Shared Gene Expression Alterations in Schizophrenia and Bipolar Disorder

Ling Shao and Marquis P. Vawter

Background: Schizophrenia and bipolar disorder together affect approximately 2.5% of the world population, and their etiologies are thought to involve multiple genetic variants and environmental influences. The analysis of gene expression patterns in brain may provide a characteristic signature for each disorder.

Methods: RNA samples from the dorsolateral prefrontal cortex (Brodmann area 46) consisting of individuals with schizophrenia (SZ), bipolar disorder (BPD), and control subjects were tested on the Codelink Human 20K Bioarray platform. Selected transcripts were validated by quantitative real-time polymerase chain reaction (PCR). The strong effects of age, gender, and pH in the analysis of differential gene expression were controlled by analysis of covariance (ANCOVA). Criteria for differential gene expression were 1) a gene was significantly dysregulated in both BPD and SZ compared with control subjects and 2) significant in ANCOVA analysis with samples that have a pH above the median of the sample.

Results: A list of 78 candidate genes passed these two criteria in BPD and SZ and was overrepresented for functional categories of nervous system development, immune system development and response, and cell death. Five dysregulated genes were confirmed with quantitative Q-PCR in both BPD and SZ. Three genes were highly enriched in brain expression (AGXT2L1, SLC1A2, and TU3A). The distribution of AGXT2L1 expression in control subjects versus BPD and SZ was highly significant (Fisher's Exact Test, $p < 10^{-06}$).

Conclusions: These results suggest a partially shared molecular profile for both disorders and offer a window into discovery of common pathophysiology that might lead to core treatments.

Key Words: AGXT2L1, antipsychotic medication, apoptosis, bipolar disorder, BUB1B, dorsolateral prefrontal cortex, EMX2, ERBB2, FGF2, FTH1, IL2RA, LGALS3, MAFG, microarray, neurogenesis, NFATC1, PVR, quantitative PCR, RERG, schizophrenia, SLC1A2, SMCY, SMO, SOX9, TU3A, TXNIP

Schizophrenia (SZ) and bipolar disorder (BPD) have traditionally been diagnosed by clinical examination of psychotic symptoms and affective dysregulation. The clinical impressions along these two dimensions coupled with historical separation into current diagnostic classifications have led to these illnesses being viewed and treated in research as independent classes (1,2). Categorization into separate classes has led to efforts for identification of separate pathophysiologies for each disorder (3). However, it has not escaped attention that the classifications share some pathophysiology, vulnerability and risk factors, genetic loci, clinical manifestations, and approximate ages of onset. Classifications have arisen that are based upon meeting clinical characteristics, and multiple criteria can be combined into discrete subgroups, which might be different in terms of pathophysiology. Recent discussions have centered on the idea that schizophrenia and bipolar are not separate illnesses (1–5), but share many criteria and differ along a dimension related to psychosis and affective symptom clusters.

Medication response can be effective in one or both disorders or equally ineffective in both. Furthermore, patients clinically present with both affective and psychotic symptoms. This study was motivated by the idea of identifying common gene-expression profiles akin to recent reports of shared candidate genes that overlap in linkage and association genetic studies of BPD and SZ, although clearly not in all studies (1).

Most reports of SZ or BPD microarray studies have not compared both disorders with a common reference control group with a few exceptions (6,7). We now present a common molecular profile of both SZ and BPD as one potential indicator of a partially shared molecular phenotype.

From the Department of Psychiatry and Human Behavior, Functional Genomics Laboratory, School of Medicine, University of California, Irvine, California.

Reprint requests to Marquis P. Vawter, Ph.D., Gillespie Neuroscience Research Facility, Functional Genomics Laboratory, Room 2119, University of California, Irvine, Irvine CA 92697-4260; E-mail: mvawter@uci.edu.

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Methods and Materials

Total RNA

The Microarray Collection Set A from the Stanley Medical Research Institute (SMRI; Bethesda, Maryland) consisted of RNA samples ($n = 105$) from the dorsolateral prefrontal cortex (DLPFC, Brodmann area 46). The SMRI sent RNA extracts to our laboratory for blind analysis consisting of 35 schizophrenia (SZ), 35 bipolar disorder (BPD), and 35 control subjects (Table 1 in Supplement 1). In the final analysis, 88 subjects were analysed, including 32 SZ, 29 BPD, and 27 control subjects; 17 samples were not included in the analysis for reasons described subsequently in Results.

Codelink 20K Oligonucleotide Microarrays

The Codelink platform was chosen as an independent microarray platform because versions of the Affymetrix microarray were run by nine other independent laboratories that received RNA or brain tissue samples from SMRI for analysis. The details and advantages to using the Codelink array platform are detailed in Supplement 1.

Microarray Data Analysis

Potential outlier microarray chips were assessed by agreement among the following procedures. The outlier chips were determined by principal component analysis plots (Partek Genomics Suite, v. 6.2, St. Louis, Missouri) of all subjects'

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Table 1. Demographic Variables for Samples in SMRI Microarray Collection A (Brodmann's Area 46)

Mean (SD) Analyzed Subjects	Age	Gender (M/F)	PMI	Brain pH	RI	cRNA	rRNA
Schizophrenia	42.9 (8.6)	23/9	30.5 (15.1)	6.48 (0.25)	5.5 (3.6)	778 (124)	2.19 (.50)
Bipolar Disorder	45.3 (9.8)	15/12	39.1 (17.9)	6.5 (0.23)	9.3 (7.8)	794 (138)	2.38 (.77)
Control	44.4 (6.5)	23/6	28.9 (12.7)	6.64 (0.26)	3.8 (2.8)	805 (181)	2.26 (.44)
Schizophrenia–Control *p* value	.452	.31	.653	**.016**	**.049**	.504	.565
Bipolar–Control *p* value	.674	**.003**	**.018**	**.041**	**.002**	.801	.487

F, female; M, male; PMI, postmortem interval; RI, refrigeration interval; SMRI, Stanley Medical Research Institute.

There were 105 RNA samples received for microarray analysis of which 88 samples passed microarray quality control analysis. The summary demographics and *t* tests for group differences are shown for those subjects analyzed. Table 1 in Supplement 1 shows each subjects' data.

good-quality discovery genes. The expression profiles of positive control probes, an average correlation index (8), and by deviations from a virtual Median Chip (calculated from the median raw data for each gene across control subjects' chips) using a linear regression plot to show profiles for each chip were also used for outlier detection.

In all analysis of covariance (ANCOVA), gender and diagnosis were considered as main effects, and age and tissue pH were considered as covariates, to estimate the adjusted mean expression for each gene using Partek Genomics software. Pairwise post hoc contrasts between least square adjusted means for BPD and control subjects, and SZ and control subjects with p value $<$ .05 were used to select significant genes for further studies. A secondary ANCOVA was performed with the same parameters; however, the subjects were restricted to those with brain pH above the median pH of 6.57. This restricted analysis was compared with the unrestricted analysis to enrich the list of genes with diagnosis effects relative to pH sensitive genes (9). The ANCOVA p values were adjusted with Benjamini-Hochberg False Discovery Rate (BH FDR) method (10) for the entire list of probes. In this study, the main effect p values of Diagnosis were not significant following ANCOVA and BH FDR; however, 17 genes passed multiple test correction BH FD for the main effect of Gender, 16 were sex chromosome localized as expected (11). Planned contrasts between adjusted means for BPD and control subjects, and SZ and control subjects with p value $<$ .05 were used to select significant genes for further studies. The effects of antipsychotic treatment on signficant gene profile was tested by linear regression.

Real Time Quantitative Polymerase Chain Reaction

Differentially expressed genes in both SZ and BPD were selected for validation with quantitative real-time polymerase chain reaction (Q-PCR). The genes for Q-PCR validation were chosen by the criteria that they meet significant differential expression after adjustment for multiple covariates ($p < .05$ by ANCOVA) with fold change greater than $\pm$ 1.25 for comparisons of both the SZ and control group, and BPD and control group.

The Q-PCR data was analyzed by ANCOVA using the Diagnosis factor and pH and age as covariates to calculate significance of the target gene. Because we have a planned direction of change, the one-tailed p value was used for significance. Two reference genes were used (CFL1 and CRSP9). The detailed protocol and primer sequences for Q-PCR are available in Supplement 1 and Table 2 in Supplement 1.

Cross-Validation in Discriminant Analysis

Multiple classification models were run to predict membership of subjects into either psychiatric disorder or control groups. The details of the cross-validation method are in Supplement 1.

Pathway Analysis

The distribution of the final list of 78 significant genes in different biochemical and functional pathways was analyzed with the Ingenuity Pathway Analysis version 4 (Ingenuity, Redwood City, California) and the ErmineJ method (12). Both sets of results were compared.

The details of the method are described in Supplement 1.

Genotyping

A validated TaqMan genotyping assay for AGXT2L1 (alanine-glyoxylate aminotransferase 2-like 1) dbSNP rs1377210 was run on the samples used for gene expression. The details of the ABI genotyping assay are shown in Supplement 1. Conventional sequencing was used to verify the accuracy of the TaqMan result.

Results

Demographics

The demographic comparisons between patient and control groups for RNA quality (28S/18S), and age were not significant ($p > .05$, Table 1; Table 1 in Supplement 1 shows each subjects' demographic data). As described in Methods and Materials, brain pH was decreased in both the schizophrenia and bipolar disorder groups compared with control groups ($p < .05$), and the refrigeration interval was significantly increased in both psychiatric groups compared with control subjects ($p < .05$). Gender was not equally balanced in the BPD and control groups, with a trend for increase in the number of female subjects in the BPD group (Pearson's $\chi^2 = 3.61$, $p = .057$). Age, brain pH, and gender have been shown previously to be significant variables in microarray studies (7,9,11,13–15) and were included in all ANCOVAs. Postmortem interval, refrigeration interval, and RNA quality, were entered as covariates for a final ANCOVA. The histograms of p values for each demographic variable of pH, age, PMI, RNA quality, refrigeration interval demonstrated the validity of selecting pH and age as primary covariates along with Gender and Diagnosis as main effects. PMI, RNA quality, and refrigeration interval (RI) were not as strong contributors to gene expression; nevertheless, these covariates were used in the final analysis since the groups were not balanced well for PMI and RI (Table 1).

Unrestricted Analysis of Subjects by ANCOVA

The first ANCOVA with all subjects was unrestricted for pH and adjusted by the covariates of pH and age. The shared molecular profile involving schizophrenia and bipolar disorder showed overlap of 327 differentially expressed genes in DLPFC (Figure 1). There were 1793 dysregulated genes not shared in both disorders with the first ANCOVA ($p < .05$, Figure 1). The total number of genes shared in both bipolar disorder and schizophrenia appeared larger compared with the expected

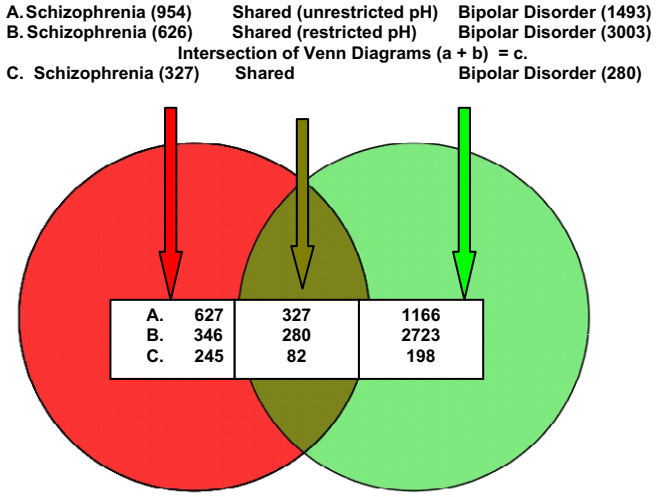

Figure 1. The Venn diagram for analysis of 88 dorsolateral prefrontal cortex RNA samples (bipolar disorder [BPD], shizophrenic [SZ], and control subjects) showing the overlap between the number of differentially expressed genes. **(A)** In schizophrenia (left circle, 627 genes) and bipolar disorder (right circle, 1166 genes). The intersection of two circles produces a set of 327 genes. **(B)** The same analysis was repeated on a restricted set of samples (brain pH > 6.57) and yielded 280 genes that were shared between both disorders. **(C)** The overlap of both analyses (intersection of Venn diagrams, A and B) shows differentially expressed genes that are robust to pH differences and shared between schizophrenia and bipolar disorder. Of 82 probes shared in the final analysis (C), 78 genes mapped unambigously to these probes. The details of list of 78 genes are shown in Table 3 in Supplement 1.

number by chance, the ratio of observed:expected was 4.57, which suggested an enrichment of shared genes (Table 2). This analysis used a common control group as a reference, which could inflate the number of shared genes in bipolar and schizophrenia because the analysis of psychiatric group differences was not independent.

Restricted Analysis of Subjects by ANCOVA

A second ANCOVA used subjects restricted to pH above the median to determine whether effects might be masked by subjects that showed the lowest pH in the study. This ANCOVA was restricted to subjects above the median pH of 6.57 and with the covariates of pH and age. Although covariate analysis approach might be useful with the strong effect of pH, it may not be linear through the lower pH range, when subjects may show a ceiling or floor effect depending on the direction of association (7–9,16–19). Therefore, we attempted to use subjects with above-median pH as an indicator of fewer residual agonal effects. After restriction of the pH, the pH was not different between groups.

The molecular profile involving schizophrenia and bipolar disorder showed overlap of 280 differentially expressed genes in DLPFC following ANCOVA. There were 3069 dysregulated genes ($p < .05$) not shared for both disorders (Figure 1) and 1.7 more genes than in the unrestricted pH analysis. The number of genes shared in bipolar disorder and schizophrenia also suggested an enrichment of shared genes, that is, the observed:expected ratio was 2.96 (Table 2). A similar caveat as in the unrestricted analysis is applicable: a common control group was used as a reference, which could inflate the number of shared genes.

Combining Both ANCOVA Results

After combining both ANCOVA gene lists for unrestricted and restricted samples, there were 78 significant genes shared between BPD and schizophrenia (Figure 1; Table 3 in Supplement 1), only 5 genes were expected by chance, and the chi-square was highly significant for 78 genes found ($p = .013$; Table 2). Two genes were represented by different probes, and two genes had ambiguous BLAST results. Important candidate genes can be contained in the list of genes that did not pass each ANCOVA filter, and the raw data are available by direct download from SMRI. There may be false negatives in our approach, for example, HOMER1, CORT, and RGS4 are examples of genes that were shown by one of our ANCOVA microarray analysis to be significant but did not pass all three ANCOVA analyses.

Restricted Gene Expression in Brain

The list of 78 genes (Table 3 in Supplement 1) for shared differential expression were next queried at Novartis Gene Symbol Atlas for brain expression levels (20). It was determined that three genes (AGXT2L1 and SLC1A2 [Figure 1 in Supplement 2], and TU3A) have expression restricted to brain regions. These three genes were highly expressed in brain samples but were not

Table 2. The Analysis of Both BPD and SZ were Conducted with ANCOVA Using Diagnosis and Gender as Main Effects with Age and pH as Covariates

	ANCOVA of Gene Expression		No. Genes Shared Between BPD and SZ in DLPFC in Both ANCOVAs
	Unrestricted pH	Restricted pH > 6.57	
SZ	954	626	
BPD	1493	3003	
Not Shared (Present in One Disorder)	1793	3069	
Shared (Significant in Two Disorders)	327	280	
Observed Number of Shared Genes	**327**	**280**	82
Expected Number of Shared Genes	**72**	**95**	4.6
Ratio (Observed:Expected)	4.57	2.96	17.81
$\chi^2 = 6.06$, df (1), $p = .013$[a]			

ANCOVA, Analysis of covariance; BPD, bipolar disorder; DLPFC, dorsolateral prefrontal cortex; SZ, schizophrenia.

The number of significant genes in each ANCOVA (column 2, without restriction of subjects by pH) and (column 3, after restriction to subjects with pH > 6.57) was increased beyond expected chance levels for BPD and SZ. The relationship was tested in a 2 × 2 chi-square analysis of the cells (boldface). The "Observed Number of Shared Genes" was 82, but because of redundant probes for the same genes that were significant, the actual number of significant genes was 78 (see Table 3 in Supplement 1).

[a]Calculated with the four boldface items in columns 2 and 3.

expressed or showed baseline levels in nonbrain samples from 56 tissues or cell lines. The following significant genes (Table 3 in Supplement 1) showed greater than 10-fold median expression levels in one or more brain regions (20): TU3A, AGXT2L1, TUBB2B, SLC1A2, SOX9, ATP6V1H, GMPR, EMX2, AHNAK, and IMPA2.

The AGXT2L1 (alanine-glyoxylate aminotransferase 2–like 1) gene was strongly dysregulated in both BPD and SZ (Figure 1 in Supplement 2). Evidence of coregulation with AGXT2L1 (Pearson Correlation p value $< .25 \times 10^{-6}$) was found for 50 other dysregulated genes. The p values for correlations of AGXT2L1 with the other genes in the final gene list is shown (Table 3 in Supplement 1), and these p values are after Bonferroni correction for all 19,980 discovery genes on the Codelink 20K chip.

The distributions of AGXT2L1 expression values in BPD, SZ, and control subjects showed that differences in AGXT2L1 levels in bipolar disorder and schizophrenia were not due to a few extreme outliers (Figure 2). In the schizophrenia and bipolar groups 48 of 59 subjects showed above the control's mode of AGXT2L1 expression levels (Figure 2), and this distribution was highly significant (Fisher's Exact Test, $p = .000001$). The odds ratio was 11.4 (95 % confidence interval [CI] = 4.0–32.5) for developing a psychiatric disorder on the basis of above control mode expression of AGXT2L1. The distributions of AGXT2L1 across all three groups appeared to have a wide expression range that varied over 5 standard deviations from minimum to maximum expression suggesting a possible genetic component in regulation. The AGXT2L1 gene consisted of one linkage disequilibrium block for all SNPs in the CEPH European sample. We tested whether there was preliminary association of one nonsynonymous AGXT2L1 SNP to schizophrenia or bipolar disorder.

The nonsynonymous coding SNP rs1377210 for AGXT2L1 (T > C) polymorphism results in an amino acid Ser > Pro at residue 185. The genotypes for Caucasian subjects (Table 4 in Supplement 1) were equally distributed across bipolar, control subjects, and schizophrenia for the nonsynonymous SNP rs1377210. There were no minor allele homozygotes to test for a genotype association with expression and an interaction with diagnosis.

Pathway Analysis

The entire distribution of p values from the ANCOVA of all subjects was entered into ErmineJ and resampled for each GO category. Three distributions of p values were analyzed by the same methodology (12): BPD–Control, SZ–Control, and the average for these two p values distribution. Three resampling distributions for common BPD and SZ Gene Ontology categories and individual distributions were significant in 16 categories (corrected $p < .005$, Table 3). The top 16 categories were regrouped into seven biological themes: apoptosis, cell growth, endogenous proteases, neuropeptide signaling, DNA replication, transmembrane receptor signaling, and humoral immune response.

The 78 genes initially found significant for both SZ and BPD (Table 3 in Supplement 1) were also analyzed in Ingenuity Pathway Analysis (IPA v. 4.0, Redwood City, California) for

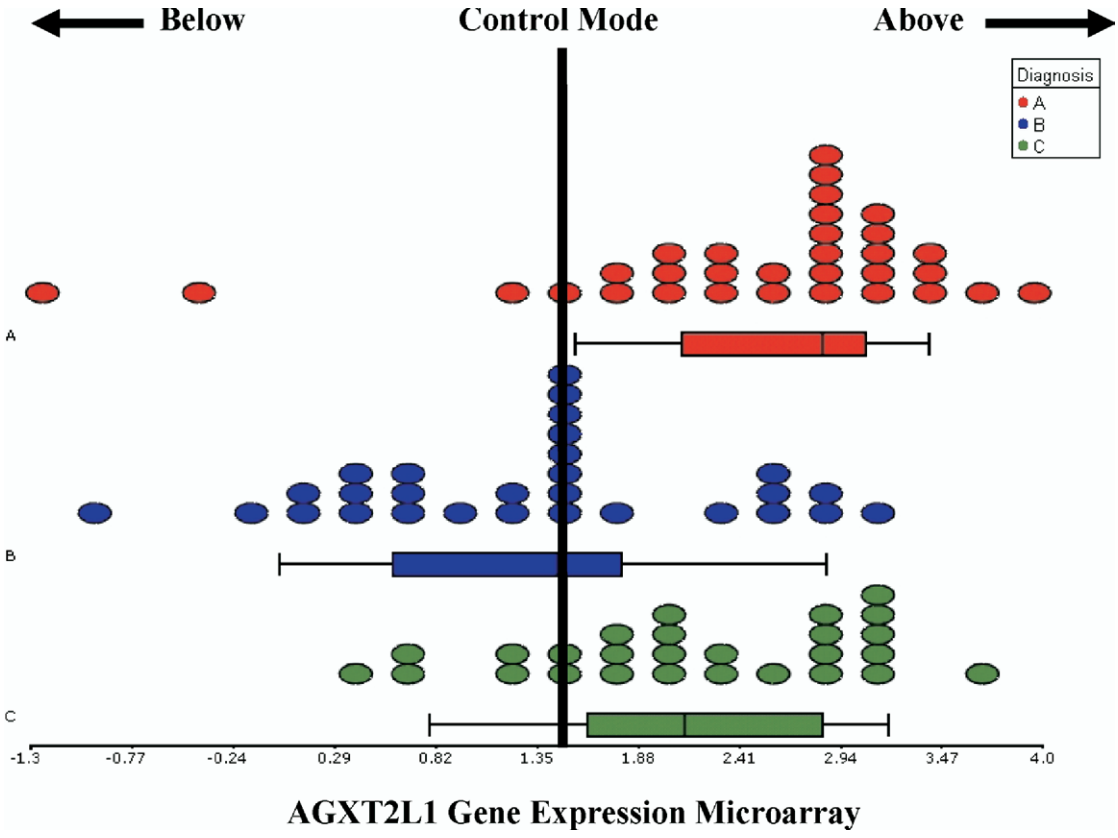

Figure 2. The distribution of gene expression values for AGXT2L1 for schizophrenia subjects (top red circles, **A**), control subjects (middle blue circle, **B**), and bipolar disorder subjects (bottom green circle, **C**). The distribution for the 88 samples combined suggests that individuals with a high AGXT2L1 value are at a higher risk of developing a psychiatric disorder. Individuals with psychiatric disorder (48) showed above the mode of control AGXT2L1 expression levels. The distribution of control subjects versus subjects with a psychiatric disorder was highly significant for the (Fisher's Exact Test, $p = .000001$), with an odds ratio of 11.4 for developing a psychiatric disorder on the basis of the above control mode expression of AGXT2L1.

Table 3. The Entire Distributions of *p* Values Were Averaged for BPD and SZ ANCOVA Analysis Using ErmineJ

Gene Ontology Category	Gene Ontology ID	Probes Codelink Array	No. of Genes	Corrected *p* Value (ErmineJ Resample)	Gene Ontology Definition
Positive Regulation of Programmed Cell Death	GO:0043068	187	152	6.45E-11	Any process that activates or increases the rate of programmed cell death; cell death resulting from activation of endogenous cellular processes
Induction of Programmed Cell Death	GO:0012502	179	145	7.07E-11	A process that directly activates any of the steps required for programmed cell death
Regulation of Growth	GO:0040008	177	143	7.31E-11	Any process that modulates the frequency, rate, or extent of the growth of all or part of an organism so that it occurs at its proper speed, either globally or in a specific part of the organism's development
Peptide Binding	GO:0042277	152	131	7.83E-11	Interacting selectively with peptides, any of a group of organic compounds comprising two or more amino acids linked by peptide bonds
Endopeptidase Inhibitor Activity	GO:0004866	143	122	8.77E-11	Stops, prevents, or reduces the activity of an endopeptidase; any enzyme that hydrolyzes nonterminal peptide linkages in polypeptides
Cell Growth	GO:0016049	152	128	9.14E-11	The process by which a cell irreversibly increases in size over time by accretion and biosynthetic production of matter similar to that already present
Regulation of Development	GO:0050793	239	199	1.04E-10	Any process that modulates the frequency, rate, or extent of development, the biological process specifically aimed at the progression of an organism over time from an initial condition (e.g., a zygote or a young adult) to a later condition (e.g., a multicellular animal or an aged adult)
Positive Regulation of apoptosis	GO:0043065	186	151	1.69E-10	Any process that activates or increases the rate of cell death by apoptosis
Transmembrane Receptor Protein Kinase Activity	GO:0019199	111	85	3.13E-10	Transmembrane receptor protein kinase activity
Second-messenger-mediated signaling	GO:0019932	225	188	5.48E-10	A series of molecular signals in which an ion or small molecule is formed or released into the cytosol, thereby helping relay the signal within the cell
Chromosome	GO:0005694	236	186	1.10E-09	A structure composed of a very long molecule of DNA and associated proteins (e.g., histones) that carry hereditary information
Humoral Immune Response	GO:0006959	207	177	2.19E-09	An immune response mediated through a body fluid
DNA Replication	GO:0006260	225	187	4.30E-03	The process whereby new strands of DNA are synthesized; the template for replication can either be DNA or RNA
Neuropeptide Signaling Pathway	GO:0007218	101	84	4.48E-03	The series of molecular signals generated as a consequence of a peptide neurotransmitter binding to a cell surface receptor
Phosphoinositide-Mediated Signaling	GO:0048015	100	86	4.67E-03	A series of molecular signals in which a cell uses a phosphoinositide to convert an extracellular signal into a response

The resulting distribution was analyzed, and the gene ontology categories that were significant (corrected *p* value after resampling $< .005$) are shown. These same categories were also significant for the individual SZ and BPD analyzed separately by the same method. ErmineJ software (36) was used for calculation of *p* values for each category shown in Table 3.

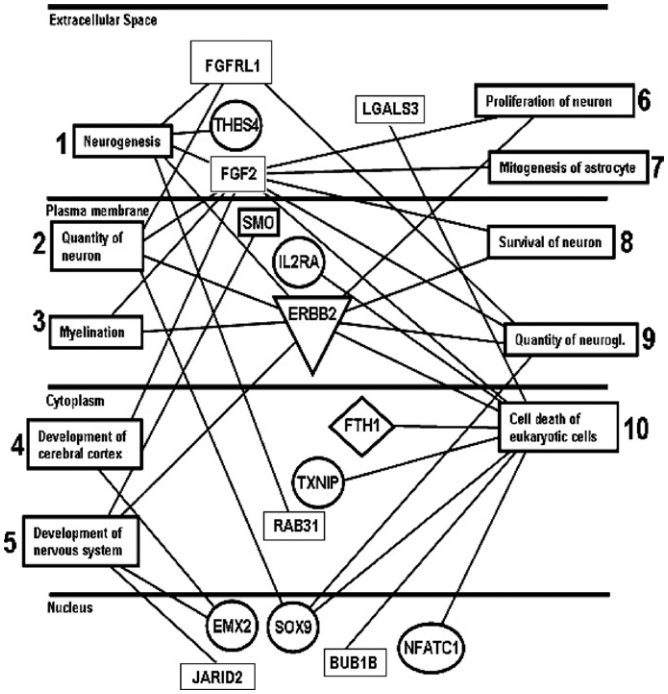

Figure 3. Genes from two categories, nervous system development (labeled 1–9) and cell death (labeled 10), were subsets of cellular growth and proliferation. The most significant functional category for genes shared in schizophrenia and bipolar disorder was cell cycle, cell stage process ($p = 9.18 \times 10^{-06}$) that contained the following genes: ERBB2, FGF2, JARID2, LGALS3, NFATC1, PPARA, PVR, SOX9, and TXNIP. Genes from two categories, nervous system development and function including quantity of neuroglia process ($p = 8.31 \times 10^{-05}$; ERBB2, FGF2, SOX9), and cell death ($p = 8.31 \times 10^{-05}$; FGF2, SMUG1, UNG) were also significant following Bonferroni correction for 546 processes.

evaluating functional pathways. The most significant functional category for genes shared in schizophrenia and bipolar disorder was cell cycle, cell stage process (Fisher's Exact Test, $p = 9.18 \times 10^{-06}$) that contained the following genes: ERBB2, FGF2, JARID2, LGALS3, NFATC1, PPARA, PVR, SOX9, and TXNIP. Genes from three categories nervous system development and function, quantity of neuroglia process (Fisher's Exact test $p = 8.31 \times 10^{-05}$; ERBB2, FGF2, SOX9), cell death (Fisher's Exact Test, $p = 8.31 \times 10^{-05}$; FGF2, SMUG1, UNG) and immune development and response (Fisher's Exact Test, $p = 1.49 \times 10^{-04}$; ERBB2, JARID2, LGALS3, NFATC1, TXNIP, NMU, PPARA, and PVR) were dysregulated in both BPD and schizophrenia. In summary, two approaches for annotation and determination of the significance of the gene lists implicated three categories in BPD and SZ: nervous system development, cell death (Figure 3 and Table 3), and immune categories.

Q-PCR Validation

The candidate gene list assayed by Q-PCR consisted of 15 genes significantly dysregulated in both BPD and SZ. The averages of two housekeeping genes (CRSP9 and CFL1) (7) were used for normalization. The delta Ct values were analyzed using ANCOVA (Diagnosis, pH, age). The overall concordance for significant genes on both microarray and Q-PCR platforms for both disorders was 5 of 15 genes tested (Table 4). The concordance of microarray and Q-PCR results ranged from 33% to 60% depending on diagnosis. Individually, nine genes were validated for bipolar disorder, and five genes for schizophrenia (Table 4) in the ANCOVA analysis (diagnosis, pH, age).

Cross-Validation in Discriminant Analysis

The initial discriminant analysis correctly identified 100% of each group membership using the 78 genes from Table 3 in Supplement 1. The Euclidean distance measure was used with K-nearest neighbor of 1 without cross-validation. The more statistically rigorous approach used a 2 × 4 two-level nested cross-validation with the 78 genes from Table 3 in Supplement 1. The two-level cross-nested validation model correctly assigned BPD and SZ to psychiatric group membership at 74.7% average across 1582 models tested. The models tested were based on varying numbers of genes in groups of 5, 10, 15, 20 . . . 70 and

Table 4. Q-PCR Analysis of Selected Genes Was Conducted Using Two Genes for Normalization, CRSP9 and CFL1

	Schizophrenia				Bipolar Disorder			
	Microarray		Q-PCR		Microarray		Q-PCR	
Gene Symbol	*p* Value	Fold Change	*p* Value	Fold Change	*p* Value	Fold Change	*p* Value	Fold Change
AGXT2L1	**8.38E-05**	2.22	5.86E-01	1.20	**1.32E-03**	1.82	**3.10E-02**	2.29
CASP6	**1.56E-02**	1.19	8.59E-01	1.08	**1.62E-02**	1.23	1.06E-01	2.15
EPHB4	**2.63E-05**	1.45	**8.32E-02**	1.71	**1.03E-02**	1.28	**4.57E-04**	3.48
GLUL	**5.97E-05**	1.50	1.70E-01	1.48	**1.81E-03**	1.40	**1.78E-02**	2.17
HMGB2	**2.05E-05**	1.39	1.82E-01	1.80	**4.37E-04**	1.40	**3.68E-02**	2.69
MAOA	**7.92E-03**	1.22	**9.85E-02**	1.62	**8.28E-04**	1.33	**4.76E-02**	1.89
MCCC2	**9.56E-04**	1.33	6.54E-01	.77	**1.73E-02**	1.22	8.50E-01	.88
NOTCH2	**2.12E-02**	1.44	7.77E-01	.91	**5.58E-03**	1.52	3.63E-01	1.43
PER2	**4.46E-02**	2.00	1.29E-01	1.57	**3.18E-02**	2.11	**7.92E-03**	2.41
SLC1A2	**8.13E-03**	1.41	3.03E-01	.79	**3.06E-03**	1.48	8.45E-01	.95
SLC1A3	**1.32E-04**	1.85	**9.15E-02**	2.43	**4.83E-03**	1.60	**5.77E-02**	2.98
SLC6A8	**1.84E-03**	1.30	**3.24E-02**	2.03	**1.68E-02**	1.21	**4.28E-02**	2.08
TNFSF10	**3.33E-05**	.46	1.15E-01	.46	**4.42E-04**	.43	2.02E-01	.50
TNFSF8	**6.90E-04**	2.91	5.09E-01	1.29	**1.90E-03**	2.87	1.24E-01	1.95
TU3A	**4.78E-03**	1.35	**4.99E-02**	1.74	**7.25E-03**	1.33	**1.49E-02**	2.14

ANCOVA, analysis of covariance; Q-PCR, quantitative real-time polymerase chain reaction.

The normalized Q-PCR data was analyed using ANCOVA with diagnosis as a main factor and pH and age as covariates. The significant Q-PCR *p* values are shown in bold ($p < .1$) for this analysis in the Q-PCR *p* value columns. The list is sorted by gene symbol, all genes showed significant gene expression differences in both schizophrenia and bipolar disorder compared with control subjects by microarray.

using classifier methods of K-Nearest Neighbor, linear discriminant analysis with equal prior probability for classifiers, support vector machine, and nearest centroid with prior probability. However, we were able to assign correctly using three-group accuracy of 51%. The top genes chosen equally most often in the best models for differentiation psychiatric disorder from control subjects were NFATC1, AGXT2L1, ZNF442, SLC14A1, NMU, EMX2, MT1X, SOX9, NOPE, ZC3HAV1, IL17RB, MGST1, and HIF3A. A frequency table for the selected best models during outer cross-validation is shown in Table 5 in Supplement 1.

Identification of Confounding Variables

The lifetime exposures to antipsychotics are estimated on the basis of historical prescription record and may not reflect recent usage or toxicological levels present in the brain. We compared two post hoc analysis of lifetime antipsychotic treatment: 1) difference in high exposure versus low exposure groups to antipsychotics by *t* test and 2) significant beta regression weight for antipsychotic exposure variable. The regression analysis showed 33 significant beta weights related to antipsychotic exposure in the top 78 genes. Furthermore, 7 of 78 genes were significant in both tests for antipsychotic effects (Table 5). In general, for genes significantly altered by antipsychotics, the directions were consistently in the same direction compared with control subjects. For example, HIF3A was significantly increased in the antipsychotic-treated group compared with no antipsychotics group, and HIF3A was also increased in both schizophrenia and bipolar analyses compared with control subjects. The summary of regression analysis with other predictors of PMI, age, rapidity of death, race, RNA 28S/18S, lifetime alcohol, gender, smoking, brain pH, refrigeration interval, and lifetime drugs are shown (Table 6 in Supplement 1, complete regression results in Supplement 3).

Discussion

A common gene-expression profile in BPD and SZ was found in this study. Moderate Q-PCR validation of genes dysregulated in both disorders suggests that this list is a reasonable starting point for evaluating a common gene expression profile in another cohort. Part of this list represents candidate genes that are brain enriched, which might contribute to common pathophysiologic mechanisms and perhaps respond to treatments that are developed in these critical pathways. Notably, some antipsychotics, antidepressants, and mood stabilizers have been shown to affect these pathways (21–25). The estimates of lifetime antipsychotic exposure were significantly related to expression for seven of the top candidate genes in a direction consistent with the direction of dysregulation compared with control subjects. This suggests that some dysregulated genes might be targets of antipsychotic treatments. A drug or gene capable of initiating the cascade of changes seen for the 78 transcripts would represent a good candidate gene.

The shared vulnerability gene profile appeared for both SZ and BPD shows a far greater proportion of nonshared to shared genes. The shared genes formed a fraction, 78 genes, compared with 443 genes that were dysregulated in either BPD (198) or SZ (245). Notably only 4 or 5 genes were expected to overlap from combined ANCOVA analyses for BPD and SZ. This 17-fold enrichment of 78 dysregulated core genes indicates a common susceptibility gene-expression profile for both disorders, represents important alterations in response to medications administered to both groups (7), or downstream events manifest during a chronic psychiatric illness. Although we are not certain about medications last taken near the time of death for each subject, we used lifetime fluphenazine equivalents to interpret additional analyses. Further animal studies to address the effects of medications on these gene transcripts that have importance in both BPD and SZ are required.

On the basis of bioinformatic research, AGXT2L1 likely interacts with SLC7A13 (solute carrier family 7, cationic amino acid transporter, y+ system) and OAT (ornithine aminotransferase). AGXT2L1 has a putative mitochondrial subcellular localization consistent with potential involvement in enzymatic amino

Table 5. Alterations in Gene Expression Related to Antipsychotic Drug Treatments (Lifetime Fluphenazine Equivalents)

Gene Symbol	Average Gene Expression: Lifetime Antipsychotic Exposure Above Median ($n = 31$)	Average Gene Expression: Lifetime Antipsychotic Exposure Below Median ($n = 28$)	Fold Change Above/Below	Lifetime Antipsychotic Exposure Gene Expression Above/Below *p* Value	Pearson's Correlation	Standardized Beta Coefficients	*p* Value for Beta Coefficients
HIF3A	1.08	.29	1.73	**.000**	.25	.44	**.000**
NOPE	1.45	1.01	1.36	**.001**	.31	.28	**.008**
LGALS3	2.00	1.60	1.32	**.005**	.28	.33	**.003**
HEBP2	.33	.04	1.22	**.009**	.32	.12	.288
NMU	−2.11	−1.51	.66	**.028**	−.34	−.22	**.041**
ERBB2	.78	.41	1.29	**.028**	.26	.22	**.042**
MT1X	5.31	4.82	1.40	**.037**	.29	.18	.108
RAB34	2.93	2.62	1.25	.066	.24	.29	**.017**
C6orf4	−.24	−.51	1.21	.071	.24	.24	**.043**
KIAA0515	1.76	1.64	1.09	.199	.28	.09	.430
ZNF442	−1.37	−1.62	1.19	.220	.23	.21	.094
CACNB1	−.22	−.08	.91	.269	−.25	−.08	.480
FTH1	4.26	4.14	1.08	.309	.24	.15	.204
ZNF254	−.17	−.25	1.06	.391	.25	.22	.069
PBX4	.14	.03	1.08	.395	.21	.23	**.030**
UNG	1.88	1.81	1.06	.406	.24	.28	**.018**

Two methods were evaluated, a *t* test comparing low and high exposure levels shown in columns 2–5. The second method was linear regression using other predictor variables in linear regression analysis along with lifetime fluphenazine equivalents shown in columns 6–8. The bold *p* values are significant ($p < .05$) in one or both tests. The list of regression results is in supplementary materials for all predictors.

acid catabolism of arginine, glutamate, histidine, glutamate, glutamine, and proline. A SAGE study of gene expression also showed the AGXT2L1 gene was expressed in only brain relevant libraries (CGAP libraries) confirming the Novartis SymAtlas query. Further experimental validation is necessary to demonstrate functions for this gene although it is upregulated by lithium treatment. It is interesting that a recent study of lithium treatment in mouse brain produced AGXT2L1 as the top candidate gene related to short-term lithium administration (26) at a significance level of $< 10^{-12}$ which passed the experimental FDR criteria.

SLC1A2 (EAAT2 or GLT, high-affinity glutamate transporter, predominantly astroglial) is a brain specific gene highly dysregulated. Alterations to the glutamatergic system in brain have been reported for expression alterations in psychiatric disorders (27–30) although not unequivocally demonstrated in all brain regions studied. Caution regarding the specific isoform that is of pathogenetic importance has been raised because both EAAT2a and EAAT2b are alternatively spliced exons for the same gene SLC1A2 (NM_004171) (31). Our primers were located at the 3′ end in exon 10, and we targeted EAAT2a in Q-PCR validation that is the predominant isoform in human brain (31). However, SLC1A2 did not validate by Q-PCR perhaps due to targeting the wrong region of the gene and could be a false negative by Q-PCR. The SymAtlas results (20) showed that the SLC1A2 gene is brain enriched, although SLC1A2 expression was shown in peripheral organs (32). An SLC1A2 genotype study did show a positive association with schizophrenia in Japanese samples (33). SLC1A2 merits further study as potential susceptibility factor in BPD and SZ.

Cell death and immune response were found to be significant in both the Ermine J and the Ingenuity Pathways analysis. A potential next step for these studies is in vivo and in vitro screening of compounds that would alter expression of genes (34–40). An example of gene alterations found in both functional categories is ERBB2 a transmembrane tyrosine kinase receptor of the ErbB family. The ErbB family includes four members, the epidermal growth factor receptor (EGF-R, ERBB1, ERBB2, ERBB3, and ERBB4). ERBB2, ERBB3, and ERBB4 mRNA appears in both gray and white matter in primate brain (41). Neuregulin binds to heterodimers composed of ERBB2 with either an ERBB3 or ERBB4 molecule (42). Receptor-ligand interaction induces the heterodimerization of receptor monomers, which then activates intracellular signaling cascades involved in proliferation, migration, differentiation, and survival or apoptosis (42). Neuregulin is an important schizophrenia/bipolar disorder susceptibility gene (43–45). Additional references for some of the 78 gene relevant genes are contained in Table 7 in Supplement 1.

We have controlled for large obvious effects on gene expression due to gender, age, pH, postmortem interval (PMI), and time to refrigeration. The impact of pH sensitive genes was reduced in stringent analyses after controlling for pH by ANCOVA and removing low pH subjects. Other studies (e.g., 15,46–48) have found that subjects with BPD or SZ have decreased mitochondrial-associated transcripts. Many authors of microarray studies acknowledge that pH will influence mitochondrial gene expression, and when the effect is strongly controlled, such as in our microarray study and others (7,9,19), the magnitude of mitochondrial gene expression differences in SZ or BPD is markedly reduced. This same effect was seen in our study in which we found mitochondrial-related genes before ANCOVA, and after removing outlier chips and samples, there was a reduction in mitochondrial genes that were significant. Microarrays are sensitive to the effect of agonal-pH differences in samples. The SMRI Microarray cases have a significantly reduced pH compared with control subjects, although most cases are rapid deaths (49). This latter observation might indicate that pH is part of the pathology in BPD and SZ. We find that after careful evaluation with ANCOVA and removal of outlier chips that mitochondrial-associated transcripts were not overrepresented in the SMRI Microarray DLPFC set. Furthermore, immune genes were significantly dysregulated in our study consistent with another recent microarray study (50) in which pH was well-balanced in case and control subjects. Thus, animal models will certainly play a role in determining whether genes that are altered in pH sensitive pathways also convey behavioral-, immune-, and plasticity-related effects.

The current categories of bipolar disorder and schizophrenia share a common gene expression profile. This makes sense because both disorders often clinically present with prominent mood and psychotic symptoms (5). A larger number of dysregulated genes are not shared across the disorders, but fold change direction generally followed similar trends in both disorders. Further work to fill in "explanatory gaps" in the common gene expression profiles of these psychiatric disorders is required at the functional level. The shared genes (Table 3 in Supplement 1) merit further consideration in future neurogenomic and cognitive studies of schizophrenia and bipolar disorder candidate genes.

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