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Research article

Landscape of intrinsically disordered proteins in mental disorder diseases

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ABSTRACT

Disrupted genes linked to mental disorders sometimes exhibit characteristics of Intrinsically Disordered Proteins (IDPs). However, few studies have comprehensively explored the functional associations between protein disorder properties and different psychiatric disorders. In this study, we collected disrupted proteins for seven mental diseases (MDD, SCZ, BP, ID, AD, ADHD, ASD) and a control dataset from normal brains. After calculating the disorder scores for each protein, we thoroughly compared the proportions and functions of IDPs between differentially expressed proteins in each disease and healthy controls.

Our findings revealed that disrupted proteins, particularly in ASD and ADHD, contain more IDPs than controls from normal brains. Distinct patterns in disorder properties were observed among different mental disorders. Functional enrichment analysis indicated that IDPs in mental disorders were associated with neurodevelopment, synaptic signaling, and gene expression regulatory pathways. In addition, we analyzed the proportion and function of liquid-phase-separated proteins (LLPS) in psychiatric disorders, finding that LLPS proteins are mainly enriched in pathways related to neurodevelopment and inter-synaptic signaling. Furthermore, to validate our findings, we conducted an analysis of differentially expressed genes in an ASD cohort, revealing that the encoded proteins also exhibit a higher proportion of IDPs. Notably, these IDPs were particularly enriched in pathways related to neurodevelopment, including head development, a process known to be disrupted in ASD.

Our study sheds light on the crucial role of IDPs in psychiatric disorders, enhancing our understanding of their molecular mechanisms.

1. Introduction

Over the past two decades, the prevalence of mental disorders has risen rapidly, along with an increasing burden on healthcare systems and society [1]. Due to the complexity of their pathogenesis and the presence of unknown etiological factors, effective therapies for mental disorders are rarely available. Although genetic variation assessments, such as Genome-Wide Association Studies (GWAS), have identified numerous genes associated with mental disorders, the underlying molecular mechanisms are still not fully understood [2–4].

In recent years, significant progress has been made in exploring the functional properties of intrinsically disordered proteins (IDPs) and unraveling their crucial role in mental disorders [4–6].

Intrinsically disordered regions (IDRs) are biologically active protein regions that exhibit high conformational variability but lack stable three-dimensional structures [7–9]. IDPs, which may contain IDRs of varying lengths or be completely disordered, play key roles in numerous biological processes, such as signaling pathways, transcription, translation, and the cell cycle [10–14]. The precise regulation of abundant IDPs in cells ensures the accuracy of signaling pathways. Mutations or changes in IDPs could lead to multiple diseases [15–17].

IDPs play a crucial role in brain development. For example, ZSWIM8, a ubiquitin ligase, is essential for the development of the embryonic nervous system [18]. Its function is attributed to the presence of

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abundant IDRs. These IDRs in ZSWIM8 and Dab1 interact with each other, correcting misfolding through the "disorder targets misorder" mechanism [19]. ZSWIM8 also promotes the proper phosphorylation of Dab1, thereby maintaining its regulatory function in the Reelin signaling pathway, which is implicated in several neuropsychiatric disorders, including Autism Spectrum Disorders (ASD), Schizophrenia (SCZ), Bipolar Disorder (BP), Major Depressive Disorder (MDD), and Alzheimer's disease [18,20].

Mutations in IDPs have been identified as major contributors to protein aggregation in the brain and are associated with plaque formation in patients with neurodegenerative disorders. Well-known IDPs, such as alpha-synuclein, amyloid-beta peptide, and Huntington's protein, are implicated in the pathogenesis of diseases like Alzheimer's disease, Parkinson's disease, and Huntington's disease [21–27].

Several studies have already demonstrated the functional importance of disorder properties by investigating one or a few genes in mental diseases [4,28]. However, the comprehensive analysis of the functional roles of IDPs in psychiatric disorders has rarely been performed [29]. With the advancement of experimental methods, along with the rising number of diagnosed psychiatric cases, there is an urgent need to conduct in-depth analyses of the disorder properties of IDPs and their involvement in the pathophysiology of major psychiatric disorders. Understanding these molecular mechanisms will provide insights into the development of more targeted therapeutic strategies.

For this purpose, we meticulously compiled proteins associated with seven psychiatric disorders—Anxiety Disorder (AD), Attention Deficit Hyperactivity Disorder (ADHD), Autism Spectrum Disorder (ASD), Bipolar Disorder (BP), Major Depressive Disorder (MDD), Intellectual Disability (ID), and Schizophrenia (SCZ)—using stringent criteria. The resulting database includes differentially expressed proteins from seven disease datasets and one control dataset (BRAIN). We then calculated the disorder properties of these proteins using the state-of-the-art disorder prediction platform, RIDAO [30].

We conducted a comprehensive comparison of the disorder properties between the disease datasets and the controls. Using protein interaction analysis, Gene Ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG), we explored the functional roles of IDPs in psychiatric disorders. Additionally, we compared the proportions and functions of liquid-liquid phase-separated (LLPS) proteins between psychiatric disorders and control data. Finally, we validated our findings by analyzing differentially expressed gene data from an ASD cohort study.

We found a higher prevalence of intrinsically disordered regions (IDRs) in ASD and ADHD compared to normal human brain proteins (p-value < 0.05). There are distinct IDR patterns across different psychiatric disorder datasets. IDPs associated with psychiatric disorders were significantly enriched in pathways related to neuron projection development, head development, cell morphogenesis, brain development, and synaptic transmission regulation. Additionally, our LLPS analysis revealed that ASD and ADHD datasets contained more LLPS proteins than controls, with these proteins also enriched in neurodevelopment and synaptic signaling pathways. A similar pattern was observed in an ASD cohort study, where proteins exhibited higher IDP proportions and greater involvement in head development and inter-synaptic signaling.

Our research aims to explore the neurobiological connections between IDPs and mental diseases, providing new insights into the pathogenesis and treatment of psychiatric disorders.

2. Materials and methods

2.1. Datasets

To create a dataset related to mental disorders, we searched the DisGeNET [31] and GeneCards [32] databases using the following keywords: 'anxiety disorder', 'attention deficit/hyperactivity disorder', 'autism spectrum disorder', 'bipolar disorder', 'intellectual disability', 'major depressive disorder', and 'schizophrenia'. Genes meeting the following criteria are included: (i) gene-disease association score > 0.1 in the DisGeNET database; (ii) gene score > 1 in the GeneCards database.

The above step selected 2388 differentially expressed genes associated with mental disorders. We focus on genes expressed in the brain, given the primary involvement of the brain in the occurrence of psychiatric disorders. To ensure this, we applied the following criteria: (i) genes expressed in either the Cerebellum, Cerebral Cortex, Hippocampus, or Lateral Ventricle tissue according to the Human Protein Atlas [33]; (ii) genes expressed in ≥ 75 % of sample slices in at least 3 of 6 brain donors from the Allen Human Brain Atlas [34]; or (iii) genes with expression RPKM (reads per kilobase of transcript per million mapped reads) values > 1 in ≥ 75 % of samples from at least one developmental stage according to the Allen Human Brain Atlas [34]. Fig. 1 illustrates the gene selection pipeline for psychiatric diseases.

We mapped the selected genes to their corresponding proteins using UniProtKB [35], resulting in a mental disorder-related protein dataset containing 2189 non-redundant proteins, referred to as the MENTAL dataset.

For comparison, we also constructed a control dataset using Brain-Specific Proteome data from the Human Protein Atlas [33]. The BRAIN dataset contains genes expressed in normal brains, selected based on the following criteria: (i) Genes with minimal expression signals in the human brain; (ii) Genes with immunohistochemical evidence of brain expression; (iii) Genes that can be mapped to proteins by the UniProt database; (iv) Genes not found in the MENTAL dataset. In the Human Protein Atlas, proteins are categorized based on their relative expression levels into three groups: (i) Elevated in brain ; (ii) Low tissue specificity but expressed in brain ; and (iii) Elevated in other tissues but expressed in brain. To ensure a fair comparison, we randomly selected 2188 non-redundant proteins from the Brain-Specific Proteome data, matching the distribution of expression categories in the MENTAL dataset. Finally, the MENTAL dataset comprised 2189 proteins, while the BRAIN dataset contained 2188 proteins.

2.2. Intrinsic disorder prediction

We used the state-of-the-art RIDAO platform [30] to predict the disorder properties of each protein in the datasets. RIDAO is a fast and efficient tool that integrates six well-established disorder prediction tools into a unified platform. Residues with scores above 0.5 are classified as disordered, while scores below 0.5 indicate ordered. In this study, protein regions with a continuous length of ≥ 30 disordered residues were classified as intrinsically disordered regions (IDRs), and proteins containing at least one IDR were classified as intrinsically disordered proteins (IDPs) [29]. Based on the presence or absence of IDRs, we categorized mental disease-related proteins into two groups: 'Low Disorder' and 'High Disorder'.

2.3. Binding sites prediction

The ANCHOR software was used to identify the binding regions [36,37]. ANCHOR uses pairwise energy estimation methods to predict disordered binding regions by identifying fragments within disordered regions that interact with globular protein partners to gain stabilizing energy. Disordered binding sites were predicted for each protein in the seven disease datasets and the BRAIN dataset.

2.4. Protein-protein interaction (PPI) network construction

We used the UniProt ID as input, set 'Protein Query' as the data source, and applied a confidence score threshold of '0.90' to completed the protein-protein interaction (PPI) networks using the STRING plugin. The construction of network edges is based on known interactions in the database, including those from curated databases, experimentally

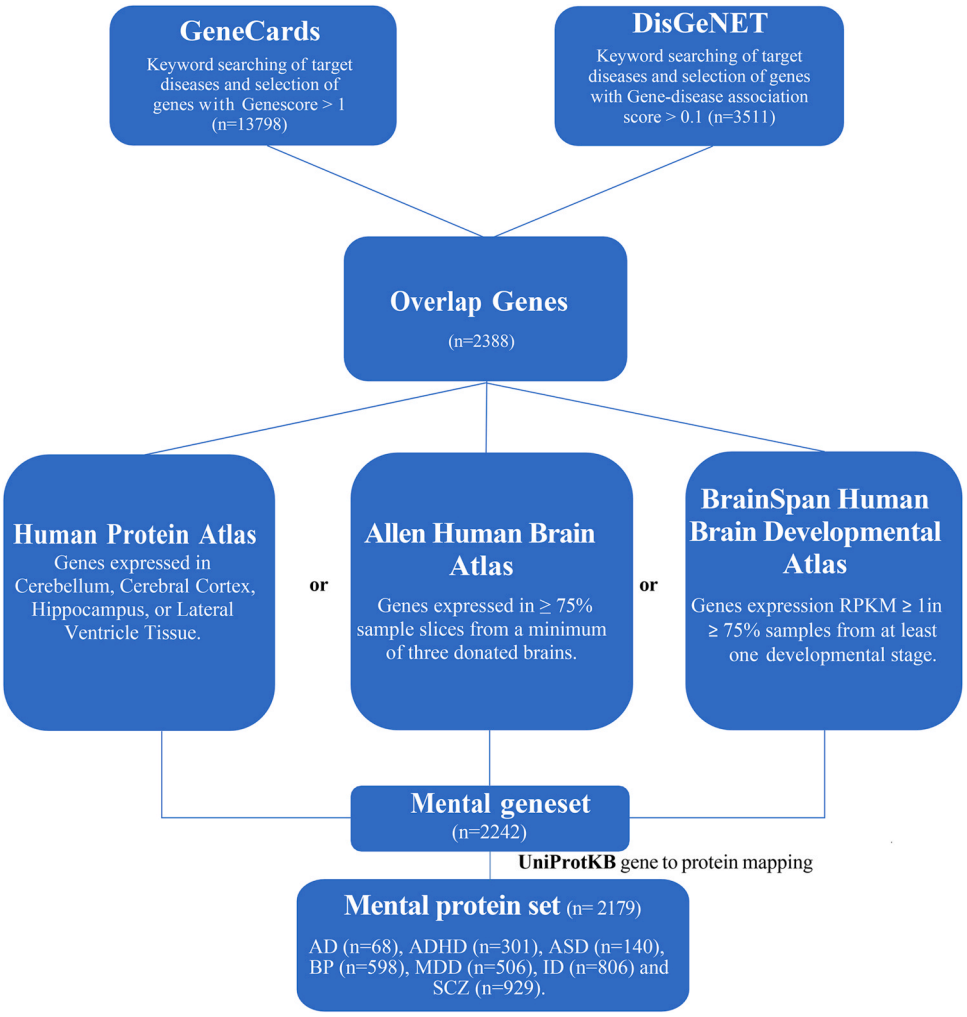

Fig. 1. The data collection processes. This figure shows how we collect the disease dataset.

validated interactions, and predicted interactions such as gene neighborhoods, gene fusions, and gene co-occurrence. Additional factors such as text mining, gene co-expression, and protein homology are also considered. The network nodes represent the input proteins, with non-interacting nodes excluded.

We applied the degree algorithm from the CytoHubba plugin to identify the top 50 pivotal proteins from each dataset. These proteins were then used to construct interaction networks through the STRING plugin. To capture a broader range of interactions, we lowered the confidence score threshold to 0.40.

2.5. Protein functional annotation

We applied Metascape [38] to perform GO and KEGG analysis on the genes in each dataset, using the following parameters: a minimum overlap of 3, a p-value cutoff of 0.05, and a minimum enrichment of 3. We identified the top 5 most significantly enriched pathways for each dataset in terms of Biological Process, Molecular Function, Cellular Component. For the KEGG analysis, we determined the top 10 pathways with the most significant gene enrichment in each dataset. R version 4.2.3 was used to visualize these observations.

2.6. Prediction of LLPS-prone proteins

We predicted the LLPS scores of psychiatric disease-associated proteins using three state-of-the-art machine learning and deep learning predictors: PSPHunter [39], PSPredictor [40], and PredLLPS_PSSM [41,42]. The final LLPS prediction score was obtained by averaging the scores from these three predictors. We applied a threshold of 0.5, based on the criteria of all three predictors, to classify proteins as either LLPS or non-LLPS. Proteins with scores equal to or above this threshold were considered as LLPS proteins.

2.7. A ASD cohort containing 1141 patients

We selected data from a cohort study that included 1141 patients with ASD [43,44]. From this study, we identified 391 genes with statistical significance (FDR < 0.1) (Supplementary Table 1). These genes were mapped to their corresponding proteins using UniProtKB [35], and we filtered the results to retain only proteins with a 'Reviewed' status and a protein existence level of 'Protein level'. For duplicate mappings meeting these criteria, we retained the protein with the longest sequence. The disorder properties of the screened proteins were assessed using the RIDAO platform [30] and IDPs and ordered proteins were categorized based on the criteria described in Section 2.2. For the identified IDPs, we performed GO functional enrichment analysis using MetaScape [45] and listed the top 5 terms across biological processes, cellular components, and molecular functions.

2.8. Statistical analysis

The statistical analysis was conducted using R version 4.2.3.

Normality tests and tests for variance homogeneity were performed on each dataset, revealing that none of the datasets followed a normal distribution and that variances were not equal across groups. Therefore, protein sequence lengths between groups were compared using the Kruskal-Wallis Test, proportional comparisons were assessed using the chi-squared test, and pairwise comparisons were adjusted using the Bonferroni correction. The correlation between the proportion of disordered residues in disrupted proteins and their LLPS scores was assessed using the Pearson correlation coefficient.

3. Results & discussion

3.1. Acquisition and characterization of differentially expressed genes in mental disorders

As described in the methods section, we created two datasets: the MENTAL dataset, consisting of disease-related proteins, and the BRAIN dataset, serving as the control. The disease dataset includes seven subsets: the anxiety disorders (AD) dataset: 68 proteins; the attention deficit hyperactivity disorder (ADHD) dataset: 307 proteins; the autism spectrum disorders (ASD) dataset: 143 proteins; the bipolar disorder (BP) dataset: 602 proteins; the major depression (MDD) dataset: 512 proteins; the intellectual developmental disorders (ID) dataset: 812 proteins; the schizophrenia (SCZ) dataset: 939 proteins. The list of these proteins is in Supplementary Table 1.

We then conducted functional enrichment analysis to examine the roles of the differentially expressed genes in the MENTAL dataset. We observed that proteins associated with psychiatric disease were significant enrichment in processes related to behavior (GO:0007610), head development (GO:0060322), neural projection development (GO:0031175), trans-synaptic signaling regulation (GO:0099177), and brain development (GO:0007420). These processes are closely related to nervous system formation and function, as well as intraneuronal communication and neurotransmitter release. Furthermore, proteins in MENTAL dataset are strongly related to transcription, signal transduction, protein-protein interactions, and gene regulatory mechanisms. They play crucial roles in dendritic, axonal, postsynaptic and presynaptic synapses. These findings are consistent with previous studies [46–48]. KEGG pathway enrichment analysis was also performed on this dataset, with the detailed results shown in Figs. 2B and 2C.

We also constructed a protein interaction network of protein in the MENTAL dataset to obtain the potential hub proteins and their interactions. The key hub proteins identified included AKT1, TP53, and ACTB. Fig. 2D depicts the comprehensive data of the protein interaction network associated with mental disorders.

3.2. Comparison of protein intrinsic disorder properties between MENTAL diseases and controls

We used the RIDAO platform [30] to predict protein disorder

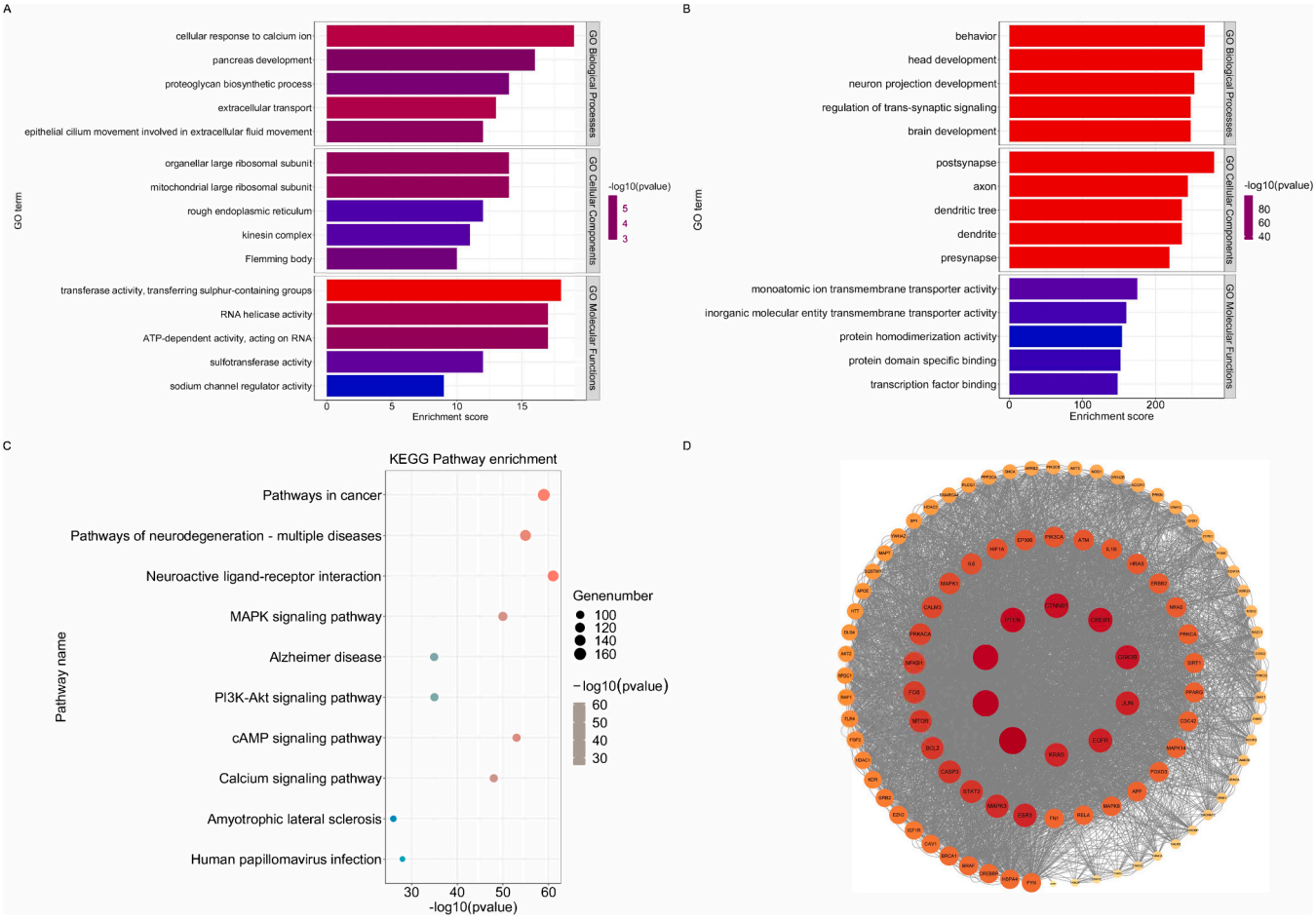

Fig. 2. Differential Gene Function Enrichment and Protein Interaction Network Analysis in MENTAL and BRAIN datasets. (**A**) Top 5 Biological Process, Cellular Component, and Molecular Function terms of genes in the BRAIN dataset. (**B**) Top 5 Biological Process, Cellular Component, and Molecular Function terms of genes in the MENTAL dataset. (**C**) Top 10 KEGG pathways in the MENTAL dataset were ranked by gene enrichment significance. (**D**) Protein-protein interaction networks of the top 100 proteins in the protein interaction network of the MENTAL dataset calculated by the degree algorithm. The inner circle lists the top 10 proteins in clockwise order.

properties of proteins in the MENTAL and the BRAIN dataset. The resulting mean disorder profile (MDP) score were used to calculate the percentage of IDPs in each dataset. Table 1 presents the details of the top 15 proteins with the highest percentage of protein disorder in the MENTAL dataset.

The proportion of IDPs in each dataset was as follows: ASD (75.40 %), ADHD (73.40 %), ID (66.70 %), BP (65.60 %), MENTAL (65.70 %), SCZ (65.30 %), BRAIN (66.20 %), MDD (64.20 %) and AD (56.50 %). As shown in Table 2, we observed that the percentage of IDPs in the ASD and ADHD dataset was higher than that in the BRAIN dataset (*P-value* < 0.05, Fig. 3B).

3.3. Binding sites prediction in MENTAL diseases and controls

We employed ANCHOR to predict disordered binding sites within the protein sequences of each dataset. Proteins with at least one disordered binding site were selected for analysis. We found that the ASD and ADHD datasets contained significantly more proteins with disordered binding sites compared to the BRAIN dataset (Bonferroni $p - value < 0.05$). The number of disordered binding sites in the ASD and ADHD was significantly higher than that in the MDD dataset (Bonferroni $p - value < 0.05$).

Proteins in the ASD and ADHD datasets exhibited a higher tendency to bind and interact with other proteins compared to those in the BRAIN and MDD datasets. This trend was closely associated with a higher prevalence of IDPs in the ASD and ADHD datasets. A summary of the disordered binding sites for each dataset can be found in Supplementary Table 2 and Fig. 3C.

3.4. Comparison of enrichment analysis between 'Low Disorder' and the 'High Disorder' proteins

After comparing the proportions of IDPs between mental disorders and healthy controls, we further investigated the potential functional pathways associated with IDPs in psychiatric diseases. To simplify the analysis, we categorized the proteins into two groups: 'Low Disorder' and 'High Disorder', based on the length of their disordered regions.

We conducted GO and KEGG functional enrichment analyses on both the Low Disorder and High Disorder datasets. We identified and listed the top 5 items for Biological Process, Cellular Component, and Molecular Function in GO, as well as the top 10 pathways in KEGG. This analysis aims to further validate the pivotal role of disordered proteins in psychiatric disorders and explore their key functions.

3.4.1. Gene ontology functional analysis

In our study, we observed significant differences in the molecular functions of these two classes of proteins. Low Disorder proteins are implicated in molecular transmembrane transport, protein-protein interactions, and oxidoreductase activity. In contrast, High Disorder proteins exhibit a strong preference for intermolecular interactions, particularly those involving proteins and DNA. Most of these interactions are related to signaling, gene expression, and transcriptional regulation.

In terms of biological processes, High Disorder proteins are more likely to be involved in neuron projection development, head development, cell morphogenesis, brain development, and the modulation of chemical synaptic transmission compared to Low Disorder proteins. Both categories of proteins play crucial roles in synaptic signaling. Additionally, these proteins are important components of the synapse in both Low Disorder and High Disorder groups, underscoring the critical role of synapses in mental disorders.

We performed functional enrichment of the ASD and ADHD datasets, which have significantly more IDPs compared to the BRAIN dataset. The results showed that the molecular functions of IDPs in ASD involved chromatin binding, DNA binding, DNA-binding transcription factors binding, etc., which were closely related to the regulation of gene expression, while the molecular functions of IDPs in ADHD were mainly related to the activities of various ion transmembrane transport proteins. In terms of biological processes, IDPs in ASD are associated with a variety of biological processes including learning and memory, chemical synaptic regulation, behavior, head development, and cognition, while IDPs in ADHD are mainly involved in the regulation of membrane

Table 1
Top 15 proteins with the highest percentage of disordered residues in the MENTAL dataset.

Protein name	Protein ID	Disease	Protein length (aa)	Amino acid residues predicted to be disordered, aa (%)	Longest disordered region (aa)	Function
Stathmin	STMN1_HUMAN	MDD/ MENTAL	149	149(100)	149	tubulin binding
Neuromodulin	NEUM_HUMAN	BP/ MDD/ MENTAL/ SCZ	238	238(100)	238	calmodulin binding
Complexin−2	CPLX2_HUMAN	ADHD/ BP/ MDD/ MENTAL/ SCZ	134	134(100)	134	calcium-dependent protein binding
Neurogranin	NEUG_HUMAN	BP/ MENTAL/ SCZ	78	78(100)	78	calmodulin binding
PRKC apoptosis WT1 regulator protein	PAWR_HUMAN	BP/ MENTAL/ SCZ	340	340(100)	340	actin binding
Protein phosphatase 1 regulatory subunit 1B	PPR1B_HUMAN	BP/ MDD/MENTAL/ SCZ	204	204(100)	204	protein kinase inhibitor activity
Complexin−1	CPLX1_HUMAN	BP/ ID/ MDD/ MENTAL/ SCZ	134	133(99.25)	123	SNARE binding
Transcription factor SOX−2	SOX2_HUMAN	ID/ MENTAL	317	306(96.53)	154	DNA binding
Protein PRRC2A	PRC2A_HUMAN	MENTAL/ SCZ	2157	2082(96.52)	1856	RNA binding
Neurosecretory protein VGF	VGF_HUMAN	BP/ MDD/ MENTAL/ SCZ	615	592(96.26)	345	growth factor activity
B-cell CLL/lymphoma 9 protein	BCL9_HUMAN	BP/ MENTAL/ SCZ	1426	1372(96.21)	943	beta-catenin binding
Transcription factor 12	HTF4_HUMAN	ID/ MENTAL	682	640(93.84)	575	bHLH transcription factor binding
Microtubule-associated protein 6	MAP6_HUMAN	MENTAL/ SCZ	813	760(93.48)	500	calmodulin binding
Methyl-CpG-binding protein 2	MECP2_HUMAN	AD/ ADHD/ ASD/ BP/ ID/ MDD/ MENTAL/ SCZ	486	452(93.00)	333	chromatin binding
Charged multivesicular body protein 2b	CHM2B_HUMAN	MDD/ MENTAL	213	196(92.02)	153	cadherin binding

AD, Anxiety Disorder; ADHD, Attention Deficit Hyperactivity Disorder; ASD, Autism Spectrum Disorder; BP, Bipolar Disorder; MDD, Major Depressive Disorder; ID, Intellectual Disability; SCZ, Schizophrenia

Table 2
Comparison of disorder properties in different mental diseases.

Name	Protein length					Intrinsic disorder estimates	
	N	Mean	SE	Maximum	Median	Overall disordered amino acids (%)	Proteins with long regions of disorder (%)
BRAIN	2188	575.8	10.9	4870	441	32.08 %	66.20 %
AD	68	695.8	72.8	2768	479.5[e]	33.87 %[e]	56.50 %
ADHD	307	995.1	54.3	8797	710.0[e]	33.89 %[e]	73.40 %[e]
ASD	143	1033	68.3	4967	708.0[e]	33.25 %[e]	75.40 %[e]
BP	602	753.1	29.2	8797	560.5[e]	29.77 %[e]	65.60 %
MDD	512	687.8	28.6	6306	485.5[e]	27.85 %[e]	64.20 %
ID	812	921.1	30.2	8797	634.5[e]	30.84 %[e]	66.70 %
SCZ	939	754.2	23.2	8797	541.0[e]	30.31 %[e]	65.30 %
P-value[a]					$P < 0.001$[c]	$P < 0.001$[d]	$P < 0.001$[d]
MENTAL	2189	791.9	15.6	8797	559.0[e]	30.43 %[e]	65.70 %
P-value[b]					$P < 0.001$[c]	$P < 0.001$[d]	$P > 0.05$

[a] *P*-value for disease-specific comparison with BRAIN dataset
[b] *P*-value of MENTAL compared to BRAIN dataset
[c] Kruskal–Wallis Test.
[d] chi-square test
[e] Values different from BRAIN dataset

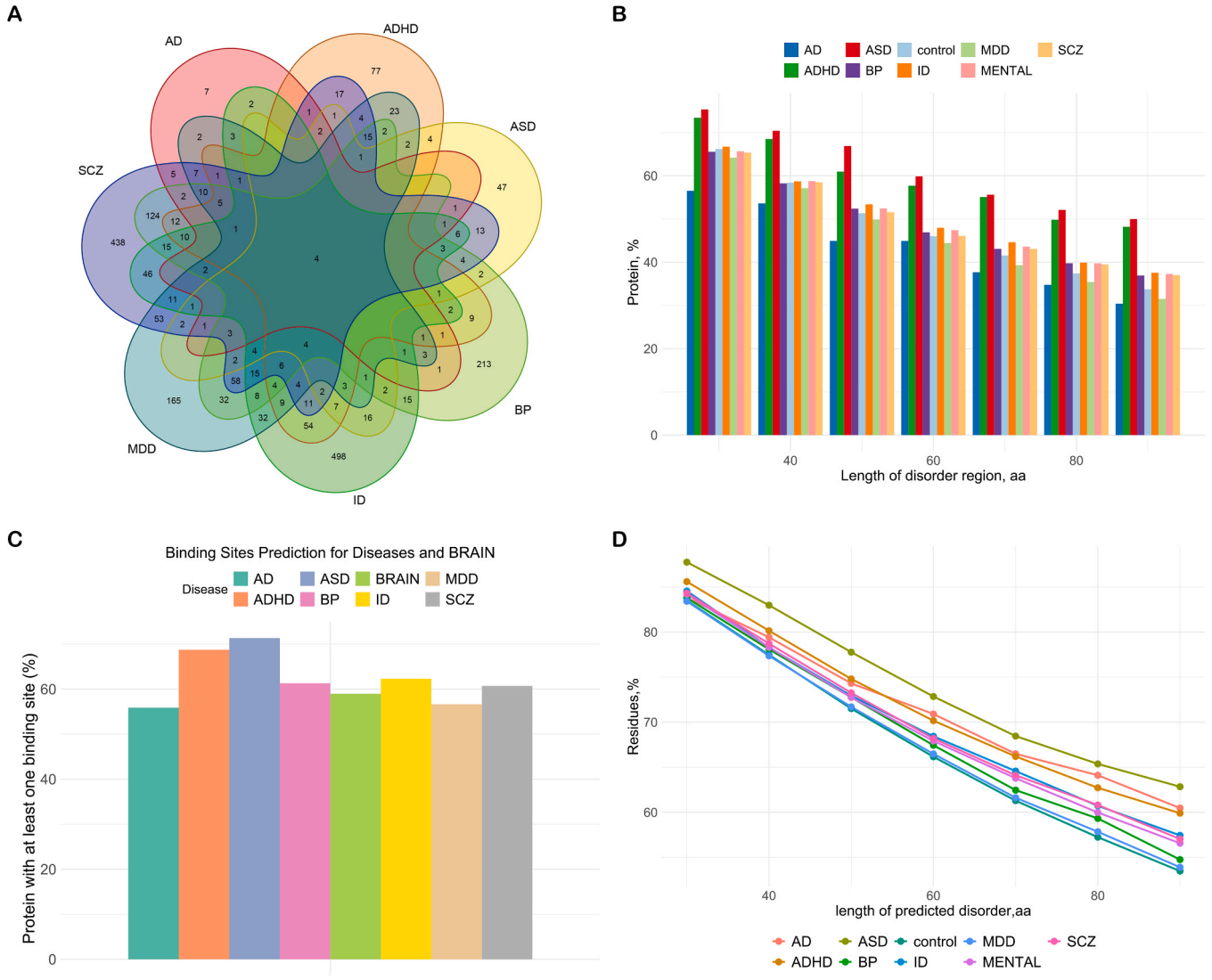

Fig. 3. Characterization and analysis of differentially expressed genes in psychiatric disorders. (**A**) Petal plots of the seven psychiatric disease datasets, with the number in each region representing the number of genes included in the corresponding disease intersection. (**B**) Percentage of proteins containing disordered residues of each length in the seven disease datasets. (**C**) Percentage of proteins containing at least one disordered binding site in the seven disease datasets and the control dataset. (**D**) Percentage of disordered residues of each length in the seven disease datasets relative to the total number of disordered residues.

potential, the regulation of ionic transmembrane transporter, and synaptic signaling, etc., which correspond to the molecular functions of IDPs in ADHD. The details of the GO analysis were shown in Fig. 4.

3.4.2. KEGG pathway enrichment analysis

The KEGG enrichment analysis showed no significant differences between Low Disorder and High Disorder proteins, nor in comparison to the KEGG pathways enriched in the MENTAL dataset. Both groups were similarly enriched in pathways associated with neurodegenerative diseases, cancer, neuroactive ligand-receptor interactions, as well as signaling pathways such as PI3K-Akt, MAPK, and cAMP. The top 10 KEGG pathways enriched for Low Disorder and High Disorder proteins are illustrated in Figs. 5C and 5D.

3.5. Comparison of PPI network between the 'Low Disorder' and the 'High Disorder' proteins

It has been reported that IDPs are important in protein interactions due to their high flexibility. To investigate whether IDPs could affect the occurrence of psychiatric disorders through protein-protein interactions, we constructed protein interaction networks for proteins in Low Disorder and High Disorder datasets, identifying key proteins in the protein interaction networks.

The Low Disorder Protein Interaction Network consists of 619 nodes and 1455 edges. We filtered the top 50 nodes using the degree algorithm, resulting in a reconstructed network of 50 nodes and 970 edges, with an average of 19.4 edges per node. The top three important proteins identified are ACTB, AKT1, and KRAS.

Similarly, the High Disorder Protein Interaction Network contains 605 nodes and 1707 edges. The reconstructed network includes 50 nodes and 1426 edges, averaging 28.5 edges per node. Key proteins here are TP53, CREB1, and CREBBP.

We briefly describe these key node proteins, especially those in the High Disorder Protein Interaction Network. The first one is KRAS, which is a significant protein in the Low Disorder network, mediates the RAS/MAPK pathway, influencing neuronal growth, proliferation, and central nervous system development [49,50]. In the High Disorder network, TP53 encodes the P53 protein, an IDP with intrinsically disordered regions crucial for its multifunctionality. The C-terminal domain of P53 interacts with GSK3, a protein linked to schizophrenia [51–53].

CREB1 and CREBBP are also vital IDPs in the High Disorder network. CREB1 encodes a transcription factor, while CREBBP encodes the CREB-binding protein, central to eukaryotic transcriptional regulation. The pKID domain of CREB interacts with CBP's KIX domain to trigger gene expression [54,55]. A meta-analysis found higher expression levels of CREB1 and CREBBP in the Brodmann Area 10 samples from schizophrenia patients compared to controls, and downregulation of CREB1 is associated with MDD, SCZ, and BP [28]. Figs. 5A and 5B illustrate the protein interaction networks for both datasets.

3.6. Results of LLPS prediction

We used the average prediction scores from three LLPS predictors to classify psychiatric disease-related proteins and control proteins into LLPS and non-LLPS proteins. The proportions of LLPS proteins in each dataset were as follows: ASD (66.20 %), ADHD (59.54 %), ID (45.01 %), BP (51.17 %), MENTAL (50.59 %), SCZ (50.59 %), BRAIN (47.41 %), MDD (51.91 %), and AD (38.28 %). We compared the LLPS proportions

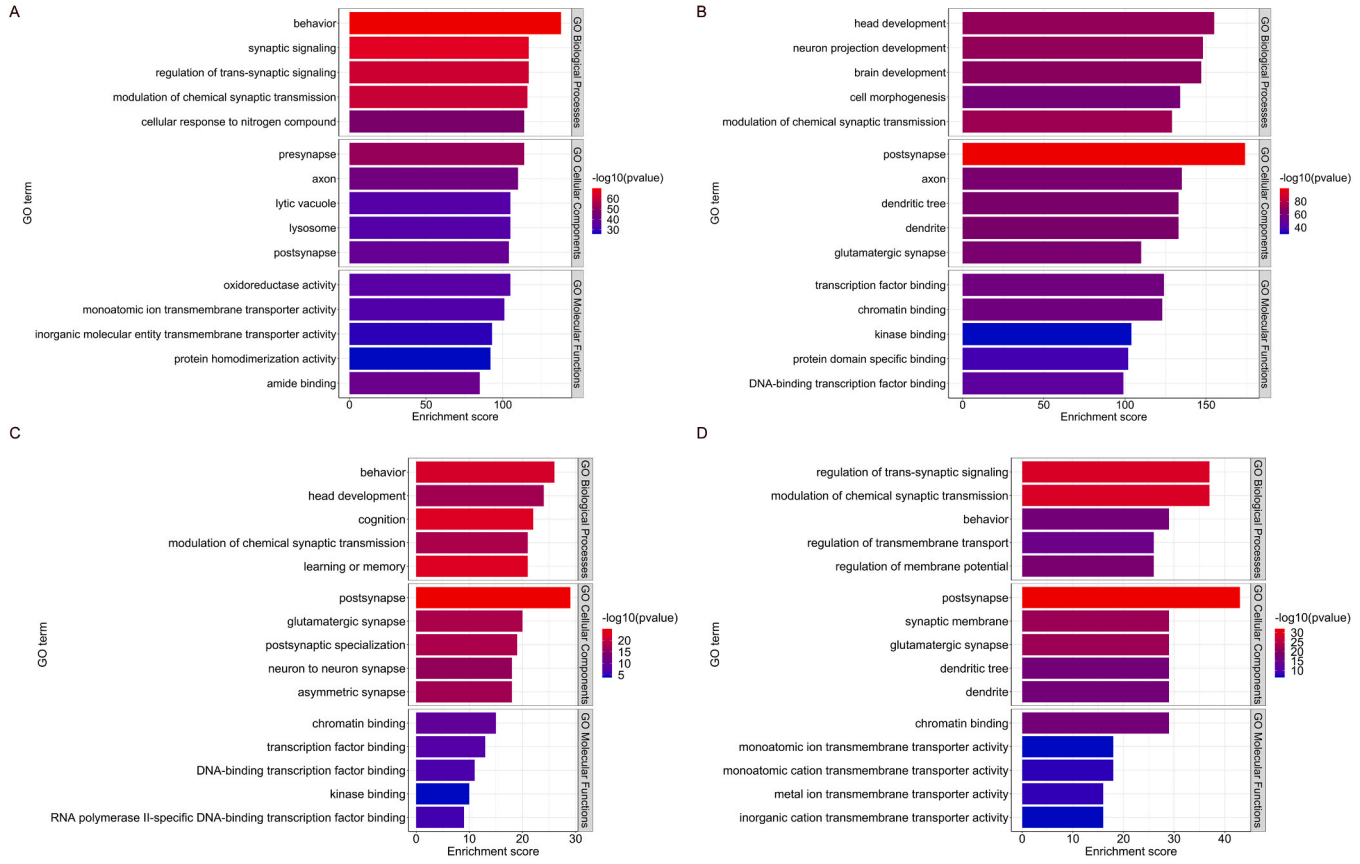

Fig. 4. GO functional enrichment of disease genes in different subgroups. (**A**) Top 5 Biological Process, Cellular Component, and Molecular Function terms for genes in the Low Disorder dataset. (**B**) Top 5 Biological Process, Cellular Component, and Molecular Function terms for genes in the High Disorder dataset. (**C**) Top 5 Biological Process, Cellular Component, and Molecular Function terms for genes in the IDPs in ASD. (**D**) Top 5 Biological Process, Cellular Component, and Molecular Function terms for genes in the IDPs in ADHD.

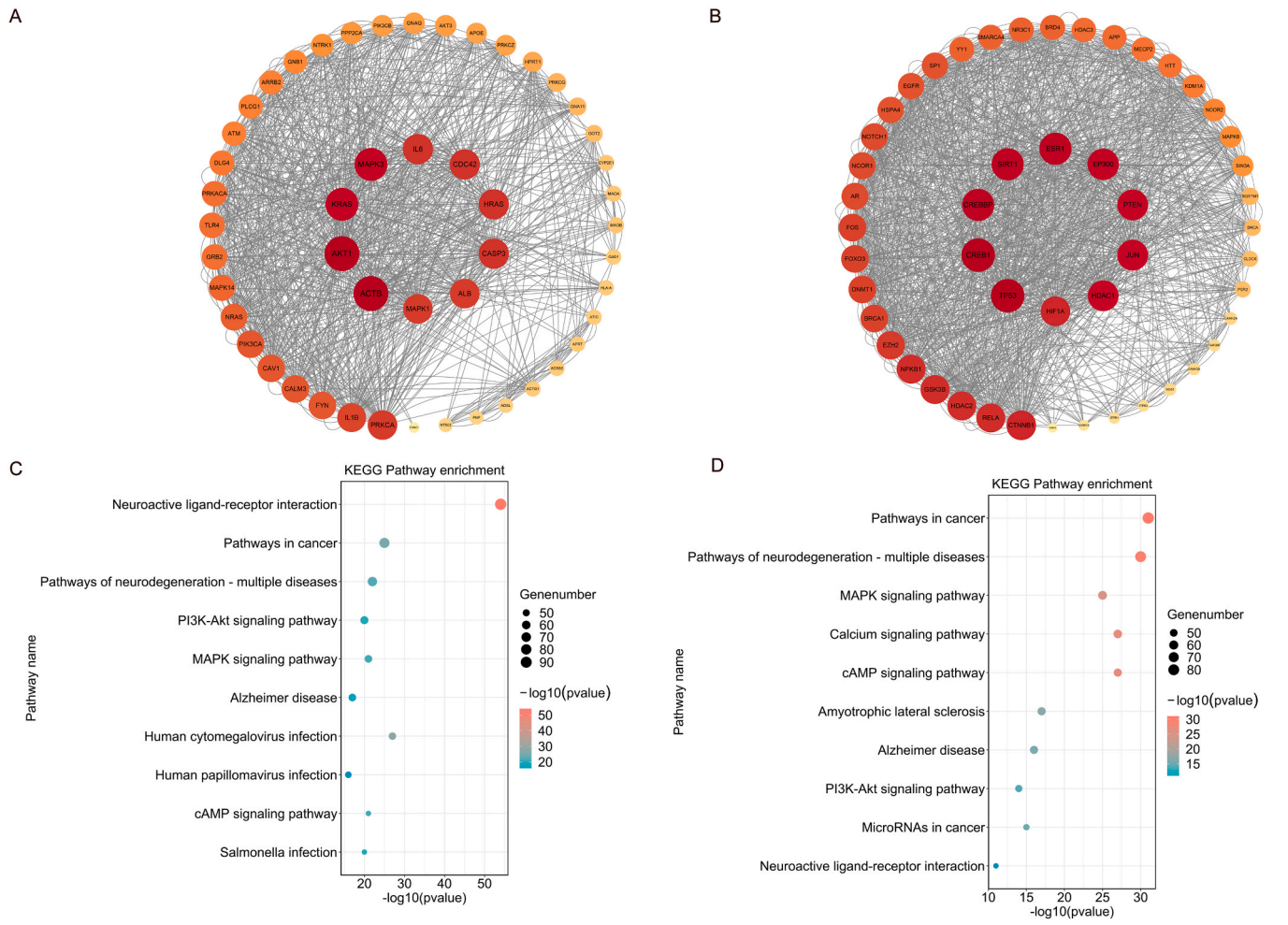

Fig. 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Protein-Protein Interaction (PPI) analysis in the Low Disorder and the High Disorder datasets. (A) Protein-protein interaction networks of the top 50 proteins in the LOW Disorder dataset, sorted in descending order by their degree scores. (B) Protein-protein interaction network among the top 50 proteins in the High Disorder dataset. (C) Top 10 KEGG pathways in the Low Disorder dataset were sorted by the number of enriched genes. (D) Top 10 KEGG pathways in the High Disorder dataset.

between psychiatric disease-related proteins and control proteins. The results indicated significant differences ($P < 0.05$), with ASD and ADHD showing significantly higher proportions of LLPS proteins compared to the control dataset ($P < 0.05$), while ID had a significantly lower proportion of LLPS proteins ($P < 0.05$) (Table 3).

3.7. Proportion of IDPs for ASD differential proteins in a cohort study

To validate our findings using real cohort data, we selected a cohort of 1141 individuals with ASD. We analyzed 391 statistically significant genes identified in this cohort (FDR < 0.1) (Supplementary Table 1). We found that the proteins encoded by these genes had a higher proportion

Table 3
Comparison of LLPS protein percentage in different psychiatric disorders.

Name	N	Mean	SE	Maximum	Median	LLPS protein percentage (%)
BRAIN	2188	575.8	10.9	4870	441	47.41
AD	68	695.8	72.8	2768	479.5[e]	38.25
ADHD	307	995.1	54.3	8797	710.0[e]	59.54[e]
ASD	143	1033	68.3	4967	708.0[e]	66.20[e]
BP	602	753.1	29.2	8797	560.5[e]	51.17
MDD	512	687.8	28.6	6306	485.5[e]	51.91
ID	812	921.1	30.2	8797	634.5[e]	45.01[e]
SCZ	939	754.2	23.2	8797	541.0[e]	50.59
P-value[a]					$P < 0.001$[c]	$P < 0.001$[d]
MENTAL	2189	791.9	15.6	8797	559.0[e]	50.59
P-value[b]					$P < 0.001$[c]	$P < 0.05$[d]

[a] *P*-value for disease-specific comparison with BRAIN dataset
[b] *P*-value of MENTAL compared to BRAIN dataset
[c] Kruskal–Wallis Test.
[d] chi-square test
[e] Values different from BRAIN dataset

of IDPs (76.68 %), which closely aligns with the proportion of IDPs in our ASD dataset (75.40 %) and was significantly higher than in the BRAIN dataset ($P < 0.05$) (Supplementary Table 4). Furthermore, functional analysis of these IDPs revealed significant enrichment in pathways such as regulation of trans-synaptic signaling, modulation of chemical synaptic transmission, head development, chromatin remodeling, and cell morphogenesis (Supplementary Figure 1). These pathways closely resemble those observed in the "High Disorder" proteins and LLPS proteins in psychiatric disorders, with head development, modulation of chemical synaptic transmission, and cell morphogenesis being significantly enriched in all three protein types. These results suggest that the differentially expressed proteins in the ASD cohort contain a higher proportion of IDPs, consistent with our findings, and further emphasize the critical role of IDPs in head development and synaptic signaling in ASD.

4. Discussion

Despite the extensive research efforts over the past few decades to uncover the pathophysiology of mental diseases [3,54–56], the precise molecular mechanisms of psychiatric disorders remain unclear. Previous studies have shown that changes in protein disorder properties may be associated with the development of a variety of diseases [15,17,57]. Due to the diversity and complexity of IDPs, current research on psychiatric disorders still focuses on neurotransmitters [58], neuronal pathways [59], and gene mutations [56], and few studies have explored the role of IDPs in psychiatric disorders. Our study is the first to systematically explore functional roles of IDPs in psychiatric disorders.

This paper analyzes the differences in the proportions and functions of IDPs across seven psychiatric disorders and normal brain controls. We found that psychiatric disorder datasets contained more IDPs than the control dataset, suggesting a potential association between IDPs and psychiatric disorders, particularly in ASD and ADHD. Previous studies indicate that ASD and ADHD share certain common neurological characteristics [60,61], with their underlying neurobiological pathways showing remarkable similarities [62–64]. However, our further analysis revealed functional differences in IDPs between ASD and ADHD, which may be linked to their distinct pathogenic mechanisms. Continued research on IDPs in ASD and ADHD will help clarify these differences and provide insights for the selection of clinical treatments.

Among the 15 most disordered proteins in our dataset, PPR1B encodes for protein phosphatase 1 regulatory subunit 1B, a regulator of kinase or phosphatase associated with glutamate and dopamine receptor activation. As a target of dopamine, PPR1B is of great relevance to the treatment of neurological and mental disorders [65–68]. Furthermore, we observed that most IDPs were associated with pathways or structures involving chromatin, nuclear membrane, cytoskeleton, neurons, and other structures. This finding is consistent with the established role of IDPs in synaptic and neuronal processes [69,70]. We also found that some IDPs are proven targets of psychiatric drugs, providing insights for further elucidating of the mechanism of action of drugs and discovering new psychiatric drug targets. These IDPs drug targets are listed in Supplementary Table 3.

In addition, our results showed that the proportion of IDPs and LLPS in ASD- and ADHD-related proteins was significantly higher than in the control dataset. Further comparative analyses revealed a strong correlation between the proportion of disordered residues in disrupted proteins and their LLPS scores ($r = 0.71$, Fig. 6A). Consistent with the functional enrichment results of 'High Disorder' proteins (Fig. 4B), LLPS proteins were also significantly enriched in pathways related to neuron projection development, head development, cell morphogenesis, brain development, and the modulation of chemical synaptic transmission (Fig. 6B). It has been demonstrated that the phase separation ability of LLPS is closely linked to its IDR region [25,71], and truncation of the IDR can disrupt the protein's phase separation function [71–74]. Therefore, we hypothesize that mutations in IDPs may contribute to psychiatric disorders by affecting liquid-liquid phase separation.

Our study has certain limitations. Although databases such as DisGeNET and GeneCards provide rich gene-protein and disease-gene likelihood data, there is still a possibility of missing newly identified genes linked to mental disorders. Furthermore, changes in gene expression levels were not considered in the diseases we studied because the databases used did not have information on gene expression levels.

Future work could investigate the aggregation properties of IDRs in psychiatric disease-associated proteins. This analysis would include parameters such as radius of gyration, end-to-end distance, polymer scaling index, and aggregate sphericity. We could also explore the impact of conformational changes of IDRs in mental disease-associated proteins on the function of full-length proteins. Furthermore, we could investigate how these conformational changes of IDRs in mental diseases are linked to cellular function, localization, amino acid sequences, evolutionary conservation, and disease variation, together with a newly released molecular model for generating IDR conformational assemblies [75,76].

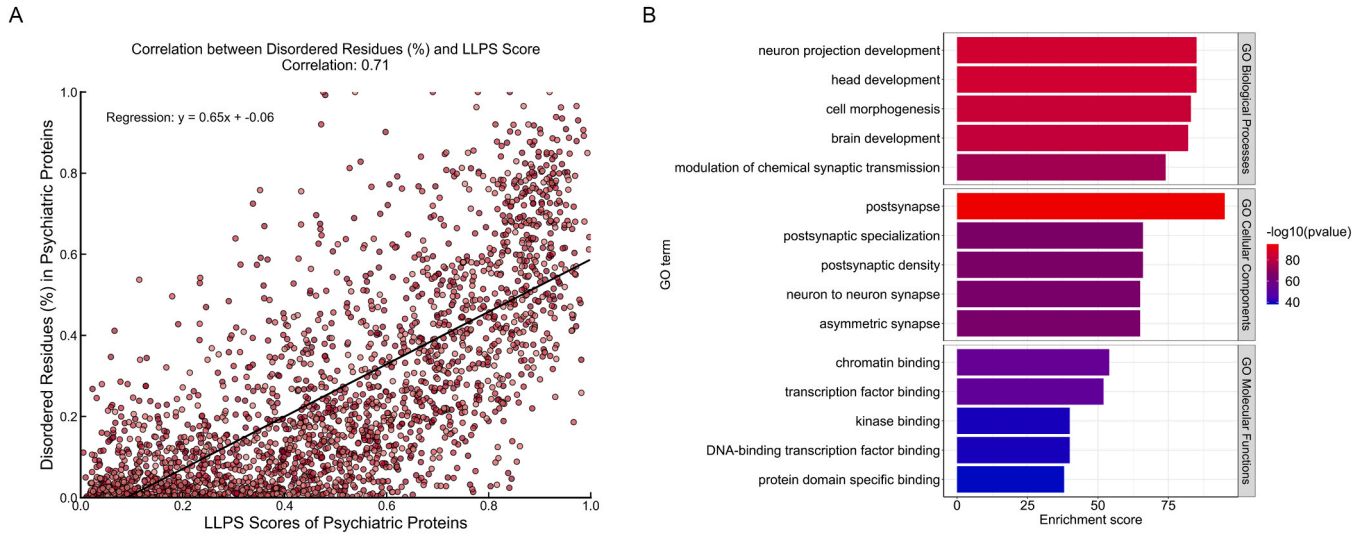

Fig. 6. Correlation analysis between LLPS Scores and Disordered Residues (%) and functional enrichment of LLPS in mental disease-related proteins. (A) Correlation analysis between the Disordered Residues (%) in psychiatric proteins and LLPS Scores of psychiatric proteins. (B) GO functional enrichment of LLPS proteins associated with psychiatric disorders.

In summary, our study contributes to exploring the neurobiological mechanisms underlying psychiatric disorders, providing a novel perspective from the standpoint of protein disorders. We discuss the specific roles and mechanisms of IDPs in psychiatric disorders.

CRediT authorship contribution statement

Qingzhen Hou: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Sankar Basu:** Writing – review & editing, Methodology. **Dongdong Qiao:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Na Zhou:** Validation, Supervision, Methodology. **Yaqing Yang:** Writing – original draft, Formal analysis, Data curation. **Ruotong Liu:** Writing – original draft, Validation, Supervision, Conceptualization. **Xixi Song:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Guangchun Hu:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Data curation. **Xinwu Zhang:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials**:** The following supporting information is attached. Supplementary Table 1: The genes involved in this study; Supplementary Table 2: Binding Sites Prediction for Diseases and BRAIN; Supplementary Table 3: IDPs in existing psychiatric drug targets. Supplementary Table 4: Comparative Analysis of the Proportion of IDPs in the BRAIN Dataset and in ASD Differential Proteins from the Cohort.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2024.10.043.

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