Integrated analysis profiles of long non–coding RNAs reveal potential biomarkers across brain regions in post–traumatic stress disorder

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Abstract

Background Post–traumatic stress disorder (PTSD) is characterized by impaired fear extinction, excessive anxiety and depression. However, underlying mechanisms, especially the function roles of long non–coding RNAs (lncRNAs) involved in PTSD is still unclear. We argued that the lncRNAs, co–expressed mRNAs, as well as the associated pathways, are altered and may thus serve as potential biomarkers and key pathways related to PTSD.

Methods The gene expression profiles of GSE68077 was downloaded from the GEO database, and the differentially expressed lncRNAs and mRNAs were identified. Gene ontology (GO) and Kyto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analysis were performed. Subsequently, protein–protein interaction (PPI) network was analyzed, and module analysis of the differentially expressed mRNAs was performed with Cytoscape software. Finally, lncRNAs–mRNAs co–expression network was constructed and core pair lncRNAs involved in PTSD were mapped.

Results A total of 45 differentially expressed lncRNAs and 726 differentially expressed mRNAs were obtained. Among of which, 17 lncRNAs and 86 mRNAs were inter–regulated, and most of the lncRNAs–mRNAs co–expression showed positive correlations. The lncRNAs–mRNAs co–expressed network suggested the potentially functional roles of lncRNAs, regulated mRNAs and related pathways in PTSD. By implication of the core pair network, lncRNA–NONMMUT010120.2 synergistically up–regulated Ppargc1a and down–regulated Cir1, Slc38a9, Atp6v0a2. Moreover, lncRNA–NONMMUT023440.2, NONMMUT034155.2, NONMMUT105407.1 and NONMMUT149972.1 were co–expressed with 10 co–expressed mRNAs, which indicated that lncRNAs involved in PTSD might work by regulating the co–expressed mRNAs.

Introduction

Post–traumatic stress disorder (PTSD) is a persistent stress response triggered after exposure to a single or a series of stressful or traumatic event directly or indirectly(Kim, et al., 2018, Ronzoni, et al., 2016). The typical clinical symptoms in PTSD patients mainly consists of re-living or avoidance the traumatic event, negative emotions and hyper vigilance(Dennis, et al., 2016). It is diagnosed when these symptoms last for at least one month and cause functional impairment and/or marked distress(Lisieski, et al., 2018).

Approximately 8.1% of Australian male war veterans suffer from PTSD(Gill, et al., 2016). A recent systematic review reported that PTSD prevalence rate among ambulance personnel amounts to 11% (Petrie, et al., 2018). Similarly, up to 36.5% secondary school students were diagnosed with PTSD after the 2008 Wenchuan earthquake in China(Hou, et al., 2011). PTSD can cause physical or psychiatric maladies, and it can bring out some negative social consequences, i.e. employment difficulties, marital difficulties. The disorders brings a considerable burden on an individual, family and the whole society. Several studies on the underlying neurobiology of PTSD have implicated hypothalamic–pituitary–adrenal (HPA) axis, autonomic nervous system (ANS), immune system and neural circuits. The

pathogenesis of PTSD has not been entirely elucidated. Hence, it is urgent to clearly understand the underlying mechanisms of PTSD in molecular level.

Long non-coding RNAs (lncRNAs) 200 nt non-protein-coding transcripts play pivotal roles in chromatin remodeling, transcription regulation, as well as epigenetic modification(Logan, et al., 2018, Spurlock, et al., 2016, Yu, et al., 2018). Mounting evidence suggested that lncRNAs participate in some important biological processes through diverse pathways, acting as critical biomarkers(Yu, et al., 2018). Although lncRNAs have been reported recently, most studies only identified differentially expressed lncRNAs associated with PTSD and few have been well characterized. There is a quite limit knowledge about the genome scale of lncRNAs and their potential functions of PTSD.

Fortunately, recent gene co-expression network analysis methods for integrating expression data across thousands of genes related with multiple diseases are currently available. These methods are often used for identifying groups of genes, whose expression levels are highly correlated, within a network i.e., co-expression modules(Breen, et al., 2015). LncRNAs–mRNAs co-expression network analysis may provide potential targets for a variety of biological processes associated with PTSD. Nevertheless, identifying key genes by constructing lncRNA–mRNA regulatory network of PTSD has not been performed.

In the current study, we identified a group of differentially expressed transcripts (lncRNAs and mRNAs) associated with PTSD-like symptoms by bioinformatics methods, then we investigated the underlying mechanisms by mapping the lncRNAs-mRNAs co-expression network. Moreover, we uncovered core pair lncRNAs involved in the developmental progression of PTSD in purpose of providing initial evidence into lncRNAs and lncRNAs–mRNAs co–expression roles in PTSD at the transcriptomic level. The findings may help us generate new insights into PTSD–associated lncRNAs and lncRNA–regulated mRNAs in PTSD.

Materials And Methods

Datasets of mRNAs and lncRNAs

The microarry dataset (mRNAs) was retrieved from the Gene Expression Omnibus (GEO) database. After screening, the expression profile number GSE68077 was retrieved based on GPL7202 platform Agilent–014868 Whole Mouse Genome Microarray 4x44K G4122F for our analysis(Muhie, et al., 2017). The dataset has ten different expression profiles among brain, blood and other tissues from male C57BL/6 mice. These mice were exposed under a social stressed condition for 5 days (T5d) or 10 days (T10d) to prepare social tress model. The samples were collected across four different time points, that is, 1 day and 10 days home cage rest after 5 days aggressor exposure (T5R1, T5R10), 1 day and 42 days home cage rest after 10 days aggressor exposure (T10R1, T10R42). The stress exposed mice elicited significant behavior characterization among various time points. The stress features included increasing freezing when locomotion (indicating fear), prolonged grooming, aggressor assaults avoidance (suggesting social avoidance), hyper-activity, jumping (suggesting hyperactivity) and no tail rattling, which suggesting to be PTSD-like phenotypes(Hammamieh, et al., 2012, Muhie, et al., 2015). The microarray data of the

hemibrain samples were downloaded for our analysis. The lncRNAs were retrieved and compared with a NCBI RefSeq transcript by adopting BLAST program.

Exploring of PTSD–associated lncRNAs and mRNAs

Differentially expressed lncRNAs and mRNAs were identified at four time points (T5R1, T5R10, T10R1 and T10R42) in social stressed (SS) group and control (C) group. Then, we overlapped differentially expressed mRNAs and lncRNAs by comparing T5R1 with T5R10, and T10R1 with T10R42, in SS and C group, respectively. By overlapping SS and C group, specific genes at T5d or T10d in SS group were obtained. After these gene profiles were compared, the overlapping mRNAs and lncRNAs at T5d and T10d were identified for further analysis.

GO annotation and KEGG enrichment

Gene ontology (GO) annotation was applied to conduct potentially biological functions. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted to screen the significant pathways of the annotated mRNAs. Both GO and KEGG analysis were conducted when *P*–value < 0.05 was considered as screening threshold by using the Database for Annotation, Visualization and Integrated Discovery (DAVID)(Huang, et al., 2007).

Protein–protein interaction (PPI) network construction and module analysis

The interaction of protein pairs were predicted by using the Search Tool for the Retrieval of Interacting Genes (STRING)(von Mering, et al., 2003). And the confidence score more than 0.4 was set as threshold criterion. The integrated regulatory networks was then mapped by Cytoscape(Shannon, et al., 2003). Finally, Module analysis was displayed from PPI network using MCODE.

Integration of the co–expression network

Differentially expressed profiles were screened by whose expression levels correlated (positive or negative). The connection matrix and Characteristic–Value were performed to measure the relationship strength among genes in the network. In briefly, we performed factor analysis to calculate the distance between the genes which can reflect the relationship strength between genes in the network, that is, Characteristic–Value. It is the contributions of gene i to lncRNA j. The model given by $\lambda x_i = a_{1i}x_1 + a_{2i}x_2 + \ldots + a_{ni}x_n$, (where “x” denotes eigenvector centrality) can be converted to $A^t \cdot x = \lambda x$ (where “A” denotes an n × n matrix). The gene with maximum value is the centrality of lncRNA or mRNA and plays a crucial role in the network. The results M (i, j) define the edges of the graph and an edge connecting the nodes.

Statistical analyses

The Bioconductor package limma v 3.26.8 and R software v 3.2.3 were used for all differential expression analysis. The *P* value was corrected by the false discover rate (FDR). The threshold of |fold changes (FC)| ≥1.5 along with *P* < 0.05 were applied to identify differential expression of lncRNAs or mRNAs. Both GO

annotation and KEGG pathway analysis were applied by Fisher's exact test and χ^2 test. And $P < 0.05$ was set as the threshold cutoff. The relationship between lncRNAs and co–expressed mRNAs were estimated by the Pearson's correlation coefficient (Pearson R). And the |Pearson R| > 0.8 was set as the cutoff value.

Results

Expression profile analysis of PTSD–associated lncRNAs and mRNAs at T5d and T10d

The lncRNAs and mRNAs expression profiles across four time points (T5R1, T5R10, T10R1 and T10R42) in SS group and C group were screen out, respectively. By the criteria of |FC|≥1.5 and $P < 0.05$, 222 lncRNAs and 1853 mRNAs were altered significantly in the SS group, and 310 lncRNAs and 1310 mRNAs in the C group at T5d were identified when comparing T5R1 with T5R10, and T10R1 with T10R42 in the SS and C groups respectively. Meanwhile, 615 lncRNAs and 6277 mRNAs were altered significantly in the SS group, and 394 lncRNAs and 1204 mRNAs were screened out in the C group at T10d. The detail of significantly expressed lncRNAs and mRNAs at T5d and T10d were shown in Table 1. The heatmaps of the regulated lncRNAs and mRNAs across hemi–brain regions at T5R1 *vs* T5R10 and T10R1 *vs* T10R42 in SS or C sample were presented in Fig. 1, respectively.

Table 1. Numbers of significantly expressed lncRNAs and mRNAs at T5d and T10d

		SS group		C group	
		lncRNAs	mRNAs	lncRNAs	mRNAs
Up–regulated	T5d	71	597	194	625
	T10d	392	2177	129	395
Down–regulated	T5d	151	1256	116	685
	T10d	223	4100	265	809
All–regulated	T5d	222	1853	310	1310
	T10d	615	6277	394	1204

Identification of overlapping lncRNAs and mRNAs at T5d vs T10d

Compared with the C group, SS group had distinct transcriptional profiles. As shown in the Venn diagram, 218 specific lncRNAs are detected at T5d and 606 specific lncRNAs at T10d. We further identified 45 overlapping lncRNAs existing in both comparisons (Fig. 2A). Meanwhile, 726 overlapping mRNAs were found in both time points (Fig. 2B). The obtained 45 lncRNAs and 726 mRNAs were underwent further analysis.

Functional and pathway enrichment analyses

Both GO annotation and KEGG enrichment were determined by Fisher's exact test and χ^2 test. And P value ≤ 0.05 was considered as the cut–off. GO analysis was performed for functional assignments by using

the 726 differently expressed mRNAs obtained. The most enriched GOs were identified, including transport (GO: 0006810), biological process (GO: 0008150), regulation of transcription, DNA–dependent (GO: 0006355), transcription, DNA–dependent (GO: 0006351) and cell differentiation (GO: 0030154). Functional enrichment analysis showed that the significant genes were in relation to Rap1 signaling pathway, cAMP signaling pathway, and mTOR signaling pathway (Fig. 3).

PPI network construction and module analysis

To further determine the PTSD–associated genes, PPI network was mapped by STRING (Fig. 4), which was composed of 144 nodes and 264 edges. After the disconnected nodes were excluded, 120 genes (16 were up–regulated and 36 were down–regulated at both T5d and T10d, and the others were up–regulated or down–regulated in T5d or T10d) were involved in our dataset and used for the depiction of the complex relationship by Cytoscape (combined score≥0.4, Fig. 5A). Moreover, 20 genes with higher node degrees were obtained as hub genes with a threshold of 'Degrees≥10'. The hub nodes included Rac Family Small GTPase 1 (Rac1), Insulin like growth factor 1 receptor (Lgf1r), Amyloid beta precursor protein (App), Mitogen–activated protein kinase 10 (Mapk10), Protein phosphatase 3 catalytic subunit beta (Ppp3cb) and Clathrin heavy chain (Cltc), Among these genes, Rac1 showed the highest degree of connectivity in the down–regulated genes (19), and App, in the up–regulated genes (14).

Subsequently, 9 clusters were selected by using MCODE, and the significant module consisted of 10 nodes and 21 edges. We also found that each module had one 'seed' gene. Ankyrin 1 (Ank1), G protein–poupled peceptor 83 (Gpr83), Fatty acid synthase (Fasn), Corepressor interacting with RBPJ, 1 (CIR1), Opioid receptor kappa 1 (Oprk1) were showed as the 'seed' genes in the respective modules (MCODE Score≥3; Fig. 5B).

Co–expression of lncRNAs–mRNAs related to PTSD

To mine the potential mechanisms of lncRNAs and the possible regulatory roles related to PTSD, co–expression network was performed. To achieve these goals, we conducted lncRNAs–mRNAs co–expression network on the 45 lncRNAs and 726 mRNAs mentioned above. In the regulatory network, based on the threshold of |Pearson R| > 0.8, 17 lncRNAs were inter–linked with 86 mRNAs, which showed a positive relationship (Fig. 6). One lncRNA was connected to many mRNAs, and various lncRNAs were also linked with one mRNA, which suggested that the complicated regulatory relationships of lncRNAs and mRNAs. It could help us further understand PTSD–associated lncRNAs and lncRNAs–regulated mRNAs in PTSD.

Additionally, we built a network of signaling pathways about lncRNAs regulating mRNAs in order to indicate their roles in the core pathways of PTSD (Fig. 7). We noted that lncRNAs–associated mRNAs in the regulatory network were mainly associated with mTOR signaling pathway, Notch signaling pathway, and TGF–β signaling pathway. We also found that they largely pointed to pivotal roles of GPR83 and Bone Morphogenetic Protein 7 (Bmp7).

Core pair lncRNAs involved in PTSD

Finally, we determined the expression patterns of the core lncRNAs and their co–regulated mRNAs. From the sub–network, lncRNA–NONMMUT023440.2, NONMMUT034155.2, NONMMUT105407.1, and NONMMUT149972.1 were closely related to 10 co–expressed mRNAs (Chst12, Gpr83, Irak2, Itga11, Mcoln1, Notch3, Pfkp, Slc22a4, Slc32a1, and Bmp7). lncRNA– NONMMUT010120.2 targeting up–regulated Ppargc1a and down–regulated Cir1, Slc38a9 and Atp6v0a2 (Fig. 8).We predicted that lncRNAs involved in PTSD might work by regulating the co–expressed mRNAs to a certain extent.

Discussion

In this current study, differently expressed lncRNAs and mRNAs at different trauma–exposed time (5, 10 days) and different rest periods (1, 10 and 42 days) were demonstrated. 45 lncRNAs and 726 mRNAs were identified among differently expression profile. Various genetic and epigenetic alterations are involved in the onset and development of PTSD. Among the hub genes we identified above, App was markedly regulated genes. The finding is consistent with an earlier study(Justice, et al., 2015). APP knock–in can cause a series of PTSD like features such as behavior and emotional disorders. Gain–of function studies have found that APP overexpression is involved in cell mobility and transcription regulation. Meanwhile, Rac1 had the highest node degree among the down–regulated genes. Rac1 is involved in hippocampus–dependent memory and acts an essential role in learning under a fearful condition(Dietz, et al., 2012, Jiang, et al., 2016). It is suggested that inhibition hippocampal Rac1 is directly related to the extinction of fear disorder(Jiang, et al., 2016). From the implication of mRNA–associated pathway, the ‘seed’ genes, i.e. Fasn and Oprk1, were captured in our study. Interestingly, we found that Fasn is related to depressive symptoms and affects susceptibility to depression(Tsuboi, et al., 2011). Oprk1 mRNA expression is found down–regulated in social–stressed disorder manner(Browne, et al., 2018). Likewise, Oprk1 is altered in chronic stress–induced behavior(Falcon, et al., 2016). In summary, the top centrality hub genes and the ‘seed’ genes identified above are mostly involved in PTSD or PTSD–like symptoms, which suggesting that these genes may serve as vital contributors to PTSD.

Using GO annotation, we found that the altered genes display a variety of functionalities, such as transport, biological process, and transcription. The results are supported by previous studies(Subhramanyam and Hu, 2017, Weiss, et al., 2018), indicating the importance and complexity of the biological processes and transport functions involved in PTSD. The significantly functional pathway focuses on the Rap1 signaling pathway, cAMP signaling pathway, and mTOR signaling pathway. The pathways are inter–regulatory in the signal–network, which provide us a molecular view into PTSD. The findings were consistence with previous studies(Chandran, et al., 2013, Li, et al., 2009, Perez, et al., 2002). Moreover, we found that the main function of lncRNAs–regulated mRNAs included mTOR signaling pathway, Notch signaling pathway, and TGF–β signaling pathway. mTOR signaling pathway is well–studied in depression(Jernigan, et al., 2011), depression–like manner(Yu, et al., 2013) or chronic stress–induced behavior(Chandran, et al., 2013, Li, et al., 2011). Mover, Notch pathway is reported rang from depressive behavior to chronic mild stress(Guo, et al., 2009). There is an evidence that TGF–β pathway

can possibly be a treatment for anxiety–related(Ageta, et al., 2008) or depressive–like behavior(Zheng, et al., 2009). Given the above evidences, we surmise that these pathways play essential roles in the pathogenesis of PTSD.

Notably, we still lack an integrated database with fuller functional lncRNAs based on experimental studies. And the functions of most lncRNAs are not well annotated. Co–expression analyses of the PTSD–related lncRNAs and lncRNAs–regulated mRNAs might provide an overall view of possible mechanisms involved in PTSD. To better understand the biological roles of lncRNAs, a functional lncRNA–mRNA co–regulatory network of PTSD was mapped with 17 lncRNAs and 86 inter–regulated mRNAs. Gpr83 and Bmp7 in our co–pair analysis were highlighted to connect with multiple lncRNAs. It showed that Bmp7 was positively related to several lncRNAs, including NONMMUT034155.2, NONMMUT149972.1 and NONMMUT105407.1. Meanwhile, Gpr83 was positively connected to two lncRNAs, namely, NONMMUT034155.2 and NONMMUT023440.2. Bmp7, as a member of TGF–β superfamily, acts a vital effect on the development of noradrenergic neurons. Previous studies demonstrated that stress–induced reduction in Bmp7 expression promote catecholaminergic disorders that associated with chronic stress and depression(Esaki, et al., 2013, Ordway, et al., 2012). Gpr83 can functionally couple with G–protein coupled receptors (GPCR)(Muller, et al., 2016). It had been revealed that GPCR signaling is highly related to anxiety, depression or social stress(Hauger, et al., 2012). Based on the signaling network analysis, we further surmised that these mRNAs are regulated by functional lncRNAs via underlying pathways.

Although we identified the differential expressed lncRNAs-mRNAs interaction and the underlying mechanisms of PTSD based on brain region in molecular and cellular level. Biomarkers from other tissue sources were currently limited. And the sex biological variable was not stated in our study, which was also an important limitation of the social stress model(Flandreau and Toth, 2018). Therefore, the gender differences to the critical pathogenesis of PTSD needed to further clarify in the follow–up studies.

Taken together, our study provided comprehensive lncRNA and mRNA profiles of PTSD in brain region. Bioinformatics methods were applied to predict the functional effects of lncRNAs and co–expressed mRNAs, as well as cooperative pathways. This results may provide new clue for the regulatory mechanisms of PTSD in brain region. However, further studies are required to develop key biomarkers from various tissue sources of rodents containing females featured with PTSD-like phenotypes, and perform common associations and lncRNA differences among them. Besides, gene knockout studies are needed to elucidate the functional roles of lncRNAs and further identify functional relationships among lncRNAs and co–regulated mRNAs associated with PTSD.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

None.

Authors' Contributions

Yaoyao Bian, Lili Yang, and Zhongli Wang contributed equally to this study. Yaoyao Bian conceived and designed the study, and conducted data analysis with Lili Yang. Zhongli Wang performed the overlap analysis. Wen Li, Qing Wang and Bin Zhang designed the flow sheet. Min Zhao, Long Wang and Yicheng Zhi optimized the graphics. Guihua Xu provided several suggestions for manuscript revision.

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Figures

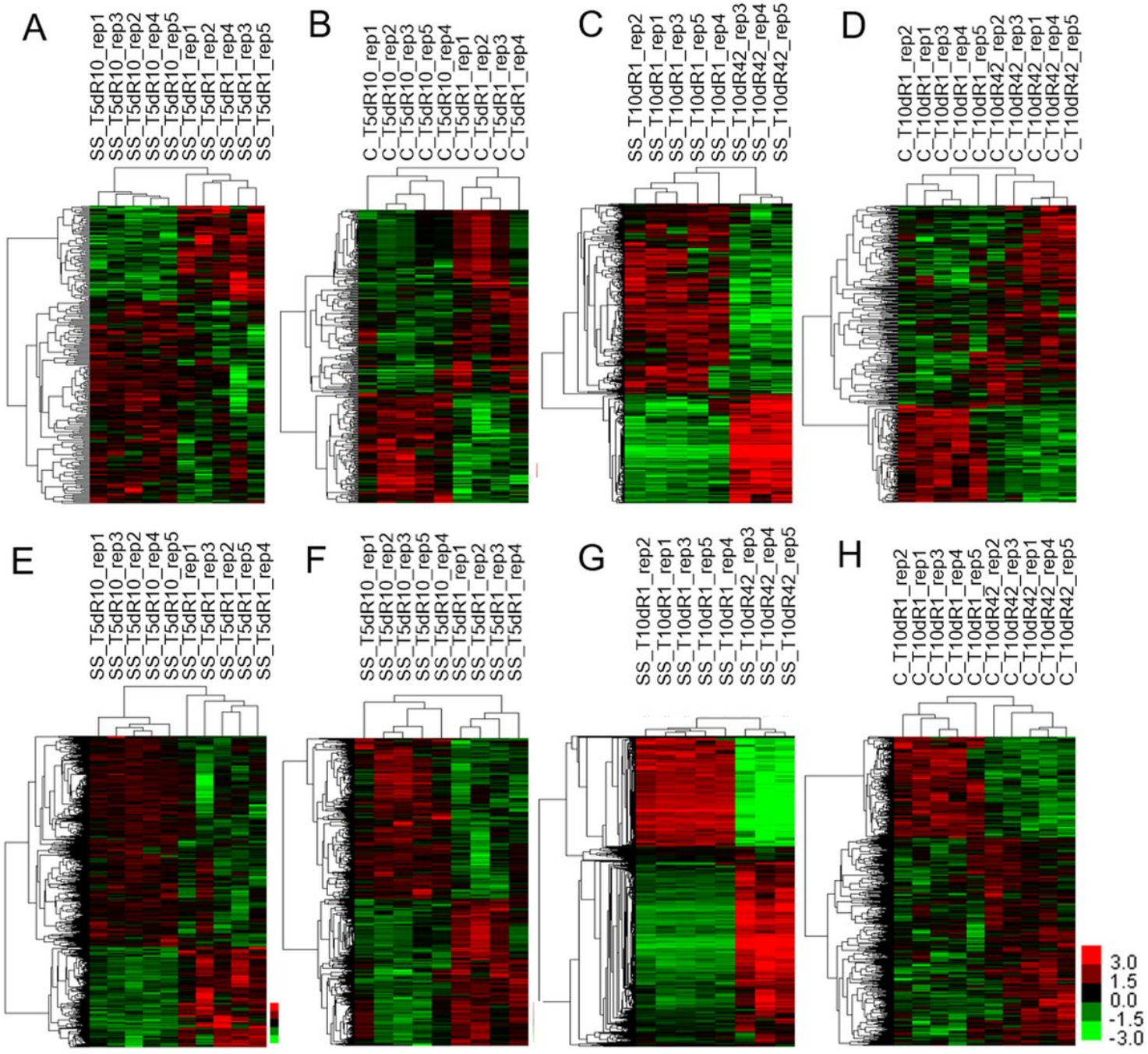

Figure 1

Differentially expressed lncRNAs and mRNAs across hemi–brain regions at time points T5R1 vs T5R10 and T10R1 vs T10R42 in SS or C group. (A and B) Heatmaps of significantly expressed lncRNAs at point of T5R1 vs T5R10 in the SS/C groups, respectively; (C and D) Heatmaps of significantly expressed lncRNAs at point of T10R1 vs T10R42 in SS/C groups, respectively; (E and F) Heatmaps of significantly expressed mRNAs at the point of T5R1 vs T5R10 in SS/C groups, respectively; (G and H) Heatmaps of significantly expressed mRNAs at point of T10R1 vs T10R42 in SS/C groups, respectively. Colors represent the relative levels of lncRNAs or mRNAs expression. The red color represents highly expressed level. The green color represents lowly expressed level. Note: T5R1, 1 day home cage rest after 5 days

aggressor exposure; T5R10, 10 days home cage rest after 5 days aggressor exposure; T10R1, 1 day home cage rest after 10 days aggressor exposure; T10R42, 42 days home cage rest after 10 days aggressor exposure.

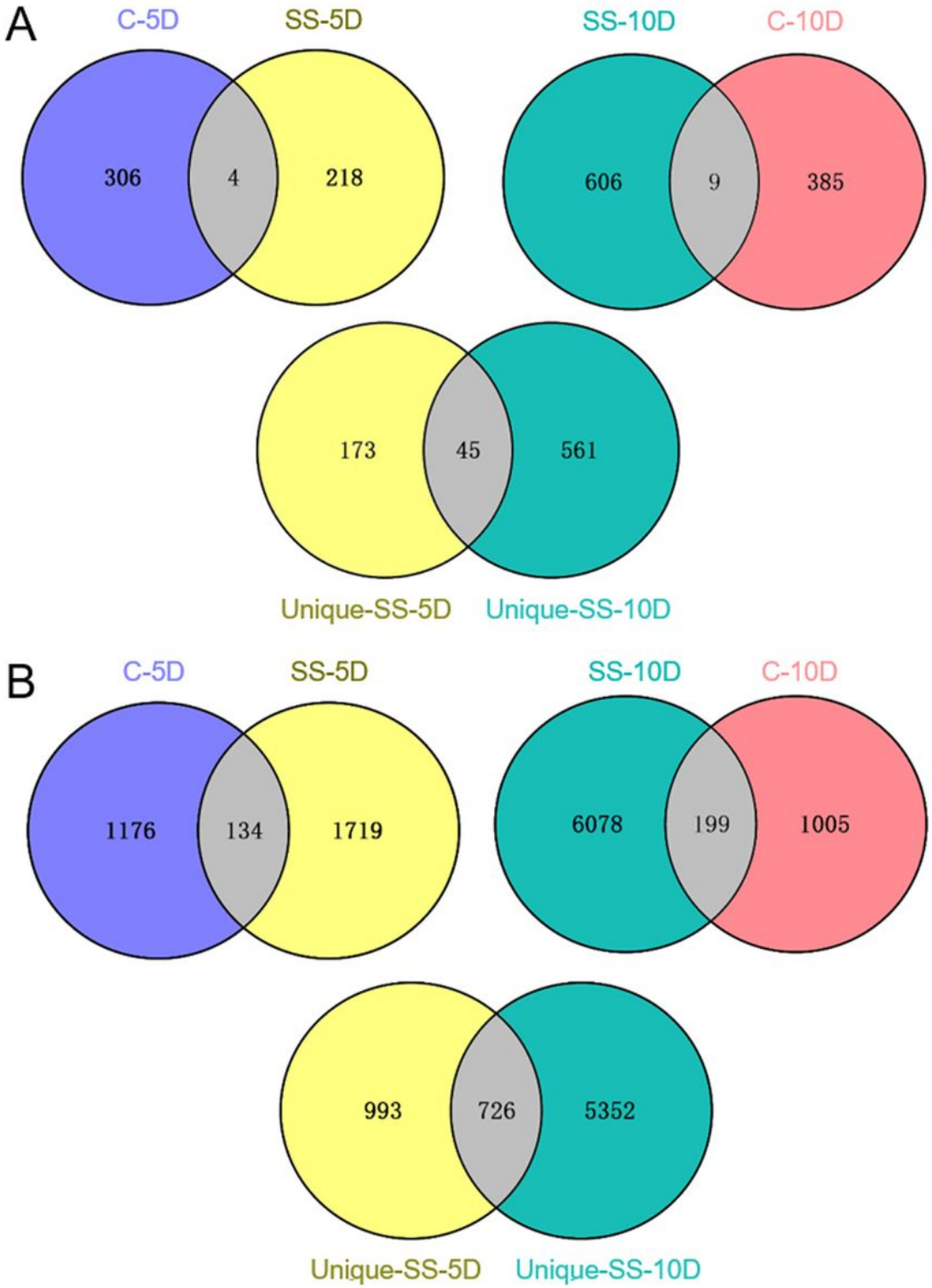

Figure 2

Overlapping lncRNAs and mRNAs at 5 days aggressor exposure (T5d) vs 10 days aggressor exposure (T10d). (A) Venn diagram of overlapping lncRNAs at T5d vs T10d; (B) Venn diagram of overlapping

mRNAs at T5d vs T10d.

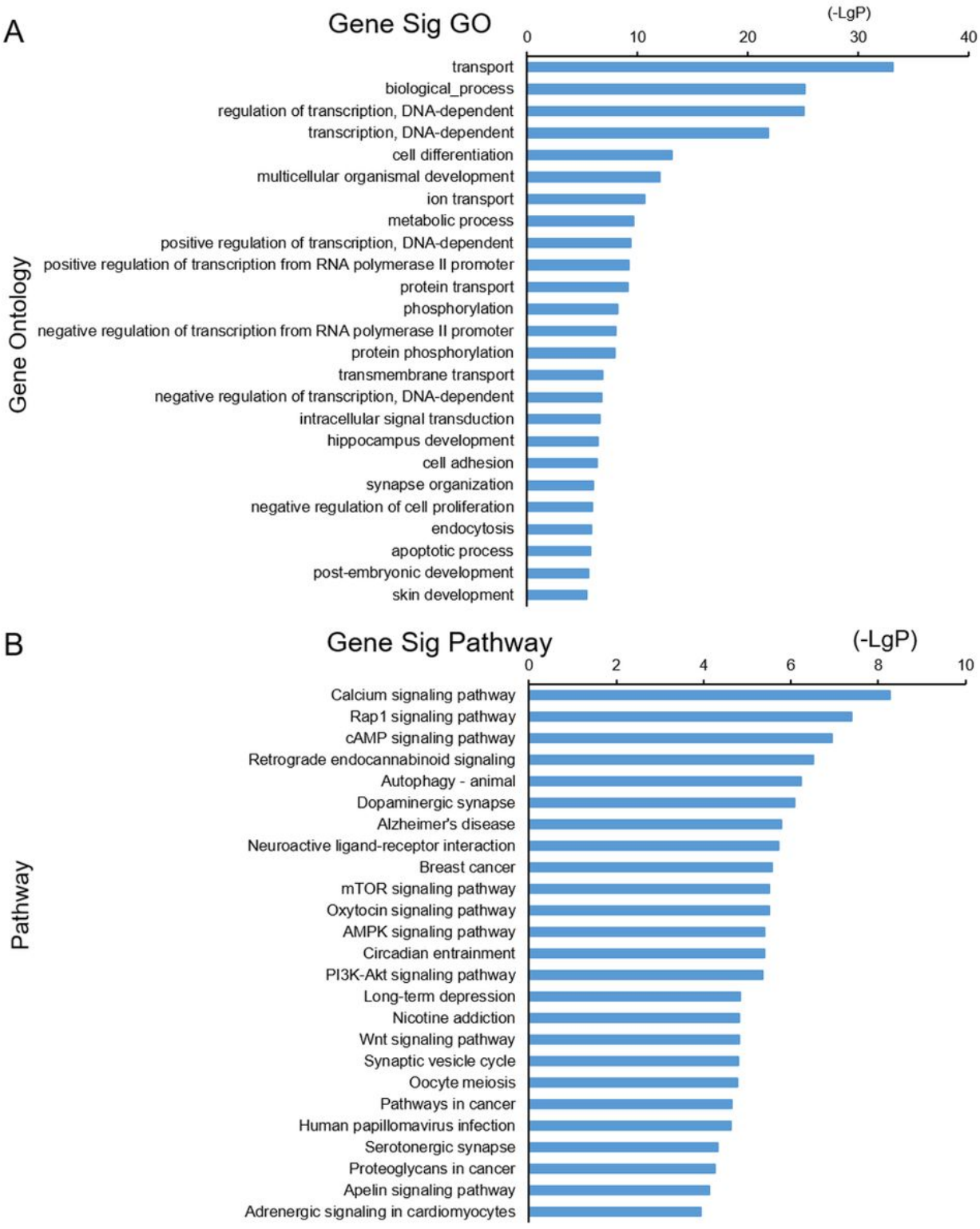

Figure 3

GO and pathway analysis of the significantly expressed mRNAs in PTSD. (A) GO annotation results of the significantly expressed mRNAs (top 25); (B) Pathway enrichment results of the significantly expressed mRNAs (top 25).

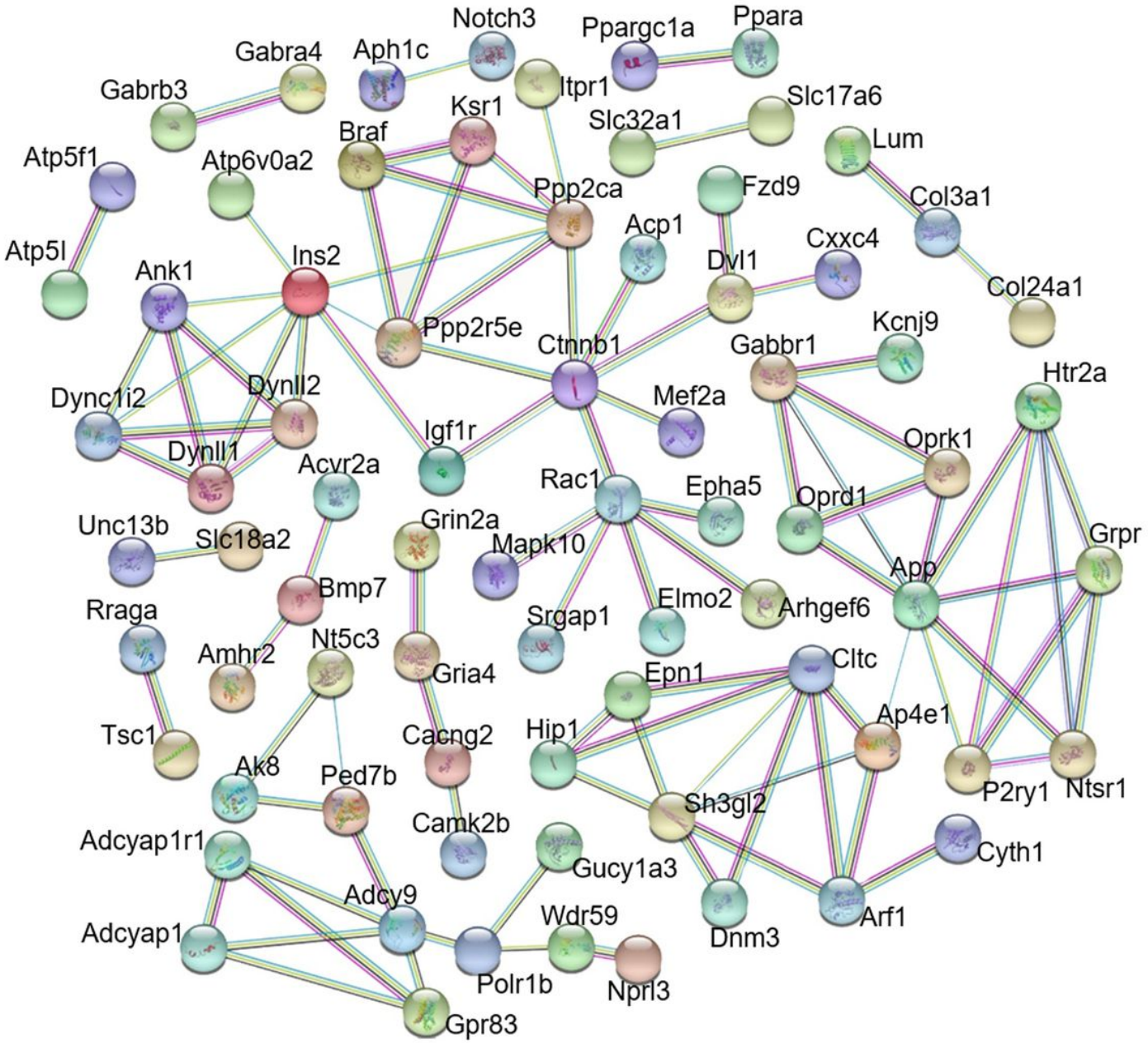

Figure 4

The PPI networks constructed by STRING. The color nodes represents proteins. The edges represents interactions.

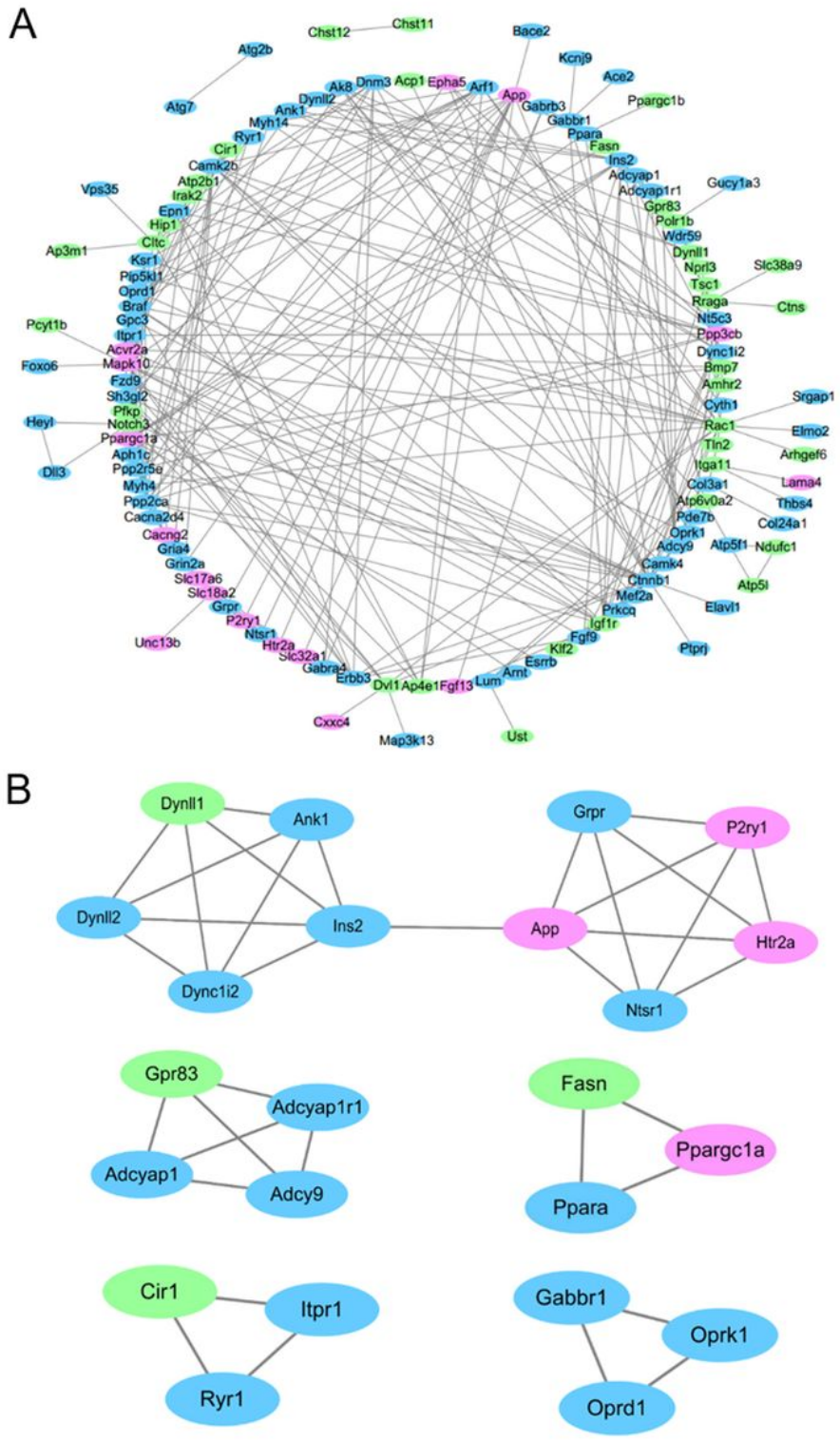

Figure 5

PPI analysis of the PTSD–related mRNAs. (A) Network of PTSD–associated mRNAs; (B) Five significant modules selected from the PPI network. Red represents highly expressed mRNAs and green represents lowly expressed mRNAs at both 5 and 10 days of aggressor exposure, and blue represents the high or low expression level of mRNAs in either of the two time sessions.

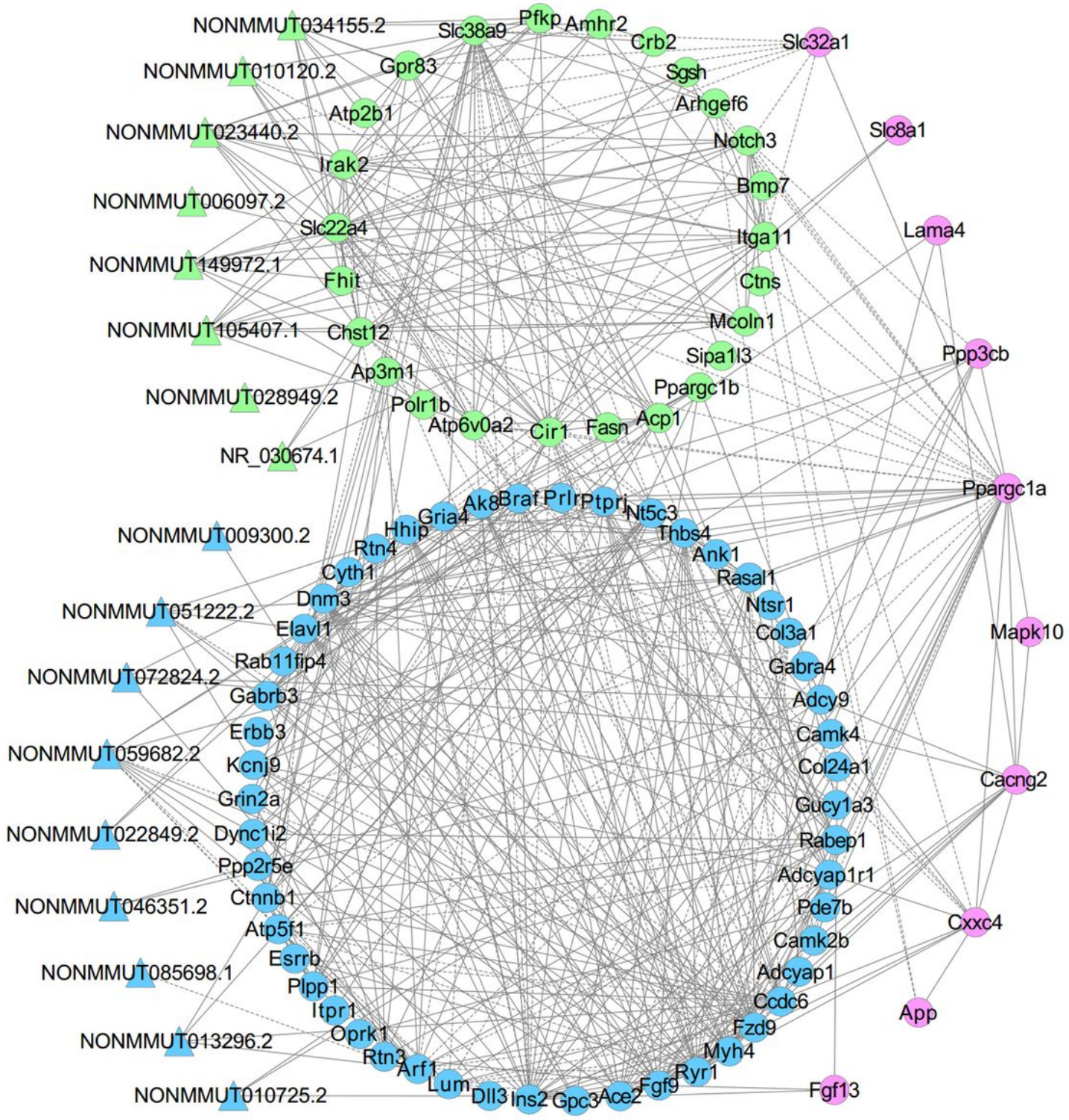

Figure 6

Co–expression pattern of lncRNAs–mRNAs involved in PTSD. Triangle nodes represent lncRNAs, circular nodes represent mRNAs, solid lines represent positive and direct connections, and dotted lines represent negative correlations. Red means up–regulated expression at both 5 and 10 days of aggressor exposure, green means down–regulated expression at both 5 and 10 days of aggressor exposure, and blue means up–regulated or down–regulated expression in either of the two time sessions.

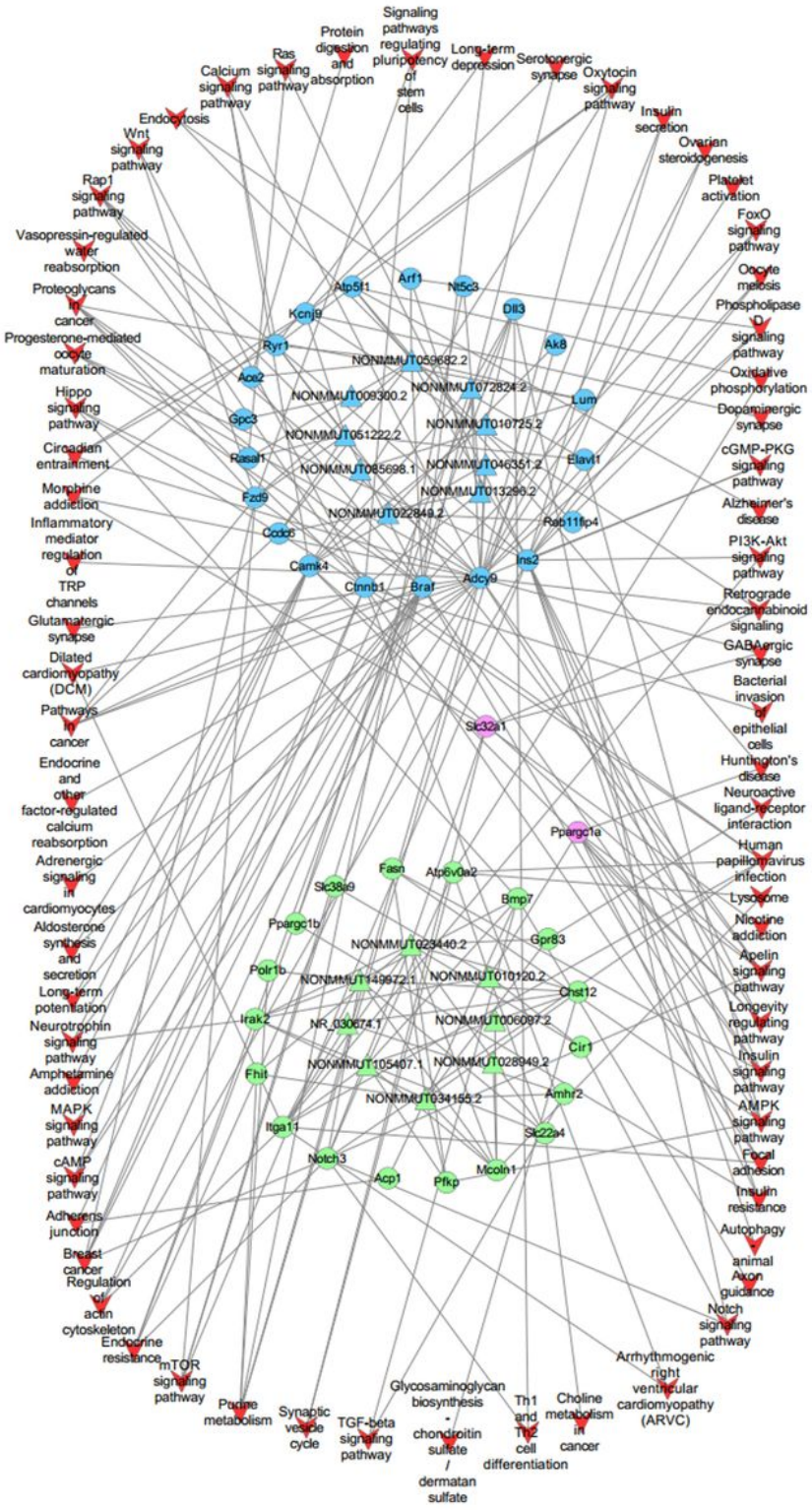

Figure 7

Signal pathway participated in lncRNA–mRNA expression. Triangle nodes represent lncRNAs, circular nodes represent mRNAs, V represents pathways. Red means up–regulated expression at both 5 and 10 days of aggressor exposure, green means down–regulated expression at both 5 and 10 days of aggressor exposure, and blue means up–regulated or down–regulated expression in either of the two time sessions.

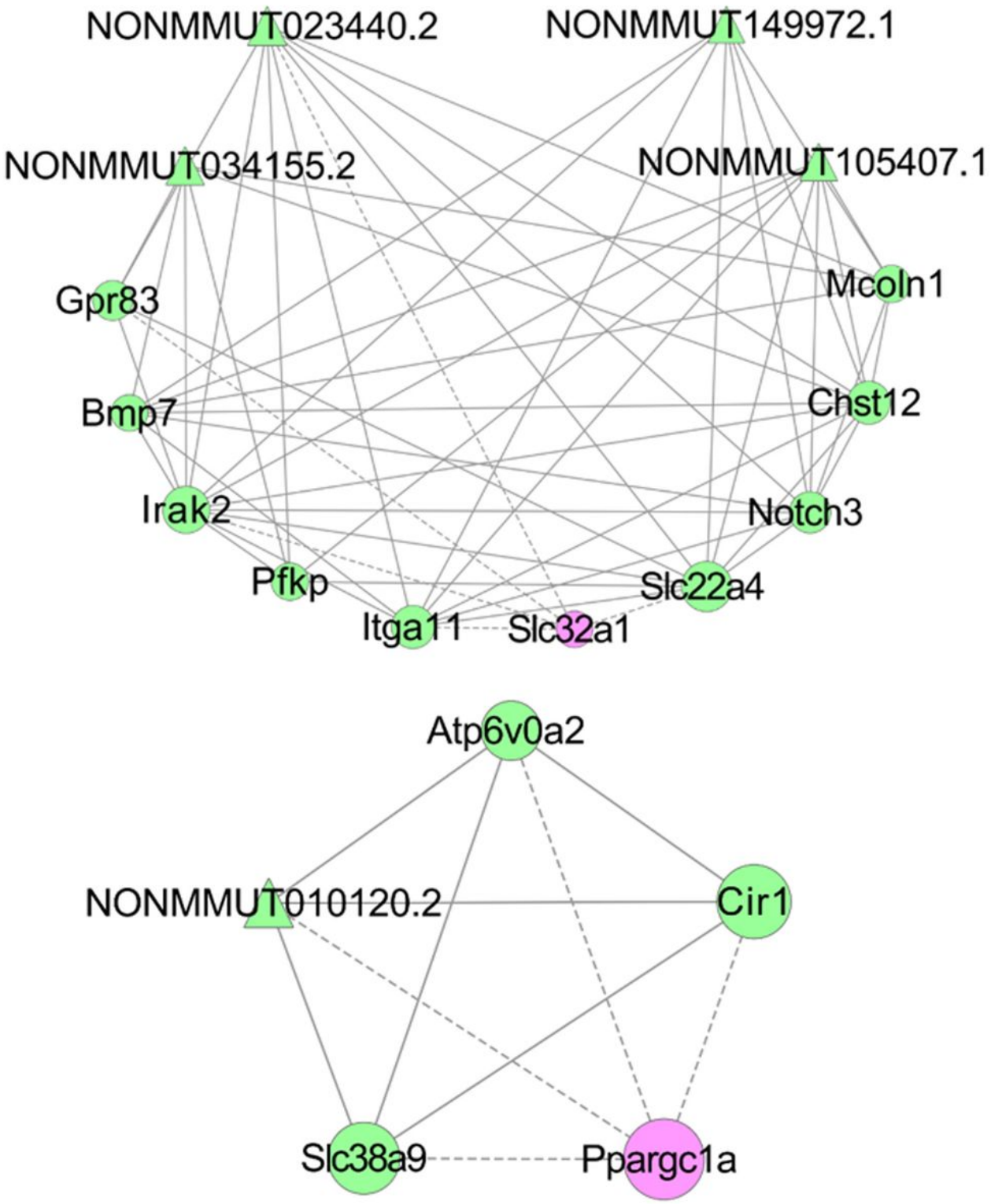

Figure 8

The sub–networks of core pair lncRNAs–mRNAs. Triangle means lncRNAs, circular means mRNAs. Red rounds represent up–regulated mRNAs, and green rounds represent down–regulated mRNAs. Solid lines mean positive and direct connections, and dotted lines mean negative correlations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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