Identification of major depressive disorder disease-related genes and functional pathways based on system dynamic changes of network connectivity

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Abstract

Objective : Major depressive disorder (MDD) is a neurologic disorder, involving complex abnormal biological functions and the neural network. In this study, we aimed to compare different network connectivity of pathological changes of brain tissues under different conditions, and dynamic analyze the biological pathways and genes significantly associated with disease progression, and further to predict potential drug targets. Methods : Datasets of mRNA expression profiles of samples of postmortem anterior cingulate cortex (ACC) and prefrontal cortex (PFC) from patients with MDD were downloaded from GEO database. We used a method based on system network analysis. Correlation networks based on co-expression were constructed. Topological properties of the networks were analyzed and compared. Results : Our results showed that the lesions of brain tissues in MDD patients were not synchronized and alterations of biological functions were not consistent either. ACC showed a greater degree of abnormality as compared to PFC suggesting a higher correlation with disease progression. We consequently analyzed the signaling pathways enriched by DEGs and further cross talk genes that bridge the multiple pathways were also identified. Through construction of the pathway-gene complex network, the genes and signaling pathways with top10 degrees were extracted, which are more likely to be the potential novel therapeutic targets. We also mined the drugbank database for the top10 cross talk genes to explore their drugable target potential. The results suggested that CACNA1A, PTDSS2 and CD19 differentially expressed in both ACC and PFC may correlate with depression progression or other cerebral nerve system related diseases, are more likely to become the new drug targets. Conclusion : Co-expression network and tissue comparing analysis are capable to identify MDD disease related signaling pathways and cross talk genes with potential to be novel drug targets.

Background

Major depressive disorder (MDD) is a common serious mental disorder, typically presenting persistent low mood, anhedonia and occasional suicidal ideation and behaviors (1). This psychiatric mood disease has an estimated 12-month prevalence of 5.3% and a lifetime disorder rate of 13.2% (2). There is a high recurrence rate (3), a heritability of 37% (95% CI=31%-42%) (4), and a higher prevalence in female (5). MDD exerts negative effects on the quality of life and is a heavy socioeconomic burden. Although currently available pharmacologic treatments are widely used, there are significant limitations including long time lag for treatment response (commonly weeks to months) and low response rates (one to two thirds will not respond to the first drug prescribed, and remain one third will not respond after multiple trials) (6-9), and thus associated with high rates of suicide. The neuropathology mechanism underlying MDD remains unclear, which makes the diagnosis and treatment of depression be challenging.

In recent years, accumulating evidence suggests that depression is not localized to a single brain area or caused by any single gene, but is a disease with complex genetic features and complicated etiology. Widespread brain areas associated with ‘emotional network’ were found abnormal in the structure, function, and coordinated activity in MDD patients, thus depression is also considered as ‘disconnection syndrome’. Disordered brain activity and impaired mood regulation were thought to be the major neuropathologies underlying depression (10). Beyond hippocampus, anterior cingulate cortex (ACC) and prefrontal cortex (PFC) are also important sites of abnormality in MDD patients (11). The ACC is involved in the modulation of negative affect, pain and cognitive control (12). It has previously been proposed that glucose metabolism in pregenual ACC is increased in patients with MDD (13). The PFC plays an important role in the regulation of the hypothalamo-pituitary-adrenal (HPA)-axis

in stress response and also depression. There is increasing evidence that MDD and chronic stress are associated with an excitatory inhibition (E:I) imbalance within PFC which is caused by a deficit of inhibitory synaptic transmission onto principal glutamatergic neurons (14). A recent functional magnetic resonance imaging study showed reduced functional connectivity of medial PFC in patients with MDD (15).Accordingly, depression is a heterogeneous syndrome with distinct causes and pathophysiologies.

Large scale gene expression analysis has generated a large amount of gene- and disease-related information regarding depression, but the discovery of disease mechanisms is still limited by heterogeneity and various sources of noise (16-18). Considering the fact that complex phenotypes manifested in mammalian systems are the result of a complex array of networks operating within and between tissues, a network perspective is necessary to explain its etiology. Tissue-to-tissue networks analysis provides a method for the identification of disease-specific genes in response to abnormalities of tissues based on genome-wide association studies (19, 20). Converging evidence indicated that gene co-expression studies offer complementary perspectives on gene changes in the context of transcriptome studies (21). Co-expression genes possibly shared similar function, and they may arise via multiple and diverse biological pathways such as common regulatory pathways (16, 19, 21). Malfunction of signaling pathways is likely to induce a variety of pathologies (22). Notably, by incorporating multiple interactions among a large number of genes, the study of gene co-expression networks provides an approach to tackle the complexity of biological changes occurring in complex polygenic disorders (16, 23).

Therefore, in this study, we systematically integrated datasets and analyzed gene co-expression links in studies with postmortem brains (ACC and PFC) of human MDD subjects and non-psychiatric control subjects. We hypothesize that genes with correlated expression patterns across tissues are more likely to function between them rather than to be regulated in specific tissue. Results will provide a novel and powerful framework to improve understanding of MDD and other complex neuropsychiatric disorders.

Methods

Expression profile analysis

Datasets of mRNA expression profiles of patients with depression were downloaded from GEO database. Six datasets were obtained with samples of postmortem anterior cingulate cortex (ACC) ('E-GEOD-54572', 'E-GEOD-54571', 'E-GEOD-54565', 'E-GEOD-54564', 'E-GEOD-54563', 'E-GEOD-54562') (24) and another six datasets were obtained with samples of postmortem prefrontal cortex (PFC) ('E-GEOD-54570', 'E-GEOD-54568', 'E-GEOD-54567', 'E-GEOD-45642', 'E-GEOD-35978', 'E-GEOD-12654') (17, 24-26). The gene ID was converted into gene symbol through the platform transformation. Multiple probes may correspond to one gene, therefore these probes were combined using ‘WGCNA’ package of R/Bioconductor (27). For multiple batches and platforms of expression profile data, to merge data under the equal variance level, we conducted Z test correction on all expression profile data, with the mean of 0, the standard deviation of 1, and following normal distribution. Afterwards, the data were integrated. Two integrated expression profile data were obtained regarding anterior cingulate cortex and prefrontal cortex. The ‘limma’ R package (28) was used to identify the differentially expressed genes (DEGs) ($P<0.05$, $|\log2 (\text{fold change})|> 0$).

Correlation analysis

Gene expression pattern changes in disease state as compared to normal cellular homeostasis. Genes with similar functions tend to correlated and dysregulated in disease state. The changes in correlations between genes can be used to identify the depression development process related feature genes (29). Therefore, we performed correlation analysis to identify significantly correlated gene pairs in both normal and disease states, using Pearson correlation coefficient with a threshold of 0.5.

Co-expression network construction

Genes with significant expression correlations in normal or disease condition were considered as co-expression. They may be regulated together and have similar functions. We obtained the gene co-expression relationships in normal and disease conditions from the two brain tissues. The co-expression network was constructed by taken co-expression relationships as edges and genes as nodes. The isolated nodes and self-interactions were removed. Cytoscape software (30) (http://www.cytoscape.org) was applied for the construction of the network.

Comparative analysis on difference of network

Commonly, the gene interaction network follows power law distribution. A few genes have a significantly higher number of connections as compared to the other genes in the network acting as hub genes. The network showed stability and robustness. Gene connections in biological networks are dynamic and may show altered co-expression under variable conditions. In disease conditions, genes may lose or gain connections in the perturbed network (29). The genes with altered connectivity or expression in disease are more likely to be involved in disease progression and may be therapeutic targets. Thus, we statistically measured the gain or loss of the node degree in normal and disease networks.

Functional pathway analysis

To further understand the biological functions of the DEGs from the two brain tissues, functional enrichment analysis was performed using KEGG (the Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis (http://www.kegg.jp/). The up-regulated and down-regulated genes were analyzed respectively. Enrichment methods adopted the Fisher's exact test, P values were adjusted by FDR (false discovery rate). Signaling pathways with $P<0.05$ were considered as significantly enriched pathways. Analysis tool DAVID (https://david.ncifcrf.gov/) was used.

Cross talk analysis

Multiple biological functions change in disease condition. Signaling proteins generally do not work in isolation but transmit signal via interaction with other proteins or biological molecules. Similar, the biological processes are not relatively independent, but reciprocally linked and regulated by the signaling proteins. Pathways influence the dynamics of each other are collectively referred to as cross-talk (31). A signaling protein belongs to more than one

signaling pathway, plays a critical role in the regulatory and cooperative relationships between signaling pathways, and probably involving with the pathogenesis of diseases. Cross-talk gene has been studied being as important drug targets and biomarkers (32). We used the DEGs and their enriched signaling pathways to construct a regulatory network. Human Protein Reference Database (HPRD, http://www.hprd.org/) was used for gene interaction relationship mapping. Based on the distribution of significant DEGs in each pathway, the crucial cross-talk genes were identified to have function in multiple significant related biological pathways.

Results

Expression profile meta-analysis

To systematically analyze the expression profile of brain tissues from patients with MDD, we integrated the expression profile datasets from anterior cingulate cortex and prefrontal cortex respectively to increase confidence in the biological reality. The integrated datasets of ACC contained 152 samples, including 76 disease samples and 76 healthy controls. While integrated datasets of PFC included 126 samples, 63 of them were disease samples and 63 healthy controls. The “limma” R package was applied for the identification of differentially expressed genes (DEGs). As a result, 586 DEGs were obtained in ACC (Fig 1a) and 616 DEGs in PFC (Fig 1b). Comparative analysis between the two brain tissues showed that the gene expression patterns in these two tissues were highly consistent, with most of the DEGs down-regulated in both tissues (Fig 1c). Up to 50 % DEGs were dysregulated in both of the two tissues. These results indicated that during depression development, there is a certain relevance in the abnormal alterations between the two brain tissues.

Co-expression network construction

The dependencies between genes and their relationships to other co-expressed genes reflected closely associations of these genes in biology (33). It is speculated that the significantly co-expressed genes may have similar biological functions and associate with similar or same biological pathways. We utilized Pearson correlation coefficient to identify the co-expression relationships of genes from the two brain tissues in both normal and disease conditions. Co-expression networks were constructed based on these relationships by cytoscape software (Fig 2).

The network topological properties were analyzed (Table 1). As compared to normal condition, the number of nodes had no significant change in the co-expression network under disease condition, but gene connections obviously loss in the network, which may result in the increase of unconnected nodes in the disease network (Table 1). The network clustering coefficient, density and centralization were significantly reduced, suggesting that the centrality and robustness of the disease network were significantly decreased as compared to the normal network. We statistically calculated the significance of the gene connections in both normal and disease conditions using Wilcox test. In PFC, Wilcox test P=0.04, and in ACC, P=1.227e-09. The results revealed that gain or loss of gene connections showed significant statistical significance in both tissues under disease condition, and more significant in ACC.

Comparative analysis on difference of networks

Through the network topology analysis, we found that as compared to normal control, in the brain tissues of patients with depression, the reduced connectivity and centrality of the disease co-expression network and the loss of gene connections were important features along with the disease development. To further compare the correlation of the two tissue lesions and the disease, we performed comparative analysis on the co-expression networks under disease condition. The nodes with gain of connections (Fig 3a) and loss of connections (Fig 3b) were compared in the two disease networks. We found that the number of nodes with gain of connections were nearly balanced with that of nodes with loss of connections in PFC (Fig 3c). Thus, in the disease condition, stability of PFC network is better than that of ACC network, and the PFC network showed better resistance to the disease signal. Our data also showed that the number of nodes with gain of connections in PFC network was higher than that in ACC network with ratio of 1.72:1, while the numbers of nodes with loss of connections tended to be similar in the two network with ratio of 1.02:1. Some genes had gain or loss of connections in both ACC and PFC cortex, which may be bridge genes connecting the two tissues by regulating similar biological functions in both parts.

To get an overall view of the gene expression pattern of the two tissues in disease condition, we statistically calculated the probability density distribution of the co-expression networks in diseased tissues compared to that in normal tissues (Fig 3d and 3e). The results showed that in ACC of depression, variance of density distribution was markedly increased (Fig 3d), suggesting that the volatility of the network was increased; while in PFC of depression, the connectivity density distribution of network tended to normal, there were no obvious deviation on mean and variance (Fig 3e). This observation indicated that in disease states, the PFC network state tended to be normal, whereas the ACC network showed drastic fluctuations, which was in line with the previous results of Wilcox test. These results revealed that the lesions of brain tissues in patients with depression were not synchronized and alterations of biological functions were not consistent either, ACC showed a greater degree of abnormal as compared to PFC suggesting a higher correlation with disease progression. Therefore, ACC is more likely to exist important therapeutic targets.

Functional pathway analysis

To gain a knowledge about the DEGs influenced biological effects, functional enrichment analysis is conducted in up- and downregulated DEGs, respectively. We found that DEGs in ACC mainly associated with circulatory system related pathways (Fig 4a), while DEGs in PFC were enriched in metabolic system related pathways (Fig 4b). The gene count and pathway P values were compared respectively (Fig 4c). The results showed that along with the increase of gene count, the P value of pathway was elevated. Thus, the pathways enriched by more DEGs were more likely to associate with disease. Moreover, the genes participated in more signaling pathways were more likely to play important roles during disease progression. We further performed cross talk analysis on these DEGs enriched pathways, to identify the cross talk genes that regulate multiple signaling pathways.

Cross talk analysis

A regulatory network was constructed using signaling pathways and genes enriched in the pathways. Two types of nodes were contained, signaling pathways and genes; three types of connections were included, signaling

pathway-gene (belong), gene-gene (co-express) and gene-gene (interact). The relationship of “belong” was extracted from KEGG pathway database; the “co-express” relationship was from co-expression network; the “interact” relationship was extracted from HPRD database (http://www.hprd.org/). As a result, a pathway-gene complex network was established including 219 relationship pairs, 16 signaling pathways and 70 genes (Fig 5a).

The network topological properties were analyzed and the degree distribution was obtained. The signaling pathways and genes with highest degrees ranking as top 10 were extracted (Table 2). These pathways were associated with more DEGs suggesting a significant correlation between the abnormal of these functions with the occurrence of depression. While, DEGs with high degrees interacted or co-expressed with each other participated in multiple signaling pathways, they may act as cross talk genes dysregulated in disease progression, and may also be potential novel therapeutic targets.

We further statistically analyzed the significance of the top10 genes through group comparison between ACC and PFC in both disease and normal conditions (Table 3). We found 9 genes significantly differentially expressed in PFC of patients with depression, while 6 genes significantly differentially expressed in ACC of patients with depression, excluding ITGA3, MAPK11, PAK6 and DUSP8 (Table 3). The results exhibited that significant difference exists between ACC and PFC during development of depression, reflecting the specificity of alteration in each tissue, but the sharing 6 DEGs in both tissues revealed the consistency of changes in both tissues during disease progression. Receiver operating characteristic (ROC) values of genes were calculated in the two tissues. All the ROC values of genes were higher than random state (0.5) (Fig 5b and Table 3). To examine whether these top10 genes are capable to be potential novel therapeutic targets, we queried these top10 genes in Drugbank database. As shown in Table 4, five genes were known targets that targeted by several drugs. Literature mining results showed that except Blinatumomab and KC706, other drugs have been reported to be related with brain tissue injury and cerebral nervous system diseases. Moreover, MAPK11 and PAK6 only differentially expressed in PFC, while CACNA1A, PTDSS2 and CD19 differentially expressed in both ACC and PFC. Therefore, these three genes may correlate with depression progression or other cerebral nerve system related diseases, are more likely to become the new drug targets.

Discussion

In the current study, we analyzed DEGs in ACC and PFC from patients with MDD. Correlation networks based on co-expression were constructed. Topological properties of the networks were analyzed and compared. Our results showed that the lesions of brain tissues in MDD patients were not synchronized and alterations of biological functions were not consistent either, ACC showed a greater degree of abnormality as compared to PFC suggesting a higher correlation with disease progression. We consequently analyzed the signaling pathways enriched by DEGs and further cross talk genes that bridge the multiple pathways were also identified. Through construction of the pathway-gene complex network, the genes and singling pathways with top10 degrees were extracted, which are more likely to be potential novel therapeutic targets. We also mined the drugbank database for the top10 cross talk genes to explore their drugable target potential. CACNA1A, PTDSS2 and CD19 differentially expressed in both ACC and PFC may correlate with depression progression or other cerebral nerve system related diseases, are more likely to become the new drug targets.

Indeed, a network perspective supports the high heterogeneity of depression, and explains how different treatment methods might take effect (10, 23). Comparisons across many datasets may show novel cross-tissue

communication and similarities in different diseases (19). Therefore, we accomplished a comprehensive analysis of gene expressions across ACC and PFC in patients with MDD and normal controls. Through analyzing and comparing the four co-expression networks, our results showed that as compared to normal condition, the unconnected nodes were increased in disease condition, which may be attributed to loss of connections. Alteration in important nodes of the network may have impact on the function of the entire network, causing depression. The topology analysis of the co-expression network indicated that in disease states, the PFC network state tended to be normal, whereas the ACC network showed drastic fluctuations. Substantial evidence from healthy subjects has linked the ACC to emotional behavior. This region uses information about punishment to manage aversively motivated actions. PFC is important in many brain functions and is a target for some neurodegenerative diseases (34). Stress could increase susceptibility to inflammation in the PFC, which shows a relative resistance to inflammation (34). We also analyzed the functional pathways enriched by DEGs in the two tissues. We found that DEGs in ACC mainly associated with circulatory system related pathways, while DEGs in PFC were enriched in metabolic system related pathways. Previous studies using postmortem brain samples have implicated dysregulated brain-derived neurotrophic factor (BDNF), gamma amino acid butyric acid (GABA), glutamate and oligodendrocyte functions in MDD (24). Glutamate and glutamine levels are reduced in the PFC.

Signaling molecules commonly do not work individually but interact with other proteins or biological molecules to realize signal transmission. Therefore, to decipher the paths along which the signaling proteins move as well as the molecules that manage these processes is crucial for gaining further understanding of MDD. Moreover, these signaling components that may co-express in a dataset and correlate across samples are predicted to reconstruct multiple signaling pathways and the map of their cross-talks for further biomedical research. Hence, co-expression links have been used to establish gene networks and to identify sets of genes with shared functions (19). Cross-talk analysis is commonly used to study the regulatory and cooperative relationship between signaling pathways, based on which to further reveal the pathogenesis of diseases (35). Therefore, through analyzing the pathway-gene network, we identified ten pathways and ten cross talk genes with highest degrees, such as Glycerophospholipid metabolism and MAPK (Ras-mitogen-activated protein kinase) signaling pathway, CACNA1A and PTDSS2, which may have important implications in pathogenesis of depression and may be new treatment targets.

Regarding to the signaling pathways identified in our study, recent studies suggest an important role for brain membrane lipids in the pathogenesis of depression and anxiety disorders, which could be exploited for improved lipid-based prevention and treatment (36). The typical glycerophospholipids (GPLs) found in mammalian membranes include phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylserines (PS) and phosphatidylinositols (PI) that are all attached through a phosphodiester linkage (36). Phosphatidylinositol-3 kinase (PI3K) activity was found significantly reduced in the PFC and hippocampus of psychiatric suicide victims as compared to non-psychiatric non-suicide subjects (37). It is worth noting that depressed patients had a significant decrease in their PI3K and Akt (serine threonine kinase or protein kinase B) activities compared with non-depressed non-suicide subjects (38). Preclinical findings have shown that the membrane-forming n-3 polyunsaturated fatty acids, glycerolipids, GPLs, and sphingolipids (SPLs) played a crucial role in the induction of depression- and anxiety-related behaviors. In alcohol abuse, a major risk factor for somatic and neuropsychiatric diseases, plasma GPL and SPL species were altered (39). These results offer new treatment options such as pharmacological interference with lipid-regulating enzymes (36). Basic and clinical studies demonstrate that BDNF and BDNF-stimulated signaling cascades, including MAPK and PI3K-Akt pathways are decreased by stress and depression, and increased by antidepressant treatments (40, 41). Moreover, BDNF-tropomyosin-related kinase

B (TrkB) downstream signaling also includes activation of the phospholipase Cg (PLCg)-Ca2+ pathways (42). These pathways activate mammalian target of rapamycin (mTOR) signaling and the mTOR complex 1 (mTORC1), increasing S6 kinase and affecting expression of synaptic proteins, postsynaptic density-95 (PSD95) as well as the subsequent signaling pathways related to synaptogenesis. Clinical studies have shown that a single sub-anesthetic dose of ketamine can induce rapid, sustained antidepressant effects(43, 44).While mammalian targets of the rapamycin (mTOR) signaling pathway mediate the antidepressant effects of ketamine by improving neurogenesis and plasticity(45).

Several cross talk genes in our study were already known drug targets for treatment of brain and neurologic diseases. For instance, the CACNA1A gene that encodes the alpha-1A subunit of neuronal Ca(v)2.1 Ca(2+) channels (46). Through mining the DrugBank database, CACNA1A is a drug target of pregabalin, which can protect against and delay epileptogenesis (47). Studies have found that there is a comorbidity between epilepsy and MDD. In addition, some antiepileptic drugs may have an inhibitory effect on mood(48-50).Our results showed high potential of these genes to be drug targets for MDD therapy. Phosphatidylserine synthase 2 (PTDSS2) is capable to convert PE to PS and shows high expression in specific tissues (e.g. brain and testis), participating in important cell signaling processes (36, 51). P21-activated kinases (PAKs) belongs to serine-threonine kinases serving as targets for the small GTP binding proteins Cdc42 and Rac1. They play important roles in morphogenetic processes of synapse formation and neuritogenesis through regulating cytoskeletal motility (52). PAK6 has been identified as a mediator for the effects of androgen receptor (AR) and glucocorticoid receptor (GR) on dopaminergic transmission (53). PAK6 was strongly co-expressed with GR in the dopaminergic regions (54), and PAK6 KO mice displayed some locomotion and behavioral deficits, possibly because the disturbed dopaminergic transmission (55). Thus, these results provide insight into the biology of complex disorders, these known drug targets have the potential to be used in the treatment of MDD.

Our research identified several critical genes and provides some interesting clues for further experiments. However, we did not conduct experimental tests on any of these selected genes, which was a limitation of this study. Subsequently, a large number of clinical samples will be needed to validate our findings investigate the function of these genes in the regulation of MDD.

Conclusions

In conclusion, this study allowed the identification of a focused set of genes for use in future genetic association studies, and together demonstrates the importance of integrating cross tissue data, gene co-expression and cross talk signaling results, paving the way for novel and complementary approaches to investigate the molecular pathology of MDD and other complex brain disorders.

Abbreviations

MDD: major depressive disorder; ACC: postmortem anterior cingulate cortex; PFC: prefrontal cortex; DEGs :differentially expressed genes ; KEGG:the Kyoto Encyclopedia of Genes and Genomes); FDR: false discovery rate; :HPRD: Human Protein Reference Database ; ROC :receiver operating characteristic ; BDNF :brain-derived neurotrophic factor; GABA:gamma amino acid butyric acid ; GPLs: glycerophospholipids; PC: phosphatidylcholines; PE:phosphatidylethanolamines; PS□phosphatidylserines□PI:phosphatidylinositols; PI3K :phosphatidylinositol-3 kinase ; SPLs:sphingolipids ; mTORC1:mTOR complex 1; PSD95:postsynaptic density-95;

PTDSS2:phosphatidylserine synthase 2; PAKs:P21-activated kinases ; AR :androgen receptor; GR:glucocorticoid receptor.

Declarations

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Availability of data and material

The GeneChip data were retrieved from the GEO data repository (http://www.ncbi.nlm.nih.gov/geo/) with the accession numbers E-GEOD-54572,'E-GEOD-54571, E-GEOD-54565, E-GEOD-54564, E-GEOD-54563, E-GEOD-54562, E-GEOD-54570, E-GEOD-54568, E-GEOD-54567, E-GEOD-45642, E-GEOD-35978, E-GEOD-12654.

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Authors' contributions

HX designed the study, analysed the data, performed computational coding. HX and GRJ involved in collecting the data, drafting the manuscript and revising it critically for important intellectual content.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. The comparison of network topological properties.

summary	Anterior_normal	Anterior_disease	Prefrontal_normal	Prefrontal_disease
nodes	388	346	465	472
edges	1604	735	2941	2287
unconnected nodes	89	111	68	92
clustering coefficient	0.295	0.181	0.301	0.275
Density	0.021	0.012	0.027	0.021
centralization	0.101	0.078	0.141	0.118

Table 2. Top10 pathways and genes in the pathway-gene complex network.

pathway	degree_path	gene	degree_gene
Glycerophospholipid metabolism	14	CACNA1A	22
MAPK signaling pathway	11	PTDSS2	19
Hematopoietic cell lineage	10	DIAPH1	15
Regulation of actin cytoskeleton	9	ITGA3	14
Focal adhesion	8	HRAS	14
Neuroactive ligand-receptor interaction	8	MAPK11	11
ABC transporters	8	DUSP8	11
T cell receptor signaling pathway	7	CD19	11
Heparan sulfate biosynthesis	6	PAK6	10
Dilated cardiomyopathy	5	NDST2	10

Table 3. Significant analysis using top 10 genes.

gene	Pvalue_anterior vs control	Pvalue_prefrontal vs control	Pvalue_anterior vs prefrontal	Pvalue_control1 vs control2	ROC_anterior	ROC_prefrontal
CACNA1A	*0.030755*	*0.030753*	0.909042	0.894571	0.6061	0.6153
PTDSS2	*0.002647*	*0.008945*	0.972924	0.961831	0.6408	0.6432
DIAPH1	0.016184	0.007884	0.848983	0.858656	0.6158	0.6441
ITGA3	0.270458	0.072082	0.719097	0.663678	0.5499	0.5996
HRAS	0.032489	0.021718	0.939288	0.816604	0.5976	0.6203
MAPK11	*0.256193*	*0.045961*	0.653705	0.612708	0.5493	0.5996
DUSP8	0.085667	0.030195	0.868925	0.726374	0.5831	0.6102
CD19	*0.017437*	*0.035308*	0.848987	0.937282	0.6121	0.6076
PAK6	*0.128097*	*0.030015*	0.703335	0.669699	0.5764	0.6193
NDST2	0.000798	0.000924	0.92247	0.8554	0.6634	0.6808

*control 1 represents the normal anterior cingulate cortex; control 2 represents the normal prefrontal cortex.

Table 4. Drugable target information for the top 10 genes.

gene	Target (yes/no)	Drug counts	drugs	PMID
CACNA1A	yes	4	amlodipine, loperamide, lyrica, pregabalin	25918454, 26390138, 26138193, 26670374
CD19	yes	1	Blinatumomab	
PAK6	yes	4	Dextromethorphan, Tizanidine, Agmatine, Moxonidine	26471212, 23648652, 26678503, 24333661
MAPK11	yes	2	KC706,Regorafenib	25563977
PTDSS2	yes	1	Phosphatidylserine	26689775
DIAPH1	no	0		
ITGA3	no	0		
HRAS	no	0		
DUSP8	no	0		
NDST2	no	0		

Figures

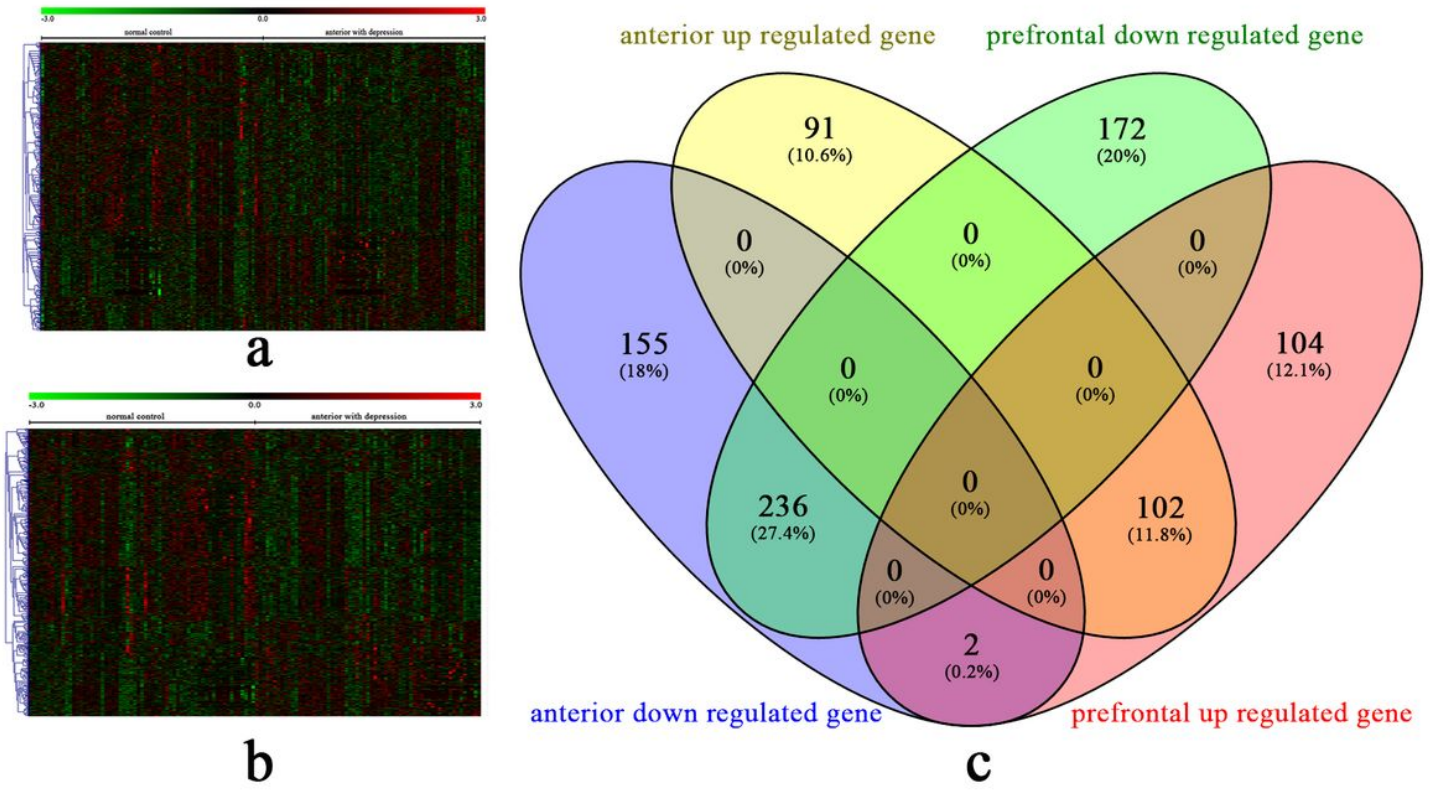

Figure 1

The differentially expressed genes (DEGs) in ACC and PFC of patients with depression. (a, b) Heatmap of DEGs in ACC and PFC, respectively. (c) The venn graph of DEGs between ACC and PFC. ACC, anterior cingulate cortex; PFC, prefrontal cortex.

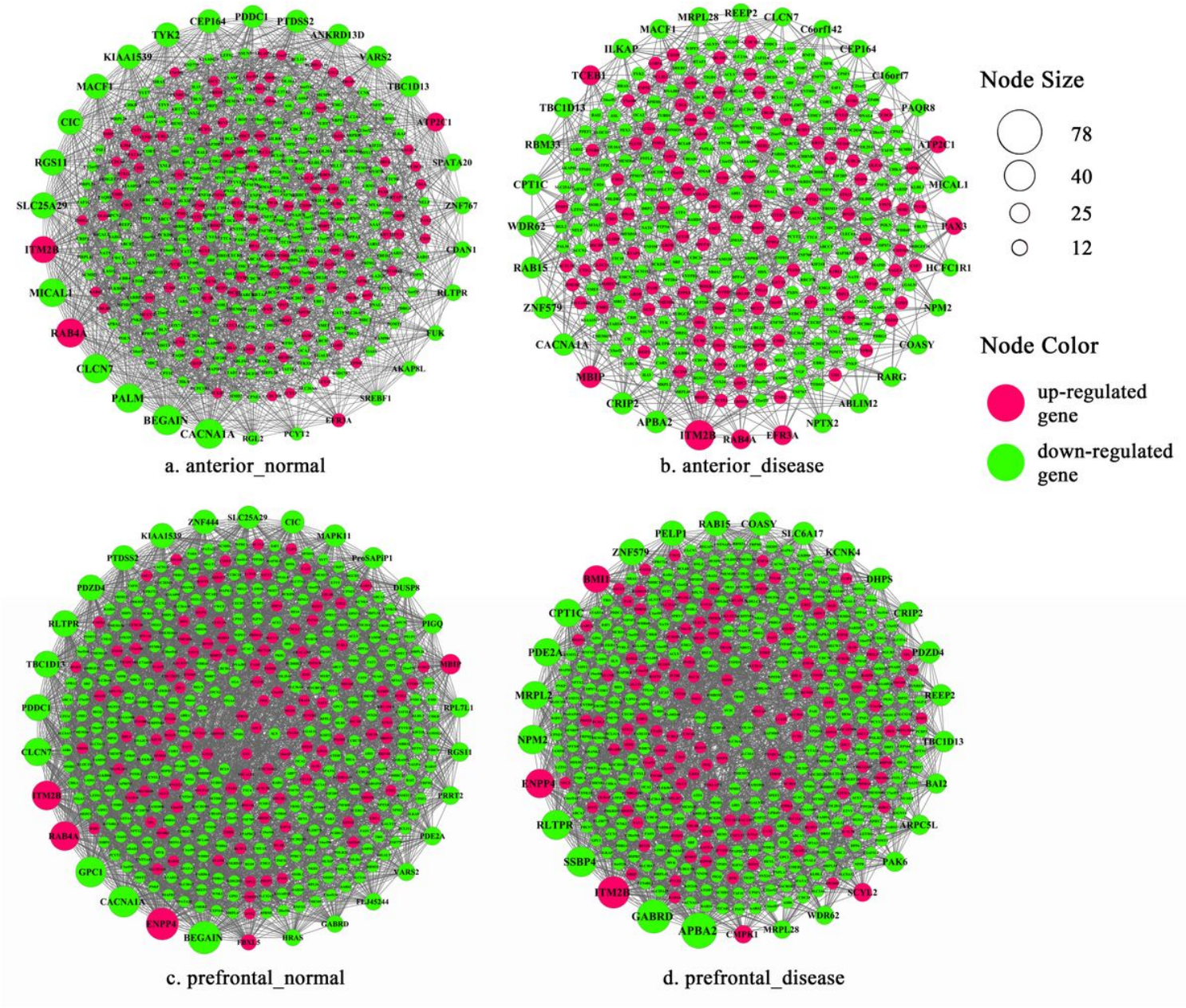

Figure 2

The co-expression network of (a) ACC normal, (b) ACC disease, (c) PFC normal and (d) PFC disease. ACC, anterior cingulate cortex; PFC, prefrontal cortex.

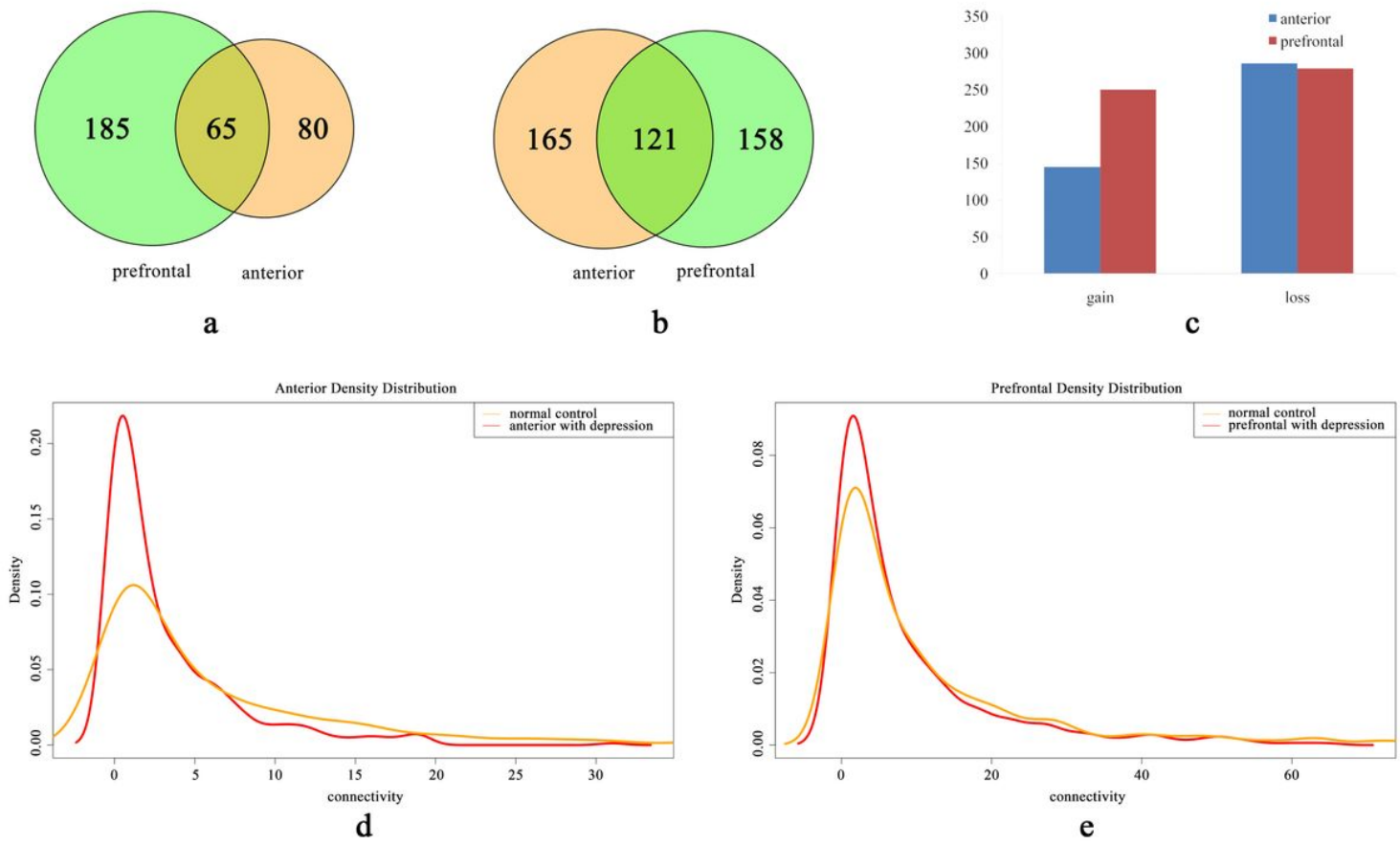

Figure 3

Comparative analysis of network difference. (a) The node counts of gain of connections between ACC and PFC; (b) The node counts of loss of connections between ACC and PFC; (c) Comparison of nodes with gain or loss of connections in ACC and PFC; (d) Probability density distribution of co-expression network in ACC under normal and depression conditions; (e) Probability density distribution of co-expression network in PFC under normal and depression conditions. ACC, anterior cingulate cortex; PFC, prefrontal cortex.

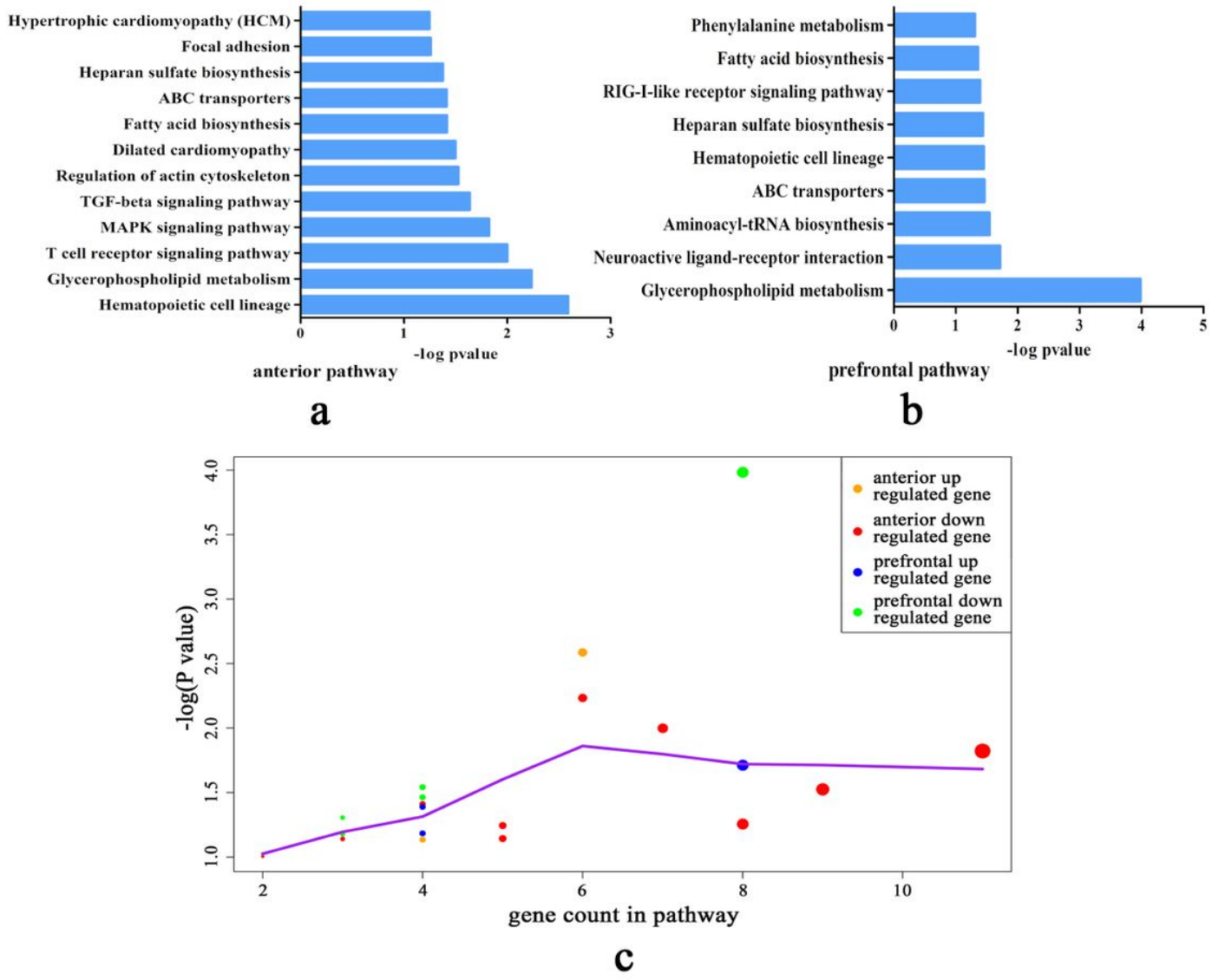

Figure 4

The pathway analysis for the DEGs of (a) ACC and (b) PFC. (c) Comparative analysis on gene count and pathway P value. The size of the nodes represents the number of DEGs that hit in the pathway; the bigger the size of node, the greater the count. The purple line represents linear fitting. ACC, anterior cingulate cortex; PFC, prefrontal cortex.

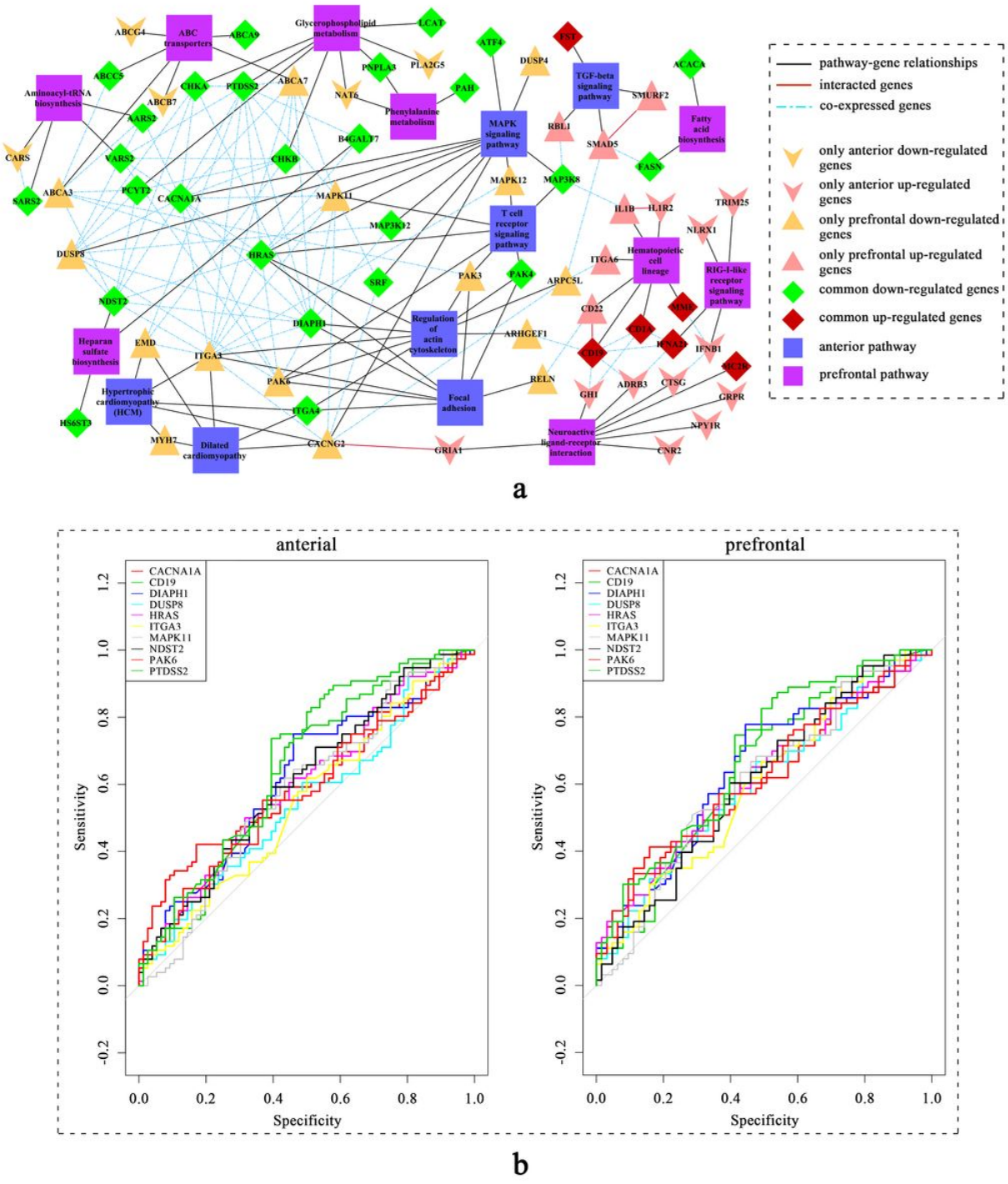

Figure 5

The pathway-gene complex network. (a) Pathway-gene complex network. (b) The ROC curve of top 10 genes in network.