JUN and PDGFRA as Crucial Candidate Genes for Childhood Autism Spectrum Disorder

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Research

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JUN and PDGFRA as crucial candidate genes for childhood autism spectrum disorder

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Abstract

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that is characterized by marked genetic heterogeneity. This study aimed to use gene expression datasets to explore candidate hub genes for ASD.

Methods: In this study, two independent microarray datasets of the cerebellum of children with ASD were integratively analysed using NetworkAnalyst to screen crucial candidate genes and related signalling pathways. Upregulation of the two hub genes was validated by other datasets and an ASD mouse model.

Results: NetworkAnalyst identified two upregulated genes as the most crucial genes in the cerebellum of children with ASD: Jun proto-oncogene (JUN) and platelet-derived growth factor receptor alpha (PDGFRA). According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, genes associated with JUN in the cerebellum highlight the pathways of Th17 cell differentiation and Th1 and Th2 cell differentiation. Genes associated with PDGFRA in the cerebellum were enriched in the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance and Rap1 signalling pathways. The analysis of all the differentially expressed genes (DEGs) of the two datasets using gene set enrichment analysis (GSEA) demonstrated the involvement of the IL-17 signalling pathway, which is related to the expression of JUN and PDGFRA. ImmuCellAI found that elevated expression of JUN and PDGFRA correlated with the abundance of Th17 and monocytes, suggesting that JUN and PDGFRA could regulate Th17 cell activation and monocyte infiltration. Our ASD mouse model of maternal immune activation demonstrated that JUN and PDGFRA were upregulated in ASD mice.

Limitations: The function of JUN and PDGFRA in patients of ASD and different ASD mouse models require explore and validation, and the results should be replicated in more patients with ASD.

Conclusions: JUN and PDGFRA are crucial candidate genes and related to the IL-17 signalling pathway in ASD patients.

Introduction

Autism spectrum disorder (ASD) is a multifactorial central nervous system (CNS) disorder that is characterized by impairments in social communication and repetitive and restrictive behaviours and

interests[1]. Although the pathogenesis is complex, ASD is considered a highly heritable disorder with an estimated heritability of 50%–90%[2]. Genome-wide association studies (GWASs) have demonstrated various risk loci for ASD[3]. However, the risk genes of ASD remain largely unknown because only a small part of phenotypic variation can be explained by GWAS-identified loci. Therefore, gathering additional evidence using other methods, such as gene expression, is vital.

The mechanisms underlying the association between ASD candidate genes and the neurobiology of autism are complex because genes encode multiple highly complex functions in different regions of the brain. To date, numerous studies have demonstrated associations between brain abnormalities and ASD. Furthermore, studies have increasingly highlighted the cerebellum as a pathological area of the brain in ASD[4-7]. Moreover, the co-occurrence of early behavioural defects in most neurodevelopmental disorders, which span motor, sensory, cognitive, and emotional domains, indicates that abnormalities in the cerebellum are the main determinant of ASD [8]. Evidence that directly supports this idea comes from the clinical characteristics of non-motor deficits in areas of executive function, memory, and language in children with cerebellar malformations[9] or those who have undergone cerebellar tumor resection[10].

Among the factors associated with CNS disorders, it is curious that non-CNS disruptions such as allergies[11], gastrointestinal disorders[12], and mitochondrial dysfunctions[13] are also associated with autism. Specifically, prenatal exposure to maternal immune activation (MIA) is considered a key environmental risk factor for ASD [14]. Animal models indicate that MIA results in offspring that have behavioural, neurological, and immunological abnormalities[15]. Recent studies have reported

the involvement of T helper 17 (Th17) lymphocyte activity and its core mechanism of inflammation, IL-17 signalling pathway in ASD processing[16]. Activation of IL-17 signalling pathway and elevated interleukin-17A (IL-17A) levels have been implicated in studies of ASD patients[17]. Moreover, in MIA mice offspring, increased populations of Th17 cells and elevated IL-17A levels have been observed[18]. Activated Th17 cells in the ASD brain activate the immune response and recruit further immune cells, such as monocytes and T cells, by elevating IL-17 secretion[19, 20]. Therefore, Th17 cells may play a vital role in the development of ASD. However, the role of Th17 imbalance in the cerebellum of ASD has not been fully elucidated.

We applied an integrated bioinformatics analysis to explore key regulators using NetworkAnalyst, a web-based visual analytics platform. The utility of NetworkAnalyst for identifying differentially expressed genes (DEGs) and pathways has recently been demonstrated. For example, network analyses have identified HNF4A and PTBP1 as effective biomarkers for Parkinson's disease[21] and ELAVL1 and APP as crucial candidate genes for Crohn's disease[22].

Here, we conducted a network-based bioinformatics analysis to screen DEGs in the cerebellum of ASD patients, which was followed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, gene set enrichment analysis (GSEA), and interaction network analysis of DEGs. We identified JUN and PDGFRA, which have previously been implicated in regulating T cell differentiation[23, 24]. Furthermore, GSEA data showed that IL-17 signalling-associated gene signatures were enriched, and ImmuCellAI suggested that Th17 cells were increased in the cerebellum, which could aid our understanding of the role of JUN and PDGFRA in

ASD.

Materials and Methods

Microarray source

The microarray studies of ASD were downloaded from the Gene Expression Omnibus (GEO) by using the terms “ASD” and “Brain”. Two microarray studies were selected for subsequent integrative analysis. The GSE28521 included 10 ASD patients and 11 typical development controls (TD). The GSE38322 included eight ASD patients and eight TD.

Integrated network-based bioinformatics analysis

We performed integrated bioinformatics analysis using NetworkAnalyst[25], in accordance with the protocol of how to perform and visualize meta-analysis on multiple gene expression data[26]. The datasets were normalized according to platform requirement and uploaded to the website. The DEGs were defined by $p < 0.05$ and a log2 fold change > 1.

The network-based bioinformatics analysis was performed by NetworkAnalyst according to the pipeline described[21]. The cerebellum-specific protein-protein interaction of the DEGs was constructed for each protocol[26], and the KEGG pathway enrichment was analyzed using a hypergeometric test.

Gene set enrichment analysis

Pre-ranked GSEA was performed using fgsea, a NetworkAnalyst module powered by the R package[25]. All DEGs ranked by fold changes and results were visualized by interactive heatmaps.

Abundance of infiltrating immune cells in the cerebellum samples

ImmuCellAI (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) can estimate the abundance and differences in the infiltration of 24 types of immune cells[27]. We analyzed all immune cell types in all blood samples. The immune scores of each dataset were calculated using ImmuCellAI.

Mice

C57BL/6 mice were purchased from HFK Bioscience (Beijing, China). Female mice were mated with male mice overnight and checked daily for pregnancy. Maternal immune activation was induced at gestational day 12.5 (GD12.5) by single intraperitoneal injection of 20 mg/kg of poly(I:C) (#P9582, Sigma). All mice were housed under specific pathogen-free conditions.

Open Field

Mice were placed in an open field (43.2 cm × 43.2 cm). Activity and position of each mouse within the open field was measured for 5 minutes. Data were analyzed by the MED Associates' Activity Monitor Data Analysis software.

Three-Chamber Test

Social behavior was measured using the three-chambered arena (40 × 60 cm). In Phase 1, the test mouse could explore the whole arena (habituation). In Phase 2, an empty cage was placed in one of

the side chambers, and one unfamiliar mouse was placed into a cage in the other side chamber. The test mouse was gently placed in the central chamber, and allowed to explore for 10 min. In Phase 3, the mouse in cage was replaced with a different unfamiliar mouse. The other cage was placed in the stranger in Phase 2. Time spent in each chamber and the sniffing zones was recorded by Ethovision XT 10 system (Noldus) with an overhead camera.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA from cerebellum of ASD mice was extracted using the TriZol Reagent (Invitrogen), and cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). qRT-PCR were achieved using a SYBR green real-time PCR kit (Toyobo, Osaka, Japan); reactions were run on a LightCycler (Bio-Rad Laboratories, Hercules, CA, USA). mRNA levels were normalized to GAPDH, and fold changes were determined using the $2^{-\Delta\Delta}$Ct method.

The primers are used as follows: 5'-GCGGACCTTATGGCTACAGT-3' and 5'-CCCGTTGCTGGACTGGATTA-3'(JUN); 5'-TCGCCAAAGTGGAAGAGACC-3' and 5'-TCACCAACAGCACCAACACT-3'(PDGFRA); 5'-TCATCCCTCAAAGCTCAGCG-3' and 5'-TGCGCCAAGGGAGTTAAAGA-3'(IL-17A); 5'-TCTCCACACCTATGGTGCAA-3' and 5'-CAAGAAACAGGGGAGCTGAG-3' (GAPDH).

Cytokine's analysis

The concentration of IL-17A in supernatants of lysed cerebellar samples was determined by the CBA Mouse Th1/Th2/Th17 Cytokine Kit from Becton Dickinson following the manufacturer's instructions.

Statistical analysis

All basic statistical analyses, which included the Mann-Whitney test, Pearson correlation, and Spearman correlation were calculated using the R software. A p-value < 0.05 was considered statistically significant. Data were presented as means and standard deviations (SDs) or medians and quantiles depending on the distribution of data.

Results

Seven DEGs were selected using integrated bioinformatics analysis

Two microarray studies (Table 1) were analyzed using NetworkAnalyst to screen DEGs in the cerebellum of ASD patients. The separate analyses using GEO2R of the two datasets revealed that no DEGs could be identified in either dataset (Figure 1A). The datasets were then processed to eliminate the batch effect, and parametric empirical Bayes frameworks provided by the ComBat function were performed using NetworkAnalyst. The PCA plots with and without batch effect adjustments indicated that the processed data were more comparable (Figure 1B).

Integrated bioinformatics analysis identified gamma-aminobutyric acid type A receptor subunit beta1 (GABRB1), long intergenic non-protein coding RNA 1561 (LINC01561), JUN, PDGFRA, and netrin G1 (NTNG1) were upregulated, and decapping exoribonuclease (DXO) and ectonucleoside triphosphate diphosphohydrolase 6 (ENTPD6) were downregulated in ASD compared with TD. Heat map visualization of these seven genes of the two datasets is provided in Figure 1C, and individual boxes for each gene expressed in ASD and TD are shown in Figure 1D.

JUN and PDGFRA are hub genes identified using network-based analysis

To explore abnormal biological processes in the cerebellum of patients with ASD, we entered all DEGs into a network-based analysis. Degree of centrality (DC) and betweenness (BC) showed that the seven identified DEGs exist as two obvious networks in the cerebellum. The most highly ranked node in the first network was JUN (DC = 85; BC = 3978.5; Figure 2A). The results of the KEGG pathway enrichment analysis of the network are shown in Table 2, which include Th17 cell differentiation and Th1 and Th2 cell differentiation.

The most highly ranked node in the second network was PDGFRA (DC = 23; BC = 253; Figure 2B). The results of the KEGG pathway enrichment analysis of the network are shown in Table 3, which include focal adhesion, EGFR tyrosine kinase inhibitor resistance, and the Rap1 signaling pathway. Moreover, the expression of JUN was strongly positively associated with PDGFRA in the two datasets (Figure 2C). These results suggest that JUN and PDGFRA both play important roles in the cerebellum of ASD patients and indicate the presence of a signaling pathway that is simultaneously regulated by genes.

IL-17 signalling pathway is enriched in the cerebellum of ASD patients

To further explore the mechanisms of ASD, pre-ranked GSEA was employed to analyse the two microarray datasets by selecting fold change as the gene ranking method. As shown in Figure 3A, the GSEA analysis suggested that the IL-17 signalling pathway was the only enriched pathway in the top 10 gene sets across the two datasets. The GSEA-generated heatmaps showed that the core enrichment genes in the IL-17 signalling pathway were upregulated in the ASD patients in these

datasets. Results revealed that the IL-17 signalling pathway may act as a crucial role in the development of ASD.

Upregulation of JUN and PDGFRA correlated with genes in the IL-17 signalling pathway

To further explore the association between JUN, PDGFRA, and the IL-17 signalling pathway in ASD patients, we analysed the correlation between JUN and PDGFRA and the major genes in the IL-17 signalling pathway using Pearson correlation. Various genes in the IL-17 signalling pathway, including IL-17D, CEBPB, TRAF3IP2, CASP3, JUND, and CCL20, were associated with the upregulation of JUN and PDGFRA (Figure 4 A and B), suggesting a direct relationship between JUN, PDGFRA, and IL-17 signalling in the cerebellum of ASD patients.

Upregulation of JUN and PDGFRA correlates with the abundance of Th17 and monocyte subsets in the cerebellum of ASD patients

The infiltration scores of immune cells were evaluated using ImmuCellAI, based on the gene expression of each sample. We analyzed the proportions of the 18 types of immune cells in the cerebellum of ASD patients and TD from GSE28521 (Figure 5 A) and GSE38322 (Figure 5 B). We found that Th17, CD4 naïve cells, and monocytes were more abundant in ASD patients than in TD in the cerebellum in GSE38322 (Figure 5 B). However, the cerebellum samples from GSE28521 differed slightly between ASD patients and TD (Figure 5 A). The inconsistency of the two datasets may be due to the marked heterogeneity of the disease. We further investigated the relationship between the expression of JUN and PDGFRA and the infiltration scores of Th17 and monocytes in the cerebellum from the GSE38322 dataset using Spearman correlation. As shown in Figure 6C,

JUN expression was positively correlated with the infiltration scores of Th17 ($r = 0.6118$, $p < 0.05$) and monocytes ($r = 0.6486$, $p < 0.01$). Similarly, PDGFRA expression was positively correlated with the infiltration scores of Th17 ($r = 0.3906$, $p = 0.1347$) and monocytes ($r = 0.5978$, $p < 0.05$). Th17 cells are activated and recruited by the brain of ASD patients to activate the immune response[16], which can recruit further immune cells, such as monocytes and T cells, by elevating IL-17 secretion[19]. These results imply that upregulation of the expression of JUN and PDGFRA is associated with Th17 cell activation, which is likely through the IL-17 signaling pathway.

Expression of JUN and PDGFRA relates to neurodevelopmental disorders and human ASD risk genes

To further explore the possible mechanisms of JUN and PDGFRA in ASD, gene–disease associations were performed using NetworkAnalyst. As shown in Figure 6 A and B, JUN (DC = 19; BC = 3965) and PDGFRA (DC = 40; BC = 3588) were associated with multiple neurodevelopmental disorders, including schizophrenia, bipolar disorder, Rett syndrome, seizures, and autistic disorder. We then analysed the association of JUN and PDGFRA with human ASD risk genes from the Simons Foundation Autism Research Initiative (SFARI). JUN (DC = 11; BC = 132.83) and PDGFRA (DC = 7; BC = 106.16) were both related to various verified ASD risk genes.

Evaluation of JUN and PDGFRA mRNAs in the brain of ASD patients

According to the information from the data of The Human Protein Atlas, JUN and PDGFRA can be detected in all brain's regions of humans and mice, and both have low region specificity (Figure 7 A and B). Thus, we explored whether the expression of JUN and PDGFRA was altered in other

241 regions of the brain in ASD. mRNA expression of JUN and PDGFRA was analysed in the frontal
242 and temporal cortices of ASD patients and TD from samples obtained from GSE28521. As shown
243 in Figure 7 C and D, JUN expression was significantly higher in the frontal and temporal cortices
244 of ASD than that of TD, whereas PDGFRA was only upregulated in the temporal cortex of ASD
245 patients. These results suggested the expression of JUN and PDGFRA may vary depending on brain
246 region. We further explored the expression of JUN and PDGFRA in two relevant animal models of
247 autism, the MIA and BTBR T+tf/J mouse models, which have been demonstrated to have increased
248 Th17 cells and IL-17. PDGFRA, but not JUN, was upregulated in the foetal brains of MIA offspring
249 (Figure 7 E). Moreover, the expression of JUN and PDGFRA in the medial prefrontal cortex (mPFC)
250 of MIA offspring and in the cerebellum of BTBR T+tf/J mice were similar to those of the control
251 mice (CON) (Figure 7 F and G). These results indicate that the expression levels of JUN and
252 PDGFRA differed between brain regions and were primarily upregulated in the cerebellum of MIA
253 offspring.
254
255 **Elevated expression of JUN and PDGFRA in MIA model ASD mice**
256 To validate the hypothesis that the expression of JUN and PDGFRA is significantly increased in the
257 cerebellum of ASD mice from MIA model. As shown in Figure 8 A, the cerebellums were removed
258 from the control and Poly(I:C) offspring at 6 weeks, and mice behaviours were recorded. Poly(I:C)
259 offspring showed greater depression and fewer social interactions than control offspring (Figure 9
260 B). qPCR assays revealed that JUN, PDGFRA, and IL-17A mRNAs and protein were significantly
261 higher in the cerebellum of Poly(I:C) offspring compared with CON offspring (Figure 8 C and D).
262 These results suggested that the increased expression of JUN and PDGFRA was related to ASD

development, which is likely via the facilitation of the IL-17 signalling pathway.

Discussion

The pathogenesis of ASD is complex, and identifying the crucial regulators in the CNS is essential for understanding and improving the clinical management of ASD. Integrated analysis provides an unbiased method to identify and prioritize biologically central biomarkers for neurodegenerative diseases[28]. Here, we identified crucial candidate genes in the cerebellum of ASD patients using a network-based analysis of GSE28521 and GSE38322 and selected seven DEGs among these datasets. JUN and PDGFRA were identified as the most crucial genes by NetworkAnalyst. In addition, differential expression of the two hub genes was validated by other datasets and an ASD mouse model, which indicated that JUN and PDGFRA play a vital role in the development of ASD.

Network-based analysis identified JUN as the most crucial hub gene. JUN encodes c-Jun, also known as activator protein 1 (AP-1), which is a transcription factor that is considered to be the main regulator of neuronal death and regeneration[29]. The activity of c-Jun is regulated by phosphorylation, which is mediated by the c-Jun N-terminal kinase (JNK) family, including JNK1, JNK2, and JNK3[30]. In the CNS, the activation of c-Jun is essential for regeneration after antiretroviral-induced peripheral neuropathy, whereas the activation of JNK3 causes neuropathic pain[31]. The activation of c-Jun caused by stressful stimuli is mediated by JNK2 and JNK3, which leads to cell death of cerebellar neurons[29]. Moreover, JNK3 is related to various neurodegenerative diseases, whereby the activation of JNK3 has impact on triggering apoptosis[32] and neuronal death

284 in several neurodegenerative disorders[33]. Recent research has indicated that JNK activation is
285 heavily involved in the pathophysiological mechanism of ASD, based on its function in regulating
286 basal dendrite development in cortical neurons[34] and cognitive impairment[35]. This is consistent
287 with our gene-disease analysis of JUN that indicated that the expression of JUN is associated with
288 mercury poisoning and brain ischemia (Figure 6 A), which are involved in neuronal death and result
289 in the development of ASD[36, 37]. Although the exact mechanism is not clearly understood, these
290 lines of evidence suggest a link between JUN and ASD.
291
292 PDGFRA, a classical proto-oncogene that encodes receptor tyrosine kinases that respond to platelet-
293 derived growth factor (PDGF), was recently identified to impact the formation of the neural crest[38].
294 Notably, mice lacking PDGFRA cannot survive and present multiple defects in CNS [39]. Activated
295 PDGFRA transduces the signals involved in multiple downstream pathways, including the
296 PI3K/Akt, MAP kinase, and EGFR pathways[40, 41], which have all been implicated in the brain of
297 ASD patients[42-44]. However, studies on PDGFRA in ASD are limited. Numerous researchers have
298 found that both adult and paediatric forms of glioblastoma (GBM) are related to the activation of
299 PDGFRA[38]. Furthermore, EGFR kinase, the most prominent oncogenic target for GBM, has been
300 disappointing after inhibited by drugs in clinical trials[45]. Notably, we found the pathway
301 enrichment of genes in the second PDGFRA network as the core EGFR tyrosine kinase inhibitor
302 resistance in the cerebellum of ASD patients (Table 3). There is an increasing recognition that GBM
303 and ASD share the same fundamental pathophysiological mechanisms at the cellular and molecular
304 levels[46]. Therefore, our work on exploring the hub genes in ASD may function as a novel mediator
305 for both ASD and GBM, which could facilitate the development of treatment strategies for ASD as

well as brain cancers.

The results from the GSEA highlight the importance of the IL-17 signalling pathway in the cerebellum of ASD patients (Figure 3 A). IL-17 is the core member of the IL-17 family of cytokines, which includes IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F[47]. IL-17, also known as IL-17A, is the signature cytokine produced by Th17 cells and is the only cytokine that is prominently upregulated in MIA offspring[20]. Recent studies have shown that IL-17 is a key contributor to neurodevelopmental abnormalities in MIA offspring[16]. Interestingly, the pathway enrichment of genes from the first network in the cerebellum of ASD patients with JUN as the core also highlighted the Th17, Th1, and Th2 cell differentiation pathways (Table 2), which play vital roles in the pathogenesis of ASD[48, 49]. Notably, the ImmuCellAI data indicated that Th17 and monocyte proportions were significantly increased in the cerebellum, which may have been caused by the function of IL-17 in immune response activation and immune cell recruitment[19]. It is reasonable to presume that the IL-17 signalling pathway may function as a vital regulator of ASD pathology in the cerebellum. In addition, our work also demonstrated the close connection of JUN and PDGFRA with genes in the IL-17 signalling pathway (Figure 4). Moreover, although the mechanisms of PDGFRA in the mediation of the IL-17 signalling pathway remain unclear, our results indicate that EGFR, which is closely related to PDGFRA in the cerebellum of ASD (Figure 2 B), plays an essential role in the activation of Th17[50]. Thus, it will be worth exploring the link between JUN and PDGFRA and the IL-17 signalling pathway and investigating their roles in the progression of ASD.

It is well-known that there are significant differences in the structure and function of various brain regions. Because of the low region specificity of JUN and PDGFRA in the brain (Figure 7 A and B), we further evaluated their mRNA in other brain regions. The relative abundance of JUN mRNA was upregulated in the frontal and temporal cortices of ASD patients (Figure 8 C and D). However, the association between the relative abundance of JUN and PDGFRA did not reach statistical significance in BTBR T+tf/J mice. One possible explanation is that the mechanism of Th17 activation in the cerebellum may differ from that in BTBR T+tf/J and MIA mice. These results indicate that the upregulation of JUN and PDGFRA mRNA in the cerebellum are specific biomarkers for MIA offspring. In our animal model, we found an abundance of JUN and PDGFRA in the cerebellum, and the increase in IL-17A further supported the role of genes in ASD (Figure 8). However, the current study could not demonstrate the regulatory functions of JUN and PDGFRA on the IL-17 signalling pathway, and further investigation on the regulator of the IL-17 pathway and their connection to abnormal behaviours in an animal model is needed.

Limitations

Brain samples of children with ASD are difficult to obtain, and the studies of relevant transcription datasets is rare. Moreover, the RNA expression of JUN and PDGFRA of ASD mice has limited validity. Another limitation is that the upregulation of JUN and PDGFRA may lead to the development of ASD in a complex mechanism, which is difficult to explored in mice or patients with ASD.

Conclusion

Our study suggests that JUN and PDGFRA are crucial candidate genes in the cerebellum of children with ASD and highlights the roles of the IL-17 signalling pathway in the activation of the immune

response in ASD. JUN may modulate the activation of Th17 cells in the cerebellum of ASD, whereas PDGFRA may be a key regulator that is associated with the EGFR pathway and Th17 activation. Our analysis provides valuable insight and further understanding of the mechanism of JUN and PDGFRA in the pathogenesis of ASD.

Ethics declarations

This study was performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of Hubei Provincial Animal Care and Use Committee. All animal protocols were approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

Heli Li, Yan Hao and Xiaoping Luo designed the study, analyzed the data, and wrote the manuscript. Xinyuan Wang, Cong Hu, Hao Li, Zhuoshuo Xu and Ping Lei analyzed the data. Yan Hao provided funding.

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Figure 1. 7 DEGs were selected using integrated bioinformatics analysis

(A) Volcano plot of DEGs between ASD patients and TD for GSE28521 (left) and GSE38322 (right). (B) PCA plots all datasets without (upper) and with batch effect adjustments (lower). (C) Heat map

representation of the DEGs across the two microarrays identified from the integrated analysis. (D) The expression of DEGs in ASD and TD from the two datasets represented by scatterplots.

Figure 2. JUN and PDGFRA are hub genes identified using network-based analysis

Network analysis of all DEGs in the cerebellum of ASD patients. The first (A) and the second (B) network of genes regulated in the cerebellum of ASD patients. (C) Scatterplots with regression lines. Correlation between JUN and PDGFRA expression in the cerebellum of ASD patients and TD.

Figure 3. IL-17 signalling pathway is enriched in the cerebellum of ASD patients according to the GSEA analysis

(A) The enrichment network of DEGs identified using network-based analysis. (B) GSEA-generated heatmaps of genes in the IL-17 signalling pathway in the two datasets.

Figure 4. Correlation of JUN and PDGFRA correlated with genes in the IL-17 signalling pathway

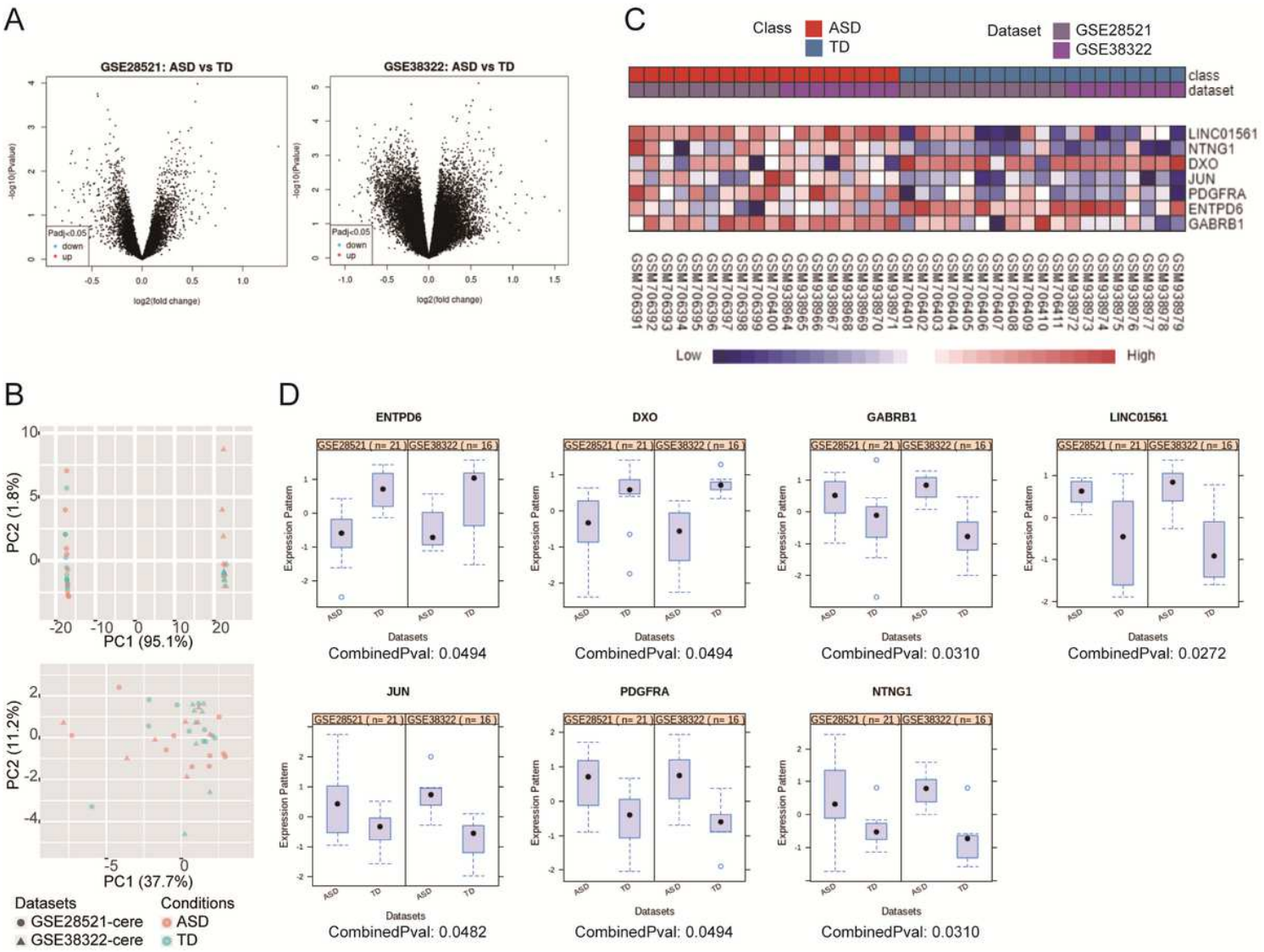
Correlation between the gene expression of JUN (left) and PDGFRA (right) and selected genes in the IL-17 signaling pathway in the cerebellum of children with ASD and TD analysed using expression data from GSE28521 (A) and GSE38322 (B) datasets are illustrated by scatterplots with regression lines.

Figure 5. Expression of JUN and PDGFRA correlated with Th17 and monocyte subsets in the cerebellum of ASD patients

518 The abundance of immune cells by group and the whole cohort of ASD patients in GSE28521 (A)
519 and GSE38322 (B) (*$p < 0.05$). The association between JUN (C), PDGFRA (D), and infiltration
520 scores of Th17 and monocytes of the cerebellum were analysed using the expression data from
521 GSE38322. Scatterplots were shown with regression lines.
522
523 **Figure 6. Expression of JUN and PDGFRA relates to neurodevelopmental disorders and**
524 **human ASD risk genes**
525 Zero-order interaction networks of JUN and PDGFAR with human diseases (A) and ASD risk genes
526 from SFARI (B).
527
528 **Figure 7. Evaluation of JUN and PDGFRA mRNAs in ASD brains**
529 The expression of JUN and PDGFRA in the brain regions of the GTEx human brain RNA-Seq
530 dataset (A) and the HPA mouse brain RNA-Seq dataset (B). Expression of JUN and PDGFRA in
531 the frontal (C) and temporal cortices (D) of samples from GSE28521. Expression of JUN and
532 PDGFRA in the fetal rat brain from GSE34058 (E) and mPFC from GSE117327 (F). (G) Expression
533 of JUN and PDGFRA in the cerebellum of BTBR T+tf/J and CON mice from GSE62594.
534
535 **Figure 8. Elevated expression of JUN and PDGFRA in MIA model ASD mice**
536 (A) Schematic representation of the procedures followed to establish the MIA model. (B) In the
537 open field and social behavior tests, the poly (I:C) group showed significantly less time in the center
538 area and socially interacting. Data are presented as means ± standard error of the mean (n = 6, *$p <$
539 0.05, **$p < 0.01$). (C) qPCR for JUN, PDGFRA, and IL-17 signalling-associated gene, IL-17A, in

the cerebellum of MIA model mice. (D) The concentration of IL-17A in supernatants of lysed cerebellar samples examined by CBA kit.

Figures



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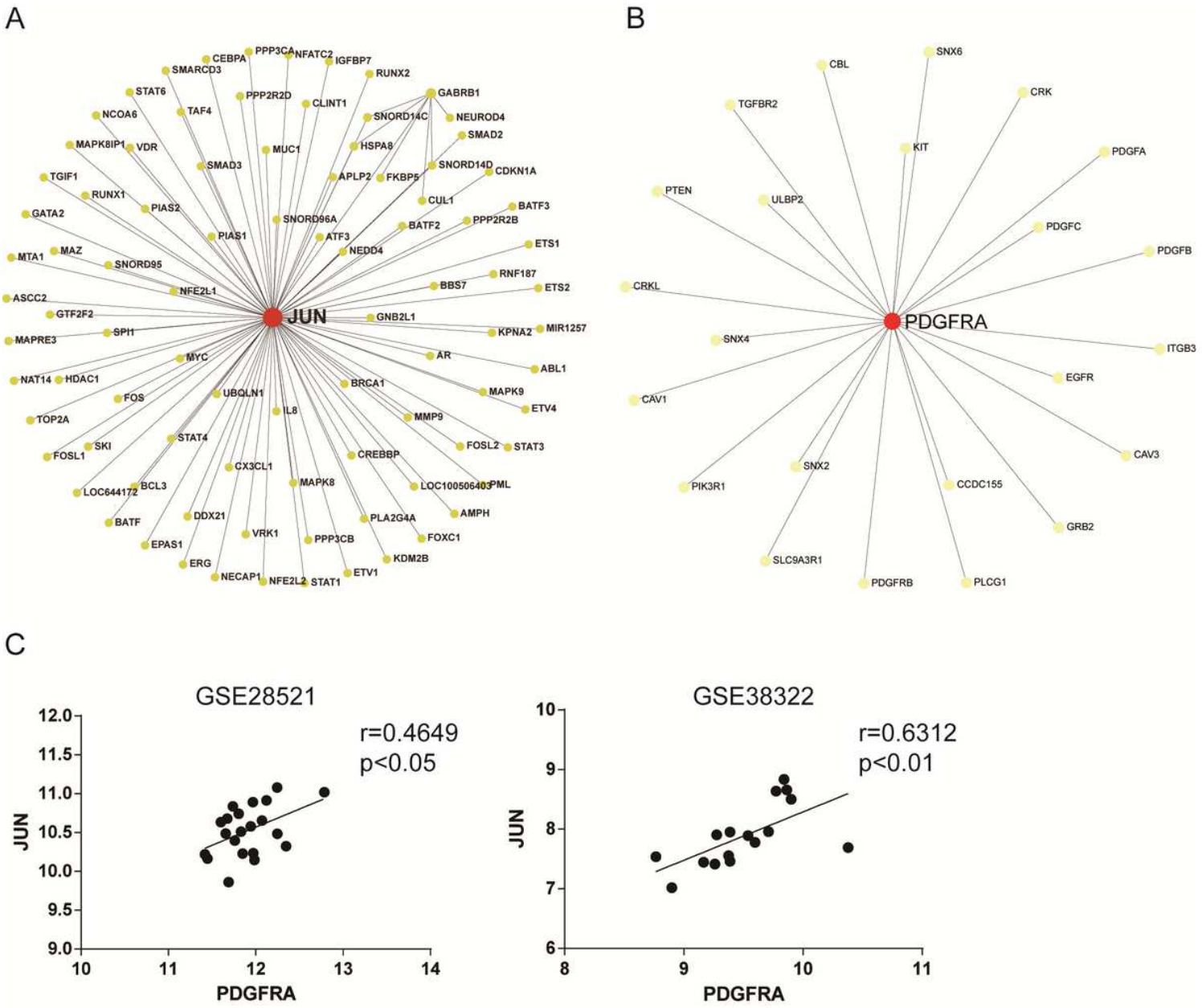

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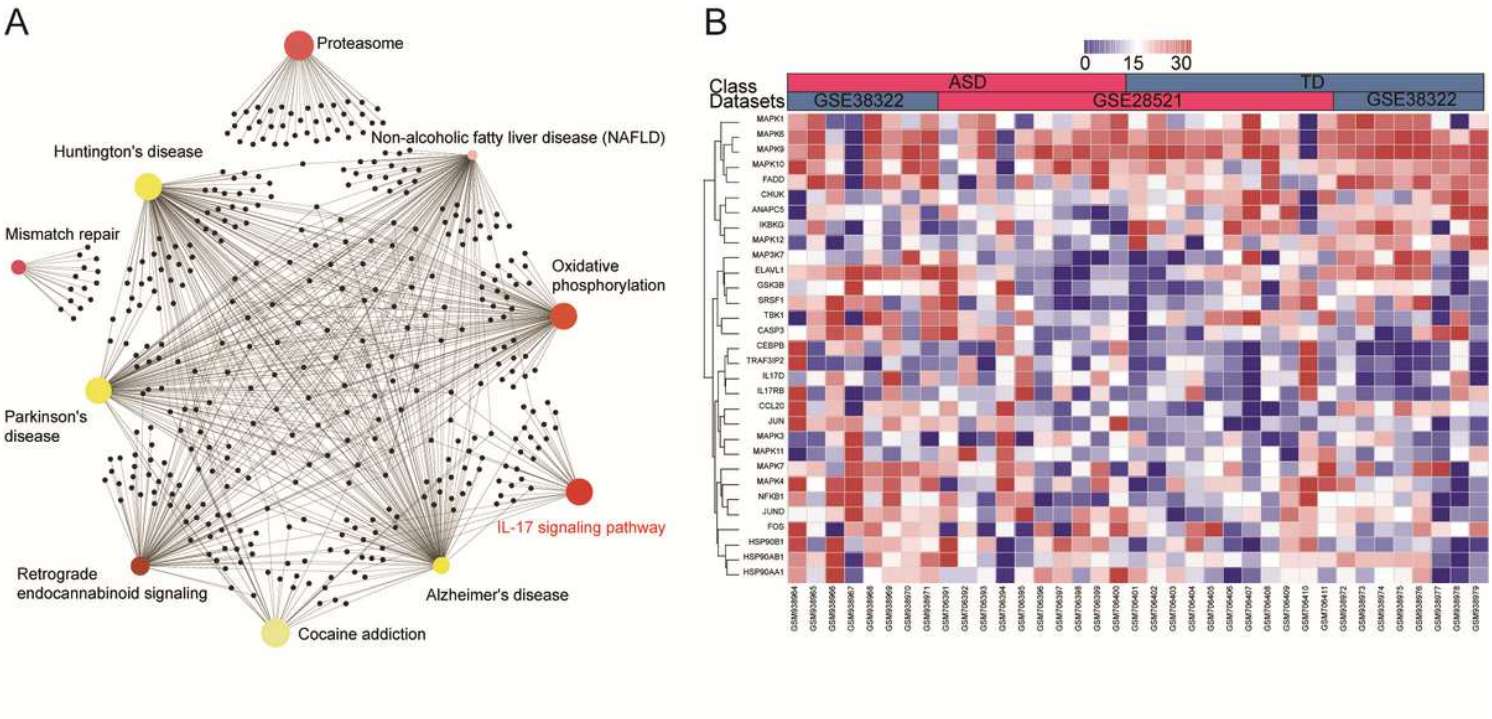

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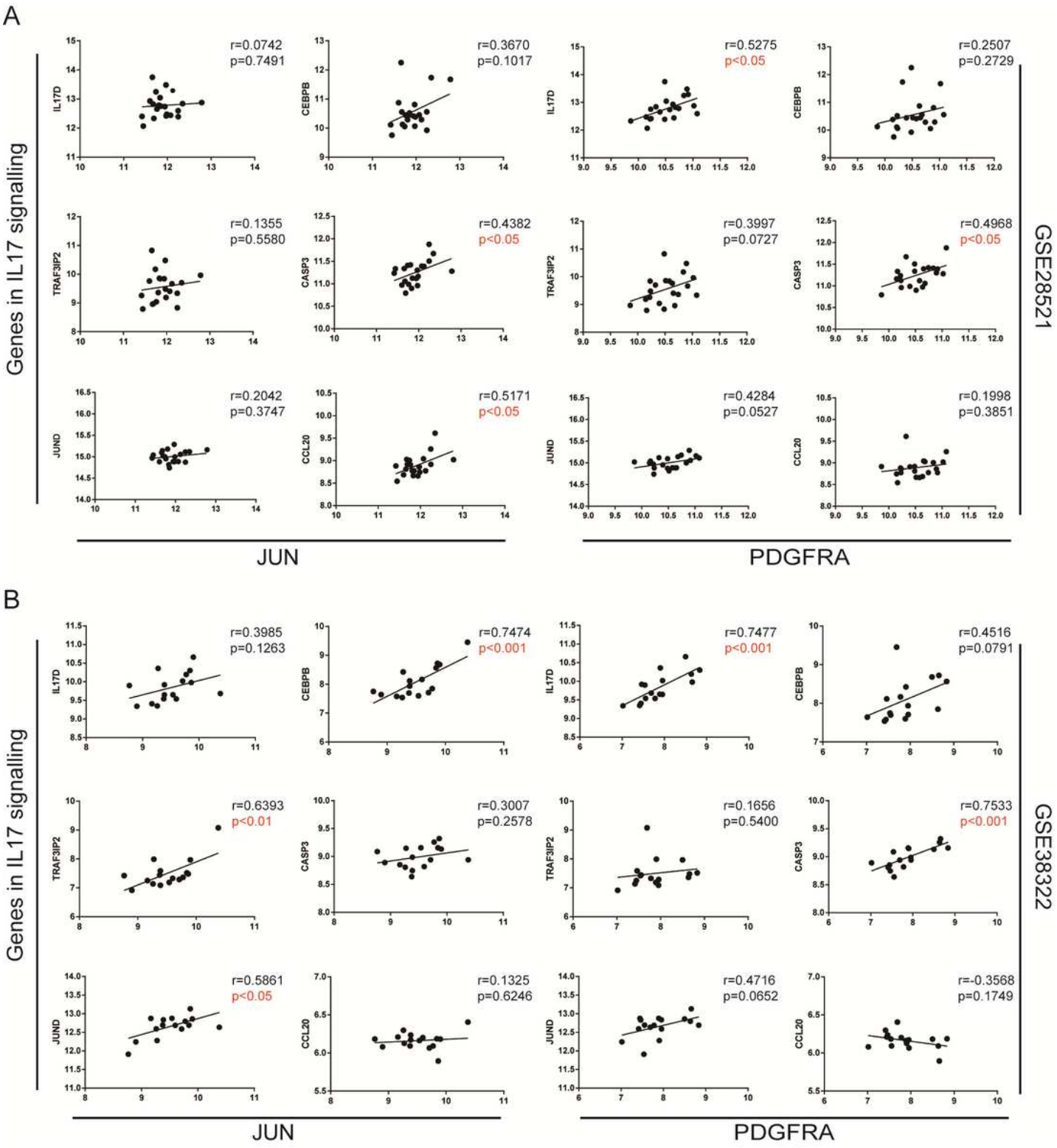

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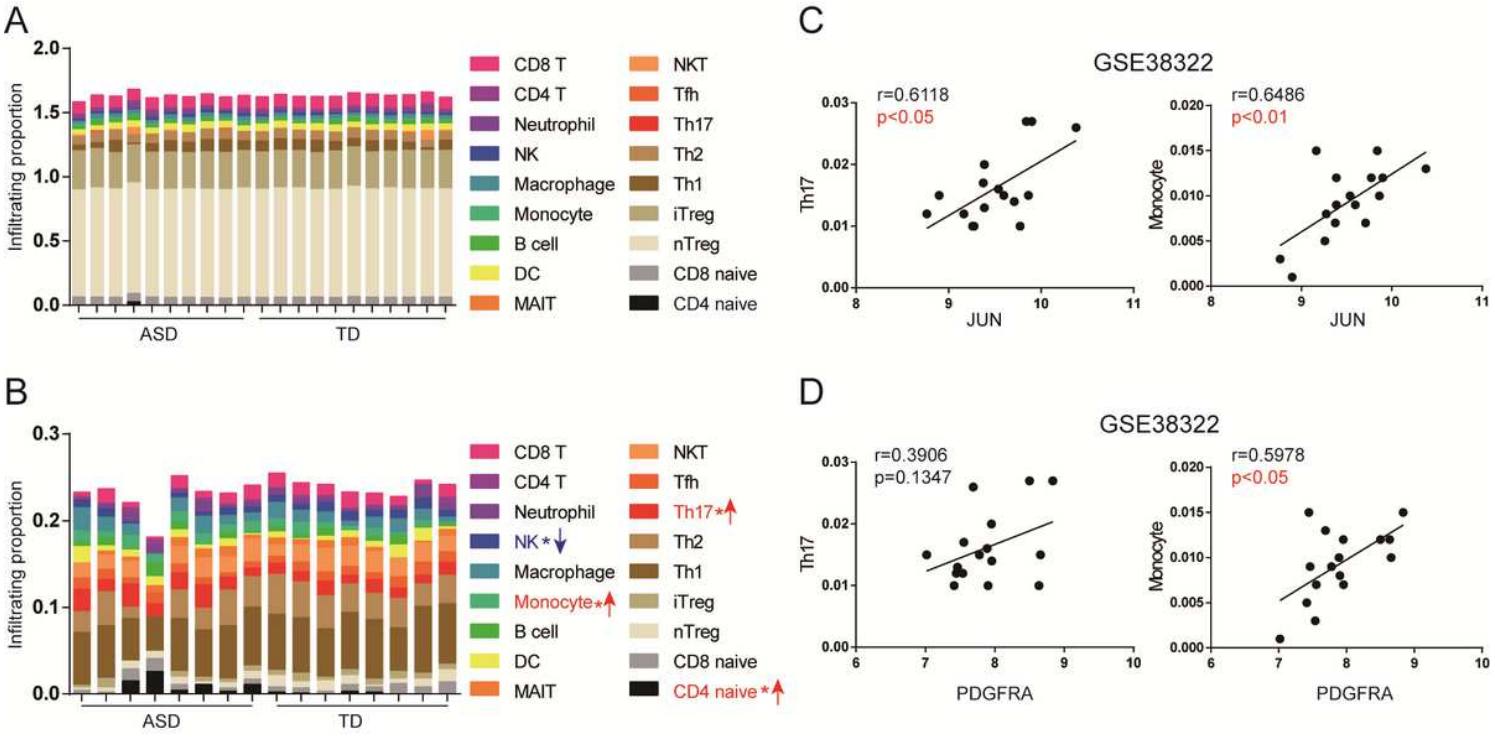

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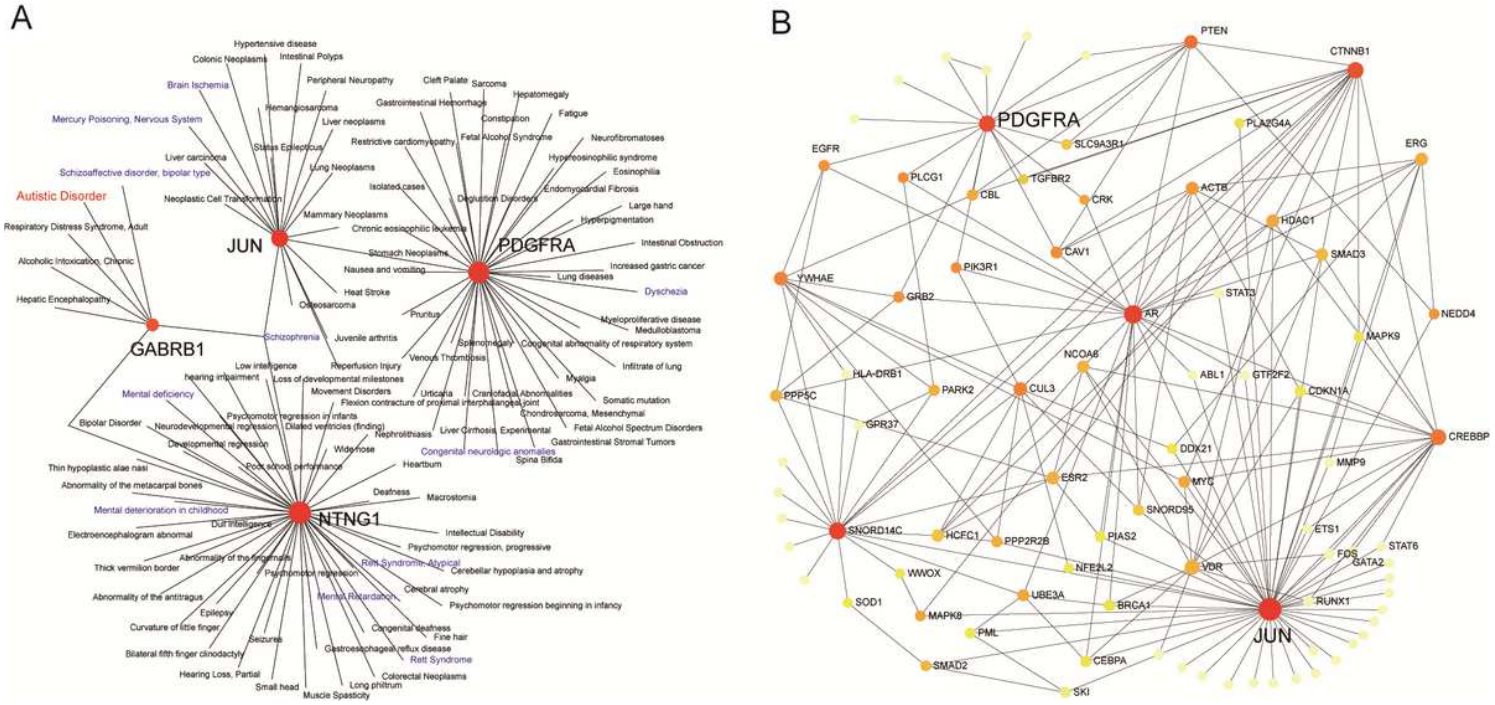

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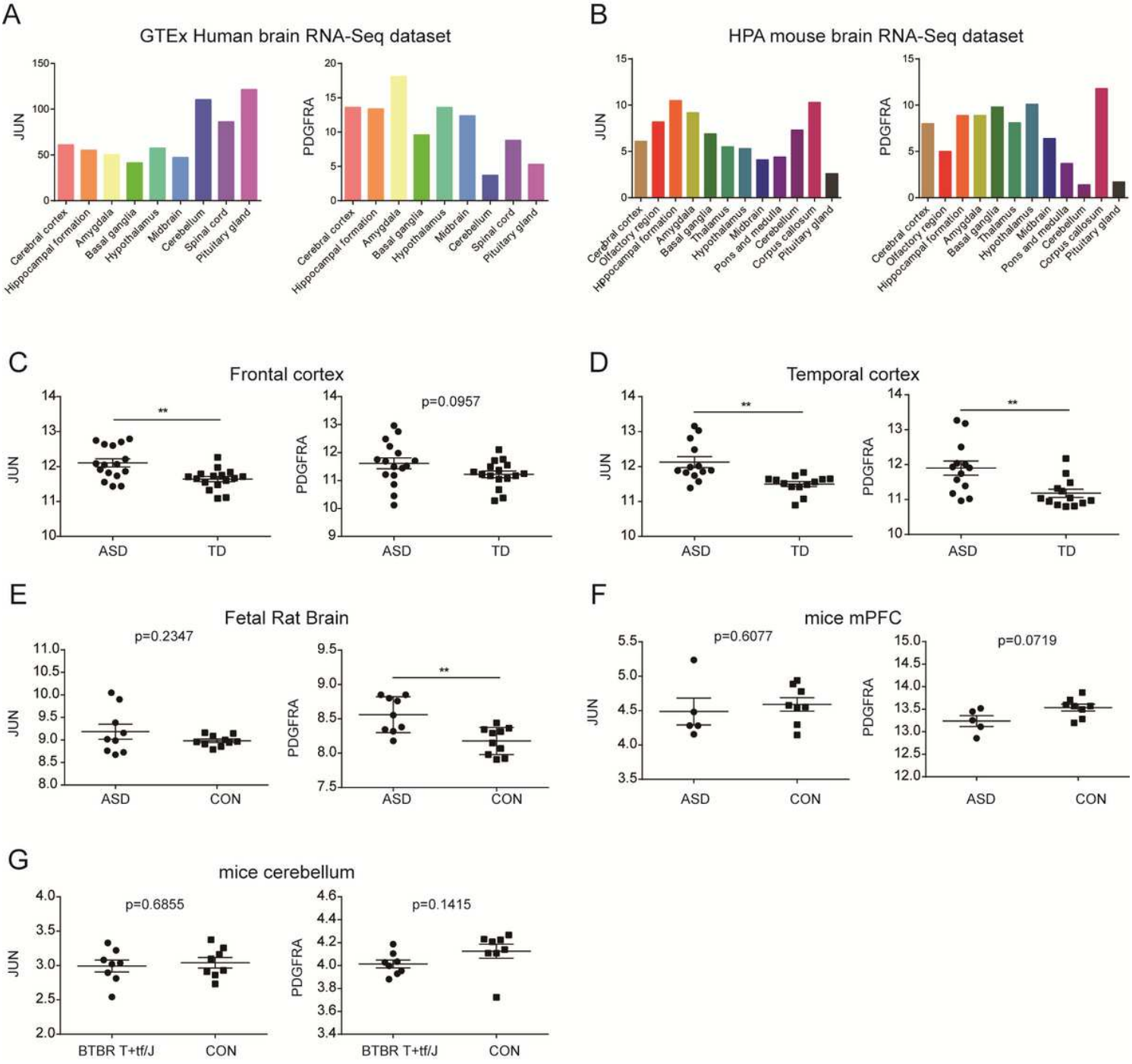

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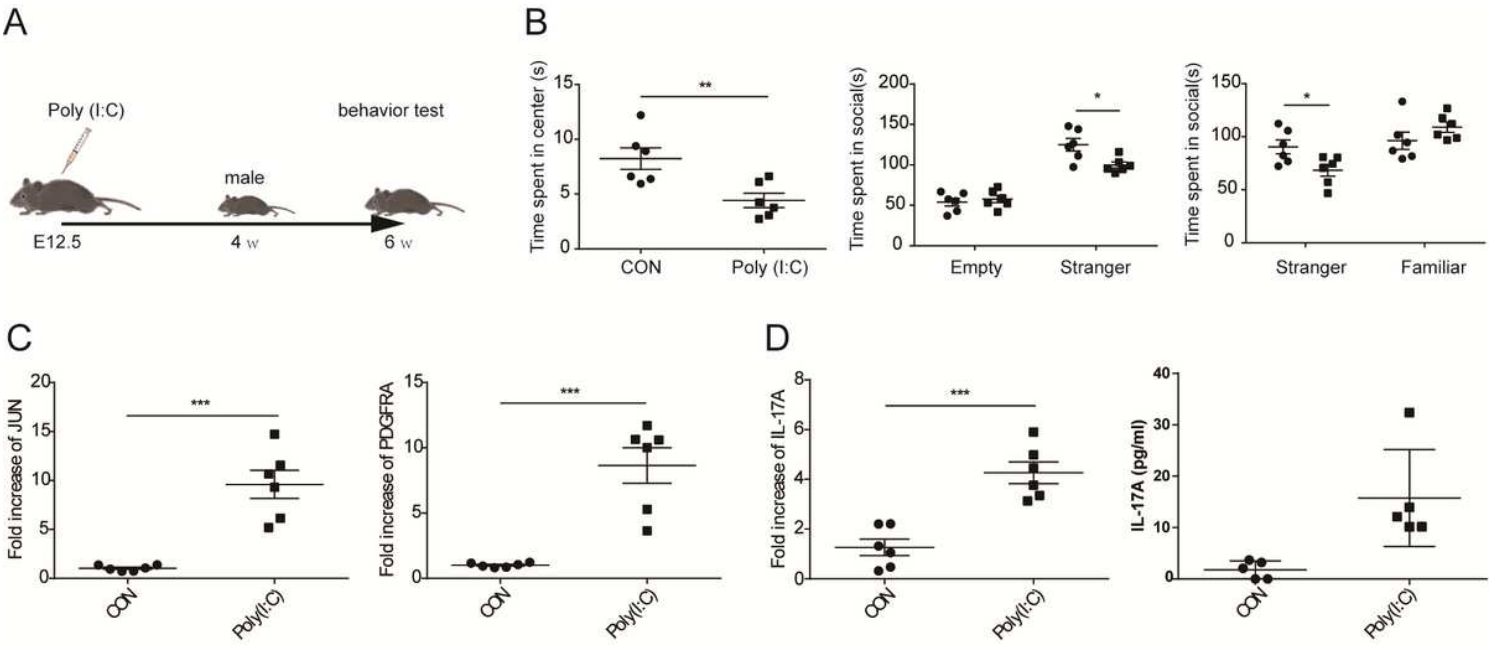

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Supplementary Files

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

- Table1.pdf
- Table2.pdf
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