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Parallel molecular alteration between Alzheimer's disease and major depressive disorder in the human brain dorsolateral prefrontal cortex: an insight from gene expression and methylation profile analyses

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Alzheimer's disease (AD) and major depressive disorder (MDD) are comorbid neuropsychiatric disorders that are among the leading causes of long-term disability worldwide. Recent research has indicated the existence of parallel molecular mechanisms between AD and MDD in the dorsolateral prefrontal cortex (DLPFC). However, the premorbid history and molecular mechanisms have not yet been well characterized. In this study, differentially expressed gene (DEG), differentially co-expressed gene and protein–protein interaction (PPI) network propagation analyses were applied to gene expression data of postmortem DLPFC samples from human individuals diagnosed with and without AD or MDD (AD: cases = 310, control = 157; MDD: cases = 75, control = 161) to identify the main genes in the two disorders' specific and shared biological pathways. Subsequently, the results were evaluated using another four assessment datasets (n1 = 230, n2 = 65, n3 = 58, n4 = 48). Moreover, the postmortem DLPFC methylation status of human subjects with AD or MDD was compared using 68 and 608 samples for AD and MDD, respectively. Eight genes (*XIST*, *RPS4Y1*, *DDX3Y*, *USP9Y*, *DDX3X*, *TMSB4Y*, *ZFY* and *E1FAY*) were common DEGs in DLPFC of subjects with AD or MDD. These genes play important roles in the nervous system and the innate immune system. Furthermore, we found *HSPG2*, *DAB2IP*, *ARHGAP22*, *TXNRD1*, *MYO10*, *SDK1* and *KRT82* as common differentially methylated genes in the DLPFC of cases with AD or MDD. Finally, as evidence of shared molecular mechanisms behind this comorbidity, we propose some genes as candidate biomarkers for both AD and MDD. However, more research is required to clarify the molecular mechanisms underlying the co-existence of these two important neuropsychiatric disorders.

Key words: Alzheimer's disease (AD), major depressive disorder (MDD), differentially co-expressed genes, differentially expressed genes (DEGs), differentially methylated genes

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INTRODUCTION

Alzheimer's disease (AD) is one of the most common neuropsychiatric disorders, with a frequency of 6% of the population over the age of 65 years. AD is a progressive disease with disabling symptoms in remembering recent events, language impairment and cognitive decrease. Recent reports have identified gender differences in hippocampus and parietal lobe development that significantly affect AD development (Benoit et al., 1999;

Perry and Hodges, 1999; Burns and Iliffe, 2009; Mielke, 2018; Guest, 2019).

Comorbidity of patients with AD and depression has been observed in up to 50% of patients. Depression is one of the highly prevalent psychiatric symptoms of AD, affecting approximately 17% of people in their lifetime. Depression and AD symptoms are often co-diagnosed, particularly in older people with impaired memory. It has been reported that older people with minor depressive disorders are more likely to develop major depressive disorders (MDD). While people with a history of depression may be at greater risk of developing AD, the history of depression in AD is not well known (Jorm, 2001; Ownby et al., 2006; Byers and Yaffe, 2011; Mendes-Silva et al., 2016).

Progress in high-throughput technology has provided new insights into the potential mechanism of depression in AD (Atkinson et al., 2014; Sulaimany et al., 2017), and emerging methods for gene co-expression studies enable the inference of gene changes in the context of transcriptomics (Ghasemi et al., 2014; Motieghader et al., 2017; Kouhsar et al., 2019). Two genes are defined as co-expressed genes in a dataset when they show a similar and correlated expression pattern across different traits (cases and controls). Differential co-expression methods are used to determine regulatory genes and identify differences in modules of genes in the various phenotypes (van Dam et al., 2018; Zhang et al., 2018; Alaei et al., 2019). The co-expression approach has been used previously to study AD and dementia. Gene co-expression network analysis was used on 329 samples to find biomarkers for AD stages (Zakeri et al., 2020). Moreover, using the co-expression approach to study neurodegenerative dementia in mice, two preserved co-expressed modules with mutations in the genes *MAPT* and *GRN* were identified (Mortezaei et al., 2017; Swarup et al., 2019). The lack of knowledge about the etiology and pathogenesis of AD and depression disorders prevents the development of more effective treatments. Although the overall mechanism of depression is not yet fully explored, a recent study indicates that immune cells and their signaling pathways play a role in the pathophysiology of major depressive disorder (Chi et al., 2014; Khundakar and Thomas, 2015).

With the use of machine learning (Masoudi-Sobhanzadeh et al., 2019b) and the gene co-expression approach, two modules in frontotemporal dementia were found that may link the impairment of neurogenesis, axon branching and synaptogenesis in the hippocampus to the pathology of MDD (Leyhe et al., 2009; Budni et al., 2015). Dysregulation in molecular signaling pathways such as ERK1/2, p38, Src family tyrosine kinases and glutamate receptors may also be related to MDD pathology. It has been reported that the progression of AD is associated with the formation of amyloid plaques and neurofibrillary tangles in the brain (Busciglio et al., 1997; Hooshmand et al., 2021). Also, the related signaling pathways in AD include Ras/ERK, PI3K/Akt and PKA/cAMP (Mizuno et al., 2012; Van Dooren et al., 2014).

Several publications have reported common mechanisms between AD and MDD (Ownby et al., 2006); one of these studies (Mendes-Silva et al., 2016) reported microRNAs as being involved in both AD and MDD biological pathways such as proteostasis control, maintenance of genomic integrity, regulation of transcriptional activity, immune-inflammatory control and neurotrophic support. Another reported shared genetic etiologies between AD and MDD in SNPs corresponding to the *SPI1* gene and the *MS4A* gene cluster and novel pleiotropic risk loci for AD conditional with MDD (Lutz et al., 2020).

Systematic reviews suggest that DNA methylation is associated with the etiology of depression and AD. Brain-derived neurotrophic factor (*BDNF*), nuclear receptor subfamily 3 group C member 1 (*NR3C1*) and serotonin transporter (*SLC6A4*) are candidate genes in association with DNA methylation in MDD (Chen et al., 2017). Tau, transmembrane protein 59 (*TMEM59*) and amyloid processing genes also have an epigenetic role in AD development (Barrachina and Ferrer, 2009; Mastroeni et al., 2011).

While the etiology of depression in AD still needs conceptual clarification, the relationship between AD and MDD suggests that they share common neurobiological abnormalities; hence, we think elucidating the cellular and molecular links between AD and MDD may be crucial to understanding the cause of comorbidity of these psychiatric disorders. This study suggests common underlying cellular mechanisms for AD and MDD. Moreover, with the knowledge of gender effects on brain development, we consider sex differences. This article addresses shared altered genes and cellular pathways by analyzing GEO datasets. Constructing a gene co-expression network and identifying differential co-expression and differentially expressed genes (DEGs) along with the view of methylated genes may be a practical approach for diagnosing parallels between AD and MDD in clinical practice.

RESULTS

Shared DEGs in AD and MDD Detecting genes with the same differential expression pattern between AD and MDD could help to find biological processes that are shared by these disorders. Accordingly, after data normalization and removing age-dependent genes, differential expression analysis was performed on AD and MDD datasets, and DEGs were identified for each trait. Furthermore, the intersection between sex-dependent genes and DEGs was checked, which revealed that sex-dependent DEGs did not overlap with disease-related

DEGs. Consequently, a total of 103 and 87 genes were differentially expressed in the DLPFC of subjects with AD and MDD, respectively, compared to control samples. Subsequently, we examined overlaps between AD and MDD DEGs. Eight DEGs including *XIST*, *RPS4Y1*, *DDX3Y*, *USP9Y*, *DDX3X*, *TMSB4Y*, *ZFY* and *E1FAY* were common between AD and MDD DEGs. *XIST* (X-inactive specific transcript) is an RNA gene on the X-chromosome of placental mammals that causes X-inactivation. Evidence shows roles for *XIST* lncRNA in MDD and for *RPS4Y1*, *DDX3Y*, *USP9Y* and *ZFY* in AD; these genes play essential roles in neural differentiation (Sun et al., 2014; Ghafouri-Fard et al., 2021). Also, the expression of *DDX3Y* (DEAD-box polypeptide 3, Y-linked) affects neuronal markers and is essential for promoting the repair of neurons, consequently contributing to neurodegenerative disease (Vakilian et al., 2015).

We also investigated gene ontology (GO) terms enriched among DEGs in AD and MDD, which indicated that three GO terms were common: cellular response to copper ion, positive regulation of chemokine production, and negative regulation of growth.

Specific disorder-related DEGs We categorized DEGs into three groups based on their presence in either disease. Genes shared by both AD and MDD are listed in Table 1, while AD-specific DEGs include *SMCY*, *CYorf15A*, *CYorf15B*, *PRKY*, *HSFY2*, *HSFY1*, *UTX*, *SMCX*, *GTPBP6*, *PLXNB3* and *PLCXD1*, and MDD-associated DEGs comprise *TTTY15*, *NLGN4Y*, *COL4A5*, *PUDP*, *NAP1L3*, *TMEM100*, *ALDH3A2*, *PAPOLA*, *CHI3L1* and *ANOS1* (Fig. 1). Specific disorder-related DEGs are major fac-

Table 1. Fold change results for common DEGs between AD and MDD in various datasets

	GSE92538	GSE33000	GSE54567-68	GSE102556	GSE84422	GSE44770
RPS4Y1	3.38	1.66	0.63	0.58	2.56	1.71
DDX3Y	1.03	1.62	0.84	−6.54	2.54	1.72
USP9Y	1.16	0.48	0.49	0.20	0.90	1.47
DDX3X	−0.30	−0.11	–	−0.50	–	−0.15
TMSB4Y	–	0.79	–	0.18	–	0.94
ZFY	0.10	0.19	0.45	0.41	–	0.20
EIF1AY	0.46	0.53	0.40	0.20	0.72	0.72
XIST	−3.68	−1.97	−1.52	−6.36	−3.46	−1.90

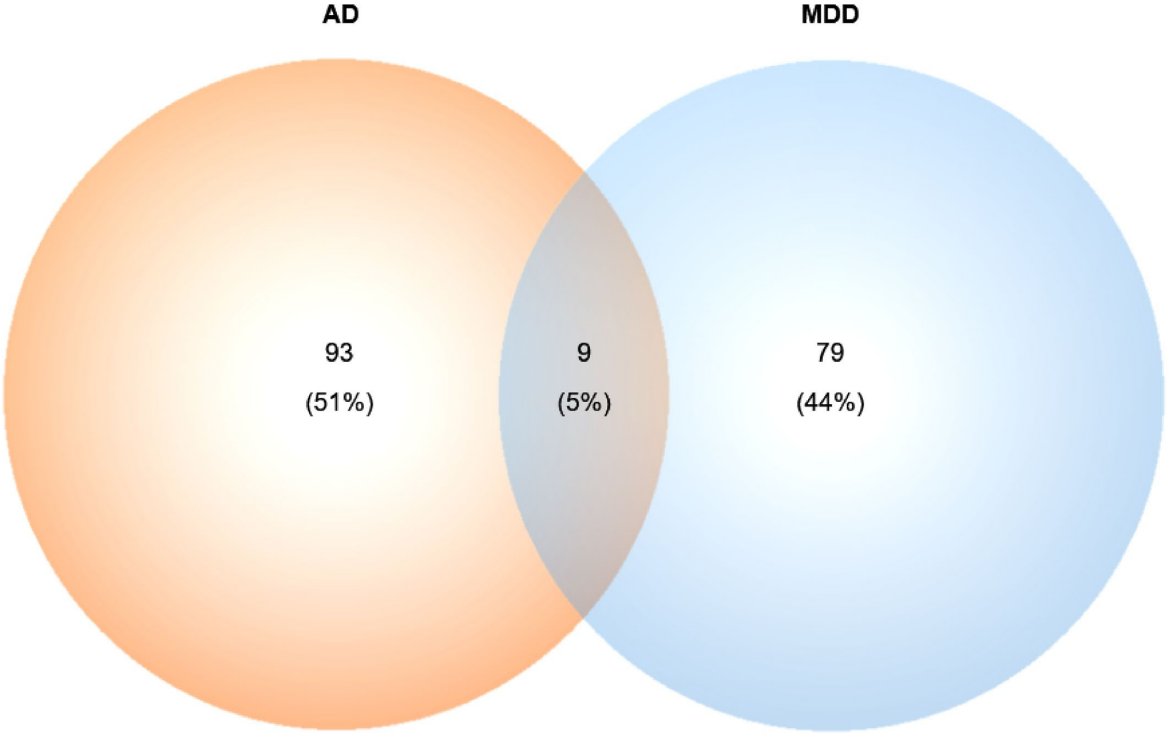

Fig. 1. Venn diagram shows the numbers of shared and disorder-specific DEGs in AD and MDD.

tors of cellular processes and biological pathways that differentiate the disorders from each other.

Disorder-related co-expression network modules Similar transcription changes of various genes between case and control subjects indicate their cooperation in carrying out particular molecular and cellular processes, which leads to the emergence of a specific trait (disorder). Hence, discovering a co-expression network and extracting co-expressed genes are important for finding

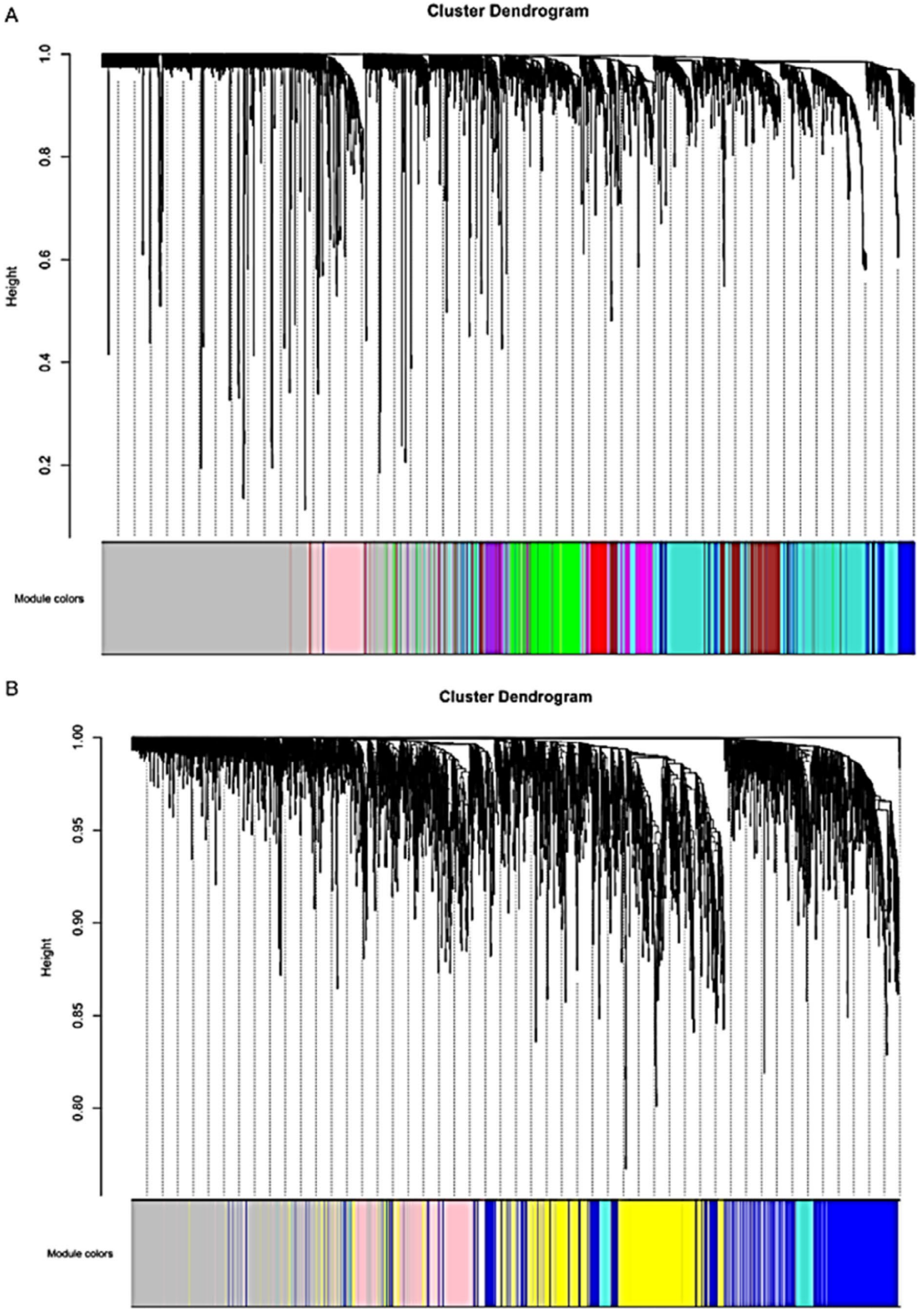

Fig. 2. Identification of gene co-expression modules in AD (A) and MDD (B). The y-axis shows the co-expression distance and the x-axis corresponds to the genes. The horizontal bar indicates modules with a different color.

altered biological processes and pathways that play key roles in disorder development. We built a co-expression network for each disorder and after scrutiny of gene expression correlations, fourteen modules were found in the AD and 21 in the MDD gene co-expression networks (Fig. 2). The most extensive module in the AD gene co-expression network contained 893 members, and the smallest had 23 members. In the MDD gene co-

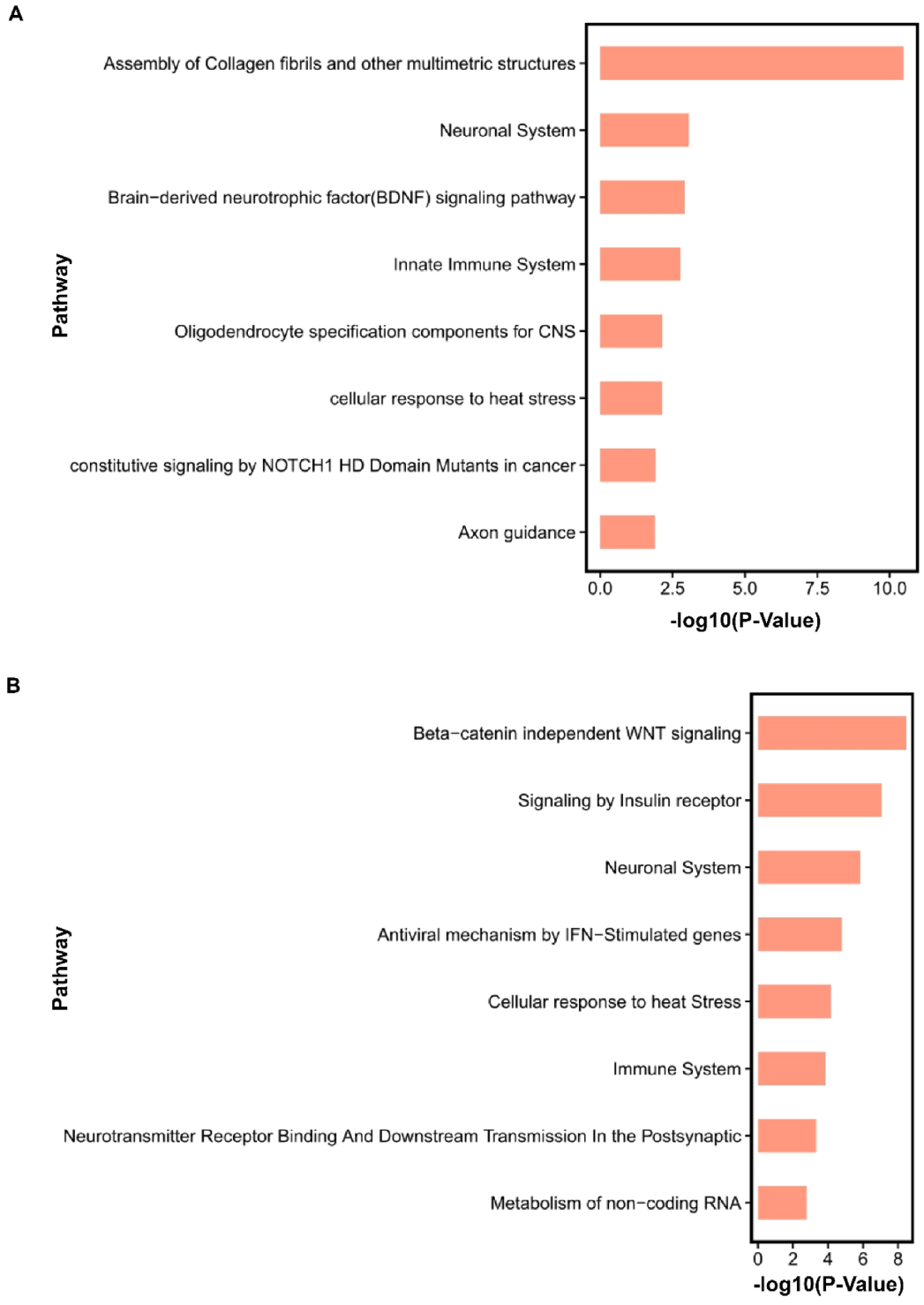

Fig. 3. KEGG pathway enrichment analysis of modular genes. (A) The significantly enriched pathways ($P < 0.05$) in the KEGG pathway analysis of the AD modular genes. (B) The significantly enriched pathways ($P < 0.05$) in the KEGG pathway analysis of the MDD modular genes.

expression network, a module with 719 compounds was the largest, and the smallest had 21 members, while 16,538 components were not assigned to any modules and are labeled with the color gray. To better understand each module's behavior, we identified the hub genes of each module. They include genes such as *RFXDC2*, *FRMD4B*, *IRF2BPI*, *SYK*, *ACTA2*, *DNAJA1*, *RIF1* and *PLLP* in AD, and *XIST*, *MLPH*, *UBE2J2*, *PRKA1A*, *ATP50*, *NOTCH2*, *ACAP1*, *ZNF148*, *EV12A*, *GAPDH*, *FAM76A*, *ACTG1*, *CANX*, *TDRG1*, *SOCS3*, *RPS24*, *EFNA5*, *SLC6A8*, *IL17RB* and *SCP2* in MDD.

To determine the functional implications of the gene co-expression networks, each module was annotated using KEGG (Kanehisa and Goto, 2000; Masoudi-Nejad et al., 2007a, 2007b), and functional enrichment analysis revealed biological functions related to each of the extracted modules in MDD and AD. Some of the top pathways are listed in Fig. 3. The enrichment analysis results are strongly associated with AD and MDD disorders, which were also enriched in shared pathways. For example, the neuronal system was the commonest shared pathway in AD and MDD. Alterations in synaptic proteins play an essential role in the neuronal system and are among the main causes of abnormal neuronal systems (Mattson, 2004). The next commonest pathway is the immune or innate immune system, which has diverse interactions with AD and Parkinson's disease neuropathology (Jevtic et al., 2017; Cao and Zheng, 2018).

A similar observation was made for the cellular response to heat stress associated with the accumulation of misfolded proteins and abnormal protein aggregation in the human brain. This is essential for various neuro-

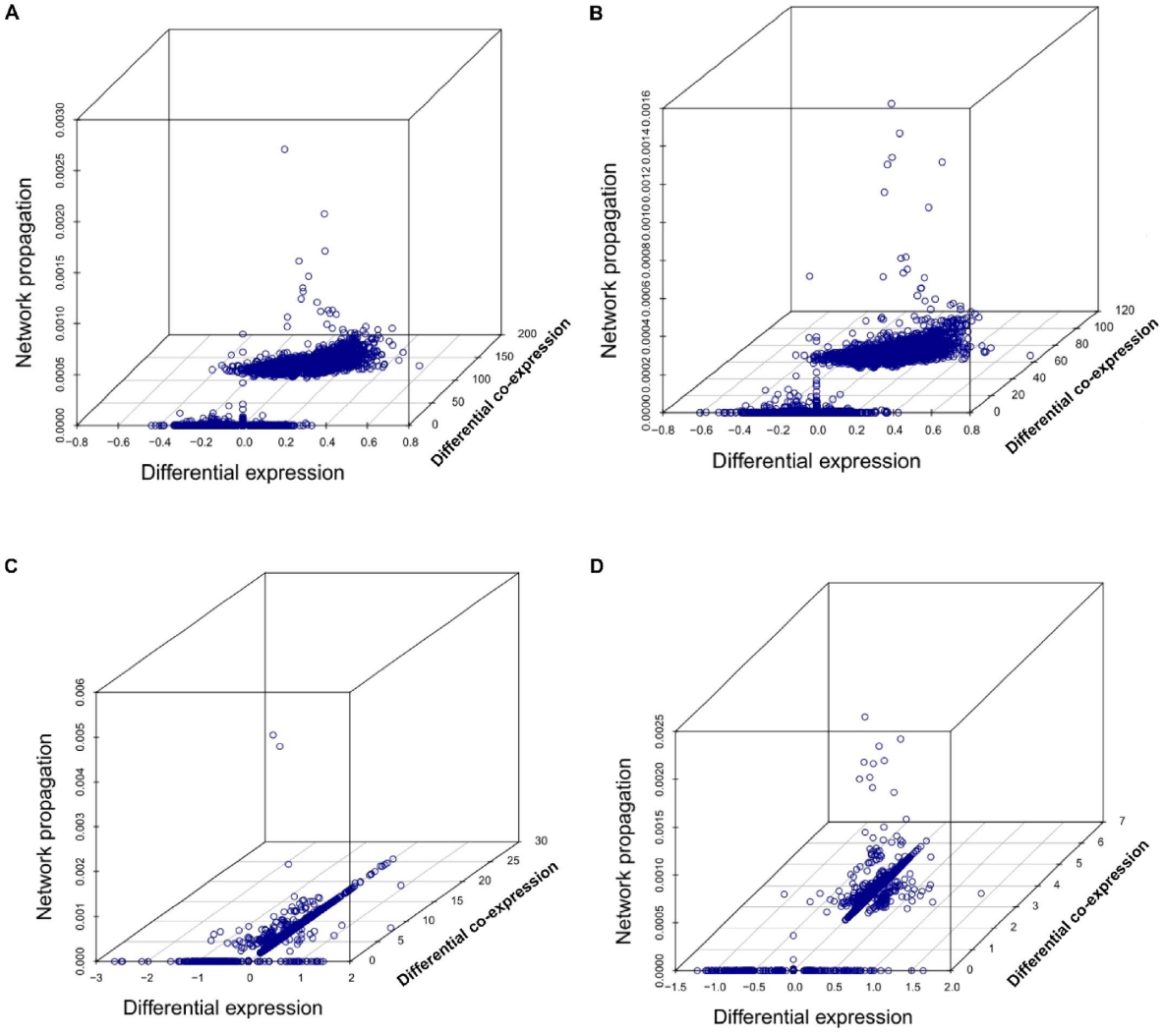

Fig. 4. Three-dimensional (3D) scatter plots of genes, modeled by 3D feature vectors. Genes that are farther from the origin may play key roles in disorder generation and progression. The x-axis shows the differentially expressed gene measure, the y-axis shows the differentially co-expressed gene value and the z-axis shows the PPI network propagation score for the gene's corresponding transcript. (A) 3D scatter plot of gene distribution for females with AD. (B) The distribution for males with AD. (C) The distribution for males with MDD. (D) The distribution for females with MDD.

degenerative diseases (Calabrese et al., 2007; Ahmadi et al., 2013; Campanella et al., 2018).

Disorder-related prioritized genes We also combined three measures, namely differential gene expression, differential co-expression and network propagation, to prioritize the genes' influences on AD and MDD progression in four separate groups (separated into male and female). Differential co-expression analysis was used to recognize highly altered hub genes that potentially play an important role in disease. Besides differential co-expression analysis, protein–protein interaction (PPI) network propagation is also a useful practical approach to surveying the influence of genes in biological processes (Ghasemi et al., 2014).

As shown in Fig. 4, males and females with AD and MDD have a similar pattern in each disease, while in each trait we observed more alterations in some genes, such as *DCTN2*, *PSMC3*, *GNG3* and *PSMD8* in the AD-female group; *PGK1*, *GNAS*, *VDAC1* and *POLR3K* in the AD-male group; *STYXL1*, *ALAD*, *ZNF141* and *SNX15* in the MDD-female group; and *PFKFB3*, *LAPTM5* and *SPTBN4* in the MDD-male group.

CpG site distribution in AD Change in the genomic distribution of CpG sites, including in the transcription start site (TSS), gene body and intergenic region, may alter some genes' expression and thus affect their associated biological pathways. Our identified CpG site distribution for AD is shown in Fig. 5 and a comparative scatter

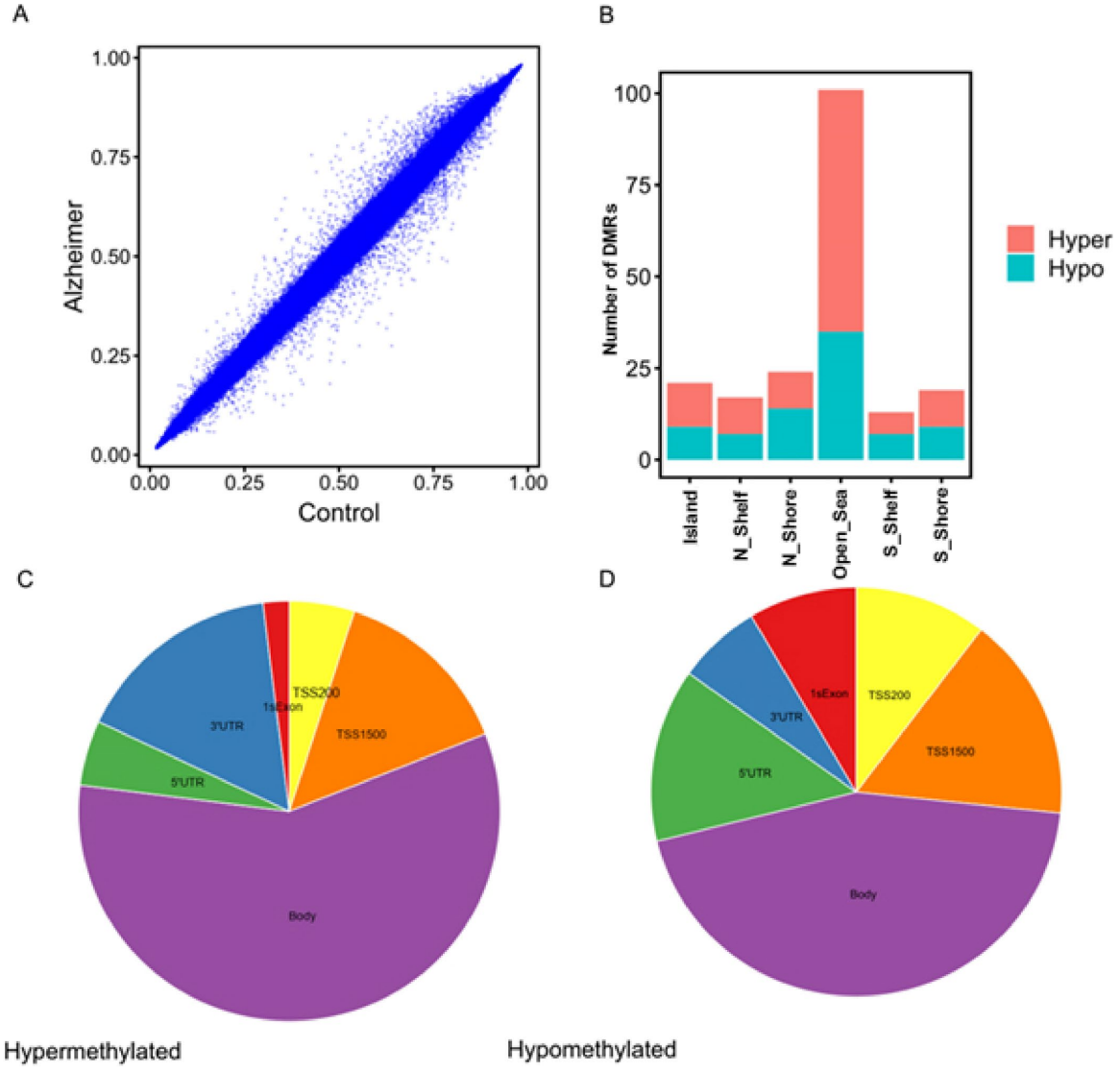

Fig. 5. AD-associated DNA methylation patterns. (A) Scatter plot of DNA methylation between AD and control samples. (B) Bar plots show CpG context distributions. (C, D) Pie charts of hypermethylated (C) and hypomethylated (D) CpGs associated with AD for the indicated gene regions.

plot of DNA methylation between AD and control is shown in Fig. 5A. TSS class is assigned to TSS1500, TSS200 or 5′ UTR, while CpG sites located in the 1st exon, body or 3′ UTR group construct the gene body. Intergenic sites are CpG sites with no annotated regions (Deaton and Bird, 2011; Edgar et al., 2014). According to our analysis, 114 differentially methylated regions (DMRs) were hypermethylated and 81 were hypomethylated in AD. Of the hypermethylated regions, 72 were located in some genes while the other 52 (which were not included in gene coding regions) were linked to several genes. The most hypermethylated genes were *PRIC285*, *HLA-DQA1*, *HDAC4* and *KIAA0319*, and the most hypomethylated ones were *HSD17B7P2*, *HLA-DRB5*, *RANBP17* and *TRIM6*. Genomic regions outside CpG islands were more likely to undergo methylation (Fig. 5B). The methylation profiles differed among genic regions. The lowest methylation level was observed for CpG sites from the 1st exon, while gene body regions were most highly methylated (Fig. 5C, 5D).

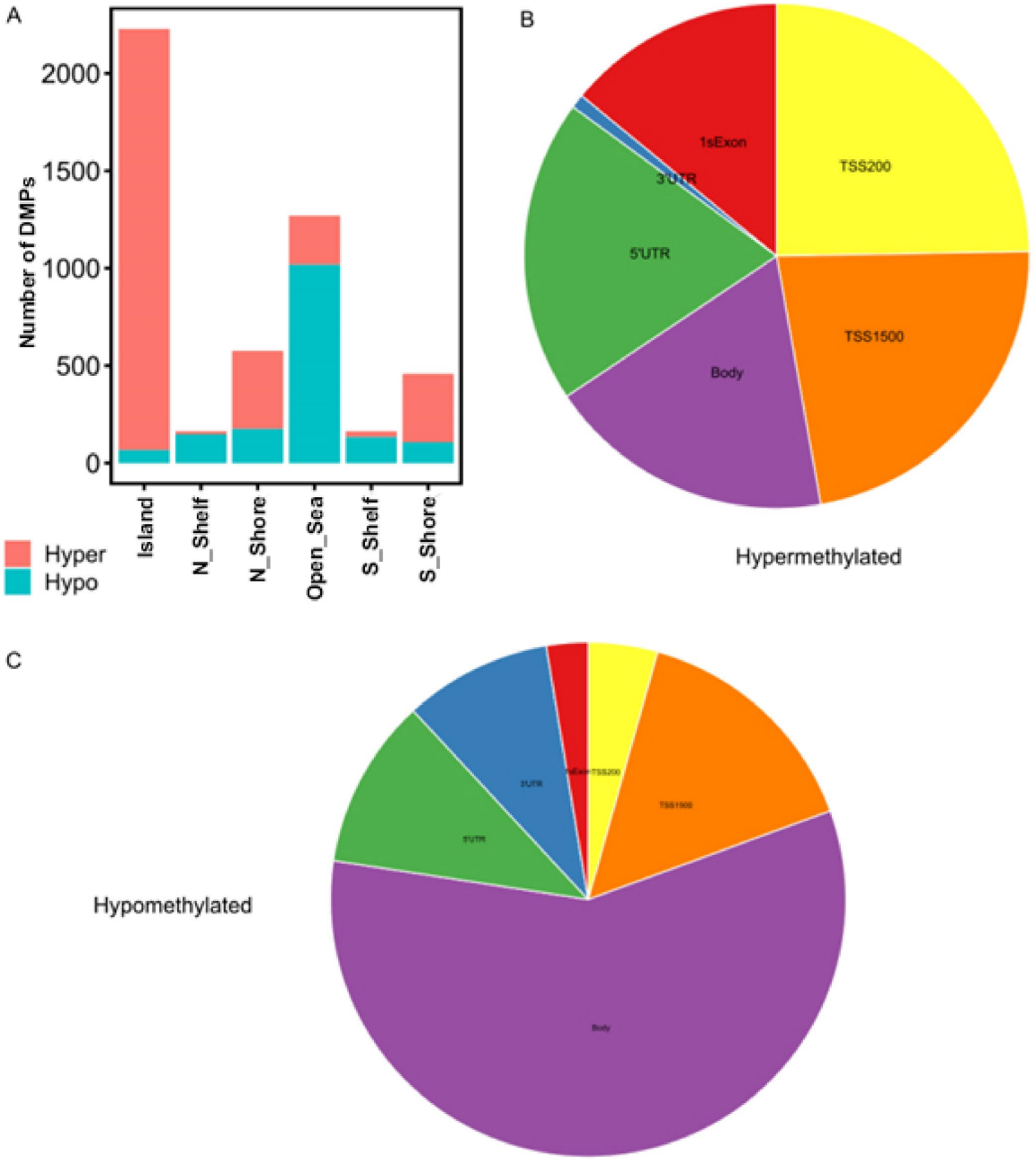

Fig. 6. MDD-associated DNA methylation patterns. (A) Bar plots show CpG context distributions. (B, C) Pie charts of hypermethylated (B) and hypomethylated (C) CpGs associated with MDD for the indicated gene regions.

CpG site distribution in MDD We also utilized differentially methylated positions (DMPs) in previously reported postmortem DLPFC of cases diagnosed with MDD (Huels et al., 2020). The CpG site distribution of DMPs in MDD is shown in Fig. 6. The overrepresented CpG sites were in islands, while N_shelf (north shelf) had the lowest distribution of methylation (Fig. 6A). Differentially methylated CpGs were more abundant in the body, TSS1500 and 5′ UTR regions in both hyper- and hypomethylated genes (Fig. 6B, 6C). The most hypermethylated genes were *ENPP6*, *RP5-998H6.2*, *RP13-895J2.7* and *RP11-162A12.2* and the most hypomethylated were *F2RL2*, *UGT8*, *AC116655.1*, *SERPINA6* and *EBPL*.

AD and MDD common differentially methylated genes We compared genes with altered DNA methylation in the postmortem DLPFC samples of individuals with AD and MDD. Seven genes are shared between the two groups, comprising *HSPG2*, *DAB2IP*, *ARHGAP22*, *TXNRD1*, *MYO10*, *SDK1* and *KRT82* (Table 2). The methylation differences included both hyper- and hypomethylated genes.

Evaluation of shared DEGs in other datasets We obtained common DEGs in AD and MDD in two test datasets (GSE33000, GSE92538). To assess the primary results in other GSE datasets that had not initially been analyzed, we compared the expression alteration pattern of the identified common DEGs with their counterparts in other datasets. The results (Table 1) showed a consistent correlation in the fold change of *ZFY*, *EIF1AY* and *XIST*, but *RPS4Y1*, *DDX3Y* and *USP9Y* showed little or no correlation with these other GSE datasets.

DISCUSSION

Comorbidity of AD and MDD symptoms in people diagnosed with one of these neuropsychiatric disorders suggests the existence of altered transcription of the same genes and shared disease-related pathways in both illnesses. With the recent advantages of high-throughput technology and advances in differential expression and co-expression analysis methods, we can expand our knowledge of the molecular and biochemical relationship between AD and MDD. In the present study, we profiled differentially expressed and co-expressed genes and functional modules of gene expression in postmortem dorsolateral prefrontal cortex samples of subjects diagnosed with AD or MDD. We also compared differentially methylated genes in postmortem DLPFC of individuals with AD or MDD. These approaches have not previously been employed integrally in MDD and AD expression datasets of the dorsolateral prefrontal cortex of the brain.

We investigated age- and sex-dependent gene expression to remove age- and sex-influenced DEGs from disorder-related DEGs. Nevertheless, some limitations could not be completely overcome. The number of males and females in the control group was not equal. The average ages considered to recognize age-dependent genes were far from the average age of cases diagnosed with the disorders. In addition, taking medicine can affect the expression of genes related to disorders. However, since information about drug consumption by the patients under investigation was not publicly available, we could not survey the exact effect of the disorders alone on gene expression alteration in this research.

Nevertheless, we found overlaps in the altered gene expression profiles in postmortem DLPFC between AD and MDD cases. Genes related to neuronal cell development and differentiation may play an essential role in neuropsychiatric disorders such as AD and MDD. They

Table 2. Common differentially methylated genes between AD and MDD

Gene	ΔBeta		Elements		*P*-value	
	AD	MDD	AD	MDD	AD	MDD
HSPG2	0.01	0.003	Body; S_Shore	Body	0.02	0.003
DAB2IP	0.03	0.004	Body; Island	Body; N_Shore	0.001	0.006
ARHGAP22	0.03	0.003	Body; Island	Island	0.000	0.007
TXNRD1	−0.01	0.008	Island; SS200; 1st Exon; Body	TSS200; Island	0.001	0.004
MYO10	0.015	−0.015	Body; Island	S_Shore	0.03	0.001
SDK1	0.02	−0.013	Body; 3′ UTR; Island	Body	0.011	0.002
KRT82	−0.01	−0.011	1st Exon; 5′ UTR; TSS200; TSS1500	3′ UTR	0.004	0.006

Note: ΔBeta is a coefficient that demonstrates differentially methylated values between AD or MDD and control samples. Genomic regions of DNA methylation alteration include gene body, CpG islands, northern/southern shores (regions up to 2 kb from CpG island, upstream and downstream), northern/southern shelves (regions from 2 to 4 kb from CpG island, upstream and downstream) and open sea (the rest of the genome).

can also have gender-specific functions and may contribute to male neurodegenerative disease (Lai et al., 2010; Gueler et al., 2012; Masoudi-Sobhanzadeh et al., 2019b).

In addition, we modeled each surveyed gene by a three-dimensional (3D) feature vector whose three features include a differentially expressed gene measure (log2(ratio) threshold), a differentially co-expressed gene value (gene neighbors alteration), and a PPI network propagation score for the gene's corresponding transcript (random walk algorithm outcome for each gene). Scatter plots of 3D gene distributions for males with AD, females with AD, males with MDD and females with MDD are illustrated in Fig. 4. Genes with the most alterations in AD and MDD included *ZNF141*, *DCTN2*, *PSMC3*, *GNG3*, *PSMD8*, *PGK1*, *GNAS*, *VDAC1*, *POLR3K*, *STYXL1*, *ALAD*, *SNX15*, *PFKFB3*, *LAPTM5* and *SPTBN4*, some of which have previously been reported as genes associated with AD and MDD (Burns and Iliffe, 2009; Naughton et al., 2015; Miyata et al., 2016).

By pathway enrichment analysis of genes associated with each co-expression module, we could divide all identified enriched pathways into five main groups (Fig. 2): signal transduction (e.g., constitutive signaling NOTCH1 HD domain mutants, opioid signaling, disease of signal transduction), endocrine control (oxidative phosphorylation, parathyroid hormone), neurotransmission (GABAergic synapse, serotonergic synapse) (Calvo-Flores Guzmán et al., 2018), neurological disorder (Parkinson's disease, addiction) and immune system (immune system, adaptive immune system) (Robson et al., 2017).

As well as identifying common differentially expressed genes between AD and MDD, we found DEGs that were associated with only one disorder. These disorder-specific DEGs determine transcriptomic changes in related conditions, which could help to distinguish genes involved in biological pathways in each disorder, as well as to identify potential disease development/progression indicator biomarkers.

There is evidence for the role of DNA methylation changes in both AD and MDD development (Irier and Jin, 2012; Pishva et al., 2017). We found that DNA methylation changes in CpG island sites and gene bodies contribute to regulation of gene expression and susceptibility to these neuropsychiatric disorders (see Table 2). Differentially methylated genes were ranked as the most hyper- or hypomethylated in AD. The eight top-ranked genes comprise *PRIC285*, *HLA-DQA1*, *HDAC4*, *KIAA0319*, *HSD17B7P2*, *HLA-DRB5*, *RANBP17* and *TRIM6*. Among these, *KIAA0319* was reported as a significant locus in the discovery analysis of AD-associated DMRs (De Jager et al., 2014). Literature data suggesting that the expression of these genes changes in AD were partially confirmed by the results of our study (Pistollato et al., 2016; Zhang et al., 2022). Also, we and others demonstrated hyper- or hypomethylated genes associated with MDD (*ENPP6*, *RP5-998H6.2*, *RP13-895J2.7*, *RP11-162A12.2*, *F2RL2*, *UGT8*, *AC116655.1*, *SERPINA6* and *EBPL*) (Huels et al., 2020).

Common differentially methylated genes provide more insights into the disorders' comorbidity etiologies. In the present study, differences in brain DLPFC genome-wide DNA methylation were observed in both AD and MDD. Our results support the importance of DNA methylation alteration in AD and MDD pathology and highlight the value of scrutinizing the exact role of some hyper- or hypomethylated genes in future studies. Our presented DNA methylation changes in *HSPG2*, *DAB2IP*, *ARHGAP22*, *TXNRD1*, *MYO10*, *SDK1* and *KRT82* in DLPFC brain tissue of cases with AD and MDD have confirmed some former research (Table 2) (Soerensen et al., 2008; Kaut et al., 2015; Roberts et al., 2017; Lutz et al., 2020). Also, DNA methylation changes of *MYO10* in three independent brain cohorts were reported before (De Jager et al., 2014).

We assessed the shared DEGs of the primary datasets in four other datasets and found that three genes (*ZFY*, *EIF1AY* and *XIST*) showed consistent differential expression patterns in all of them (Table 1). These genes are worthy of further investigation as potential biomarkers of AD and MDD comorbidity. The other five common DEGs do not have consistent expression behavior in all analyzed datasets. As they were not age- or sex-dependent genes, these DEGs may be related to unknown specific characteristics of the other datasets.

MATERIALS AND METHODS

Data collection and preprocessing Microarray gene expression datasets including GSE54567, GSE54568, GSE92538 and GSE102556 for postmortem DLPFC of cases with and without MDD along with GSE33000, GSE84422 and GSE44770 for postmortem DLPFC of subjects with and without AD, as well as the GSE125895 raw methylation dataset for DLPFC of cases with and without AD, were downloaded from the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) database. Moreover, we utilized previously reported differentially methylated positions (DMPs) in the postmortem DLPFC of individuals diagnosed with MDD (Huels et al., 2020) due to the lack of publicly available MDD methylation data. Table 3 describes these datasets in detail. In addition, we utilized human protein–protein interaction data from String (von Mering et al., 2003) and disease-associated genes from eDGAR (Babbi et al., 2017; Masoudi-Sobhanzadeh et al., 2019a) databases.

Human postmortem DLPFC transcriptome samples of subjects with AD or MDD were normalized using the RMA-Express tool (Bolstad et al., 2003; Irizarry et al., 2003a, 2003b). The SVA package (Leek et al., 2012) was then

Table 3. Characteristics of GSE datasets used in this work

GSE number	Disease	Platform	Number of participants							
			Male		Female		Patients		Controls	
			No.	Age	No.	Age	No.	Age	No.	Age
GSE54567	MDD	Affymetrix	28	–	0	–	14	–	14	–
GSE54568	MDD	Affymetrix	0	–	30	–	15	–	15	–
GSE92538	MDD	Affymetrix	177	52.00±15.74	59	59.00±10.72	75	41.20±13.56	161	59.30±12.31
GSE102556	MDD	Illumina HiSeq	26	44.19±13.54	22	49.28±15.70	26	45.68±13.15	22	47.63±16.48
GSE84422	AD	Affymetrix	24	78.11±10.81	41	89.10±7.41	34	87.82±8.06	31	63.50±9.90
GSE33000	AD	Rosetta/Merck	258	71.12±12.16	209	79.45±10.81	310	80.60±8.99	157	63.51±9.90
GSE44770	AD	Rosetta/Merck	144	68.55±13.01	86	78.38±11.76	129	80.14±9.26	101	62.11±10.86
GSE125895	AD	Illumina Human Methylation 450 Beadchip	39	67.31±11.08	29	67.10±13.05	21	79.95±9.46	47	61.54±7.71

Note: GSE102556 and GSE125895 are datasets of whole RNAseq; the others are microarrays.

employed to remove batch effects. Probes with missed values were adjusted using multiple regression. Hierarchical clustering demonstrated GSM1319087, GSM1319094 and GSM1319095 samples as outliers in the merged GSE54567 and GSE54568 datasets; therefore, they were removed from further analyses. Two datasets, GSE33000 and GSE92538 (with the largest numbers of samples), were picked out as the primary investigation input datasets and the others were used to validate DEG analysis results.

The GSE125895 dataset, consisting of raw human postmortem DLPFC methylation data of cases with AD, was preprocessed using the minfi package (Aryee et al., 2014). Probes with low signal detection containing control probes, probes with P-value > 0.01 in more than 50% (n = 110), cross-reactive probes, non-CpG probes and sex chromosome probes were filtered out by minfi (with n = 485,512 probes as input). Beta values were calculated as the ratio of methylated signal to the sum of unmethylated and methylated signals at each CpG site (ranging from 0 for the unmethylated site to 1 for a fully methylated site) and normalized by the Funnorm normalization method for background correction and adjusting probe types I and II.

DEG analysis: identifying disorder-related DEGs for AD and MDD Limma tools (Smyth, 2005) were utilized to identify DEGs between case and control samples. Genes with adjusted P-value ≤ 0.05 were selected for further analyses. To reduce the effects of individuals' age on the final results, we treated DEGs for samples with age greater than the average age of the dataset and samples with age less than the dataset average age separately, and common DEGs were considered age-dependent DEGs. A similar approach was applied to identify and remove gender-dependent DEGs (DEGs between normal males and females calculated and considered as gender-specific DEGs). After removing age- and gender-dependent DEGs, disorder-related DEGs, which were calculated separately for each of the datasets, were integrated to form a set of obtained DEGs for each of the AD and MDD disorders. The number of final DEGs between AD cases and controls was 103, whereas 87 MDD-related DEGs were identified.

Construction of gene co-expression networks and module identification Using the WGCNA (weighted gene co-expression network analysis) package (Langfelder and Horvath, 2008), we created signed networks for male and female samples of GSE33000 and GSE92538 for AD and MDD, respectively. Pearson's correlation for pairwise genes was calculated to construct an adjacency matrix. This was then replaced with a weighted adjacency matrix by raising the correlations to the power β, which was chosen to emphasize strong correlations between genes and penalize weak correlations. Next, the weighted adjacency matrix was transformed into a topological overlap matrix (TOM), which could measure the network connectivity of a gene with all other genes. Network features are clustered into co-expressed modules that have strong interconnectivity patterns. TOM empowers us to create more robust co-expression relationships by identifying modules.

Differentially co-expressed gene analysis We measured the change for each node's neighborhood in disease and control co-expression networks to identify highly differentially co-expressed genes. This value is considered a differential co-expression measure for each gene. Highly differentially co-expressed genes may have a great impact on disease generation and progression (Chen et al., 2008).

PPI network propagation gene ranking The RandomWalkRestartMH package (Valdeolivas et al., 2019) implemented in R was used to establish network propagation from a given seed (protein) using the random walk algorithm. We obtained PPI networks for AD- and MDD-related co-expression networks from the String database (von Mering et al., 2003). For each gene in the disorder-related co-expression network, corresponding transcripts and all their directly linked transcripts with a combined score of more than 700 were obtained from the String database and assumed as a disorder-related PPI network. The random walk algorithm was then used on AD and MDD PPI networks with each disease gene's transcripts as algorithm seeds, to rank transcripts (genes) based on their interaction with genes proven to cause a genetic disorder. Disease-associated genes (seeds) for each of AD and MDD were downloaded from the eDGAR database (Babbi et al., 2017). Eventually, PPI network propagation top-ranked genes were identified and assumed to be disorder-related genes.

Gene set enrichment analysis and function annotation To determine the functions of the genes in the modules, genes were submitted to Enrichr web servers (Raudvere et al., 2019) for annotation. Adjusted P-value < 0.05 was the threshold used to define significant terms.

Identification of DMRs The seqlm package (Kolde et al., 2016) was applied with a maximum 500-bp distance between each segmentation to identify DMRs. Regions with a false discovery rate (FDR) < 0.05, methylated regions with more than three probes, and absolute methylation value ($\Delta\beta$) ≥ 0.01 were identified as candidate DMRs.

DMPs in MDD To identify the differentially methylated sites in MDD, we utilized previously reported DMPs in the DLPFC in MDD individuals (Huels et al., 2020), because the methylation data of MDD in DLPFC samples were not publicly available. Huels and colleagues studied methylation profiles in DLPFC samples of late-life MDD in 608 participants from The Religious Orders Study and Rush Memory and Aging Project (ROS/MAP) using Illumina Infinium Human Methylation 450K.

CONCLUSION

In this study, we analyzed differentially expressed and co-expressed genes, PPI network propagation and DNA methylation alteration in datasets of DLPFC samples of postmortem human brain tissue data to find important distinct and shared disease-causing genes in and between AD and MDD, two of the most globally burdensome neuropsychiatric disorders. Moreover, by identifying co-expression modules and employing pathway enrichment analysis, we suggest some molecular biological mechanisms that play key roles in the development and progression of either AD or MDD, or of both disorders. Several common DEGs were also proposed as potential biomarkers of AD and MDD comorbidity.

DECLARATIONS

Availability of data and materials: The datasets analyzed during the current study are freely available from GEO under the accession numbers GSE54567, GSE54568, GSE92538, GSE102556, GSE84422, GSE33000, GSE44770 and GSE125895.
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