Alternative splicing in mouse brains affected by psychological stress is enriched in the signaling, neural transmission and blood-brain barrier pathways

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Abstract

Psychological stress increases the risk of major psychiatric disorders. Psychological stress on mice was reported to induce differential gene expression (DEG) in mice brain regions. Alternative splicing is a fundamental aspect of gene expression and has been associated with psychiatric disorders but has not been investigated in stressed brain yet. This study investigated changes in gene expression and splicing under psychological stress, the related pathways, and possible relationship with psychiatric disorders. RNA-seq raw data of 164 mouse brain samples from 3 independent datasets with stressors including chronic social defeat stress (CSDS), early life stress (ELS), and two-hit stress of combined CSDS and ELS were collected. There were more changes in splicing than in gene expression in the ventral hippocampus and medial prefrontal cortex, but stress-induced changes of individual genes by differentially spliced genes (DSGs) and DEGs could not be replicated. In contrast, pathway analyses produced robust findings: stress-induced DSGs were reproducibly enriched in neural transmission and blood-brain barrier systems, and DEGs were reproducibly enriched in stress response-related functions. The hub genes of DSG-related PPI networks were enriched in synaptic functions. The corresponding human homologs of stress-induced DSGs were robustly enriched in AD-related DSGs as well as BD and SCZ in GWAS. These results suggested that stress-induced DSGs from different datasets belong to the same biological system throughout the stress response process, resulting in consistent stress response effects.

Introduction

Psychological stress is ubiquitous in human life experience and excessive stress results in biological changes that increase the risk for psychiatric illness. The hypothalamic–pituitary–adrenal (HPA) axis is the primary system responding to psychological stress [1–3]. Psychological stress stimuli are mainly processed in the limbic brain regions [4–7] that regulate the HPA axis [8–10]. Limbic brain regions intersect with reward circuit-related brain regions, including the ventral tegmental area (VTA), nucleus accumbens (NAc), amygdala, hippocampus, etc. Research suggests that modulation of the reward system could help cope with stress [11, 12]. Animal models revealed that chronic psychological stress may alter the volume of reward-related brain regions [13–16] and elevate anxiety- and depression-like behavior [17–20]. Human studies also showed that chronic psychological stress increased susceptibility to psychiatric disorders [21–23]. How chronic psychological stress increases the risk for psychiatric disorders remains poorly understood.

Mouse model studies have found chronic psychological stress induced changes in gene expression [19, 20, 24]. Chronic social defeat stress (CSDS) and early life stress (ELS) paradigms are widely used in mouse models since they produce certain phenotypes related to psychiatric disorders in human [19, 25]. In CSDS, each defeated male mouse is placed into a cage of an aggressor mouse for 5–10 min for 10 days. In ELS, each mouse is treated by maternal separation for 3–4 hours and limited nesting for 24 hours each day for 10 days. Laine et al. [24] observed increased anxiety-like behavior and changes in myelin-related gene expression in the medial prefrontal cortex (mPFC) and ventral hippocampus (vHIP)

after CSDS. Peña et al. [19] found that ELS-induced differential gene expression was associated with latent depression-like behavior.

Alternative splicing is an essential biological process in gene transcription, producing multiple proteins from one gene, and it is highly abundant in the brain [26–28]. Previous studies identified possible relationships between chronic psychological stress and alternative splicing in several candidate genes. For example, Uchida et al. [29] reported an overexpressed splicing isoform of the transcriptional repressor REST in the mPFC after ELS. Nair et al. [30] discovered altered BDNF transcripts in the hippocampus in response to ELS. In addition, recent studies reported aberrant mRNA splicing in the transcriptome studies of brains of psychiatric disorders [31–33].

How chronic psychological stress affects splicing in the brain transcriptome and whether stress-induced alternative splicing could mediate the effects of psychological stress on the risk of psychiatric disorders have not been investigated. RNA-seq data of vHIP, mPFC, NAc, and VTA from previous publications, which are part of the reward-related regions, were available for this analysis. This study hypothesized that psychological stress leads to alterations in mRNA splicing in the brain. It also hypothesized that alternative splicing may mediate the impacts of psychological stress on the risk of psychiatric disorders. DSGs in response to three stress conditions in four brain regions, and DSG-associated pathways, networks, and relevance to psychiatric disorders were analyzed. Because of concerns about reproducibility [34], the data from different sources were compared to assess the robustness of the findings. The results provided new insight into the biological effects of psychological stress on the brain. It also provided a foundation for future research testing the mechanistic role of molecular pathways implicated in psychological stress.

Methods And Materials

Study cohorts. RNA-seq raw data retrieved on Gene Expression Omnibus were required to meet the following conditions: (1) chronic psychological stress/CSDS/ELS; (2) brain tissue. Since this study aimed to assess the reliability of the findings, datasets with the same stress model in the same brain regions were required.

Data of C57BL/6 mice brain samples were obtained from four independent studies, Peña et al. (GSE89692) [35], Bagot et al. (GSE72343) [20], Laine et al. (GSE109315) [24], and Marrocco et al. (GSE131972) [36]. GSE89692 was set as the discovery dataset with 132 brain samples with stressors, including ELS, CSDS, and two-hit stress (ELS during postnatal days and then the second stress in adulthood), assessed in the vHIP, mPFC, NAc, and VTA. GSE72343 (n = 18) and GSE109315 (n = 35) were the independent replication datasets for CSDS in vHIP and mPFC. GSE131972 with five brain samples was used to replicate ELS in vHIP. Detailed sample size information is provided in Table 1.

Table 1
Sample information and the numbers of DSGs and DEGs

Datasets	Brain regions	Stress conditions	Case vs Control	DSCs/DSGs		DEGs	
				adj.*p* < 0.05	p < 0.05	adj.*p* < 0.05	p < 0.05
GSE89692	vHIP	ELS	6/6	111/109	1224/1111	1	625
		CSDS	6/6	154/149	1378/1208	1	473
		EC	6/6	81/75	1080/975	0	90
	mPFC	ELS	11/12	45/45	1534/1391	0	785
		CSDS	6/12	21/21	1133/1051	0	254
		EC	6/12	70/67	1467/1303	49	1295
	NAc	ELS	15/7	1/1	262/256	93	1165
		CSDS	11/7	1/1	235/231	7	650
		EC	20/7	1/1	291/280	12	1340
	VTA	ELS	4/4	0/0	245/235	0	83
		CSDS	6/4	3/3	256/242	0	84
		EC	6/4	1/1	312/302	5	224
GSE131972	vHIP	ELS	3/2	10/10	277/263	0	7
GSE72343	vHIP	CSDS	6/3	0/0	224/219	2	6
	mPFC	CSDS	6/3	1/1	362/356	1	175
GSE109315	vHIP	CSDS	11/6	21/20	182/176	0	217
	mPFC	CSDS	12/6	75/74	824/752	2	782

GSE89692 is the discovery dataset. GSE131972, GSE72343, and GSE109315 are replication datasets. vHIP, ventral hippocampus; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; VTA, ventral tegmental area; ELS, early life stress; CSDS, chronic social defeat stress; EC, early life stress + chronic social defeat stress; DEG, differentially expressed genes; DSC, differentially spliced clusters; DSG, differentially spliced genes. *Adj.p* Benjamini–Hochberg adjusted p value.

RNA-seq data preprocessing. All sample FASTQ files were trimmed for adapter sequence and low base call quality using Trimmomatic [37] (version 0.36). The trimmed reads in FASTQ format were inspected by FASTQC program (version 0.11.2), then the reads were aligned to the GENCODE release M23 (GRCm38.p6) using STAR [38] (version 2.6.0). Bam files were produced and sorted using Samtools [39] (version 1.7). For differential splicing analysis, Leafcutter (version 0.2.7) was applied to estimate the 'percentage spliced-in' (PSI) values for local alternative splicing events. The spliced reads with a shared splice site were clustered and filtered to keep an intron cluster supported by at least 50 split reads,

retaining introns of up to 500 kb. For differential gene expression analysis, RSEM (version 1.3.1) was used to obtain the expression matrix. Genes were filtered to include those with the counts > 10 in at least 50% of the samples. Outlier samples with standardized sample network connectivity Z-score < -2 were removed.

Covariate correction. Quality control metrics were calculated using RNA-SeQC [40] (version 1.1.8), bamdst, PicardTools (version 1.119), and Samtools. The potential covariates for all samples included sex, age, library preparation batch, experiment duration, experiment time point, the interval between the end of experiment and the death of the subjects, and sequence quality control metrics. ComBat function of sva package [41] was employed to correct batch effects. The weighted average proportion of variance explained by each covariate was estimated using PVCA. Surrogate variable analysis on differential splicing and differential expression measurements was used to remove the effect of hidden factors.

Differential gene expression analyses. DESeq2 R package was used to calculate differential gene expression between stress conditions and control group by the multiple regression linear model. The covariate model specified in the previous section was included in the model. The significant DEG was set at Benjamini–Hochberg-corrected $adj.p < 0.05$.

Differential splicing analyses. Stress-induced differential splicing events in each brain region were identified by jointly modeling intron clusters using the Dirichlet-Multinomial generalized linear model from Leafcutter [42], adjusting for the selected covariates. The significant differentially splicing clusters (DSCs) was set at Benjamini–Hochberg-corrected $adj.p < 0.05$. The genes harboring the DSCs were called Differentially spliced genes (DSGs).

Function and pathway enrichment of stress-induced DSGs and DEGs. Since the numbers of DSGs and DEGs that can survive $adj.p < 0.05$ were too small for the geneset enrichment analyses, the DSGs and DEGs with nominal $p < 0.05$ across three stress conditions in the four brain regions were extracted for this enrichment analysis (Fig. 1). Gene Ontology (GO) of biological processes and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to annotate the function of the DSGs and DEGs. This analysis was performed by gProfileR R package without hierarchical filtering and an ordered query. The first and second parent GO terms and a term with the intersection less than 2 were removed. Benjamini–Hochberg FDR correction was applied for multiple testing.

PPI network analyses. DSGs and DEGs with nominal $p < 0.05$ across three stress conditions in the four brain regions were used to construct the DSG-related PPI networks by STRING [43] (version 11.5). The STRING database contains 19566 human proteins, with an interaction score of 0.7. The CytoHubba plugin [44] in Cytoscape was employed to find the top 50 hub genes with a high degree of connection based on the maximal clique centrality method.

Enrichment in risk genes of psychiatric disorders. Corresponding human homologous genes of the DSGs and DEGs with nominal $p < 0.05$ across three stress conditions in four brain regions were used for enrichment test against GWAS signals, including that of Alzheimer's disease [45], autism spectrum

disorder [46], major depressive disorder [47], bipolar disorder [48], and schizophrenia [49]. The enrichment test was performed by MAGMA v1.07 [50], using the European subset of the 1000 Genomes as a reference panel for LD.

For the enrichment test against psychiatric candidate genes, the candidate genes contained the results of differential expression, differential splicing, and non-GWAS genetics as below:

1. ASD-related gene sets included: (1) genes with rare, de novo, loss of function or missense single-nucleotide variants from the NP de novo database [51]; (2) FMRP (Fragile X mental retardation protein) binding targets [52]; (3) candidate genes from the gene reference resource for ASD research database, AutDB [53]; (4) disease DEGs from a recent meta-analysis [54] and the PsychENCODE project [32]; (5) disease DSGs in ASD from PsychENCODE project [32].
2. SCZ-related gene sets included: (1) genes affected by copy number variants (CNVs) [55]; (2) genes identified by linkage and association study [56–58]; (3) genes with de novo variants from NP de novo database [51]; (4) genes identified by convergent functional genomics (CFG) [59]; (5) genes identified by Sherlock integrative analysis (60,61); (6) genes identified by Pascal gene-based test [60]; (7) DEGs in SCZ [54]; and (8) DSGs in SCZ from PsychENCODE project [32].
3. BD gene set. DSGs in BD from PsychENCODE project [32].
4. MDD gene set. DEGs in MDD [54].
5. AD gene set. DSGs in AD [31].

Enrichment was performed by two-tailed Fisher's exact test in combination with Bonferroni correction.

Results

Poor reproducibility of individual genes induced by stress as differentially expressed genes (DEGs) and differential splicing clusters (DSCs). Stress-induced DEGs based on these same data have been studied previously, but the reproducibility of the findings has not been assessed. The data were reprocessed and analyzed using a consistent procedure so that results could be compared directly. Replication analyses could only be performed for CSDS and ELS in vHIP, and CSDS in mPFC due to the limited amount of data. Hundreds of DEGs were detected in each stress-brain region combinations. More DEGs in NAc were detected than the other regions at different threshold (Table 1, Supplementary Table 1). However, the detected DEGs were inconsistent between the discovery and replicate data (Supplementary Fig. 1, Supplementary Table 2). At the nominal $p < 0.05$, there were no overlapped DEGs in different data.

In contrast, there were more alterations in splicing in vHIP and mPFC than other regions in the discovery dataset (Table 1). The significant DSCs under CSDS, ELS, and EC in vHIP, mPFC, NAc, and VTA are shown in Supplementary Table 3. The alterations of individual genes in splicing were also inconsistent between discovery and replicate data; at nominal $p < 0.05$, only the introns in DSCs under ELS in vHIP showed even a weak correlation (rho = 0.19, p = 0.04) that did not survive correction for multiple testing (Supplementary Fig. 2).

The data from vHIP and mPFC showed fewer expression changes than splicing alterations (Table 1). None of the significant DSGs (BH *adj.p* < 0.05) were differentially expressed, suggesting that alternative splicing was independent of differential expression.

The heterogeneity in different studies was further explored by closely examining the effects of covariables (Table 2). The experimental age and intervals between the end of experiment and death were strongly correlated with principal components of the PSI matrix. (Supplementary Fig. 3, Supplementary Fig. 4).

Table 2
Comparison of experimental design in different study cohorts

	GSE72343	GSE109315	GSE89692		GSE131972
Conditions	CSDS	CSDS	CSDS	ELS	ELS
Experimental age	56 days	38 days	60–70 days	10 days	2 days
Durations	5 min for 10 days	Max 10 min for 10 days	5 min for 10 days	7–10 days	10 days
Time points	?	3–6 pm.	Randomly in the light cycle	Whole day	Whole day
Intervals	48 h	6–8 days	At least 60 h	50–62 days	58–78 days
Time of death	?	8–11 am.	?	?	?

ELS in GSE89692 includes maternal separation for 4h each day and limited nesting in the whole day; ELS in GSE131972 are only limited nesting in the whole day. "?" refers to unknown information.

DSGs and DEGs were reproducibly enriched in stress response-related functions and pathways. Two databases were used to annotate the functions and pathways of the DSGs and DEGs (nominal p < 0.05): (1) gene ontology (GO) of biological processes enrichment, and (2) KEGG pathways enrichment. Organelle organization, cellular protein metabolic process, establishment of localization, system development, and macromolecule modification were the top five GO terms significantly enriched in DSGs under all twelve conditions (three stress conditions × four brain regions) (Fig. 2A). A similar situation was observed in the pathway analysis. Although individual DSGs were distinct in the twelve conditions, most of them were enriched in the same pathways, including cAMP signaling pathway, endocytosis, axon guidance, dopaminergic synapse, circadian entrainment, etc. (Fig. 2A, Supplementary Table 4). Most of the enriched GO terms and pathways of DSGs in different studies were consistent (Fig. 2A, Supplementary Fig. 5A-F, Supplementary Table 4). Pathways related to signaling, neural transmitting, and blood-brain barrier (BBB) were especially well-replicated.

In contrast to DSGs, analysis of GO enrichment showed that DEGs from different datasets were mainly enriched in response to stimulus (Fig. 2B, Supplementary Table 5). For pathway enrichment analysis, fewer than five pathways were enriched for CSDS-induced DEGs in vHIP and mPFC in the replication datasets. ELS-induced DEGs in vHIP did not show enrichment in any pathway. (Supplementary Fig. 5J-K).

The hub genes in DSG-related PPI networks were robustly enriched for synaptic functions. The PPI networks were constructed using DSGs (nominal $p < 0.05$) under all twelve conditions. The top 50 genes according to node scores calculated by the maximal clique centrality method were defined as hub genes. In vHIP, only two hub genes could be replicated under CSDS and six hub genes under ELS (Fig. 3A, D, G, Supplementary Fig. 6-S10). These replicated genes in vHIP had distinct differential splicing events in the discovery and replicate data. There were no overlapped hub genes under CSDS in mPFC.

The hub genes of these DSG-related networks were significantly enriched in many GO terms, including the top terms of synapse organization, synaptic signaling, and protein-containing complex assembly in the discovery dataset (Fig. 3B, E, H, Supplementary Fig. 6–10, Supplementary Table 6). 37%-52% of the enriched biological process terms for hub genes from different data were consistent (Fig. 3C, F, I, Supplementary Fig. 8–9, Supplementary Table 6), including the top terms above.

The hub genes of DEG-related PPI networks were robustly enriched for response to stimulus DEGs (nominal $p < 0.05$) under all twelve conditions were used to construct PPI networks. Like DSGs, the hub genes of DEG-related PPI networks were distinct across different data. These hub genes were primarily enriched in response to stimulus and cell communication (Fig. 4A, Supplementary Fig. 11–13). 12% of these functional annotations for hub genes under CSDS in vHIP and mPFC from different studies were consistent (Fig. 4B, Supplementary Table 8), including the response to stimulus and cell communication functions. Functional annotation for hub genes under ELS in vHIP were not available for assessment due to the small number of DEGs in this condition.

Stress-induced DSGs were associated with psychiatric disorders. To test whether stress-induced DSGs were associated with psychiatric disorders, DSGs with nominal $p < 0.05$ were matched into human homologs, then tested for the enrichment of the candidate genes and GWAS signals of AD, ASD, MDD, BD, and SCZ.

Several enrichment tests of candidate genes in disease-related genes were consistently shown in at least two independent datasets. CSDS-induced DSGs in vHIP and mPFC, and ELS-induced DSGs in vHIP were enriched in AD-related DSGs and ASD-related FMRP target genes (Fig. 5A).

The genes that were both stress-induced DSGs and AD or ASD candidate genes were further annotated at the pathway level. These genes related to ASD were reproducibly enriched in glutamatergic synapse pathway. The genes associated with AD were enriched in tight junction and adherens junction. (Supplementary Table 8).

DSGs under CSDS in vHIP and mPFC were enriched in GWAS of BD. The CSDS- and ELS-induced DSGs in vHIP were enriched for GWAS of SCZ (Fig. 5B). Despite the inconsistent splicing events from different datasets, there were many common DSGs connected to GWAS genes. Based on this, 58 DSGs under CSDS in vHIP were associated with SCZ and BD in GWAS, including *DLG2, SYNGAP1, KALRN, RAPGEF4, NFASC*, etc.

Unlike DSGs, only CSDS-induced DEGs were reproducibly enriched in SCZ-related DEGs (Supplementary Fig. 14A). The enrichment of DEGs in psychiatric GWAS was not replicated (Supplementary Fig. 14B).

Discussion

This study systematically analyzed psychological stress-induced differential splicing and differential expression in four brain regions of reward circuitry in mice. The changes of individual genes for DSGs and DEGs had poor reproducibility. In contrast, these genes were enriched in pathways and were well-replicated. The stress-induced DSGs were also enriched in candidate genes of brain disorders.

The primary goal of this study was to evaluate the robustness of transcriptome changes in animal stress models. Datasets with the same stress models were collected, and the results were expected to be comparable. However, the results at the individual gene level were not reproducible. Comparing experimental methods across datasets revealed differences in experimental design for the same stress model. Previous studies revealed that stress response probably varies depending on the time of daily experiment [62] and the intervals between the end of the experiment and death [30, 63]. This study also showed that experimental age and intervals may be the primary contributors to the heterogeneity or inconsistency in different datasets. However, the mice in different datasets showed similar behaviors after stress, suggesting stress-induced DSGs and DEGs from different datasets probably participate in the same pathways.

The most important finding of this study was the reproducible convergent enrichment in functions and pathways for DSGs and DEGs. The GO terms such as synapse organization, dendrite development and axon development were reproducibly enriched in DSGs. The GO terms related to stress and immune response were robustly enriched in DEGs.

There were many common enrichment pathways in DSGs from different datasets, including endocytosis, dopaminergic synapse, tight junction, etc. This suggested that the DSGs in each independent dataset belong to the same biological system throughout the stress response process, resulting in consistent stress response effects. For example, in the tight junction system (Fig. 6), DSGs from different datasets perform the same functions related to cell polarity, cell survival, tight junction disruption.

Analysis of enriched pathways of DSGs revealed commonality across brain regions and stress conditions. Pathways that have been hypothesized to be involved in stress responses previously [12, 64–66] include the neural transmission and BBB related pathways. The DSG pathway analyses in the current study supports these findings.

Dopaminergic synapse was one of the significantly enriched pathways in the four brain regions, especially in the VTA. The brain's reward circuits were reported to be activated by releasing dopamine from the VTA [67, 68]. Interestingly, there were fewer DEGs and DSCs in VTA. If the early-response brain regions have fewer changes than the downstream circuitry in a cascade reaction, VTA may be the first stress-responding area among the four brain regions. Some DSGs across three stress conditions in vHIP, mPFC, NAc, and VTA were members of CAMKII and MAPK families, participating in neuronal activity in the postsynaptic cells within the system. The altered splicing of these genes in response to chronic psychological stress may either activate or disrupt dopamine signaling, thus influencing the reward system.

The current study indicated that aberrant splicing of genes caused by chronic stress may lead to BBB disruption. Stress-induced DSGs were significantly enriched in the tight junction. *Arhgef2* was differentially spliced under CSDS in mPFC in different datasets. It participates in decreased paracellular permeability. Previous studies also found that chronic stress caused disruption of tight junctions, thus increasing permeability of the BBB [69–71].

Pathway enrichment for DEGs could not be replicated because there were fewer enriched pathways for DEGs in the replication datasets than in the discovery dataset. This may be due to the difference in daily experimental time in different datasets. The random timing of the daily experiment in the discovery dataset increased the unpredictability of the mice's response to stress, thus causing more pathways to be affected.

PPI networks were used to evaluate the binding partners of psychological stress-induced DSGs. Although the individual hub genes in the DSG-related PPI networks could not be replicated, the enriched functions and pathways of the hub genes were reproducible. Stress-induced DSGs from different datasets were significantly enriched in synapse organization and synaptic signaling, indicating the impacts on synapses in response to chronic psychological stress.

The human homologous genes of a few stress-induced DSGs showed consistent enrichment in specific psychiatric disorders in humans. Although there was no evidence to demonstrate that stressors can induce the same splicing changes in human homologous genes, the results suggested that stressors may increase the risk of psychiatric disorders through alternative splicing. A few examples are particularly interesting.

Data from CSDS and ELS in vHIP and mPFC showed that DSGs in mice were significantly enriched in human AD-related DSGs. These DSGs were robustly enriched in tight junctions. For example, *Dlg1* and *Myl6* were DSGs under both CSDS and ELS, which are involved in cell polarity at tight junctions [72, 73]. The disruption of tight junction may lead to BBB dysfunction. Mouse models indicated that a chronic BBB dysfunction leads to progressive neurodegeneration [74–76]. Imaging studies in human brain have shown BBB damage in AD [77–79].

Some FMRP target genes were differentially spliced in response to different stress conditions. These DSGs across studies and stress conditions were commonly enriched in the glutamatergic synapse pathway. The involved genes include glutamate receptors, such as *Grik5, Grin1*, and *Grm5*. Previous studies demonstrated that the loss of functions of FMRP protein results in overexpression of glutamate receptors [52]. Abnormalities in glutamate receptor genes and deregulation of the glutamatergic synapse pathway were found in ASD patients [80].

BD and SCZ GWAS genes were significantly enriched in human homologous genes of DSGs under CSDS and ELS in vHIP and mPFC. It is unknown whether human homologous genes are responding the same way to stress, but this result suggested a potential relationship between stress-induced differential splicing events and genetic variants in psychiatric risk genes. How genetic variants interact with stress to increase risks of BD and SCZ through altered splicing will be an exciting future research project.

The alteration in splicing under stress conditions is evidently independent of changes in gene expression. The significant DSGs from different datasets were not differentially expressed. There were more differential splicing events than differential gene expression in vHIP and mPFC. In the discovery dataset, NAc was the key responding region for DEGs. In contrast to DSGs, the hub genes in DEG-related PPI networks were enriched in response to stimulus, and stress-induced DEGs were associated with human homologs that are SCZ-related DEGs. Therefore, differential expression and differential splicing appear to be parallel mechanisms that act together on the stress system and lead to the behavioral response to chronic psychological stress.

This study is a re-analysis of existing data collected from the limited datasets in public databases for mice and is therefore restricted by the limitation of the original study designs. Inconsistent DEGs and DSGs were observed. Datasets to evaluate reproducibility are available for only a few stress conditions and brain regions. These four brain regions are critical to stress response, but stress responses may occur throughout the brain. Samples from other brain regions that may be affected by psychological stress are not available.

Taken together, this study showed inconsistent results for individual genes for both DEGs and DSGs under stress in vHIP and mPFC, but changes were reproducibly significant in pathways related to neural transmission and blood-brain barrier for DSGs in these brain regions. Future research will benefit from studies with standardized, consistent protocols that cover more time points during stress conditions. Besides, transcriptional changes to stress in all brain regions should be studied in the future.

Declarations

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Conflict of Interest

The authors report no biomedical financial interests or potential conflicts of interest.

Author contributions: C.C. and C.L. designed the study and interpreted the results. F.W., X.Y, and Z.R. collected the datasets. F.W. preprocessed the datasets and performed all the analyses in this study. All authors read and approved the final version.

Supplementary information is available at MP's website

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Figures

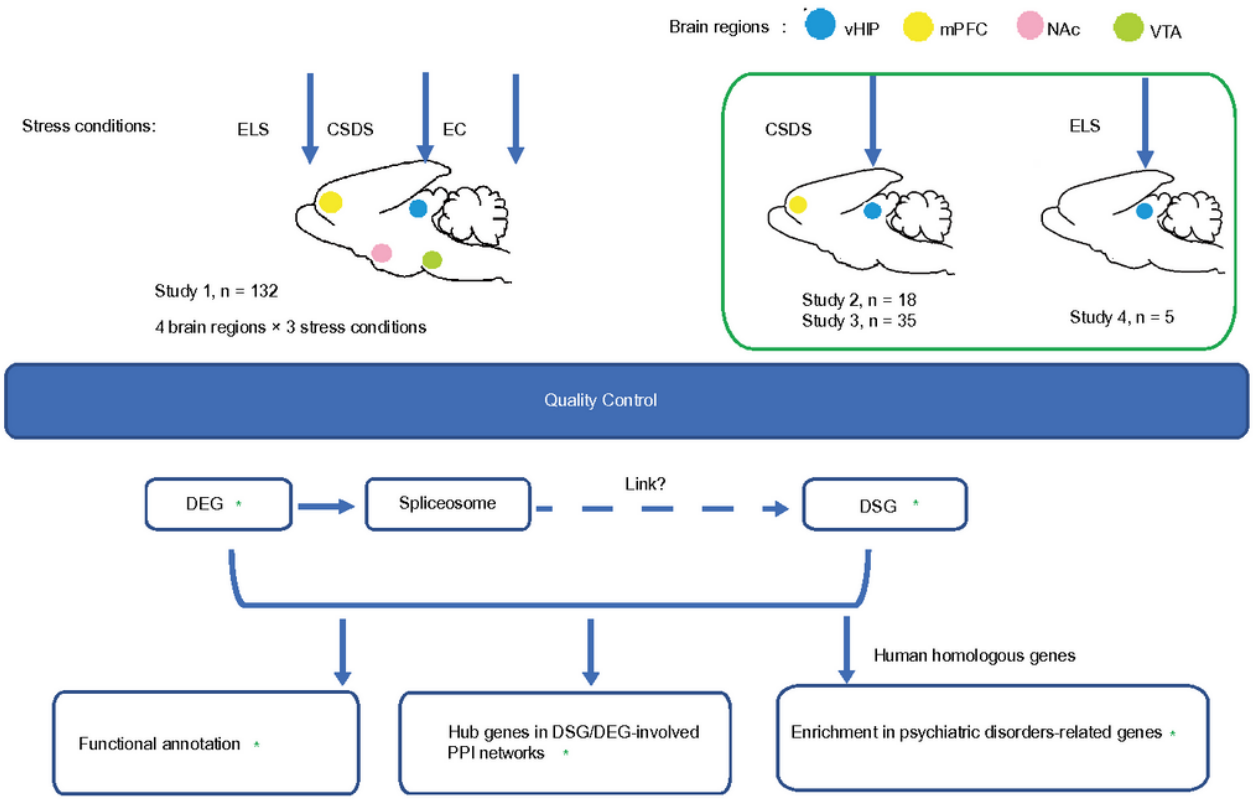

Figure 1

Overview of the study. Study 1 is the discovery dataset collected from GSE89692. The replication datasets are in the green box. Study 2, study 3, and study 4 are collected from GSE72343, GSE109315, and GSE131972, respectively. “*” indicated that the analysis in the box is replicated. vHIP, ventral hippocampus; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; VTA, ventral tegmental area; ELS, early life stress; CSDS, chronic social defeat stress; EC, early life stress + chronic social defeat stress; DEG, differentially expressed genes; DSG, differentially spliced genes.

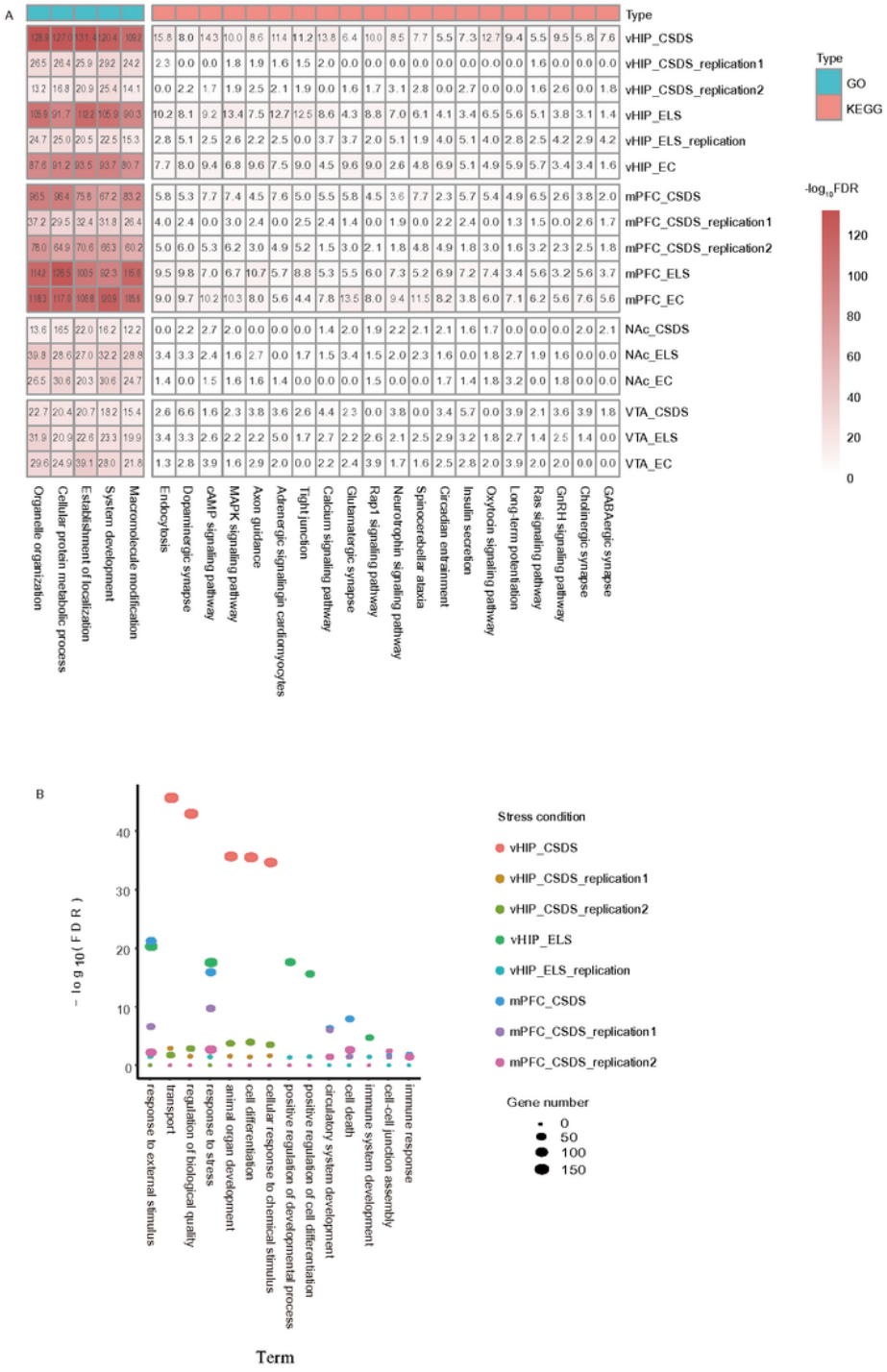

Figure 2

Top enriched biological processes for DSGs and the enriched KEGG pathways for DSGs. (A) The enrichment of DSGs across three stress conditions in the four brain regions in GO biological processes (top 5) and KEGG pathways (top 20). The values in the box are -$\log_{10}$FDR. (B) The common enriched biological processes in different datasets.

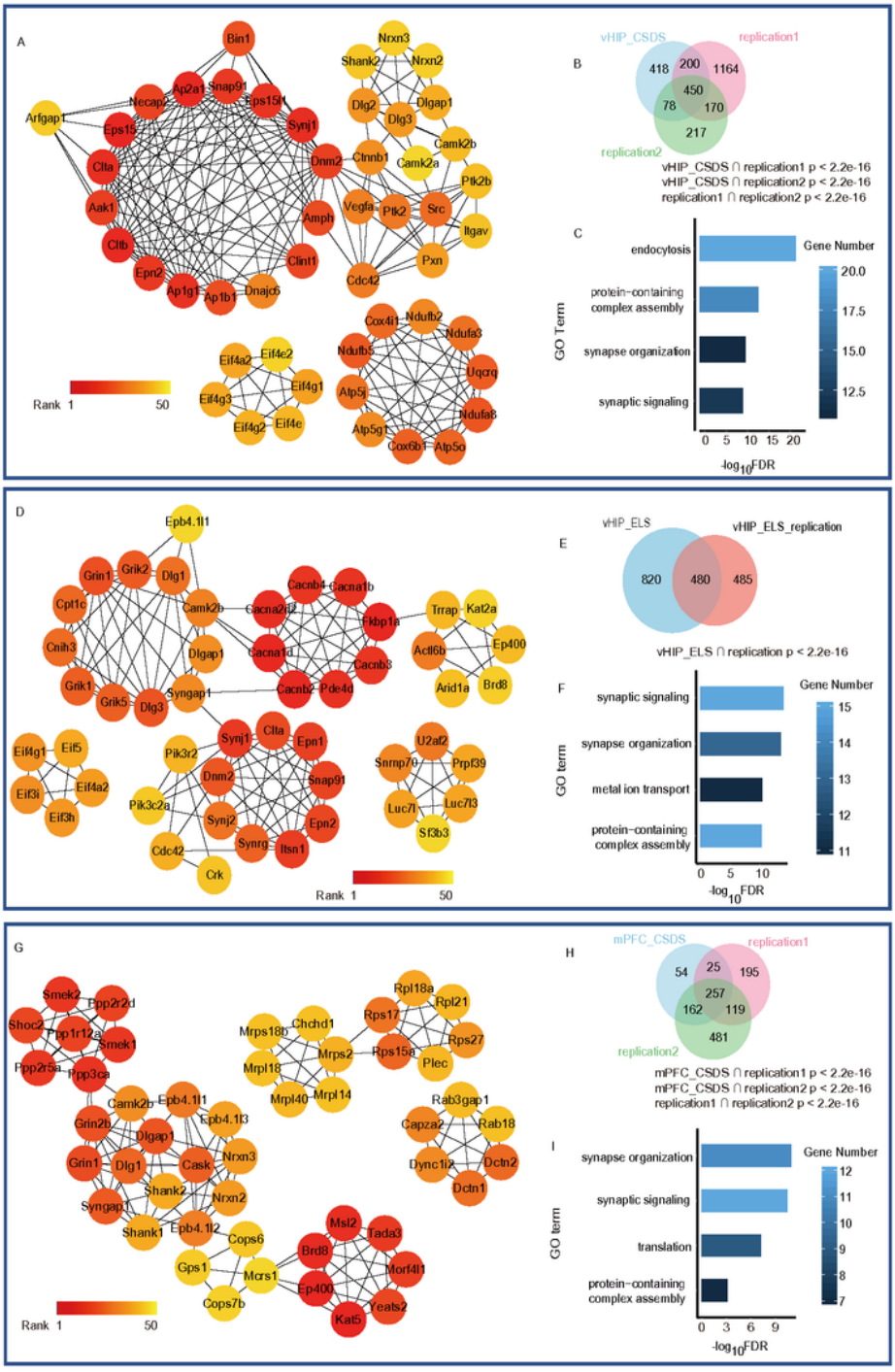

Figure 3

The hub genes in DSG-related PPI networks and their functional annotations. The hub genes in PPI networks constructed by CSDS-induced DSGs in vHIP (A), ELS-induced DSGs in vHIP (D), and CSDS-induced DSGs in mPFC (G), respectively. Ranking of the hub genes is denoted using the key at the end of each panel. (B), (E), (H) The overlaps in GO terms of biological processes in different cohorts under CSDS in vHIP (B), ELS in vHIP (E), and CSDS in mPFC (H), respectively. *P* values for hypergeometric tests of

pairwise overlaps are shown at the bottom. (C), (F), (I) The top four enriched biological processes for the hub genes under CSDS in vHIP (C), ELS in vHIP (F), CSDS in mPFC (I). The histograms are colored by the gene numbers.

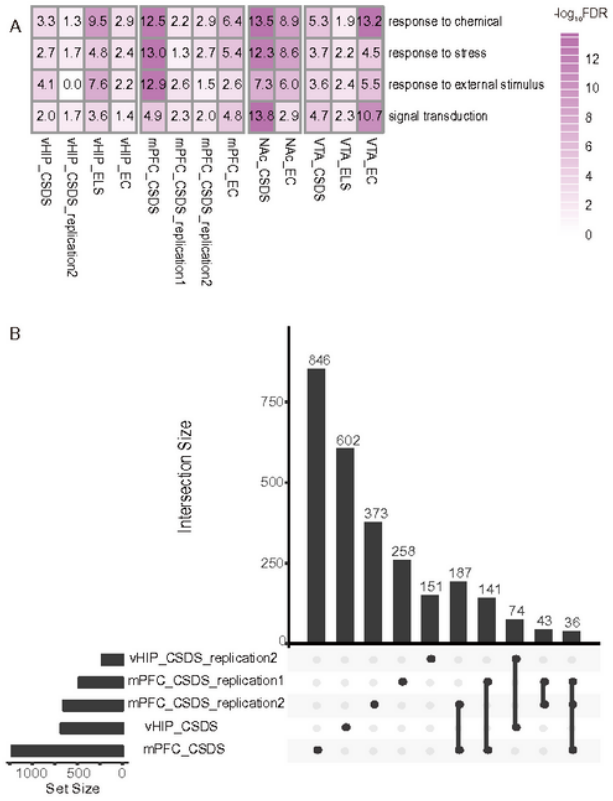

Figure 4

Functional annotations for hub genes in DEG-related PPI networks. (A) The common enriched GO terms of biological processes for the hub genes in DEG-related PPI networks. The values in the box are -$\log_{10}$FDR. (B) The overlapped biological processes in different datasets. Replication 1 is data from GSE72343; replication 2 is data from GSE109315.

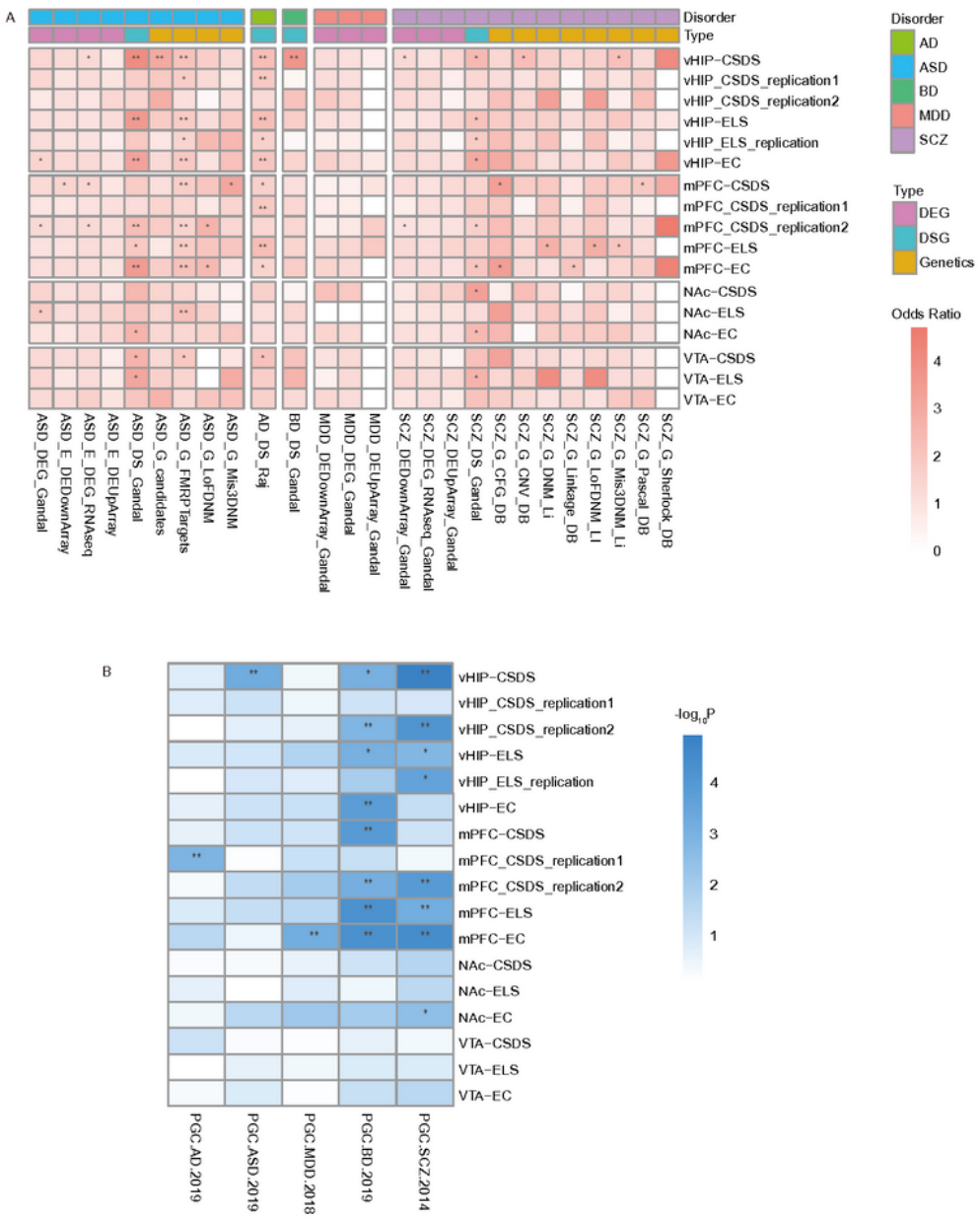

Figure 5

Enrichment of DSGs in psychiatric disorder-related candidate genes and GWAS genes. (A) The enrichment of DSGs across three stress conditions in the four brain regions in psychiatric disorder-related candidate genes. The *x*-axis shows 26 gene sets divided based on the psychiatric disorder and labeled by type; the *y*-axis shows DSGs across three stress conditions in vHIP, mPFC, NAc, and VTA. The color of the box shows the odds ratio for enrichment. For the discovery dataset, "*" indicates enrichment is

statistically significant ($p < 0.05/12$), "**" indicates $p < 0.05/ (12\times26)$. For the replication dataset with CSDS in vHIP and mPFC, "*" indicates enrichment is statistically significant ($p < 0.05/5$), "**" indicates $p < 0.05/ (5\times26)$. (B) The enrichment of stress-induced DSGs in GWAS genes. For the discovery dataset, "*" indicates enrichment is statistically significant ($p < 0.05/12$), "**" indicates $p < 0.05/ (12\times5)$. For the replication dataset with CSDS in vHIP and mPFC, "*" indicates enrichment is statistically significant ($p < 0.05/5$), "**" indicates $p < 0.05/ (5\times5)$. The box is colored by $-\log_{10}$FDR.

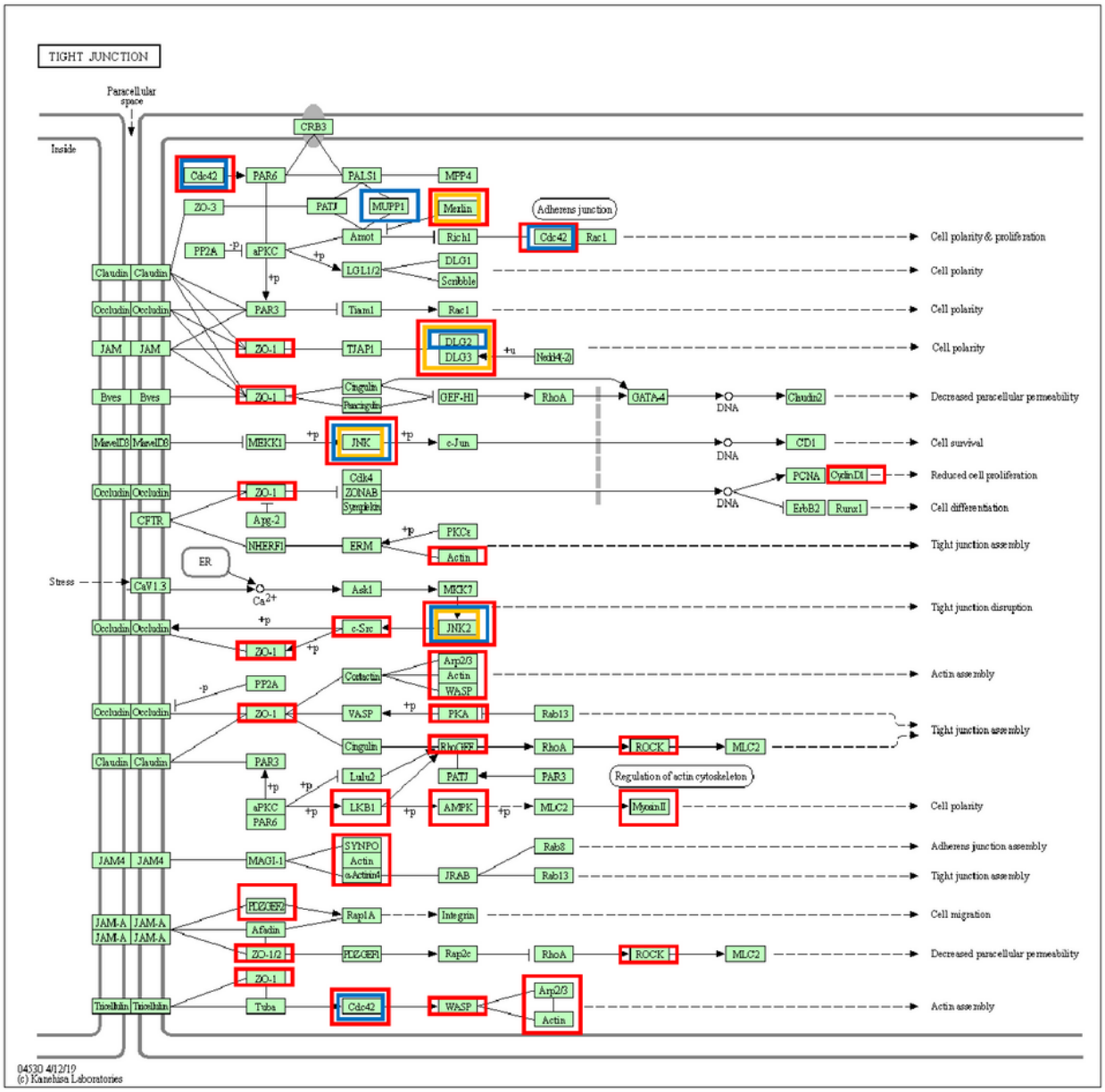

Figure 6

The enrichment of CSDS-induced DSGs in tight junction pathway from different datasets in vHIP. The red box refers to DSGs from GSE89692, the yellow box refers to DSGs from GSE72343, and the blue box refers to DSGs from GSE109315.

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