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ORIGINAL ARTICLE

Differential activation of immune/inflammatory response-related co-expression modules in the hippocampus across the major psychiatric disorders

S Kim[1], Y Hwang[2], MJ Webster[1] and D Lee[2]

The Stanley Neuropathology Consortium Integrative Database (SNCID, http://sncid.stanleyresearch.org) is a data-mining tool that includes 379 neuropathology data sets from hippocampus, as well as RNA-Seq data measured in 15 well-matched cases in each of four groups: schizophrenia, bipolar disorder (BPD), major depression (MD) and unaffected controls. We analyzed the neuropathology data from the hippocampus to identify those abnormalities that are shared between psychiatric disorders and those that are specific to each disorder. Of the 379 data sets, 20 of them showed a significant abnormality in at least one disorder as compared with unaffected controls. GABAergic markers and synaptic proteins were mainly abnormal in schizophrenia and the two mood disorders, respectively. Two immune/inflammation-related co-expression modules built from RNA-seq data from both schizophrenia and controls combined were associated with disease status, as well as negatively correlated with the GABAergic markers. The correlation between immune-related modules and schizophrenia was replicated using microarray data from an independent tissue collection. Immune/inflammation-related co-expression modules were also built from RNA-seq data from BPD cases or from MD cases but were not preserved when using data from control cases. Moreover, there was no overlap in the genes that comprise the immune/inflammation response-related modules across the different disorders. Thus, there appears to be differential activation of the immune/inflammatory response, as determined by co-expression of genes, which is associated with the major psychiatric disorders and which is also associated with the abnormal neuropathology in the disorders.

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INTRODUCTION

Many neuropathology studies have been conducted on postmortem samples from individuals with schizophrenia, bipolar disorder (BPD) and major depression (MD).[1–3] Although these studies have identified a wide variety of abnormalities in multiple brain regions, many of the studies have focused on the hippocampus where numerous abnormalities have been identified in markers related to synaptic function[4–7] the GABAergic system[8–10] and central nervous system development.[9–12] However, the results from many of the studies in the hippocampus are inconsistent and the relationship between the deficit in the markers of neuropathology and the molecular mechanisms that may underlie these deficits remains to be determined.

To facilitate the number and quality of neuropathology studies for the major psychiatric disorders and to identify possible targets for drug development, the Stanley Medical Research Institute (SMRI) has been providing postmortem brain tissue for research since 1994.[13] The Stanley Neuropathology Consortium (SNC) contains 15 well-matched cases in each of four groups: schizophrenia, BPD, MD and unaffected controls and has been widely used in numerous psychiatric research studies. SMRI also provides samples from the Array Collection (AC), which is an additional collection with 35 cases in each of three groups: schizophrenia, BPD and unaffected controls. The diagnostic groups in both collections are matched for the descriptive variables, age, gender, race, postmortem interval, mRNA quality (RIN), brain pH and hemisphere.[13] In addition, SMRI has developed the Stanley Neuropathology Consortium Integrative Database (SNCID; http://sncid.stanleyresearch.org) to allow exploration and mining of all the data generated from the many independent studies of these two collections.[14] The database includes data from neuropathology studies, genome-wide gene expression microarray data, single-nucleotide polymorphism data[14] and more recently RNA-seq data from several different brain regions. Integrative analysis using the neuropathology data and genome-wide expression data from brain samples all derived from the same individuals provides a unique opportunity to explore the biological processes that may be associated with abnormalities in the various neuropathology markers.[15]

Periodically SMRI summarizes and conducts meta-analysis of the numerous neuropathology data sets and more recently has begun to explore the molecular mechanisms that may underlie the deficits in the neuropathology markers.[15–18] Multiple biological processes including cellular metabolism, central nervous system development and apoptosis were found to be associated with the decreased number of perineuronal oligodendrocytes and with the decrease in density of calbindin-positive interneurons in the frontal cortex of people with major psychiatric disorders.[15] A co-expression network analysis utilizing RNA-sequencing data from the hippocampus found a module related to immune/inflammation response that was associated with both schizophrenia and with the density of parvalbumin-containing neurons

[1]Stanley Brain Research Laboratory, Stanley Medical Research Institute, Rockville, MD, USA and [2]Department of Bio and Brain Engineering, KAIST, Yuseong-gu, Daejeon, Korea. Correspondence: Dr S Kim, Stanley Brain Research Laboratory, Stanley Medical Research Institute, 9800 Medical Center Drive, Rockville, MD 20850, USA.
E-mail: kims@stanleyresearch.org
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in the hippocampus.[19] We have now updated the database and included additional data from multiple brain regions including hippocampus of all subjects in the SNC. In this study, we summarize the abnormalities found in the hippocampus of each psychiatric disorder group as compared with the controls using the 260 neuropathology data sets. Then using the RNA-seq data from the hippocampus, we identified co-expression modules and biological processes associated with each major psychiatric disorder and also with neuropathology traits that were abnormal in the hippocampus. Finally, using the hippocampal microarray gene expression data from the second collection (AC) we attempted to replicate the schizophrenia-associated co-expression networks and the BPD-associated co-expression networks that we identified in the first collection (SNC).

MATERIALS AND METHODS

Neuropathology data sets and RNA-seq data in the SNCID

Nine hundred twenty-three neuropathology data sets have been added to the SNCID[14] since the last update in August, 2009. As of December 2014, there are 379 neuropathology markers measured in the hippocampus and available for analysis. These neuropathology markers include RNA levels measured by quantitative PCR and *in-situ* hybridization, protein levels measured by western blot and enzyme-linked immunosorbent assay, receptor-binding studies and cytoarchitectural studies that measure cell size, number and density as identified by histological stains and immunohistochemistry. For this study, only data sets with continuous variables were included and only if data for $>80\%$ of the cases in each diagnostic group ($n \geqslant 12$) were available. Thus of the 379 hippocampal data sets, 260 met criteria and were included in the analysis.

The raw RNA-sequencing data (FASTAQ files) from the hippocampus of the SNC are also available from the SNCID and were downloaded for use in this study. Demographic and clinical variables of SNC samples used for neuropathology studies are listed in Supplementary Table S1.

Read mapping and quantification of gene expression

In our previous study, we mapped and quantified the RNA-seq data from the hippocampus of the schizophrenia cases and controls.[19] We now map and quantify the RNA-seq data from the hippocampus of the BPD and MD cases using the same methods as described in our previous study.[19] In brief, all reads were mapped to H. sapiens reference genome using TopHat v2.0.0 with UCSC refFlat gene model annotation file (build hg18) on the –G parameter.[20] We used the expected mean inner distance between mate paired-ends as –r parameter. TopHat calls Bowtie v0.12.7 (http://bowtie-bio.sourceforge.net/index.shtml) to perform the alignment with no >2 mismatches. The quantification of gene expression was accomplished by HTseq v0.5.3p9 and edgeR package[21] (http://www.bioconductor.org/packages/release/bioc/html/edgeR.html). All mapped read counts of the genes were counted by htseq-count (subprogram of HTseq) with UCSC refFlat gene model annotation file, no strand-specific option, and intersection-nonempty option.

Analysis of covariance of the neuropathology data

The effect of each psychiatric disorder on the neuropathology data were examined by using a permutation analysis of covariance (ANCOVA). First, descriptive variables were tested for identification of potential confounding factors. The effects of the continuous variables such as age, brain pH and postmortem interval on the individual data sets were examined by Pearson correlation test and the categorical variable, sex, was tested by variance analysis. *P*-values <0.05 were considered significant. Significant differences of markers between each disease groups and unaffected controls were then tested using a permutation ANCOVA implemented in lmperm R package (lmPerm v1.1–2, R. E. Wheeler, www.r-project.org). A permutation test is known to be more powerful than a parametric analysis when sample sizes are small or data distribution is not normal. As the three disorder groups and the unaffected controls were measured in all data sets, *post-hoc* Bonferroni tests were conducted to adjust for multiple testing. It should be noted that because we analyzed all available data sets by permutation ANCOVA test with significant covariance and we did not run pre-processing of the data, our results may not be consistent with the previously published analyses that were conducted by the individual researchers who produced the original data sets. Adjusted *P*-values <0.05 were considered significant.

Gene co-expression network analysis

We performed unsupervised weighted correlation network analysis (WGCNA) to build the co-expression networks[22] using RNA-Seq data of all the genes in the hippocampus and pooled data from each disease group independently with the controls. Before generating the co-expression network, we used surrogate variable analysis (SVA)[23] to identify the potential confounding effects in the RNA-Seq data. The resulting surrogate variables from the SVA were then used as covariates in the linear regression to adjust the confounding effects on the gene expression data. The standardized residuals from the linear regression were used to generate gene co-expression networks using WGCNA. To compare the co-expression networks across the three psychiatric disorders, the parameters were set the same as previously used in our study for schizophrenia and controls.[19] The correlation between co-expression modules and traits, such as diagnosis, demographic or clinical variables, were performed to identify modules that were associated with disorder and/or confounding factors. The raw data for all pathology marker measurements in the hippocampus that were available in the SNCID were used as additional trait markers to identify modules that may be associated with these markers. The network connections with topological overlap above the threshold of 0.1 were visualized using VisANT.[24]

Network comparisons

To further explore gene–gene interactions that may differ between disease group and controls, we generated separate co-expression networks for each disease group and for controls by using only the disease group data and only control data. Module preservation was examined using a permutation test procedure implemented in the WGCNA.[25] Summary preservation Z score ($Z_{summary}$) was used to determine whether a co-expression module was preserved in a test network as compared with a reference network or not. Previous threshold guidelines were followed: if $Z_{summary} < 2$, no evidence of the module preserved. If $2 < Z_{summary} < 10$, weak-to-moderate evidence of the module preserved. If $Z_{summary} > 10$, strong evidence of the module preserved.[25]

Replication analysis of co-expression network using microarray data of AC samples

As an independent replication of the co-expression network analysis (WGCNA) in the SNC using the RNA-seq data, we performed a similar WGCNA using the gene expression microarray data from the hippocampus of the AC samples. We adjusted batch effect and covariates on the microarray data as described previously[26] with slight modifications. Briefly, the fRMA-normalized microarray gene expression data were first adjusted for batch effect using ComBat[27] and then outlier samples were excluded. The probes were filtered out if the coefficient of variation of the probes were <0.05. The potential confounding effects were adjusted by using SVA[23] as described above. Demographic and clinical variables of AC samples used for the replication studies are listed in Supplementary Table S1.

Functional annotation

DAVID (http://david.abcc.ncifcrf.gov/home.jsp) was used to identify the biological processes that were significantly over-represented by differentially expressed genes between BPD and controls, as well as genes included in the co-expression modules that were correlated with the disease status: schizophrenia, BPD and MD,[28] as well as in the non-preserved modules. *P*-values <0.05 were considered significant.

RESULTS

Neuropathology markers significantly altered in the hippocampus of the major psychiatric diseases

Of the 379 neuropathology data sets measured in the hippocampus of the SNC and deposited in the SNCID, 260 met inclusion criteria for our analysis. Of the 260 data sets, 37 showed a significant group differences without covariates (adjusted $P<0.05$) and of these, 20 showed a significant abnormality in at least one disorder group as compared with unaffected controls using

permutation ANCOVA (Supplementary Table S2). A similar number of abnormal markers were identified in schizophrenia ($n = 9$), BPD ($n = 11$) and in MD ($n = 11$). We then identified the functional categories to which the abnormal neuropathology markers were involved and then visualized the results using heat maps (Figure 1). The density of parvalbumin-containing neurons in CA4 and dentate gyrus were only altered in schizophrenia, whereas synaptic-related markers were mainly abnormal in BPD and MD (Figure 1). In contrast, several markers were significantly altered in all three disorders, for example, the density of parvalbumin-containing neurons in CA1, *5-HT2A* (5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled) and GAD1 (glutamate decarboxylase 1 (brain, 67 kDa)) mRNA levels in CA4, and *CPXL2* (complexin 2) mRNA levels in parahippocampal gyrus. To visualize the relative level of each marker in individual cases, we generated a heat map using the z score of each marker in each individual case (Figure 2). The heat map shows that the markers were generally decreased in the hippocampus across all three disorder groups as compared with controls (Figure 2), with the exception of the *SNAP25* (synaptosomal-associated protein, 25kDa) protein levels, which were increased in the MD cases as compared with controls.

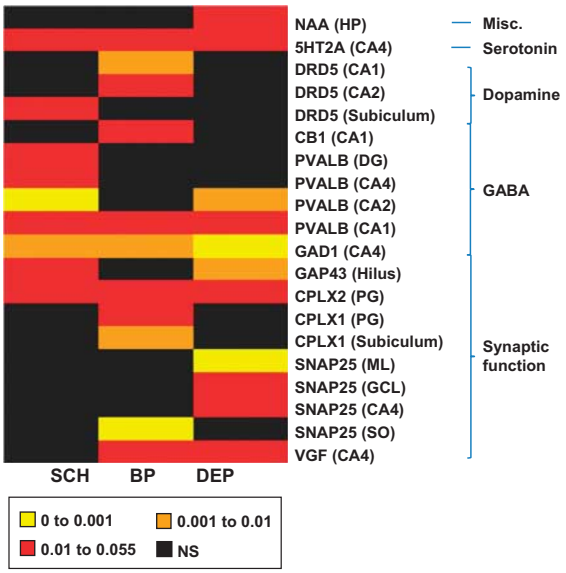

Figure 1. Heat map for neuropathology markers in the hippocampus that were significantly altered in at least one diagnostic group. The color code represents adjusted *P*-values of neuropathology markers. BP, bipolar disorder; DEP, depression; DG, dentate gyrus; GABA, gamma-aminobutyric acid; GCL, granule cell layer; HP, whole hippocampus; ML, molecular layer; NS, not significant; PG, parahippocampal gyrus; SCH, schizophrenia; SO, stratum oriens.

Gene expression profiling in the hippocampus of individuals with BPD and depression

We previously performed whole-genome expression profiling using RNA-Seq data from the hippocampus of individuals with schizophrenia and from controls.[19] Here, we performed the same analysis using RNA-Seq data from the hippocampus of BPD and controls, as well as from MD and controls. Although we were unable to identify any gene that was significantly differentially expressed between BPD and controls in the hippocampus at false discovery rate < 0.05, we did find three significantly differentially expressed genes, *GREM1* (gremlin 1, DAN family BMP antagonist), *C9orf84* (chromosome 9 open reading frame 84) and *C2orf77* (coiled-coil domain containing 173), in the hippocampus of individuals with MD as compared with controls. Although the expression of *GREM1* was downregulated, *C9orf84* and *C2orf77* were upregulated in the hippocampus of the MD cases.

Co-expression network analysis in the hippocampus of individuals with schizophrenia, BPD and depression

We next performed unsupervised co-expression network analyses using the hippocampal RNA-Seq data to identify co-expression modules associated with schizophrenia, BPD and MD. We combined the RNA-Seq data, which were adjusted for covariates, from an individual disease group with that from the controls. We previously used normalized RNA-Seq data for the co-expression network analysis of the hippocampus of individuals with schizophrenia and controls.[19] Here we reanalyze all the data using the SVA adjusted gene expression data.

Of the 16 co-expression modules that were generated with data from the schizophrenia cases and controls, 5 were significantly associated with schizophrenia (Supplementary Table S3). Two

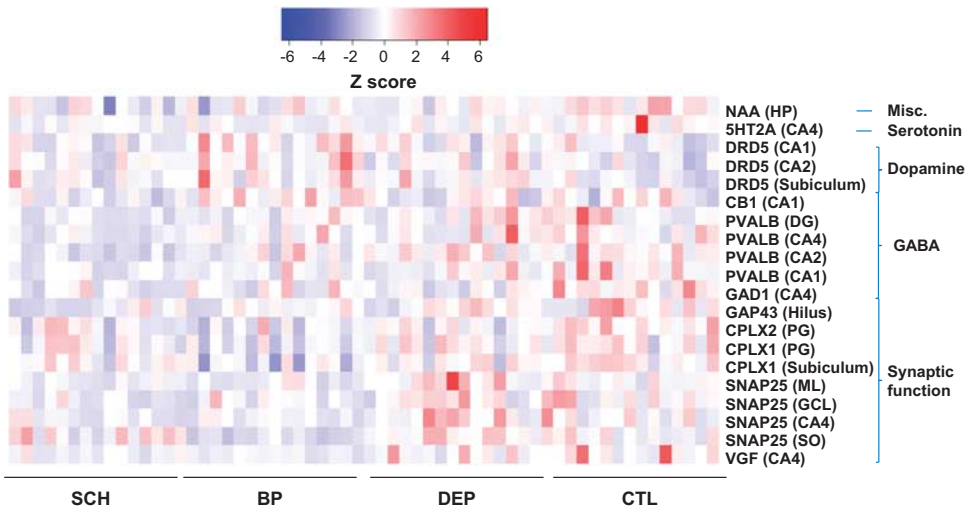

Figure 2. Heat map of z score for neuropathology markers in the hippocampus that were significantly altered in at least one diagnostic group showing data for each individual case. BP, bipolar disorder; CTL, control; DEP, depression; DG, dentate gyrus; GABA, gamma-aminobutyric acid; GCL, granule cell layer; HP, whole hippocampus; ML, molecular layer; PG, parahippocampal gyrus; SCH, schizophrenia; SO, stratum oriens.

Table 1. Neuropathology markers significantly correlated with the modules associated with schizophrenia in the hippocampus

Pathology marker				*Module*[a]			
Marker	*Marker type*	*Layer*	*Method*	*S_M3*	*S_M8*	*S_M13*	*S_M16*
Parvalbumin	Cell	DG	IH (density)	0.54	−0.37	NS	−0.43
Parvalbumin	Cell	CA4	IH (density)	0.54	NS	−0.42	−0.42
Parvalbumin	Cell	CA2	IH (density)	0.5	NS	−0.4	−0.38
Parvalbumin	Cell	CA1	IH (density)	0.48	−0.38	NS	−0.4
GAD1	RNA	CA4	ISH	0.44	−0.45	−0.44	NS
5-HT2A	RNA	CA4	IH (density)	NS	NS	NS	−0.46

Abbreviations: DG, dentate gyrus; GAD1, glutamate decarboxylase 1 (brain, 67 kDa); ISH, *in-situ* hybridization; IH, immunohistochemistry; NS, not significant; *5-HT2A,* 5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled. [a]Correlation coefficients between modules associated with schizophrenia and cellular markers. Correlation analysis was performed using the WGCNA package. *P*-values < 0.05 were considered significant. Correlation coefficients only represent significant correlations between the module and the cellular marker.

modules, S_M2 and S_M3, were negatively correlated with schizophrenia. However, S_M2 was excluded from downstream analysis because the module was also negatively correlated with lifetime antipsychotic treatment. Module S_M3 was a large module containing 628 genes. Genes related to neuron development, vesicle-mediated transport and mitochondria-related metabolism were significantly enriched in this module (Supplementary Table S4). Three modules, S_M8, S_M13 and S_M16, were positively correlated with schizophrenia. S_M8 is a large module that includes many of the differentially expressed genes and is significantly enriched for processes related to inflammation and immune response, apoptosis and regulation of angiogenesis (Supplementary Table S5). Although response to virus, immune response and blood vessel development were enriched in the S_M13 module (Supplementary Table S6), inflammation-related processes were not. Translation-related processes such as translational elongation, ribosome biogenesis and ribosomal RNA processing were only enriched in the S_M16 module (Supplementary Table S7). Confounding effects of demographic and clinical variables on the co-expression modules were examined (Supplementary Table S3). RIN was significantly correlated with the three modules, S_M3, S_M13 and S_M16, and brain pH was correlated with S_M8 and S_M13.

A similar analysis using data from BPD cases and controls and from MD cases and controls was performed. A total of 23 co-expression modules were generated for BPD but none were significantly associated with disease (Supplementary Table S8). Similarly, 32 co-expression modules were generated for MD and none were associated with disease (Supplementary Table S9).

Co-expression modules associated with schizophrenia and with pathology markers in the hippocampus

In our previous analysis of the RNA-seq data in the hippocampus of schizophrenia, we performed a correlation analyses between two co-expression modules that we found were associated with schizophrenia and the density of parvalbumin-containing neurons measured in the hippocampus.[19] Here, we extend the correlation analyses to include the four disease-associated co-expression modules that we found associated with schizophrenia using the SVA adjusted data, and we include all neuropathological markers that were significantly altered in schizophrenia as compared with controls. We found six markers in the hippocampus that were significantly correlated with at least one schizophrenia-associated co-expression module (Table 1). Module S_M3 was related to neuron development, vesicle-mediated transport and mitochondria-related metabolism and was positively correlated with GABAergic interneuron markers including *GAD1* and parvalbumin. The two immune-related modules, S_M8 and S_M13, were negatively correlated with the GABAergic interneuron markers. S_M16, which was associated with translation and ribosomal RNA processing, was negatively correlated with the GABAergic markers and RNA level of *5-HT2A*.

Comparisons of co-expression networks between BPD and unaffected controls

Although we identified four modules that were associated with schizophrenia, we were unable to identify co-expression modules associated with BPD or MD when we constructed the networks by combining data from the diagnostic group cases and controls as we did for the schizophrenia analysis. This may indicate that there are fundamental differences in gene–gene interactions in the hippocampus of individuals with a mood disorder as compared with controls.[25] To explore this possibility further, we constructed co-expression networks for BPD and controls separately. We then compared the co-expression networks for BPD with those for controls, or vice versa and found that almost all the modules were conserved between the BPD cases and controls indicating that there is no difference in the gene–gene interactions between cases and controls. However, there were six modules that were only weakly conserved indicating that there are some possible differences in the gene–gene interactions between the cases and controls (Supplementary Table S10). Two modules, C_only1_M6 and C_only1_M10, were weakly conserved in BPD as compared with controls, whereas four modules were weakly conserved in controls as compared with BPD ($2 < Z_{summary} < 10$; Supplementary Table S10). Of the 15 co-expression modules generated with the control data, 2 were only weakly conserved in BPD. The C_only1_M6 module was significantly enriched for genes related to glucocorticoid metabolic process and cell signaling (Supplementary Table S11). Regulation of cell cycle is the main biological process associated with C_only1_M10 module (Supplementary Table S12). A total of 28 co-expression modules were generated from the BPD data, of which 4 modules were weakly preserved in controls. Immune/inflammation response was significantly enriched in the genes of the BPD_only_M18 module (Figure 3, Supplementary Table S13) and *IL23R* (interleukin 23 receptor), *IL-10* (interleukin 10), *CXCL13* (chemokine (C-X-C motif) ligand 13), *Cd40LG* (CD40 ligand) and *GBP6* (guanylate-binding protein family, member 6) were genes related to the immune/inflammation response. *IL23R* was a hub gene in the module. Glycoprotein metabolic process, cell signaling and transcription were the main biological processes that were significantly enriched in the genes of the other weakly conserved modules, BPD_only_M7, BPD_only_M23 and BPD_only_M28 (Supplementary Tables S14-S16). Confounding effects of demographic and clinical variables on the co-expression modules were examined. RIN was negatively correlated with C1_only1_M6 and BPD_only_M7. All other variables were not correlated with the modules.

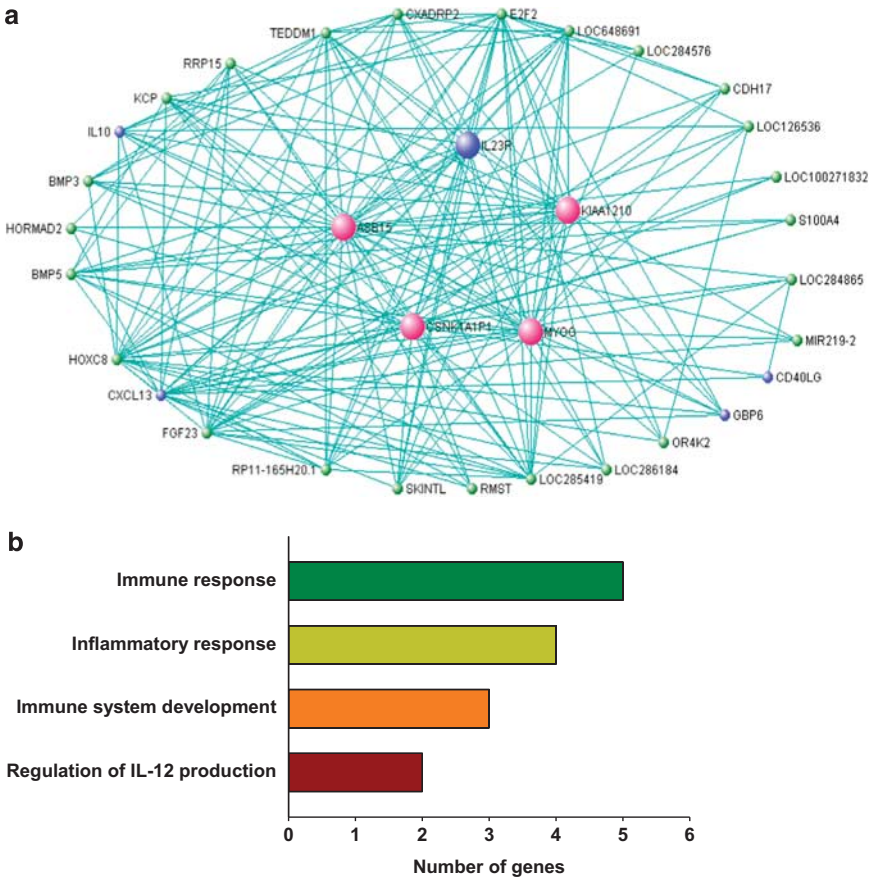

Figure 3. Co-expression network for bipolar disorder (BPD) in the hippocampus. (**a**) The co-expression module (BPD_only_M18) was built from BPD data and was weakly preserved in unaffected controls and (**b**) major biological processes (gene ontology) enriched in the genes in the co-expression module. All network connections with topological overlap above the threshold of 0.1 were visualized using VisANT.[24] The hub genes are larger circles in the center of the network. Genes related to immune/inflammation responses are blue. IL-12, interleukin-12.

Comparisons of co-expression networks between depression and unaffected controls

We also constructed co-expression networks for MD cases and controls separately. Again most modules were conserved between the MD cases and controls indicating that there is no difference in the gene–gene interactions between cases and controls. However, two modules were only weakly conserved in MD as compared with controls, and three modules were weakly conserved in controls as compared with MD ($2 < Z_{summary} < 10$; Supplementary Table S17). As seen in the comparison between BPD and controls, we found a module (C_only2_M6) containing genes related to glucocorticoid metabolic processing and cell signaling (Supplementary Table S18). Biological processes related to neuron development and glial cell migration were associated with the other module that was weakly conserved in MD, C_only2_M10 (Supplementary Table S19). Of the 16 co-expression modules generated from MD data, one was not preserved at all and 3 were only weakly preserved in controls. The module that was unique to MD, MD_only_M9, consisted of genes related to the regulation of the immune system and blood vessel development (Supplementary Table S20). Of the weakly preserved modules, MD_only_M11 consisted of the genes related to immune/inflammation response (Figure 4, Supplementary Table S21). Of the five top hub genes in this module, three (*C1QA* (complement component 1, q subcomponent, A chain), *C1QB* (complement component 1, q subcomponent, B chain) and *C1QC* (complement component 1, q subcomponent, C chain)) are related to complement activation. Protein maturation, response to extracellular stimulus and transcription were the main biological processes significantly enriched in the genes of the remaining modules that were weakly preserved in controls (Supplementary Tables S22 and S23). Confounding effects of demographic and clinical variables on the co-expression modules were examined. Age was positively correlated with C1_only1_M6 and BPD_only_M7. All other variables were not correlated with the modules.

Replication of co-expression modules associated with schizophrenia and BPD using independent data

To replicate the co-expression modules associated with schizophrenia or BPD, we used an independent data set from the AC samples. We built co-expression networks by combining data from gene expression microarray data from hippocampus from schizophrenia cases and controls and from BPD cases and controls. A total of 33 co-expression modules were built using data from schizophrenia and controls (Supplementary Table S24). Of the 33 co-expression modules that were generated, two modules (S_MA_M13 and S_MA_20) were significantly positively associated with schizophrenia (Supplementary Table S24). The S_MA_M13 module consisted of 310 genes. The small nuclear RNA metabolic process, immune system development and ion transport were significantly enriched in this module (Supplementary Table S25), which is functionally similar to S_M8 module built using the RNA-seq data (Supplementary Table S5). The S_MA_M20 was also positively associated with schizophrenia and protein

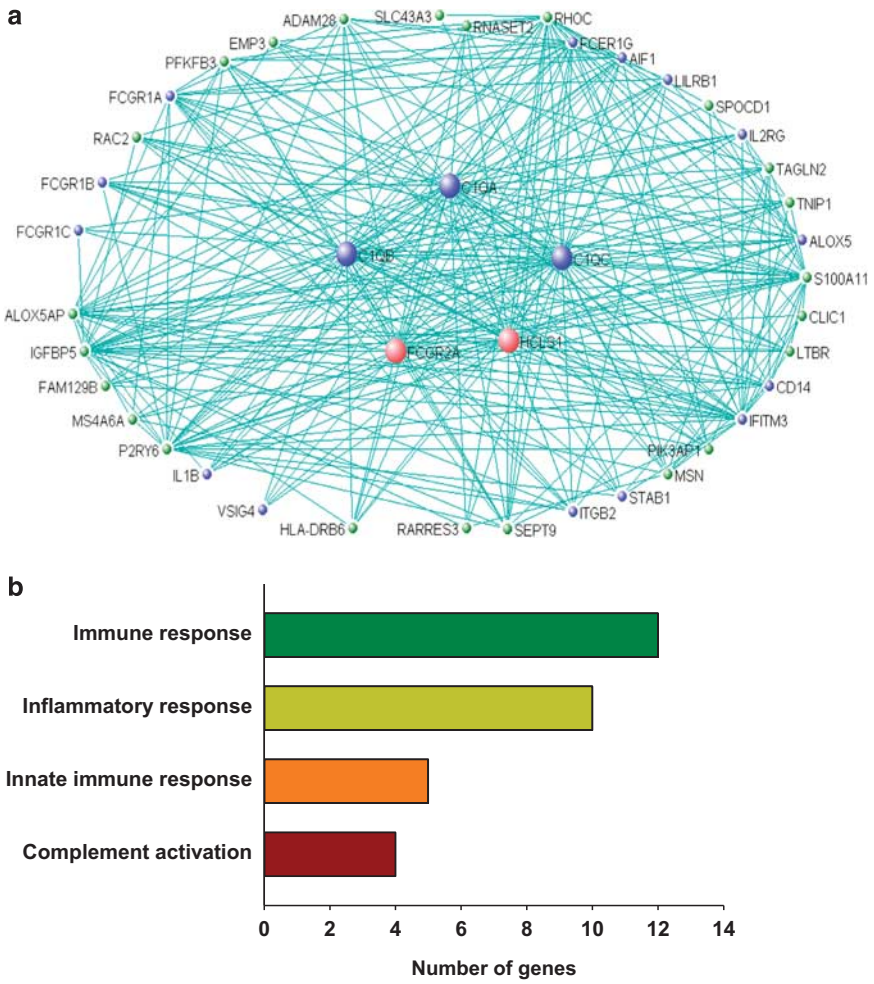

Figure 4. Co-expression network for major depression (MD) in the hippocampus. (**a**) The co-expression module (MD_only_M11) was built from data from MD cases and is weakly preserved in unaffected controls and (**b**) the major biological processes (Gene ontology) enriched in the genes in the co-expression module. All network connections with topological overlap above the threshold of 0.1 were visualized using VisANT.[24] The hub genes are larger circles in the center of the network. Genes related to immune/inflammation responses are blue.

transport, ubiquitin cycle and synaptic transmission and RNA metabolic process were enriched in this module (Supplementary Table S26), and thus appears functionally similar to the S_M3 and S_M8 modules built using the RNA-seq data (Supplementary Table S4 and S5).

A similar analysis for BPD built a total of 29 modules, however, none of the modules was significantly associated with BPD (Supplementary Table S27), which is consistent with the result of our network analysis using combined RNA-seq data of BPD and controls (Supplementary Table S8).

DISCUSSION

Neuropathology studies of the major psychiatric disorders has been ongoing for many years, however, the pathophysiology of schizophrenia, BPD and MD remains unclear.[1–3] The SNCID provides a unique opportunity to extensively profile thousands of neuropathology data sets from various brain regions that were measured in the same individuals with major psychiatric disorders.[14] In this study, we analyzed all the neuropathology data from the hippocampus that had been deposited in the database to date and summarized the results. The hippocampus is involved in memory, cognition, mood and stress response[29] and has therefore been implicated in the symptomatology that manifests in psychiatric disorders. Consequently, the hippocampus has been a major focus for neuropathology research, which has generated a large number of data sets. We find a similar number of abnormally expressed neuropathology markers in the hippocampus of each disorder; however, the majority of the abnormalities are specific to one disorder. Although markers of GABAergic cells were mainly altered in schizophrenia, those related to synaptic function were mainly abnormal in the mood disorders. However, the pattern of synaptic marker abnormality was quite different between the two mood disorders, for example, SNAP25 was significantly increased in CA4 and in the molecular and granular layers in MD, whereas complexin 1 mRNA levels were significantly decreased in the parahippocampal gyrus and subiculum in BPD. Complexin 1 is located primarily in the inhibitory axosomatic synapses of the cortex and because the deficit in complexin 1 is in the parahippocampal gyrus and subiculum, which supply input to the dentate gyrus and CA4, there may be synaptic dysfunction in earlier processing areas of the hippocampal circuitry in individuals with BPD. In contrast, in schizophrenia the abnormalities were primarily confined to the GABAergic inhibitory neurons and the parvalbumin positive in particular, in the dentate gyrus and the CA subfields of the hippocampus. Interestingly, there were four markers, *5-HT2A* and *GAD1* in CA4, parvalbumin-containing neurons in CA1 and *CPLX2*

in parahippocampal gyrus that were significantly altered in the three disorders and may contribute to the overlap in symptoms that are associated with these disorders. However, overall it appears that the fundamental neuropathology in the hippocampus of the three disorders is quite different.

Using the hippocampal RNA-seq data of the SNC, we performed gene expression profiling of the hippocampus of BPD and MD in order to compare the results with those obtained from a similar previous analysis in schizophrenia and to identify co-expression modules associated with mood disorders. In contrast to the 144 differentially expressed genes that we found previously in schizophrenia,[19] there were very few genes differentially expressed in the mood disorders. There were no genes differentially expressed in BPD and only three genes differentially expressed in MD. Previous microarray studies found many genes that are differentially expressed in the brain of individuals with mood disorder as compared with unaffected controls.[30–38] Genes involved in mitochondrial function,[30,31] apoptosis,[32] development of the nervous system[33] and ubiquitin cycle[34] were differentially expressed in brain samples from individuals with BPD. Genes involved in synaptic transmission were differentially expressed in brain samples from cases with MD.[35,36] Expression levels of genes involved in oligodendrocyte function and myelination were changed in the both diseases.[37,38] In contrast, a recent RNA-seq study using a subset of cases from the same collection as used here found only five genes that were downregulated in the dorsolateral prefrontal cortex of BP subjects as compared with controls.[39] However, most of the abnormally expressed genes that have been found in the various studies have not been replicated across studies. Several factors including heterogeneity of samples, platform differences and confounding factors may be responsible for the inconsistencies. Furthermore, the variation in the standard methods of analysis used to compare gene expression levels between two or more groups may be contributing to the inconsistency, particularly because the changes in gene expression are usually quite subtle and there are often large variations among samples in the same diagnostic group. Moreover, standard statistical analyses do not examine the gene–gene interactions (that is, connectivity between genes), which may have an important role in the pathophysiology of psychiatric diseases. Gene co-expression network analysis has been used as an additional and/or alternative statistical method to overcome the limitations of standard statistical methods and to detect possible abnormalities in gene–gene interaction in neuropsychiatric diseases.[40-45] We, therefore, performed gene co-expression analysis to identify modules of co-expressed genes that were correlated with psychiatric diseases and also with pathology markers. We also wanted to detect gene–gene interactions that differed between disease cases and unaffected controls.

The strength of the SNCID is that numerous neuropathology and genome-wide expression data sets from multiple brain regions have been collected from the same individuals. Thus, biological processes associated with the abnormalities in the neuropathology markers can be identified by integrating both types of data and then the molecular mechanisms that underlie the pathological deficits can be explored. In our previous study in the hippocampus of schizophrenia, we found the co-expression module that was related to the immune/inflammation response was also associated with deficits in the parvalbumin-containing neurons.[19] Here, we extended such association analysis to all the neuropathology markers that were significantly altered in the hippocampus of the three psychiatric disorders.

Recent studies show that systematic confounding factors often affect RNA-seq data.[46,47] Thus, we adjusted such factors in our RNA-seq and microarray data using the SVA package and then constructed co-expression networks. We then re-built co-expression networks using combined RNA-seq data from schizophrenia and controls. We found the deficits in parvalbumin-containing interneurons and *GAD1* RNA levels were associated with multiple co-expression modules that were also associated with schizophrenia, indicating that multiple mechanisms are associated with these pathological deficits. For example, the processes enriched in the modules that are positively correlated with schizophrenia (for example, immune response) and negatively correlated with the interneuron markers may be contributing to the interneuron deficits in schizophrenia. In contrast, the processes enriched in the module that is negatively correlated with schizophrenia but positively correlated with the interneuron markers may be a consequence of the interneuron deficit. We found two immune/inflammation response-related modules positively correlated with schizophrenia, which is consistent with results from our previous co-expression network analysis that used unadjusted RNA-seq data.[19] We also replicated the positive correlation between an immune-related module and schizophrenia using microarray data from an independent tissue collection, the AC. Thus, the association between the immune/inflammation response-related modules and schizophrenia is unlikely to be affected by any confounding factors. Although the two modules were positively correlated with schizophrenia they were negatively correlated with the GABAergic cell markers, which indicates that an activated immune/inflammation response by some environmental factors may lead to the reduction of these interneuron markers in the hippocampus of individuals with schizophrenia. Although the two co-expression modules were both enriched with genes related to the immune response, there was no overlap in individual genes between the two modules indicating that specific gene sets are contributing to each co-expression network, for example, genes related to response to virus such as *MX1* (myxovirus (influenza virus) resistance 1, interferon-inducible), *MX2* (myxovirus (influenza virus) resistance 2) and *IFI35* (interferon-induced protein 35) were significantly enriched in the S_M13 module, suggesting that the activated immune/inflammation response by viral infection may be specifically associated with schizophrenia, as well as with the decreased GABAergic interneuron cell density in the disease.

Co-expression network analysis that uses combined data from disease cases and controls may have more power to detect modules that are associated with some clinical traits than a similar analysis that uses data from cases and controls separately. However, if there are significant differences in gene–gene interaction (that is, gene connectivity) between disease cases and controls, co-expression modules that are associated with disease may not be efficiently built from the combined data.[25] In this study, we were unable to detect modules associated with BPD or MD from network analysis that included data from the controls. Thus, there may be differences in gene–gene interaction in the hippocampus between the mood disorder groups and the controls and therefore, we conducted a network analysis using data from the disease groups and controls separately to detect possible diagnostic group-specific modules. Co-expression modules that included genes related to glucocorticoid metabolic processes such as *CRH* (corticotrophin-releasing hormone) and *STAR* (steroidogenic acute regulatory protein) were detected in controls but were not conserved in BPD or MD. *CRH* is known to be involved in the acute stress response. The association between CRH and mood disorder is controversial, however, an increase in CRH levels has been reported in individuals with depression and BPD, and there is impairment in the cortisol response to CRH in patients with stress-related depression.[48] In the hippocampus, CRH has both positive and negative roles in stress-mediated hippocampal function depending on the stress type, intensity and duration.[49] Detecting a co-expression module in controls that involves CRH but which is not conserved in BPD or MD suggests that abnormal CRH-mediated stress response may be a common pathophysiology underlying both mood disorders. Although speculative, it is possible that the mechanism disrupting this module in the mood disorders may also contribute to the

abnormalities in the neuropathology markers that are common to both disorders.

Co-expression modules related to inflammation/immune response were built in BPD and MD but were not conserved in controls, indicating that immune/inflammation response may be associated with the mood disorders, as well as with schizophrenia. However, the individual genes included in each module did not overlap and thus the precise biological function associated with the co-expression modules may be different in each disorder. Interleukin 23 receptor (*IL23R*) was a hub gene in the co-expression module for BPD. The *IL23R* gene encodes a subunit of the receptor for interleukin 23 (IL-23), which is a pro-inflammatory cytokine. Genetic variations in the gene are associated with both Crohn's disease[50] and with Alzheimer's disease.[51] IL-23 induces production of several cytokines including IL-10 in cell culture.[25] *IL-10* is also involved in the same co-expression module in which *IL23R* is a hub gene. A co-expression module related to immune/inflammation response was also generated for MD cases, but was not conserved in controls. The module includes the complement genes *C1QA*, *C1QB* and *C1QC* as the hub genes in the module. C1q is the first subcomponent of the classic complement cascade. Beside key roles in the innate immune system, C1q also mediates synapse elimination in the central nervous system during development.[52] This raises the possibility that synapses may be reduced in the hippocampus of people with depression by abnormal activation of C1q. However, studies examining synaptic function and number in the hippocampus of individuals with depression have not been consistent. One study reports a decrease in synapses and reduced expression of several synapse-related genes, *CALM2* (calmodulin 2 (phosphorylase kinase, delta)), *SYN2* (synapsin II) and *RAB3A* (RAB3A, member RAS oncogene family) in the prefrontal cortex of cases with depression.[53] In contrast, our results and those of the previous original study[5] report no difference in RNA levels of the synaptic marker, *CPLX1* (complexin 1) between MD and controls in the hippocampus. Moreover, protein levels of SNAP25 in multiple areas of the hippocampus were increased in MD. Thus, it is possible that the activated C1q is involved in a classic immune response rather than mediating synaptic elimination in the hippocampus in depression. This module also included the pro-inflammatory cytokine interleukin-1 beta (*IL1B*), which has been implicated in the etiology and the pathophysiology of depression. Chronic stress induces elevated IL-1b levels in the hippocampus and reduces hippocampal neurogenesis in a mouse model of depression[54] and IL-1 regulates C1q production from macrophages.[55] Thus, these distinct co-expression modules related to the immune/inflammation response are specific to each disorder and may relate to specific molecular mechanisms that underlie the abnormal neuropathology markers that are also specific to each disease.

Although this large-scale data analysis shows many interesting avenues for further exploration and data mining, it should be viewed as exploratory. Rigorous replication studies are needed to validate our results, particularly for our co-expression networks constructed separately for the mood disorders and controls. Although we could successfully replicate the co-expression networks for the combined data from disease cases and controls, we were unable to replicate the co-expression networks when using data from cases and controls separately. We discontinued the co-expression network analysis using BPD and control data separately because only a small number of genes from the total input microarray data were involved in co-expression modules as compared with those in our RNA-seq data. From a total of 10 708 genes in the microarray data set, we found 291 genes involved in the co-expression modules in controls and 274 genes involved in the co-expression modules in BPD. In contrast, of the total of 16 940 genes in the RNA-seq data, we found 1421 genes involved in the co-expression modules in controls and 2762 genes involved in the co-expression modules in BPD. This is possibly due to less sensitivity and more noise in the microarray data than in the RNA-seq data.[56,57] Thus, large-scale RNA-Seq studies using independent brain samples are needed to confirm our results in the future. One of the main limitations of this analysis is the possible confounding effects of descriptive and clinical variables on individual data. Such effects are often more serious on data generated using postmortem tissues and can be difficult to control in a statistical analysis. Several possible confounding variables often affect neuropathology data, as well as gene expression profiles. Antipsychotic medications have been known to affect gene expression profiling in various brain regions. However, in our data, antipsychotic treatment unlikely affected immune/inflammation-related co-expression modules associated with schizophrenia because there was no significant correlation between a total lifetime intake of antipsychotics and any of the immune/inflammation-related co-expression modules associated with schizophrenia. In our replication study, the immune response-related module (S_MA13) that was associated with schizophrenia was negatively correlated with the antipsychotic treatment, suggesting antipsychotic treatment may normalize activated immune/inflammation-related genes rather than contribute to upregulation of the genes in schizophrenia. A previous study shows that antipsychotic treatment suppresses the levels of pro-inflammatory cytokines and raise the levels of anti-inflammatory cytokines.[58–60] Other variables such as brain pH, postmortem interval and RIN have also been known to confound gene expression profiles. RIN appeared to be associated with most co-expression modules associated with psychiatric disease. Three modules out of four that were significantly associated with schizophrenia were also associated with RIN. However, there were no significant differences between any psychiatric disease group and the unaffected controls in any of these variables, including RIN, indicating that the contribution of RIN to the disease-associated co-expression modules is likely to be very low. A final limitation is that our results indicate correlational relationships between gene expression modules and neuropathology markers and not causal relationships. Nevertheless, the genes and the biological processes enriched in the co-expression modules associated with psychiatric diseases, as well as with various neuropathology markers may provide novel insights into the mechanisms that underlie the pathophysiology of these disorders. These types of studies may eventually lead to mechanism-based novel drug development for the major psychiatric diseases.

Gene symbols of each co-expression modules are listed in Supplementary Table S28.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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