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Integrative network analysis identifies differential regulation of neuroimmune system in Schizophrenia and Bipolar disorder

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ABSTRACT

Background: Neuropsychiatric disorders such as Schizophrenia (SCZ) and Bipolar disorder (BPD) pose a broad range of problems with different symptoms mainly characterized by some combination of abnormal thoughts, emotions, behaviour, etc. However, in depth molecular and pathophysiological mechanisms among different neuropsychiatric disorders have not been clearly understood yet. We have used RNA-seq data to investigate unique and overlapping molecular signatures between SCZ and BPD using an integrative network biology approach.
Methods: RNA-seq count data were collected from NCBI-GEO database generated on post-mortem brain tissues of controls (n = 24) and patients of BPD (n = 24) and SCZ (n = 24). Differentially expressed genes (DEGs) were identified using the consensus of DESeq2 and edgeR tools and used for downstream analysis. Weighted gene correlation networks were constructed to find non-preserved (NP) modules for SCZ, BPD and control conditions. Topological analysis and functional enrichment analysis were performed on NP modules to identify unique and overlapping expression signatures during SCZ and BPD conditions.
Results: We have identified four NP modules from the DEGs of BPD and SCZ. Eleven overlapping genes have been identified between SCZ and BPD networks, and they were found to be highly enriched in inflammatory responses. Among these eleven genes, TNIP2, TNFRSF1A and AC005840.1 had higher sum of connectivity exclusively in BPD network. In addition, we observed that top five genes of NP module from SCZ network were downregulated which may be a key factor for SCZ disorder.
Conclusions: Differential activation of the immune system components and pathways may drive the common and unique pathogenesis of the BPD and SCZ.

1. Introduction

Mental disorder are mainly characterized by some combinations of abnormal thoughts, emotions, behaviour, and relationships with others (Watson et al., 2005). Apart from social and cognitive impacts, it also affects the global economic growth at a higher rate than other health issues such as cardiovascular diseases, cancer, and diabetes (Bloom et al., 2011). Mental disorders are known to be multifactorial in nature. Brain structure and its function, environmental factors, malnutrition before birth, problems during birth, psychosocial factors, familial history, genetic components (certain genes) and immunological responses are some of the factors which contribute to mental disorders (Craddock et al., 2006; Felger et al., 2016; Meyer et al., 2011). SCZ and BPD are the most prevalent mental disorders (Charlson et al., 2019). BPD is commonly known as manic depressive disorder which causes unusual shifts in mood, energy, activity level and ability to carry out day-to-day activity (Gitlin et al., 1995). SCZ is a chronic and severe mental disorder which affects a person's thoughts, feelings, and behaviours. The major

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treatments for this disorder are antipsychotic drugs, psychosocial therapies and coordinated special care (De Hert et al., n. d.; Geddes and Miklowitz, 2013; Lieberman et al., 2005).

Anterior Cingulate Cortex (AnCg) is the front part of cingulate cortex, located towards the front of Corpus callosum in the medial frontal lobe of the human brain (Gabriel et al., 2002). AnCg is responsible for detection of errors, detection of conflicts, reward-based learning, emotional regulation, experience of sadness, anxiety, ironic rebound and source of hemisity etc.(Bush et al., 2000). Abnormalities in AnCg region have been implicated in several neuropsychiatric disorders such as SCZ, depression, BPD, obsessive-compulsive disorder, autism and post-traumatic stress disorder etc. (Anticevic et al., 2015; Benes, 1993; Rauch et al., 1994). Reduced functional connectivity at resting stage of ventral anterior cingulate cortex was found in BPD patients and also showed similar pattern of connectivity with SCZ (Anticevic et al., 2015), which suggested a common mechanism behind the psychotic symptoms of these disorders. In a recent study, it was noted that pro-inflammatory cytokines viz. TNF-α, IL-6, IL-8, IL-2, IL-4 and IL-6 were elevated in BPD. Similarly, TNF-α, IFN-γ, IL-12 and sIL-2R were also found to be elevated in SCZ (Najjar et al., 2013). In the AnCg region, it was observed that increase in interleukin-1β (IL-1β) and IL-6 gave rise to suicidal thinking (Holmes et al., 2018).

Computational psychiatry is a novel field which uses integrative approaches to draw inferences on brain functions by studying gene expression deviations, interaction among genes and roles of genes in biological functions (Maia et al., 2017; Moutoussis et al., 2017; Pergola et al., 2019). Ramaker et al. compared molecular signatures across different disorders and brain regions (Ramaker et al., 2017). They have observed majority of disease associated gene expression changes were in the AnCg region. However, their work was limited to only differential expression analysis of the genes. Several studies have reported that AnCg region was associated with multiple biological functions such as cognition, error detection, conflict resolution, motivation, and modulation of emotion (Carter et al., 1998; Paus, 2001; Weissman et al., 2005). Significant overlap in gene expression changes in BPD and SCZ of AnCg region and their findings were reported in literature (Thompson et al., 2009; Woo et al., 2008). Such findings motivated us to explore further about the overlapping and unique molecular signatures by performing co-expression analysis, preservation analysis, topological analysis and gene set enrichment analysis on significant DEGs identified from BPD and SCZ samples of the AnCg region in post-mortem brain samples.

To this end, we have analysed a homogeneous RNA-seq dataset generated from post-mortem brain tissues of AnCg region in BPD (n = 24), SCZ (n = 24) patients and controls (n = 24) using multiple benchmarked algorithms. We have carried out differential expression analysis, co-expression analysis, preservation analysis, topological analysis and enrichment analysis of the dataset. Using an integrative approach, we have identified the overlapping and unique set of genes, components of core and specific pathway for BPD and SCZ. By comparing co-expression networks of control and disease using preservation analysis, we identified two non-preserved (NP) gene co-expression modules from the control samples, one NP gene co-expression module from each BPD and SCZ samples. Network topological analysis supported by literature and database mining could identify overlapping and unique genes with known or potential functional association with BPD and SCZ. Identification of overlapping and disease-specific set of genes and pathways will be advantageous for developing effective therapeutic intervention and possible prevention measures.

2. Methods and materials

2.1. Dataset collection and preprocessing

We have collected the publicly available RNA-seq data GSE80655 from NCBI Gene Expression Omnibus (GEO) database (Ramaker et al., 2017). It was collected from three different brain regions AnCg, nucleus accumbens and dorsolateral prefrontal cortex from 281 persons diagnosed with SCZ, BPD, major depressive disorder (MDD) and normal controls. We have extracted 96 samples from AnCg region and used count data of AnCg region of two different disorders, i.e., BPD (n = 24), SCZ (n = 24) patients and normal controls (n = 24).

Before performing differential expression (DE) analysis on RNA-seq count data, it is necessary to perform pre-processing steps such as low read counts removal, normalization and transformation (Chowdhury et al., 2019). Initially, we removed genes that had very low read counts under each sample as these genes do not contain much information. A gene was removed from downstream analysis if the gene did not contain on an average 10 read counts across the whole samples of control and corresponding disorder (Love et al., 2014). Widely used differential expression analysis tools such as DESeq2 (Love et al., 2014) and edgeR (MD et al., 2009) have built in strategy to remove low read counts genes. RNA-seq count data contains various types of biases due to the different sequencing depth and transcript sizes (Chowdhury et al., 2018). To handle such biases, normalization is important. We used two normalization methods, i.e., DESeq and TMM available with DESeq2 and edgeR packages, respectively. RNA-seq count data follow discrete data distribution (Chowdhury et al., 2018). Therefore, it is necessary to perform transformation to use statistical or computing methods to analyse RNA-seq count data. We used *log2* transformation to transform the datasets into normal like distribution before performing co-expression analysis on the selected differentially expressed genes (DEGs).

2.2. Differential expression analysis

Differential expression analysis was performed to compare the expression patterns of a gene between two different group of samples. It is used to find genes which are differentially expressed across groups of samples or in diseased conditions. Most differential expression analysis tools work in two steps (Love et al., 2014; MD et al., 2009): (1) estimation of fold change by considering sequencing depths and variability in sequencing depths and (2) estimation of significance using test statistics.

After removal of low read counts and normalization of count data, two widely used DE analysis tools viz., DESeq2 and edgeR were employed to perform DE analysis. Consensus of the results of these tools were built for selection of more accurate list of DEGs for further analysis. These two tools also support different test statistics such as Wald-test, *t*-test, LRT to compute the significant of differential expression. A gene having low read counts may become more significant falsely. To control this, we used Benjamini-Hochberg (BH) method to compute the *Padjusted* value for each gene and then we used *Padjusted* value < 0.05 as a cut-off criterion to select DEGs. Our consensus selects those genes as differentially expressed which are selected by both the tools.

2.3. Co-expression network analysis

Co-expression network analysis identifies groups of tightly correlated genes responsible for biological process or pathways or phenotypic variations (Chowdhury et al., 2019; Kakati et al., 2019, 2016). Most widely used workflow of co-expression analysis comprises of four steps (Langfelder and Horvath, 2008)'(Chowdhury et al., 2019): (1) Computation of pairwise similarity of genes, (2) construction of co-expression network, where the network may be signed or unsigned or weighted or unweighted, (3) clustering to identify modules and (4) functional and topological analysis of modules to associate modules with external biological knowledge. WGCNA is the most widely used co-expression analysis tool to find functionally interesting modules of genes (Langfelder and Horvath, 2008).

Before performing co-expression analysis using WGCNA tool, we performed *log₂* transformation of the RNA-seq count data. WGCNA recommends excluding outlier samples before estimation of β value which is later used to construct biologically relevant scale free network topology. If the dataset contains outlier samples then estimation of β value may be

inappropriate. We performed sample hierarchical clustering to detect and remove outlier samples, using to compute the appropriate β value for the construction of scale free topology for co-expression analysis. WGCNA is the most widely used co-expression analysis method for construction of co-expression networks by applying some power to the correlation value computed in step (1) using the equation $Adj_{ij}=(abs(cor(g_i, g_j)))^{\beta}$, where β is called the soft threshold(Langfelder and Horvath, 2008).

Where each Adj_{ij} indicates connection strengths between gene g_i and g_j. The power β is chosen based on criteria of approximating scale-free topology of the network (Ravasz et al., 2002). For disorder and control appropriate β were chosen. This β value is the lowest integer which satisfies approximate scale-free topology (linear regression model fitting index R^2 that should be larger than 0.8). Topological overlap matrix (*TOM*) was created from Adj_{ij}, and later *TOM* was converted into a dissimilarity $TOM, {}_{disimilarity} = (1 - TOM {}_{similarity})$ and that is considered as co-expression network (Langfelder et al., 2011). We used hierarchical clustering and dynamic tree cut method to identify different modules present in the constructed network. Each module contained densely interconnected genes. The detailed workflow is presented in Fig. 1.

2.4. Preservation analysis

Preservation analysis helps in evaluating whether a module defined in the reference network can also be found in test network or not (Langfelder and Horvath, 2008). It also helps in finding hidden information presents in the two compared networks by quantifying the structural changes in the modules. A module may be preserved in test network due to natural selections or disrupt due to some changes in pathways or biological processes (Chowdhury et al., 2019). We hypothesized that, those modules, which were weakly preserved in diseases, might influence the pathogenesis process compared to the control network. Two widely used preservation statistics named *Z-summary* and *medianRank* provide overall significance of the preservation of a module based on density and connectivity (Langfelder and Horvath, 2008). When a module contains a smaller number of genes than the module may show large value of *Z-summary* falsely because of the permutation test used while calculating *Z-summary* (Langfelder and Horvath, 2008). To handle this problem, *medianRank* statistics is used as it provides relative preservation ranking of modules. The calculated *Z-summary*<2 indicates no preservation, 2≤*Z-summary*≤10 indicates weak to moderate preservation, and *Z-summary*>10 means strong preservation (Langfelder and Horvath, 2008),(Chowdhury et al., 2019). Most NP modules usually get the worst *medianRank* values.

2.5. Topological and differential connectivity based analysis

We performed topological analysis of weakly preserved module using R package igraph (Csárdi and Nepusz, n. d.). Topological analysis allows to study different network features such as degree and connectivity to find interesting genes along with characterization of the network (Langfelder and Horvath, 2008) (Chowdhury et al., 2019). It also helps to find interesting genes, responsible for the non-preservation of a module. The highly connected genes in the network can be considered as hub gene. We considered degree centrality, change of degree centrality, sum of connectivity and change in sum of connectivity as topological analysis parameters on NP modules for further analysis. The degree of a node is the number of connections it has with other nodes in the module. While finding degree we considered those edges which have connection strength greater than the mean connection strength of the module in the reference network. We used degree and sum of connectivity to identify the hub genes present in the module. Differential connectivity analysis help in finding genes which changes significant number of degree or sum of connectivity in test network (Chowdhury et al., 2019). We have used degree difference and sum of connectivity difference as differential connectivity parameter to quantify how much a gene can be considered as border or central while going from reference to test sub-network.

2.6. Gene set enrichment analysis (GSEA)

GSEA is a method to identify a set of genes that are overrepresented in a large set of genes which may have an association with diseases (Chowdhury et al., 2019, 2018; Kakati et al., 2016). We used Database for Annotation, Visualization Integrated Discovery (DAVID) for elucidating the biological insights of the NP modules, by Gene ontology and pathway analysis (Hosack et al., 2003). The significance of the enrichment terms was considered by applying p-value < 0.05. To further understand the relationship of biological processes in which NP module genes are involved, we used BiNGO (Maere et al., 2005), a Cytoscape plugin (Langfelder and Horvath, 2008), which overrepresented gene ontology in a set of genes or a subgraph of biological network. It also maps the predominant function of a given gene set.

3. Results

3.1. Common and unique differentially expressed genes among BPD and SCZ

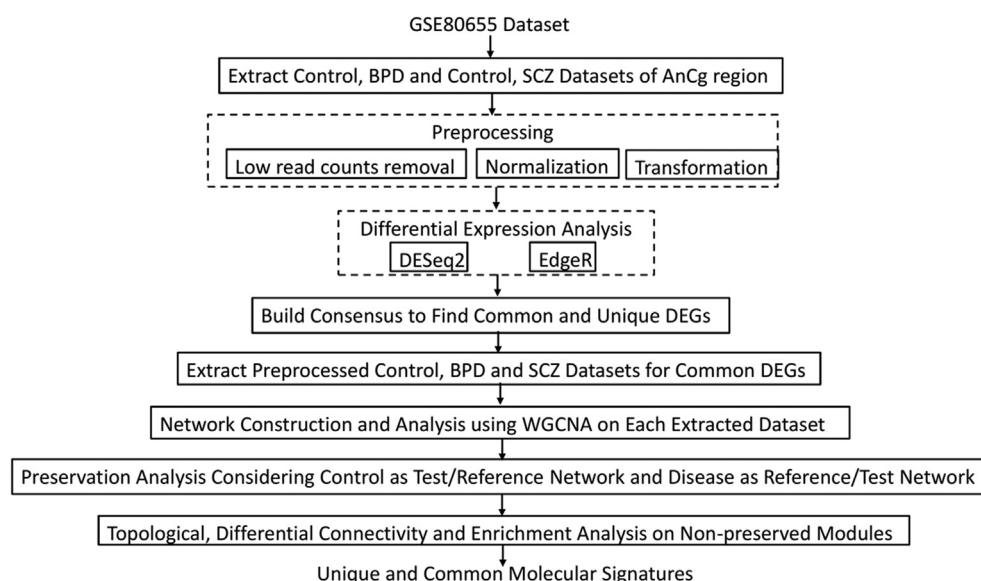After removal of low read counts 14,717 genes in BPD and 14,753

Fig. 1. Workflow. After extracting the control and diseases dataset separately, required pre-processing steps was performed. Then, differential expression analysis on both the datasets was carried out and results were combined through a consensus. After finding common and unique DEGs, co-expression network construction and analysis was performed on control, BPD and SCZ dataset considering common DEGs. Next, preservation analysis was carried out to identify all the NP modules present in all the constructed networks by considering control and disease network as reference or test network. Finally, topological, differential connectivity and enrichment analysis was performed to identify interesting signatures.
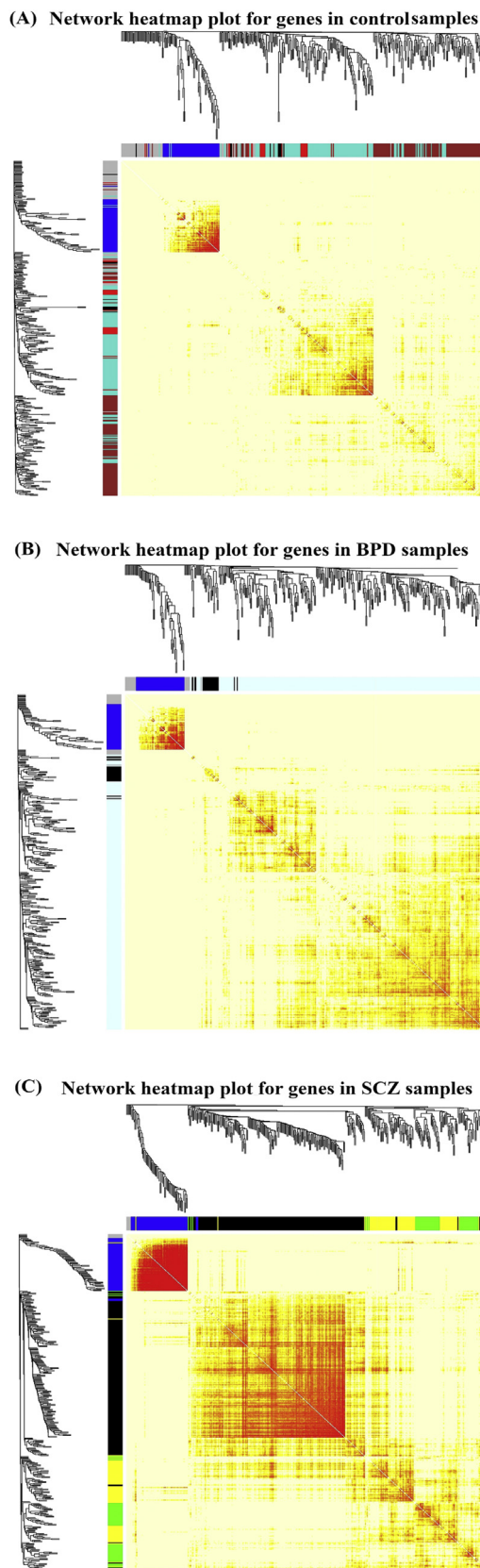
(A) Network heatmap plot for genes in control samples

(B) Network heatmap plot for genes in BPD samples

(C) Network heatmap plot for genes in SCZ samples

Fig. 2. Clustering dendrogram Heatmap with dissimilarity based on topological overlap, together with assigned module colours. (A) for genes in control samples, (B) for genes in BPD samples and (C) for genes in SCZ samples. Distinct co-expression signatures are visible among control, BPD and SCZ.

genes in SCZ were remained. To identify common and unique DEGs among SCZ and BPD through comparison of gene expression levels between normal and diseases, we used DESeq2 and edgeR tools. The number of DEGs detected by DESeq2 were 1,617 and 1,307 from BPD and SCZ, respectively. On the other hand, significant number of DEGs from edgeR were 415 and 1,524 from BPD and SCZ, respectively (Supplementary Table 1). We observed a drastic change in number of DEGs for BPD cohort. It may be due to the false negative DEGs detected by EdgeR. We have used consensus of both EdgeR and DESeq2 tools for selecting the list of DEGs for further downstream analysis. The cut-off threshold value i.e., *P-adjusted* < 0.05 was used to select significant DEGs. In Supplementary Fig. 1A, we show the comparison of DEGs detected by EdgeR and DESeq2 tools in BPD and SCZ conditions. We found that there were 787 and 325 DEGs identified by DESeq2 and edgeR, respectively which were common in BPD and SCZ as shown in Supplementary Figs. 1(B and C). In total, 830 DEGs were unique to BPD and 520 DEGs were unique to SCZ obtained from DESeq2 tool. On the other hand, 90 DEGs were unique to BPD and
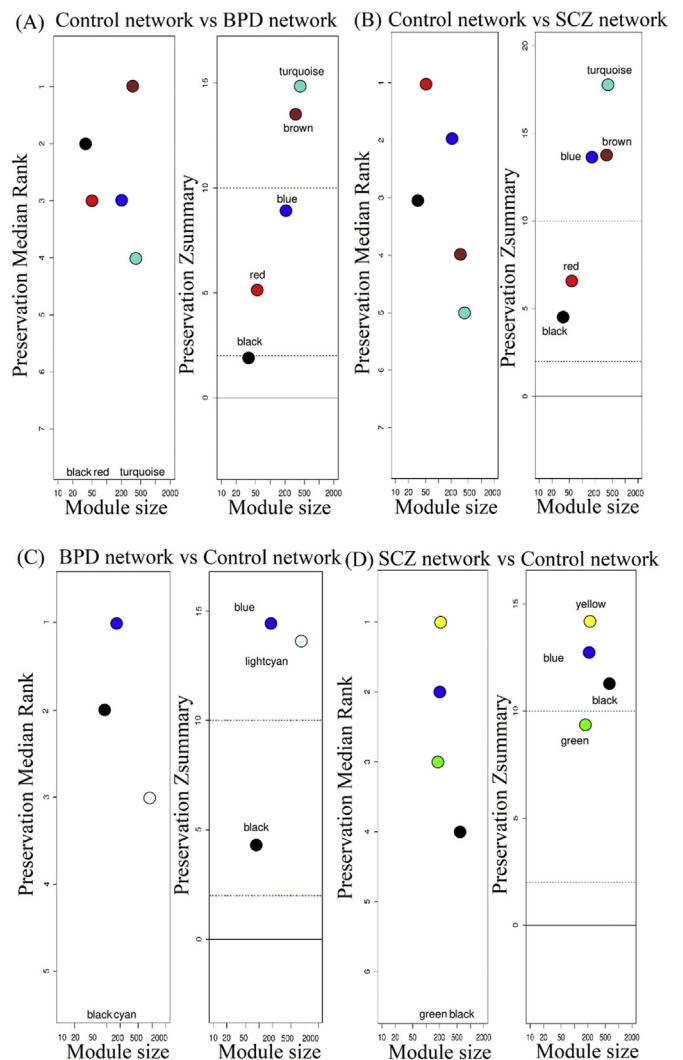

Fig. 3. Module preservation Chart of control, BPD and SCZ samples. (A) Using the five modules of control network we compared with the Bipolar disorder network. *Black* and *Red* are non-preserved module that consists of 37 and 54 genes, respectively. (B) Using the five modules of control network we compared with the Schizophrenia disorder network. *Black* is non-preserved module that consists of 37 genes. (C) Using the three modules of Bipolar Disorder network we compared with the control network. Here, *Black* module is found to be NP. *Black* module consists of 87 genes. (D) Using the four modules of Schizophrenia network we compared with the control network. Here, *Green* module is found to be NP. *Green, Black* module consists of 179 genes.

1199 were unique to SCZ as obtained from edgeR tool. DEGs that are common between BPD and SCZ given by DESeq2 and edgeR tool (398 and 1,283 respectively) are presented in Supplementary Figs. 1(D and E).

3.2. Co-expression network construction and module finding

To perform co-expression network analysis, we considered 1283 common significant DEGs for BPD and SCZ from DESeq2 and edgeR tool. We applied log_2 transformation to transform the data distribution into normal like distribution. Sample clustering was performed to detect the outliers. The samples X1834_AnCg_C_SL31501, X4063_AnCg_C_SL6003, X3281_AnCg_C_SL5883 and X3523_AnCg_C_SL7800 were found to be outliers in the group of control samples in (Supplementary Fig. 2A), X3452_AnCg_B_SL6414 was identified as an outlier in the group of BPD
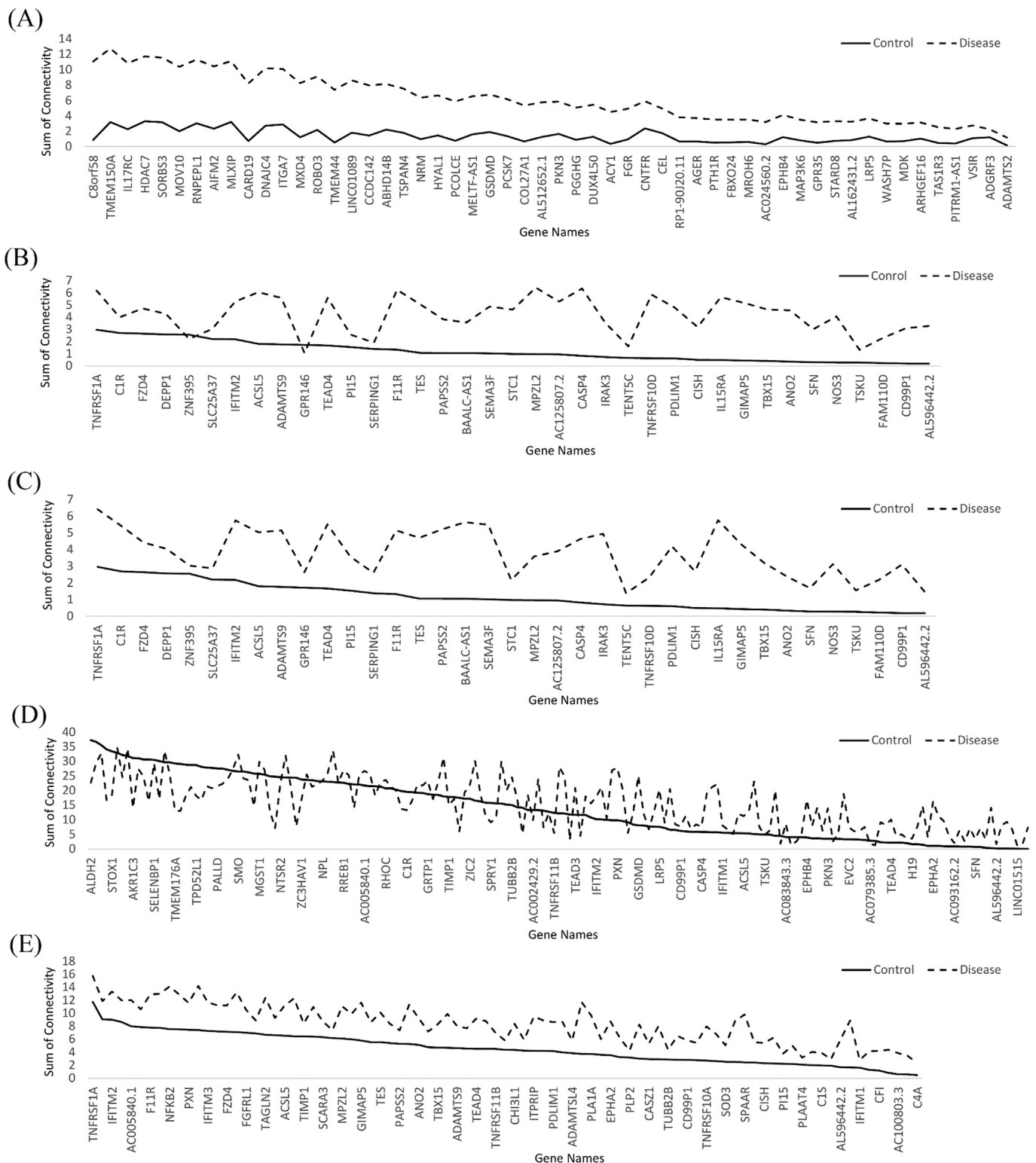

Fig. 4. Network Topology Analysis. The chart shows the sum of connectivity differences of: (A) *Red* NP module between control vs BPD, (B) *Black* NP module between control vs BPD, (C) *Black* NP module between control vs SCZ, (D) *Green* NP module between SCZ vs control, and (E) *Black* NP module between BPD vs control.

samples in (Supplementary Fig. 2B) and X4385_AnCg_S_SL6346 was as an outlier in the group of SCZ samples in (Supplementary Fig. 2C). These samples were removed in order to make the dataset more homogeneous. Finally, we have 20 control, 23 BPD and 23 SCZ samples.

The construction of weighted gene network needs the choice of soft thresholds power β. Adjacency (Adj_{ij}) was calculated by raising power to the co-expression similarity which fulfils the criteria of approximate scale-free topology. While constructing weighted gene co-expression network for each disorder along with control the suitable β values were 16, 26 and 14, for control, BPD and SCZ dataset, respectively . The figures for β estimation for control, BPD and SCZ can be observed have been presented in Supplementary Figs. 3(A, B, C), respectively. We performed hierarchical clustering to identify modules from each of the constructed networks. If the correlation between a pair of eigen genes are ≥ 0.75 then we merged them to form single modules. For control network, we had 8 modules before performing merging operation. After merging, we obtained 5 modules excluding the grey module of 177 genes for further analysis. Similarly, for BPD and SCZ, we had 14 modules each before merging, and after merging, we obtained 3 and 4 modules excluding grey modules, respectively. In case of BPD and SCZ, the grey module contained 55 genes and 13 genes, respectively. The *dissTOM* heatmap for control, BPD and SCZ network before and after merging modules are presented in Fig. 2(A, B, C). The detailed clustering dendogram of the genes and modules for control, BPD, and SCZ are presented in Supplementary Figs. 4(A, B, C).

3.3. Identification of NP modules

The module preservation analysis was carried out for both BPD and SCZ by the following two methods: (i) the control modules vs. the disorder modules; and (ii) disorders modules vs. control modules. Those modules which were weakly preserved might influence the unique pathological signatures of the two disorders compared to the control network. Two modules in control network were found to be NP in BPD (shown as *Black* and *Red* colour in Fig. 3(A), and of them the *Black* module is also NP in SCZ network in Fig. 3(B). Similarly, 1 module each from BPD (*Black* in colour) and SCZ (*Green* in colour) were found to be NP in control network as shown as in Fig. 3(C and D) respectively. *Black* and *Red* NP modules from control network contained 37 and 54 genes, respectively. The *Black* NP module, which was found in the BPD network contained 87 genes. *Green* non-preserved module from SCZ network contained 179 genes.

3.4. Topological analysis of non-preserved modules

We performed network topological analysis of all the identified NP modules to identify interesting genes and to study different network parameters for each gene. In Fig. 4(A, B, C, D, E), we reported the

comparison of sum of connectivity for each gene of NP modules in control and diseased condition (Supplementary Table 2). In all the figures we see that many genes have changed their sum of connectivity strength significantly and top 5 of such genes along with their sum of connectivity were reported in the Table 1. In Table 1, we observed that the NP modules found from control and BPD network have significantly lower sum of connectivity than the disease network. It may happen due to the activation or represssion of some genes in the diseased condition. On the other hand, the NP module found from SCZ network had significantly higher sum of connectivity in control condition. It may be due to the abnormal changes of expression values of some genes in diseased condition. These analyses give a clear idea that some genes of NP modules acting abnormally due to some external factors.

3.5. GSEA of the NP modules

The *Black* and *Red* NP module from control samples consisting of 37 and 54 genes and *Black* and *Green* NP module from BPD and SCZ samples consisting of 87 and 179 genes, respectively were analysed using DAVID tool and BiNGO, a Cytoscape plugin. The significance of the enriched GO terms and KEGG pathways were considered by applying threshold of *p-value*<0.05. GSEA results consisted of the top 5 significantly enriched Biological processes and KEGG pathways obtained from the DAVID tool have been reported in Tables 2 and 3 for each of the NP modules. Genes in the NP modules were also analysed using the BiNGO tool to understand the enriched biological processes and their interactions. As shown in Fig. 5(A and B), the immune related biological processes were highly enriched in the NP modules of both BPD and SCZ.

4. Discussion

Co-expression analysis on DEGs followed by non-preservation analysis of the modules can provide novel predictions of molecular signatures. This analysis can help to understand the molecular insights of neuropsychiatric disorders. Although the pathogenesis between SCZ and BPD has been reported in earlier studies but the earlier inferences were mostly based on differential expression analysis (Craddock et al., 2006; Hu et al., 2016). Our approach considers interactions among the DEGs to identify biologically relevant genes. Topological and enrichment analysis on NP module exerting different molecular functions and signatures in BPD and SCZ patients in AnCg brain region. Previous studies suggested that the nervous system has an important role associated with the immune system in BPD and SCZ (Kerr et al., 2005; Sainz et al., 2013). Neuroinflammation within the central nervous system which has been proven to have a role in the etiology of these disorders (DiSabato et al., 2016; Hong et al., 2016; Thibaut, 2017; Trépanier et al., 2016). On pathway and biological processes analysis of NP module genes, we observed that most of the genes were related to neuroinflammation

Table 1
Top five genes from each NP module based on sum of connectivity in control and disease.

Control vs BPD and SCZ *Black* Module				Control vs BPD *Red* Module		
Gene Symbols	Control	BPD	SCZ	Gene Symbols	Control	BPD
TNFRSF1A	2.9708	6.196312	6.417682	C8orf58	0.8782	10.19323
C1R	2.7036	3.994709	5.448868	TMEM150A	3.204	9.541098
FZD4	2.6486	4.71635	4.429367	IL17RC	2.267	8.582103
DEPP1	2.5764	4.28416	4.046879	HDAC7	3.295	8.42896
ZNF395	2.5586	2.211981	3.036811	SORBS3	3.173	8.377483

BPD vs control *Black* Module			SCZ vs control *Green* Module		
Gene Symbols	Control	BPD	Gene Symbols	Control	SCZ
TNFRSF1A	11.764	15.74675	ALDH2	37.232	22.67699
TNIP2	9.0549	11.85779	EMX2	36.690	29.46347
IFITM2	9.0014	13.34243	PLCD1	35.541	32.73036
TRAF3IP2	8.6595	11.99204	ADHFE1	34.073	16.72638
AC005840.1	7.9882	12.02869	STOX1	33.383	18.50491

pathway and processes. There were pathways and processes which were related to *Staphylococcus aureus* infection, pertussis and skeletal system development. These pathways contain genes related to immune response. These components of the infection-induced immune response system overlaps with neuroinflamatory responses.

Among the highly enriched genes, 11 genes were found to be enriched in both BPD and SCZ network (Table 2). TNFRSF10D and GSDMD were uniquely enriching inflammatory responses for BPD and SCZ, respectively. TNFRSF10D belongs to the TNFR superfamily and it acts as negative regulator of NF-kappa-B pathway (Marsters et al., 1997). GSDMD is a part of the innate immune system and found to be involved in autophagy. Out of eleven genes, three genes viz., TNIP2, TNFRSF1A and AC005840.1 were found to have high sum of connectivity in BPD network (Table 1). TNIP2 mainly found to inhibit NfkB signalling pathway (Banks et al., 2016). TNFRSF1A, an important receptor of the cytokine TNF-a which activates the caspase pathway leading to apoptosis in BPD (Pandey et al., 2015). AC005840.1 gene is a ribosomal protein and its exclusive function is not so clear in neuropsychiatric disorders. Whereas, TRAF3IP2 and IL17RC genes were exclusively found in two different NP modules related to BPD (Table 1). TRAF3IP2 which encodes Act1, a positive signalling adaptor in IL-17-mediated immune responses (Perricone et al., 2013) and IL-17 proteins are found in breaking the integrity of the blood brain barrier (Setiadi et al., 2019). Thus, this interaction indicates that neuroinflamatory responses can have a prominent role in the BPD pathogenesis.

We have observed list of genes related to autoimmunity and autophagy in control vs BPD and SCZ network. DEPP1 which acts as a critical modulator of FOXO3 induced autophagy via increased cellular ROS. These genes participate in neurodegenerative disorders associated with autophagic clearance (Stepp et al., 2014). C1R gene encodes a protein which acts as a proteolytic subunit in the complement system C1 complex. This complement system gets activated during autoimmune attack. We found another gene, ZNF395, to be upregulated in the SCZ network and downregulated in the BPD network. ZNF395, under hypoxic condition was found to be involved in inflammation and cancer progression by mediating the genes participating in innate immune responses (Jordanovski et al., 2013) (Table 1).

We observed top five genes from SCZ module (Table 1). Of these 5 genes (i) ALDH2 acts as a protector against Oxidative stress, in addition it acts as neuroprotector by clearing 4-HNE (Guo et al., 2013); (ii) EMX2, whose deficiency results in abnormal forebrain structure formation, (iii) PLCD1 gene is known as a tumour suppressor gene in different types of cancer; (iv) ADHFE1 gene is used to metabolize the biological alcohol and ethanol beverage (Deng et al., 2002); and (v) STOX1 gene

Table 2
GSEA results obtained from DAVID shows the top five enriched Biological Processes in the NP modules, together with P-value, Fold Enrichment and Gene Symbols contributing in the corresponding Biological processes. [*Area of our interest].

Term	P-value	Fold Enrichment	Gene symbols
***Red* Module (control)**			
cell surface receptor signalling pathway	0.022	6.451	PTH1R, ADGRF3, AGER, TSPAN4
osteoblast development	0.0368	51.987	PTH1R, LRP5
***Black* Module (control)**			
cell-cell adhesion	0.0091	8.851	PDLIM1, TES, F11R, SFN
cytokine-mediated signalling pathway	0.0186	13.733	TNFRSF1A, CISH, IL15RA
negative regulation of calcium ion transport	0.020	92.263	STC1, NOS3
inflammatory response*	0.0223	6.329	TNFRSF1A, F11R, TNFRSF10D, CASP4
immune response	0.029	5.697	TNFRSF1A, C1R, TNFRSF10D, IFITM2
***Black* Module (BPD)**			
inflammatory response*	0.00000059	7.283	TNFRSF1A, NFKB2, TNFRSF10A, AC005840.1, CHI3L1, EPHA2, F11R, TNFRSF11B, TNIP2, TNFRSF10D, CASP4, C4A
response to lipopolysaccharide	0.000072	9.818	TNFRSF1A, NFKB2, EDN1, TNFRSF10A, AC005840.1, TNFRSF11B, TNFRSF10D
regulation of cell proliferation	0.00014	8.703	FGR, TNFRSF1A, TNFRSF10A, AC005840.1, TES, TNFRSF11B, TNFRSF10D
skeletal system development	0.00030	10.074	GLI2, FGFRL1, EPHA2, TNFRSF11B, TEAD4, PAPSS2
extrinsic apoptotic signalling pathway via death domain receptors	0.00057	24.213	TNFRSF1A, TNFRSF10A, BAG3, TNFRSF10D
***Green* module (SCZ)**			
smoothened signalling pathway	0.00025	10.504	PAX6, EVC, GLI2, HES1, SMO, EVC2
inflammatory response*	0.00029	3.824	TNFRSF1A, NFKB2, GSDMD, TNFRSF10A, AC005840.1, CHI3L1, EPHA2, F11R, TNFRSF11B, TNIP2, CASP4, C4A
cerebellar cortex morphogenesis	0.00065	72.483	GLI2, RFX4, SMO
regulation of cell proliferation	0.00084	5.224	FGR, TNFRSF1A, TNFRSF10A, AC005840.1, TES, AGT, TNFRSF11B, PLCD1
skeletal system development	0.00094	6.172	EVC, GLI2, FGFRL1, EPHA2, TNFRSF11B, TEAD4, PAPSS2

Table 3
GSEA results obtained from DAVID shows the top five enriched KEGG pathways in the NP modules, together with P-value, Fold Enrichment and Gene Symbols contributing in the corresponding KEGG pathway. [*Area of our interest].

| Term	P-value	Fold Enrichment	Gene Symbols
***Red* Module (control):** Enrichment results not found			
***Black* module (control):** No Significant results available			
***Black* module (BPD)**			
Complement and coagulation cascades	5.85E-04	12.461	VWF, C1R, C1S, CFI, C4A
Staphylococcus aureus infection	0.003	12.738	C1R, C1S, CFI, C4A
Apoptosis	0.005	11.095	TNFRSF1A, TNFRSF10A, CASP7, TNFRSF10D
Pertussis	0.008	9.172	C1R, CASP7, C1S, C4A
Hippo signalling pathway	0.010	5.694	TEAD3, GLI2, LIMD1, FZD4, TEAD4
***Green* module (SCZ)**			
Complement and coagulation cascades	0.0004	8.927	VWF, SERPING1, C1R, C1S, CFI, C4A
Fatty acid degradation	0.0006	12.222	CPT1A, ALDH2, ACADS, ADH1B, ACSL5
Pertussis	0.005	6.844	SERPING1, C1R, CASP7, C1S, C4A
Cytokine-cytokine receptor interaction*	0.008	3.380	IL17RB, TNFRSF1A, TNFRSF10A, AC005840.1, CNTFR, IL15RA, INHBB, TNFRSF11B
Hippo signaling pathway	0.014	4.079	TEAD3, GLI2, TP53BP2, TCF7L1, FZD4, TEAD4
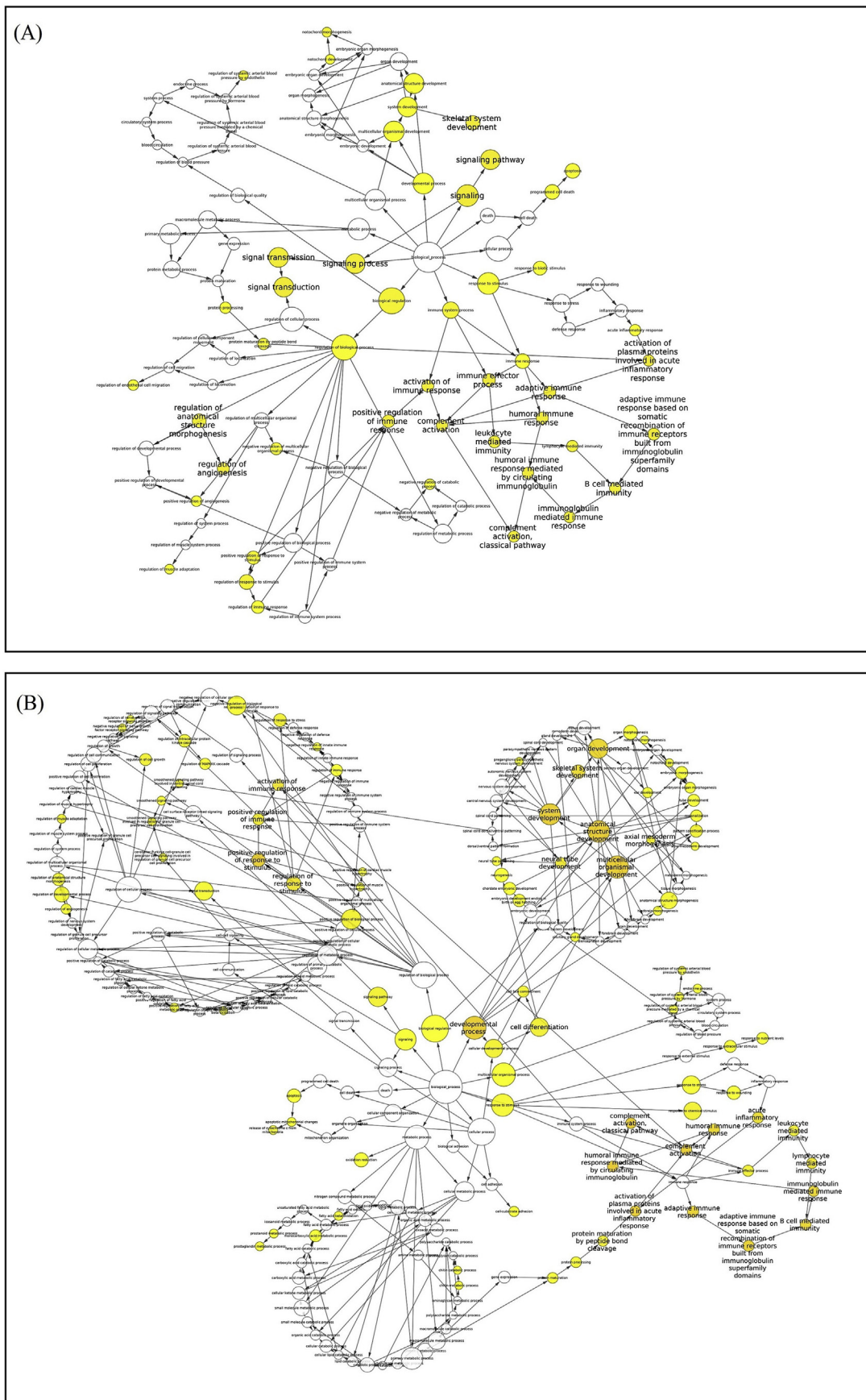
Fig. 5. GSEA using the BiNGO Cytoscape plugin. (A) BPD vs control NP *Black* module and (B) SCZ vs control NP *Green* module. Yellow colour indicates the highly enriched processes.

predominantly found in brain, its role is to transactivate the LRRTM3 gene which plays a role in the maintenance of nervous system (Fig. 4(D)). Interestingly, all of these top five genes which are beneficial in the CNS were found to be downregulated in the SCZ disorder. This gene may be a vital factor for the progression of SCZ disorder.

We observed clear overrepresentation of the pro-inflammatory cytokine receptors which supports that cytokine-mediated immunological responses play a vital role in the BPD and SCZ. In addition, other genes associated with activation of autoimmunity and autophagy indicates that there is an urge of balancing energy sources and genomic instability at critical times such as neurodegeneration (Glick et al., 2010). Thus, investigating the BPD and SCZ by focusing on the above processes can serve as a potential therapeutic strategy based on immune-neural system. Our study presented in this manuscript has mainly been focused on neuroinflammation. However, there is scope to explore the other response signatures during BPD and SCZ of other domains. Anti-inflammatory medication as an adjunctive therapy can be implemented for the clinical treatments of BPD and SCZ (Radtke et al., 2017). Our results will contribute to the ongoing efforts in the domain of immune-based therapies.

Declaration of competing interest

The authors declare that they have no known conflicting or competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2019.100023.

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