (DeRisi *et al.* 1997).

RNA amplification

Universal Human Reference RNA (UHRR; comprised of 10 different human cell lines) was purchased from Stratagene (La Jolla, CA, USA). Both reference and brain RNA were amplified as described (Mayfield *et al.* 2003). Briefly, total RNA (1.0 µg) was denatured together with T7-oligo dT primer (0.2 µg) at 70°C for 5 min and then chilled on ice. Reverse transcription (20 µL reaction) was catalyzed by SuperScript II (Invitrogen, Carlsbad, CA, USA) at 42°C for 2 h. Reagent mix (80 µL) for the 2nd cDNA strand synthesis was added and incubated at 16°C for 2 h. Double-stranded cDNA was purified and dissolved in 8 µL of DEPC-H₂O. *In vitro* transcription was performed in a 20-µL reaction at 37°C for 6 h using T7 MegaScript kit (Ambion, Austin, TX, USA). Amplified antisense RNA was purified using the RNeasy Mini kit (Qiagen, Valencia, CA, USA). The quality and concentration of amplified antisense RNA was determined using the Agilent 2100 Bioanalyzer. Approximately 29 µg of amplified RNA was generated from 1.0 µg of original brain total RNA.

CDNA labeling, hybridization and array scanning

Amino allyl-dUTP chemistry was used to indirectly label cDNA as described (Mayfield *et al.* 2003). Amplified RNA (2 µg) and random hexamer primer (5 µg, Amersham, Piscataway, NJ, USA) were denatured at 70°C for 10 min and then chilled on ice. The reverse transcription reaction containing amino allyl-modified dUTP was incubated at 42°C for 2 h. Then the RNA strand was degraded and cDNA was purified and concentrated to 9 µL using YM-30 Microcon filtering tubes (Millipore, Bedford, MA, USA). Cyanine-3 or -5 (Amersham) was resuspended with 9 µL of purified cDNA together with 1 µL fresh NaHCO₃ buffer (1.0 M, pH 9.0). Brain samples (test RNA samples) were always labeled

Table 1 Case information. The average age of control and alcoholic cases used in this study are 64 years (average PMD 30 h) and 55 years (average PMD 24 h), respectively. Control and alcohol groups are not significantly different in age or PMD (Student's *t*-test, both $p > 0.1$)

Case group	Controls							Alcoholics						
	C1	C2	C3	C4	C5	C6	C7	A1	A2	A3	A4	A5	A6	A7
Age (years)	85	55	54	56	58	62	78	34	29	70	36	58	79	70
PMD (h)	38	38	56	10	27	18	22	31	24	15	29	29	20	17
Sex	M	M	M	M	M	M	F	M	M	M	M	M	M	F

'A' indicates alcoholic cases and 'C' indicates control cases. PMD: postmortem delay.

with cyanine 3 (Cy3, channel 1), and amplified UHRR with cyanine 5 (Cy5, channel 2). The dye coupling reaction was carried out in the dark for one hour at room temperature. The labeled cDNA was then purified using the QIAquick PCR purification kit (Qiagen). Equal amounts of Cy5- and Cy3-labeled cDNA were combined and concentrated in YM-30 Microcon tubes. Blocking reagents (10 μ g human Cot-1 DNA, 5 μ g polyA, and 5 μ g yeast tRNA) were used to decrease non-specific hybridization. Hybridization buffer (20 \times SSC and 10% SDS) was added to the labeled cDNA solution at a final concentration of 3.5 \times SSC, and 0.26% SDS. The hybridization solution was adjusted to 70 μ L with H₂O, denatured at 100°C for 2 min, and cooled to room temperature. The hybridization solution was then loaded between a postprocessed microarray slide and a Lifterslip™ (Erie Scientific, Portsmouth, NH, USA). Hybridization was carried out in a humidified chamber in the dark at 65°C for 16 h. Slides were washed with solution containing 0.6 \times SSC and 0.03% SDS followed by a second wash with 0.06 \times SSC only at room temperature. Slides were dried under centrifugation at room temperature, and then scanned with an Axon GenePix 4000 scanner (Axon, Union City, CA, USA). Images were subsequently analyzed using Axon GenePix 4.0 software.

Feature detection and array normalization

GenePix 4.0 default feature detection parameters were used for spot detection. In addition, the pixel homogeneity within a spot was determined by calculating the Pearson correlation coefficient of pixel intensities from both channels in each spot on the array. The resulting regression correlation was set at 0.6 and above. Channel 2 (Cy5) was normalized for each array in the Longhorn Array Database (LAD) at the University of Texas at Austin (Killion *et al.* 2003). The normalization was based on the assumption that the log-transformed ratio of net channel 2 intensity to net channel 1 intensity for each spot was centered on zero. The normalized ratio of medians was the raw ratio of medians divided by the calculated normalization factor.

Brain RNA hybridization design

Each amplified brain sample (test sample, channel 1) was hybridized against UHRR (reference, channel 2), resulting in a total of 28 arrays. The normalized log₂ intensity ratios (net median Ch2 intensity to Ch1 intensity) were compared between the control and alcoholic groups within each brain region.

Bayesian statistics

Confidence in the interpretation of microarray data with a relatively small number of replicates can be improved by using a Bayesian statistical approach. The Bayesian strategy relies on prior information of treatment measurement. This prior information assumes that genes of similar expression level have similar measurement error. The Bayesian posterior probability is defined as the probability of an event occurring after empirical data has been considered. In the current study, the selection criteria for differential expression was a Bayesian posterior probability of greater than 95% or 99% for mean expression (relative to reference) being different by at least 1 standard deviation. Priors were normal, based on previous estimates of random pairing of control tissue. Bayesian statistical procedures were performed according to (Baldi and Long 2001; Long *et al.* 2001) using IGOR Pro (WaveMetrics, Inc.).

Differentially expressed genes

Differentially expressed genes consisted of two types, class I and class II. Class I genes were qualitatively different, that is, they were predominantly detected in one group but not the other, while class II genes were consistently detected in both groups. Thus, class I genes represent those that were more likely ‘turned off’ or ‘turned on’ as a result of alcohol abuse, whereas class II genes represent consistently expressed genes for which quantitative differences in expression could be determined (see ‘Bayesian statistics’ above for details). A standardized criterion was developed for selection of differentially expressed genes: class I down-regulated genes included those that were detected on at least four more arrays in the control group than in the alcohol group within the same brain region. In addition, only genes with net channel 1 intensities of greater than 300 fluorescent units were included in this class. Class II down-regulated genes were those that were detected on at least four arrays in both the control and alcohol groups, and had Bayesian posterior probabilities of greater than 95% for differential expression. The up-regulated genes were defined in the same manner with opposite direction. A more stringent criterion was developed to select genes for hierarchical clustering analysis. Class I genes having a difference of at least five in detection frequency between the control and alcoholic groups were selected. In addition, the threshold of Bayesian posterior probability for class II genes was raised to greater than 99%.

Functional grouping

Differentially expressed genes were uploaded into the Database for Annotation, Visualization and Integrated Discovery (DAVID) to obtain functional annotations (Dennis *et al.* 2003). The Gene Ontology (GO) database (Ashburner *et al.* 2000) was used for classification of genes into functional groups (GO, Biological Process).

Results

A total of 28 array hybridizations were performed on frontal and motor cortex samples from each of seven control and seven alcoholic cases. The average number of features detected in each experimental group ranged from 30 061 to 33 820 per array. In the frontal cortex, fewer features (~3700) were detected in the alcoholic group compared to controls. This difference was not observed in the motor cortex (Fig. 1).

Class I and II differentially expressed genes in each brain region are summarized in Table 2. The number of class I down-regulated genes was significantly greater in the frontal cortex compared to motor cortex. This brain regional difference was consistent with the data shown in Fig. 1, in which fewer class I features were detected in the frontal cortex of the alcoholic group. Significantly fewer class I up-regulated genes were detected than down-regulated ones in the frontal cortex. A greater number of class I up-regulated genes were identified in the motor cortex than in the frontal cortex. The total number of class II genes up-regulated genes was similar between brain regions, while fewer class II genes

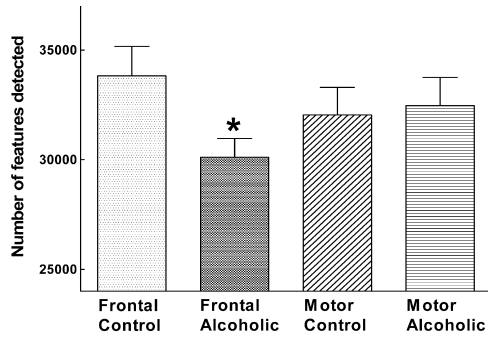


Fig. 1 Average numbers of features detected in the frontal and motor cortices for control and alcoholic groups. Mean ± SEM is shown for seven arrays per group. *Different from control ($p < 0.05$, single factor ANOVA followed by t -test).

Table 2 Summary of differentially expressed genes. See Methods for selection criteria

	Class I	Class II	Subtotal
<i>Standard selection criteria</i>			
Frontal cortex			
Increased	32	1281	1,313
Decreased	893	598	1,491
Motor cortex			
Increased	208	1278	1,486
Decreased	317	1071	1,388
<i>Stringent selection criteria</i>			
Frontal cortex			
Increased	1	534	535
Decreased	163	259	422
Motor cortex			
Increased	29	577	606
Decreased	53	448	501

were detected in the frontal cortex. Figure 2 shows a volcano plot of the Bayesian posterior probability of differential expression as a function of the percent change in gene expression. The plot illustrates that not all of genes with large average expression differences were associated with high probability of differential expression. The high average expression changes may only result from a single outlier. On the other hand, when changes are consistent across all cases, even small changes may show high probability of differential expression. A similar pattern was observed in the motor cortex (data not shown).

The degree of overlap in differentially expressed genes in the frontal and motor cortex is shown in Fig. 3. Of the features that were common to each brain region, a group of seven genes belonging to the ubiquitin-proteasome system was identified. Ubiquitin-conjugating enzyme E2L 3 and ubiquitin-conjugating enzyme E2-like genes were both up-regulated. Down-regulated genes included F-box and

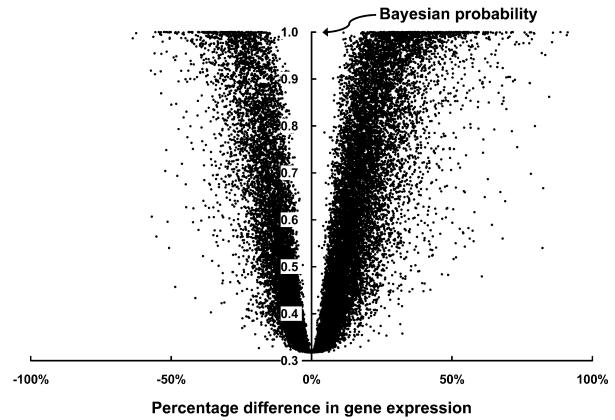


Fig. 2 Volcano plot of genes detected in the frontal cortex. The Bayesian probability of differential expression (y-axis) is plotted as a function of difference in gene expression (% change) between the control and alcoholic group. x-axis indicates the percentage of over- (positive values) or under- (negative values) expression of a gene in alcoholic group relative to the control group. A total of 30 479 genes are plotted.

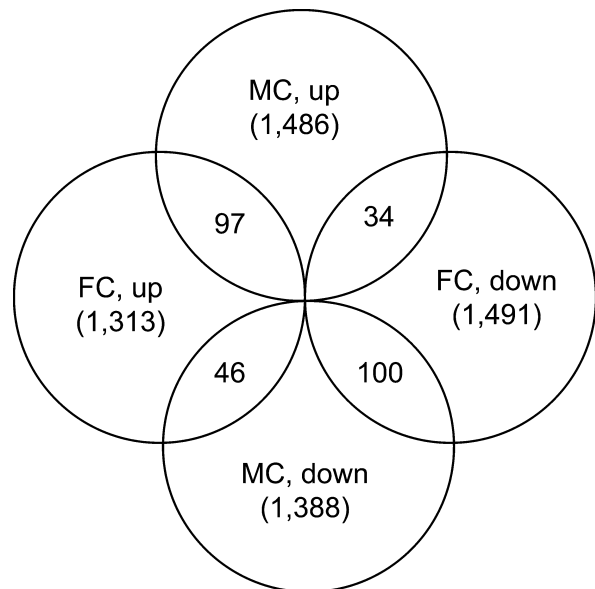


Fig. 3 Venn diagram of the differentially expressed genes in the frontal and motor cortex. Both class I and class II differentially expressed genes are included. The overlapping areas represent the numbers of genes that were differentially expressed in both brain regions (regardless of direction of changes). FC, frontal cortex; MC, motor cortex.

leucine-rich repeat protein 2, F-box and leucine-rich repeat protein 3 A, ring finger protein 14, ubiquitin specific protease 8, and ubiquilin 2.

The biological significance of transcriptional profiling can be revealed by functional grouping of differentially expressed genes. Differentially expressed genes were

uploaded into DAVID for functional analysis (gene selection based on standard criteria). Differentially expressed genes are classified into functional groups based on biological processes in which they are involved (Table 3). Most functional groups contain both up- and down-regulated genes. The functional groups identified primarily fall into six major categories including metabolism, immune response, cell survival, cell communication, signal transduction and energy production. A number of genes are also suggested to be involved in the development of neurodegenerative diseases, including Alzheimer's disease, schizophrenia, Parkinson's disease, and Huntington's disease. A list of myelin-related genes which were identified in previous studies (Lewohl *et al.* 2000; Mayfield *et al.* 2002) also showed altered expression levels (Table 4). A total of 10 and 11 genes in

frontal and motor cortex, respectively, were differentially expressed in the current study.

Hierarchical clustering was performed on differentially expressed genes to investigate individual variation in expression and the extent to which gene expression differentiates control and alcoholic cases. We hypothesized that differentially expressed genes do not necessarily distinguish all alcoholic cases from controls due to individual variability and relatively small differences in expression. Clustering of 793 class II differentially expressed genes (stringent criteria) in the frontal cortex completely distinguished all alcoholic cases from controls (Fig. 4a). In motor cortex, the 1025 class II genes (stringent criteria) also distinguished between the groups with the exception of one alcoholic that clustered with the control cases (Fig. 4b). In addition, cases are more

	Frontal cortex		Motor cortex	
	Increased	Decreased	Increased	Decreased
Metabolism				
Alcohol metabolism		6	8	
Amine metabolism		9	8	9
Amino acid and derivative metabolism			7	8
Biosynthesis	20	26	35	32
Carbohydrate metabolism	6	17	9	9
Catabolism	24	43	38	33
Lipid metabolism	15	8	16	12
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	54	80	100	96
Organic acid metabolism	5	10	8	10
Phosphorus metabolism	19	29	26	32
Protein metabolism	50	87	87	100
Immune response				
Response to abiotic stimulus	7	5		5
Response to biotic stimulus	23	21	29	30
Response to pest/pathogen/parasite	13	14	13	8
Response to wounding	8	9	8	7
Cell survival				
Cell death	7	14	13	7
Cell proliferation	24	42	38	34
Programmed cell death	7	12	13	6
Cell communication				
Cell adhesion	18	21	22	13
Cell-cell signaling	9	18	10	12
Signal transduction	43	72	62	82
Energy production				
Electron transport	6	7	5	6
Energy pathway		5		5
Other				
Organogenesis	20	23	14	28
Cell organization and biogenesis	20	22	25	24
Transport	28	61	36	56
Neurodegenerative disease related genes	6	8	9	7
Unclassified	1099	1164	1160	1051

Table 3 Functional grouping of differentially expressed genes (class I and class II, standard selection criteria). Only the functional groups containing at least five genes per group are shown

Table 4 Myelin-related genes differentially expressed in each brain region. Class I and class II genes are included. Each gene is preceded by the corresponding UniGene cluster ID. Asterisk indicates genes that also passed the stringent criteria

<i>Frontal cortex (10)</i>	
Down-regulated (7)	
*Hs.103724	Peripheral myelin protein 22
Hs.172619	Myelin transcription factor 1-like
*Hs.174185	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)
Hs.194772	Oligodendrocyte myelin glycoprotein
Hs.396489	Transferrin
Hs.406397	Glial fibrillary acidic protein
Hs.42945	Acid sphingomyelinase-like phosphodiesterase
Up-regulated (3)	
Hs.69547	Myelin basic protein
Hs.78687	Neutral sphingomyelinase (N-SMase) activation associated factor
*Hs.55235	Sphingomyelin phosphodiesterase 2, neutral membrane (neutral sphingomyelinase)
<i>Motor cortex (11)</i>	
Down-regulated (8)	
Hs.42945	Acid sphingomyelinase-like phosphodiesterase
Hs.74571	ADP-ribosylation factor 1
Hs.75290	ADP-ribosylation factor 4
Hs.183153	ADP-ribosylation factor 4-like
*Hs.287820	Fibronectin 1
Hs.55235	Sphingomyelin phosphodiesterase 2, neutral membrane (neutral sphingomyelinase)
Hs.85112	Insulin-like growth factor 1 (somatomedin C)
Hs.405998	Human insulin-like growth factor 1 receptor mRNA, 3c sequence
Up-regulated (3)	
Hs.399736	ADP-ribosylation factor-like 2
Hs.75736	Apolipoprotein D
*Hs.29117	Purine-rich element binding protein A

similar to each other in the frontal cortex than motor cortex, as indicated by shorter branches in the dendrogram. Cluster analysis also distinguished the groups when performed on a 30 signal transduction genes that were down-regulated in the frontal cortex (Fig. 5a). In the motor cortex, clustering failed to separate cases using 27 genes from the same functional group (Fig. 5b). We did not observe a strong relationship between hierarchical clusters and functional groups as in Table 3 when clustering was performed in two-dimensions. Nevertheless, visual inspection suggested that genes in the same functional group tended to be close in the clustering structure.

Discussion

Gene expression profiles were compared in postmortem human brains of seven control and seven alcoholic cases using cDNA microarrays. The arrays used in this study consisted of 46 448 clones (31 271 unique UniGene clusters), thus providing a more comprehensive coverage of genes than in previous studies (Lewohl *et al.* 2000; Mayfield *et al.* 2002; Sokolov *et al.* 2003). This is of particular importance for alcohol research due to the

widespread effects of alcohol on gene expression in the nervous system.

Valid statistical methods are required to identify regulated genes, particularly in brain where gene expression changes are often small and may be diluted by cellular heterogeneity (Wurmbach *et al.* 2002; Hoffman *et al.* 2003). In this study, Bayesian statistics (Long *et al.* 2001) and hierarchical clustering were used to identify differentially expressed genes and their relationships among individual cases.

Myelination in brain has been an area of interest in human alcohol studies due to the observed brain damage observed in the frontal cortex of alcoholics and the potential role of demyelination in this process (Krill and Halliday 1999; Lewohl *et al.* 2001). Myelin-related genes were altered in the current study, most of which were down-regulated. The number of myelin genes that passed the stringent selection criteria was significantly less than that observed using the standard criteria. This may reflect individual differences in the rate of demyelination. One of the alcoholic cases in the study was abstinent from alcohol for two years prior to death, which might reverse the loss of white matter (Harper and Krill 1990). In the frontal cortex, oligodendrocyte myelin glycoprotein, peripheral myelin protein 22, and glial fibrillary

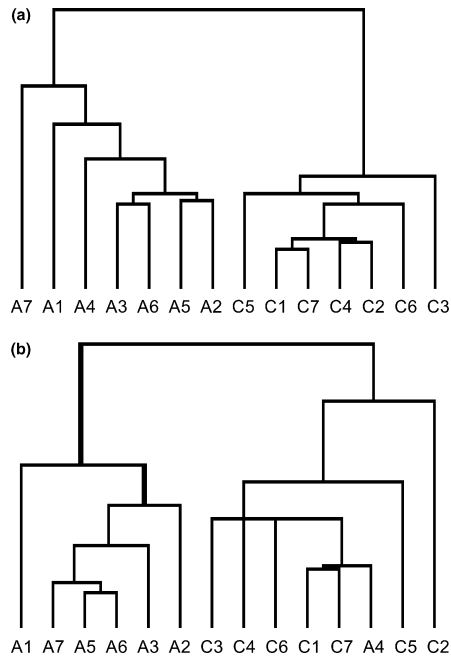


Fig. 4 Hierarchical clustering of 14 cases by all class II differentially expressed genes. Clustering was performed on genes selected from the frontal cortex (a, 793 genes) and motor cortex (b, 1025 genes). A indicates alcoholic cases and C indicates control cases; the number corresponds to the list of cases listed in Table 1.

acidic protein were changed in previous human studies (Lewohl *et al.* 2000; Mayfield *et al.* 2002). Myelin basic protein was up-regulated in the frontal cortex, which is consistent with a previous study showing increased transcript level of myelin basic protein induced by demyelination (Kristensson *et al.* 1986). Sphingomyelin is an abundant constituent of the plasma membranes of myelin sheath in brain. Plasma membrane-bound neutral sphingomyelinase (nSMase) is one of the two sphingomyelinases that determine the major route of sphingomyelin degradation. It serves as a key enzyme in the regulated activation of the sphingomyelin cycle and cell signaling (Tomiuk *et al.* 1998). Increased expression of this enzyme in the frontal cortex while decreased in the motor cortex indicates the increased degradation of this glycolipid in the frontal cortex. This also suggests the potential role of sphingomyelin cycle in the observed brain damage in the frontal cortex.

Widespread changes in gene expression were detected in both the frontal and motor cortex. Despite the brain regional differences in expression, differentially expressed genes in both brain regions were involved in similar cellular functions. A recent microarray study suggested that temporal cortex also showed alcohol-induced gene expression changes, including mitochondrial genes as well as genes involved in signal transduction, cell adhesion, and other functions (Sokolov *et al.* 2003). This suggests that alcohol abuse can cause an overall regulation of cellular machinery in different

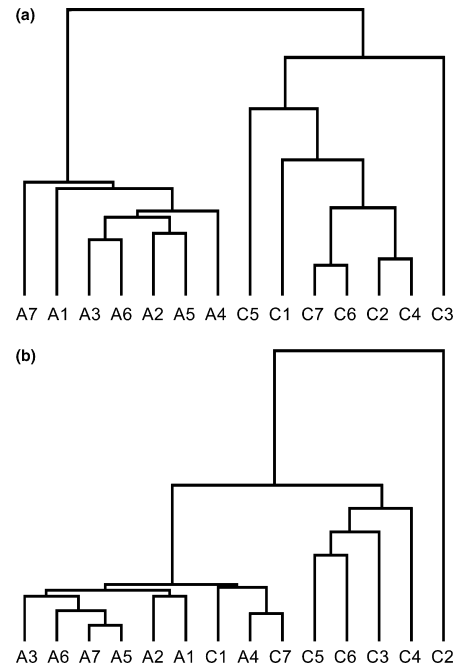


Fig. 5 Hierarchical clustering of 14 cases by class II differentially expressed genes in the signal transduction functional group. (a) Clustering of cases by 30 genes in the frontal cortex; (b) clustering of cases by 27 genes in the motor cortex.

human brain regions, such as metabolism, signal transduction, and cell communication.

A remarkable down-regulation of genes in the ubiquitin-proteasome system was observed in both brain regions. Components of this system have been shown to be essential for the regulation of synaptic transmission and plasticity (Speese *et al.* 2003; Zhao *et al.* 2003). Proteasome function is impaired in several neurological diseases (Cline 2003). This system has also been shown to be altered in postmortem temporal cortex of alcohol abusers (Sokolov *et al.* 2003). Altered expression level of components in this system in both brain regions studied suggests a universal impairment of ubiquitin-mediated protein degradation in human brain; and in turn, dysfunction of this system may lead to impaired synaptic plasticity.

A comparison between our results and previous studies of schizophrenia in the frontal cortex revealed a large overlap between two brain diseases. Schizophrenia, like alcoholism, is a complex mental disorder and multiple genes have been proposed to predispose individuals to susceptibility to schizophrenia (McGuffin *et al.* 1995). Patients with schizophrenia have significantly impaired neuronal function and a loss of oligodendrocytes in prefrontal cortex (Hof *et al.* 2002). Myelination-related genes decreased in alcoholics, for example, transferrin, were also down-regulated in patients with schizophrenia, suggesting a common impairment of oligodendrocytes in this brain region (Hakak *et al.* 2001).

Transcriptional levels of glutamate decarboxylase 1 (67 kDa, GAD67) has been consistently shown to be decreased in schizophrenics in prefrontal cortex, indicating reduced GABAergic function in both diseases (Mirmics *et al.* 2000; Vawter *et al.* 2002; Hashimoto *et al.* 2003). Increased expression level of genes in both ubiquitin-proteasome system and cytochrome P450 family in the present alcoholism study, an opposite direction of change in schizophrenia, may represent the unique increased oxidative stress from alcohol consumption (Vawter *et al.* 2001; Middleton *et al.* 2002; Vawter *et al.* 2002).

Gene expression profiling has been successfully applied to cancer studies for the purpose of classification or discovering new subclasses of tumors (Dyrskjot *et al.* 2003; Rozovskaia *et al.* 2003; Lapointe *et al.* 2004). Compared to these cancer studies, our gene expression changes are relatively small. This raised the question of whether they are sufficient to separate alcoholics and controls based on gene expression profiles. Our hierarchical clustering results clearly demonstrate the feasibility of distinguishing alcoholics from controls by the expression level of a set of selected genes, especially in the frontal cortex. Separation of alcohol and control cases by even a subset of genes in the frontal but not motor cortex further supports our finding that the frontal cortex shows more consistent changes in gene expression across case individuals. This is consistent with histological studies which indicate that this brain region is more susceptible to alcohol abuse (Kril *et al.* 1997). Alcohol and control cases can still be accurately separated by clustering of genes selected by the standard criterion (over 2000 genes) in the frontal cortex but not in motor cortex (data not shown). This suggests that the frontal cortex is of more interest for further investigation compared to motor cortex, at least, at the transcriptional level.

In summary, the present study compared gene expression changes in two regions of postmortem alcoholic human brains with nonalcohol cases. A comparison between two brain regions reveals that the frontal cortex showed more consistent expression changes than the motor cortex. Despite this brain regional difference, similar functional groups of genes were altered at transcriptional level in both regions. Changes in gene expression reliably distinguished between the alcohol and control cases and suggest that molecular detection classification and diagnosis of alcoholism may be feasible.

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