Dysregulated Spliceosome Gene Expression May Be a Common Process in Brains of Neurological and Psychiatric Disorders

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Abstract

Alternative splicing (AS) contributes to the increased cellular and functional tissue complexity that is substantial in the brain. AS is tightly regulated because it is critical to many biological processes. Defective splicing is observed in several neurological and psychiatric disorders. While exonic mutations usually affect the splicing of an individual RNA, mutations in the splicing factors (components of spliceosome) frequently produce widespread disruption in the processing of many precursor-mRNAs. Thus, we tested the hypotheses that expression changes of spliceosome genes may be a common process and shared splicing pathways may be involved in complex polygenic brain disorders. We searched for expression changes of spliceosome-related genes (SGs) using a transcriptome database of several brain regions in 6 neurological and psychiatric disorders, namely Alzheimer's disease, and autism spectrum, bipolar and major depressive disorder, Parkinson's disease, and schizophrenia. Out of 255 SGs detected in brain, 138 showed excessive, significant changes in one or more of these disorders. Dysregulation of 10 SGs was shared in 4 disorders, and they were mostly downregulated. Six associated pathways were over-represented in all 6 disorders, including the major and the minor mRNA splicing pathways and RNA metabolism. Therefore, we found that aberrations in the mRNA splicing process may be a common trajectory to many complex brain disorders involving the spliceosome complex.

Introduction

Precursor (pre)-mRNA alternative splicing (AS) is a complex posttranscriptional mechanism that produces multiple functional alternative transcripts from identical pre-mRNA by combining different choices of exons. We have just recently started to understand the magnitude of alternative isoform expression in higher eukaryotes [1, 2]. Currently, it is estimated that more than 97% of human genes have multiple exons [3], while 95-100% of multiexon human genes express several splicing isoforms [4]. In addition, about 86% of human genes have substantial levels of more than one discrete mRNA isoform population [5]. AS is a key biological process that increases cellular and functional complexity in human tissues, particularly the brain [6], which is remarkably diverse. Brain region-specific expression patterns of splicing factors and other RNA-binding proteins have been described [7-9]. The functional role of alternative transcripts has been reported to vary from little evidence for protein isoforms due to AS [10-12] to extensive production of protein isoforms in mammals using quantitative proteomics [13, 14] or ribosome-associated transcripts [15].

To date, publications have reported differentially expressed transcripts or changed splicing in postmortem brains of multiple brain diseases, including major depression (MDD)[16], schizophrenia (SCZ)[17, 18], bipolar disorder (BD)[18, 19], autism (ASD)[18], and Alzheimer's disease (AD)[20-22]. Such splicing changes cannot be observed at the gene level, as exemplified by *TREM2* in Alzheimer's disease cases that show altered usage of an isoform lacking the 5' exon despite showing no overall expression differences at the gene level[22].

The spliceosome, a macromolecular ribonucleoprotein complex, performs the splicing reaction by removing introns from the pre-mRNAs and joining exons to produce mature mRNAs. Two types of spliceosomes have been described: the major spliceosome removes 99.5% of introns, and the remaining 5% is processed by the minor spliceosome [23]. More than 200 spliceosome-related proteins are involved in AS activity and regulation in humans [24]. Changes in the composition and regulation of the spliceosome support a diverse range of alternative splicing.

Under physiological conditions, AS is strictly regulated because it is critical for the precise function of many biological pathways. One specific transcript is expressed at a higher level (85% of the total mRNA from a given protein-coding gene). In most conditions, this dominant transcript is often the primary transcript in many tissues [11]. AS enables the cells to respond and adapt to distinct stimuli fine-tuning their protein composition in the central nervous system. Aberrant splicing contributes to brain aging [24] and many pathogenic conditions, including cancer, neurological, autoimmune, and psychiatric [25]. It has been predicted that one-third of diseases are caused by genetic variants that modulate AS [26, 27].

Recently, AS dysregulation was reported in AD, where several mis-splicing events in the brain have been associated with amyloid burden and neurofibrillary [21], ASD, SCZ [28-31], and Huntington's disease [32, 33], furthermore, emerging splicing therapeutics are promising therapeutic approaches in aberrant/deregulated AS [34-38], and clinical trials are currently underway for spinal muscular atrophy (<https://clinicaltrials.gov/ct2/show/NCT04240314>).

We hypothesize that the excessive splicing changes observed in brain conditions may be related to changes in their regulators, spliceosome-related genes (SGs). Therefore, this study aims at identifying SG expression changes in multiple brain disorders using postmortem brain transcriptome data.

Methods

Brain transcriptome database and differential expression analyses

We developed a database to systematically explore the consistency and reproducibility of gene expression changes and gene-gene interaction networks in six neurological and psychiatric disorders, including AD, ASD, BD, MDD, Parkinson's disease (PD), and SCZ. It included 48 human brain datasets from five sources: GEO, ArrayExpress, Stanley, PsychENCODE, and ROSMAP. The original brain donors comprised individuals with AD (N=151), ASD (N=187), BD (N=345), MDD (N=295), PD (N=150), SCZ (N=454), as well as controls unaffected by such disorders (N=2,238), totaling 3,820 samples. The sample size for each disorder and control comparison in each brain region is listed in Table 1.

The samples from microarrays were used as discovery data, and those from RNA sequencing were used as the replication data. Raw data included multiple adult brain regions of both sexes. The detailed analyses were described in the reference [39].

Each dataset was first preprocessed according to a consistent pipeline, including quality control, quantile normalization, gene annotation, and covariate correction. Then samples in each dataset were separated into different brain regions. Data of each brain region of different datasets were combined for mega-analysis with each dataset as a batch and corrected by the ComBat program [40]. Next, differentially expressed gene (DEG) mega-analyses were

conducted in brain region for each disorder. A linear regression model in the limma package [41] was chosen to detect the case-control differences. Benjamini & Hochberg's (BH) method [42] was used for multiple testing corrections. The threshold for significance is $\text{adj_P_Val} < 0.05$. Finally, we created a database BrainEXP-NPD (<http://brainexpnpd.org:8088/BrainEXPNPD/index.html>) to disseminate the results.

Catalog of spliceosome-related genes (SGs)

We used a total of 255 genes for analysis of SGs, as previously described [43]. This list combined 158 genes from the major and minor spliceosome family from the HUGO Gene Nomenclature Committee (HGNC) database (<https://www.genenames.org/>) and 109 core spliceosome component genes [44]. Additional splicing factors regulated by cellular senescence (*SF2*, *SRSF3*, *SRSF1*, *HNRNPA1*, and *HNRNPA2*) [45-49] obtained from the transcriptome annotation file were also included (Online Resource 1). Duplicated genes from multiple sources were removed.

Differentially expressed spliceosome genes (dSGs)

We queried the 255 spliceosome genes against the BrainEXP-NPD DEG database and identified the significantly differentially expressed spliceosome genes (dSGs) (BH adjusted $P < 0.05$) in each disorder in each brain region for downstream functional annotation and network analyses. Given that the sample size of microarray data is much larger than the RNA-seq data, the downstream analyses used only results from the microarray data.

Reactome analysis

We used the free, open-source, manually curated, and peer-reviewed pathway database Reactome v77 (<https://reactome.org/>) [50] to analyze the dSGs, including interactors, to identify significantly over-represented pathways (False discovery rate, $\text{FDR} < 0.05$).

Functional annotation and network analysis of the dSGs

STRING v11.5 (<https://string-db.org/>) [51] was used to perform Gene Ontology (GO) functional annotation and detect protein-protein interaction (PPI) networks among the significant dSGs.

GWAS signals and expression quantitative trait loci (eQTL) related to dSGs

The significant dSGs were also searched against genome-wide association studies (GWAS) of about 25,025 genes from the 6 disorders' latest public GWAS summary statistics [52-57]. A hypergeometric distribution test was used to evaluate the significance. We further analyzed whether the dSGs have significant brain eQTL SNPs (single nucleotide polymorphisms) that can relate to the GWAS SNPs expression regulation of the dSGs, based on the PsychENCODE eQTL results [58-60]

Co-expression networks related to dSGs.

We used the co-expression results from PsychENCODE [58] to reveal the genes co-expressed with dSGs and their association with psychiatric disorders. A robust version of weighted gene correlation network analysis (rWGCNA) was conducted on 2160 brain samples, including 1232 control, 593 SCZ, 253 BP, and 82 ASD samples. Network analysis was performed 100 times by resampling 2/3 samples to ensure the robustness of the module. Consensus network analysis was used to define final modules [58]. In total, 34 co-expression modules were identified. Disease association test was performed on module eigengene (the first principal component of the module) and disease trait. LD score regression (s-LDSR) was used to investigate the enrichment of GWAS signal in the co-expression module. Finally, cell type enrichment was performed with cell type-specific marker genes using the Fisher's exact test.

Transcriptome-wide association analysis (TWAS) analysis for the dSGs.

We performed TWAS using S-PrediXcan[61] based on the PsychENCODE eQTL results (psychencode.db) [58]. The input data were from the 6 disorders' latest public GWAS summary statistics [52-57].

Results

1. Differentially expressed spliceosome genes (dSGs) across neurological and psychiatric diseases

A total of 138 dSGs were identified as the union of significantly differentially expressed spliceosome genes from all the brain regions of all the six diseases (Online Resource 2). The dSGs have a significantly excessive presence ($P = 8.52\text{E-}23$) in all the 6 diseases' DEGs. Besides, we found 10 dSGs (*FAM50A*, *HNRNPAB*, *LSM5*, *LSM7*, *PPWD1*, *SF3A1*, *SF3B5*, *SNRPB*, *SNRPD1*, and *YBX1*) (Fig. 1, Table 2) shared by four disorders among all brain regions, based on the query results (Online Resource 3). The detailed summary statistics of the 10 overlapped genes are shown in Table 2. No dSG was shared by five or six disorders.

The number of significant dSGs varied in different brain regions for each disorder. Some regions did not show any significant dSGs. The top 2 brain regions affected the most by aberrant splicing were: hippocampus ($n=17$) and neocortex ($n=9$) in AD; cerebellum ($n=52$) and temporal cortex ($n=13$) in ASD; frontal cortex ($n=47$) and cerebellum ($n=1$) in BD; frontal cortex ($n=20$) and anterior cingulate cortex ($n=3$) in MDD; substantia nigra ($n=25$) and striatum ($n=24$) in PD; temporal cortex ($n=32$) and frontal cortex ($n=22$) in SCZ (Online Resource 3). The frontal cortex is the most affected region across diseases (AD, BD, SCZ, and MDD). This uneven distribution of dSGs across brain regions may provide helpful insights into which brain regions are most disrupted by AS and spared in each disease, which could be further studied in each brain region.

Out of the 138 dSGs, 116 were also dSGs in the RNA-seq replication datasets, with only 5 being in opposite directions in microarray and RNA-seq results (Online Resource 4), which showed robustness of our results.

2. Functional annotation of dSGs

Functional annotation was performed for the dSGs of each disorder in each brain region in Online Resource 3. The results showed 18 significantly enriched (FDR < 0.05) GO terms including spliceosomal complex, ribonucleoprotein complex, RNA binding and regulation of RNA splicing shared by 6 disorders (Fig. 2, Table 3). In addition, all the 8 genes (*HNRNPAB*, *LSM5*, *LSM7*, *SF3A1*, *SF3B5*, *SNRNPB*, *SNRPD1*, and *YBX1*) (Online Resource 5) were involved the 18 GO terms shared by the 6 disorders were part of the 10 dSGs shared by the 4 disorders.

3. Reactome analyses of the dSGs

Reactome analyses revealed unique and common over-represented pathways to more than 2 disorders (Fig. 3, Table 4). The following 6 pathways were over-represented in all 6 disorders: mRNA splicing, major and minor pathway of mRNA splicing, processing of capped intron-containing pre-mRNA, metabolism of RNA, and SLBP (stem-loop binding protein) independent processing of histone pre-mRNAs. Additionally, the two genes (*SNRNPB* and *YBX1*) (Online Resource 5) shared by the 6 disorders in the 6 pathways were also part of the 10 dSGs.

(place for Fig. 3 Six-way Venn diagram and UpSet plot showing the numbers of Reactome pathways significantly over-represented in 6 disorders (FDR<0.05) using a list of dSGs and interactors. a. Venn diagram shows the numbers of shared Reactome pathways. b. UpSet plot shows the numbers of Reactome pathways shared across disorders.)

4. Gene networks of the dSGs

PPI network analyses were performed in the 6 disorders using the significant dSGs in each disorder and in different brain regions. The results revealed the unique and shared over-represented protein complexes in multiple disorders (Fig. 4, Table 5). Two protein complexes were over-represented in all the 6 disorders: U2-type spliceosomal complex, and mRNA cis splicing, via spliceosome; U2-type precatlytic spliceosome.

The 3 overlapped matching genes (*LSM7*, *SF3A1*, *SF3B5*) (Online Resource 5) shared by at least 4 disorders in the 2 PPI terms shared by 6 disorders were in the 10 significant dSGs.

5. Excessive GWAS signals around the significant dSGs.

The 138 dSGs were compared to the list of GWAS significant genes in the latest largest public GWAS summary statistics of the 6 disorders (Online Resource 6) [52-57]. Ten dSGs also had SCZ GWAS associations (Online Resource 7). No significant dSG was found in the other 5 disorders. According to the brain eQTL data from PsychENCODE [58], 3 of these 10 dSGs had 18 SNPs associated with their gene expressions (Table 6), which were the very same SNPs identified in the SCZ GWAS.

6. Co-expression patterns of the 3 significant dSGs with both GWAS and eQTL signals

Two of three significant dSGs were co-expressed with other genes in PsychENCODE co-expression modules [58]. *IK* was in the M11 module related to RNA processing, spliceosome, and ribonucleoprotein complex functions. M11 was enriched for marker genes of astrocytes (FDR=0.0002). *SF3B1* was in the M14 module, which was related to nuclear speck, regulation of stress-activated MAPK cascade, and Wnt-activated signaling pathway involved in forebrain neuron fate commitment. M19 was enriched for GWAS signals of SCZ (FDR=1.75e-05), BD (FDR=0.03), ASD (FDR=0.05) and Years of Education (FDR=9.70e-06).

7. TWAS analysis of the 138 significant dSGs

Nine unique genes (Table 7) were found significantly associated with brain disorders based on the PsychENCODETWAS analysis [52-57]. *IK* was significantly associated with AD, MDD, SCZ (p.adjust = 0.0208, 0.0377, 7.09E-06, respectively). *SF3B1* was significantly associated with BD, MDD, SCZ (p.adjust = 0.000305, 0.0440, 1.18E-08, respectively). *LSM1* was significantly associated with BD, SCZ (p.adjust = 0.00759, 1.98E-06, respectively). No genes were found significantly associated with ASD or PD.

Discussion

1. Significant dSGs detected in the brains of neurological and psychiatric diseases

This study analyzed transcriptome data sets from several brain regions in 6 different neurological and psychiatric disorders, namely AD, ASD, BP, MDD, PD, and SCZ, and identified significant dSGs in all these conditions. No single gene with significant dSGs was found in all 6 conditions; however, SGs were enriched in the differentially expressed genes in all disorders. Moreover, 10 dSGs overlapped in 4 disorders, and 9 out of these 10 genes were downregulated in the brain regions we analyzed. Furthermore, 6 pathways were over-represented in all 6 disorders, including the major and minor mRNA splicing pathways and RNA metabolism. Therefore, we found that aberrations in the mRNA splicing process may be a common trajectory to many brain conditions, as it was dysregulated in all queried disorders.

The spliceosome, a macromolecular complex consisting of several proteins and small nuclear (sn) ribonucleoproteins (RNPs), distinguishes specific sequences in the intron-exon borders to promote splicing. Several splicing activator and repressor proteins attached to enhancers and silencers regulate the

spliceosome activity, affecting AS of different pre-mRNAs that share common regulatory elements, resulting in AS patterns [62-64]. Pre-mRNA splicing is performed by 2 types of spliceosomes, the major, U2-dependent, and the minor, U12-dependent, that identify and delete U2- and U12-type class of introns, respectively [65]. We found the U2-type (major) spliceosomal complex to be the most shared system. Based on the PPI network, this complex has been connected to dSGs in brains of all 6 diseases analyzed in this study.

Majority of the dSGs are disease specific indicating the complexity of the splicing regulation and the relationships between spliceosome and each disorder. Even though each of these SGs work in the “same” so-call spliceosome complexes, their individual expression changes lead to distinct downstream effects, including changes of splicing in sets of genes and ultimately various symptoms and disorders. The mechanistic details remain to be uncovered.

Ten overlapping dSGs in neurological and psychiatric conditions

The 10 overlapping dSGs found in 4 studied disorders are associated with pre-mRNA processes, especially pre-mRNA splicing. Seven dSGs are components of the major U2-dependent spliceosome, 2 are splicing factors (*SF3A1* and *SF3B5*), 2 are snRNA Sm-like proteins (*LSM5* and *LSM7*), 2 are snRNP (*SNRPB* and *SNRPD1*), and 1 is a DNA binding protein (*FAM50A*). The paragraphs below briefly summarize each of the 10 dSG.

FAM50A (Family with sequence similarity 50 member A; Chromosome (Chr) Xq28) is a nuclear protein that functions as a DNA-binding protein involved in mRNA processing; it has a role in the major spliceosome C-complex [66], and its allelic variants have been identified in males with the Armfield type of X-linked syndromic intellectual development disorder [66, 67].

HNRNPAB (heterogeneous nuclear ribonucleoprotein A/B; Chr 5q35.3) is associated with pre-mRNAs, and binds to one of the components of the multiprotein editosome complex that performs RNA editing [68].

LSM5 (U6 snRNA-associated Sm-like protein LSm5; Chr7p14.3) and *LSM7* (U6 snRNA-associated Sm-like protein LSM7; Chr 19p13.3) contain the Sm sequence motif. These proteins are important for pre-mRNA splicing as a component of the U4/U5-U6 tri-snRNP complex in the major spliceosome assembly and as part of the pre-catalytic spliceosome B complex [69].

PPWD1 (Peptidylprolyl isomerase domain and WD repeat-containing 1; Chr 5q12.3) belongs to the cyclophilin family of peptidyl-prolyl isomerases, it catalyzes the conversion cis-trans isomerization of proline[70] and may be involved in pre-mRNA splicing [71].

SF3A1 (Splicing factor 3a subunit 1; Chr 22q12.2), a component of the mature U2 snRNP, plays a critical role in the spliceosome assembly and pre-mRNA splicing as a pre-catalytic spliceosome ‘B’ complex [72-74].

SF3B5 (Splicing factor 3B subunit 5; Chr 6q24.2), a component of the SF3B complex, is a major spliceosome subunit required for “A” complex assembly shaped by the binding of U2 snRNP to the branchpoint sequence in pre-mRNA [75].

SNRPB (Small nuclear ribonucleoprotein polypeptides B and B1; Chr 20p13) and *SNRPD1* (Small nuclear ribonucleoprotein polypeptide D1; Chr 18q11.2) encode nuclear proteins found in U1, U2, U4/U6, and U5 snRNPs, the five snRNAs in the core of the major spliceosome. *SNRPB* allelic variants have been described in the cerebrocostomandibular syndrome [76-78].

YBX1 (Y-box binding protein 1; Chr 1p34.2) functions as a DNA and RNA binding protein and has been implicated in many cellular processes, including pre-mRNA splicing and RNA dependent processes [79].

It is estimated that at least 20% of disease-causing mutations affect pre-mRNA splicing [80]. Spliceosomopathies are human diseases caused by mutations in the components of the major and minor spliceosomes, such as retinitis pigmentosa, myelodysplastic syndromes, spinal muscular atrophy, and craniofacial malformations [81-83]. Mutations in RNA-binding proteins involved in splicing regulation and disruptions in RNA metabolism, including mRNA splicing, have been associated with diseases, such as ASD [29], age-related disorders (frontotemporal lobar dementia [84], PD [85], and AD [21, 86, 87]). In AD, it has been suggested that the core splicing machinery may be altered due to the increased aggregation of insoluble U1 snRNP [88]. Raj *et al.* [21] found ribosomal binding protein (RBP) sites enriched among splicing quantitative trait loci (sQTL). The binding targets for 18 RBPs were among the lead sQTL. Furthermore, sQTL SNPs were significantly enriched for several hnRNP, and they were correlated with the intronic excision level of hundreds of genes, including several AD susceptibility loci. Therefore, indicating that altering the sequence-specific binding affinity of splicing factors can change the probability of a splicing event in vivo.

2. Cross-disease comparisons highlighted genes that contribute to all six brain diseases.

Five overlapped genes (*SNRPB*, *YBX1*, *LSM7*, *SF3B5*, and *SF3A1*) either shared by six brain disorders in the 6 pathways or shared by at least four disorders in the 2 PPI terms shared by six disorders were all in the overlapped genes shared by the six disorders in the 18 GO terms and the 10 significant dSGs. These genes may hold the key connecting all the seemingly unrelated hundreds of risk genes and their changed splicing patterns in patient brains. Their regulation targets and biological processes should be the foci of future functional studies.

3. Genetic regulators of spliceosome genes contribute to brain disease risk

Out of the 255 SGs tested, 10 genes were significant dSGs and GWAS genes of one of the brain diseases. Three genes have both significant GWAS and eQTL signals. There are 18 overlapped SNPs (Table 11) between the GWAS signals from the 10 dSGs and eQTL signals from the 3 dSGs. The 3 genes were significantly differentially expressed in ASD, BD, and MDD comparing to healthy controls.

Among the 3 genes, *SF3B1* was a significantly down-regulated dSG in ASD and MDD (FDR = 0.031, 0.043, respectively) and with a nominally significant down-regulation in cerebellum, parietal cortex and striatum of SCZ (P = 0.033, 0.016, 0.045, respectively). It has GWAS signals related to SCZ and brain eQTL signals. *SF3B1* encodes subunit 1 of the splicing factor 3b protein complex and is mainly related to the mRNA splicing pathway [89]. The *SF3B1* related SNP rs788021 is a very strong risk SNP for cognitive ability, years of educational attainment (both at P_Value = 1.00E-09P)[90], and SCZ (pleiotropy) (P_Value = 5.92E-14)[57]. Our results indicate a potential mechanism that a SNP may disturb expression of spliceosome gene *SF3B1* and lead to downstream changes of splicing of its target genes, and increased risks of psychiatric disorder(s).

4. Current limitation and future experiments

Our DEG analyses on the spliceosome were performed using available microarray and RNA-seq data. The brain sample size is still relatively small. It is possible that more dSGs will be detected and be shared across disorders when sample size increase. Future studies should focus on functional experiments to validate the relationships between altered expression of spliceosome-related genes and changes of splicing patterns in brains.

Conclusion

In summary, AS regulation in the human brain is distinct and highly complex [91, 92], and it may have central roles in brain development and physiological function. We detected the excessive changes of SG expression with both disease-specific and disease-shared patterns in brains of six neurological and psychiatric disorders. Our data support the notion that dysregulated AS processing, especially involving the major spliceosome, may have a dominant role in these disorders.

Declarations

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Conflict of interest/Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

Data availability

The dataset accession ID and gene differential expression analysis results are available from the BrainEXP-NPD database at <http://brainexpnpd.org:8088/BrainEXPNPD/index.html>.

Code availability

Not applicable.

Authors' contributions

Chunyu Liu and Ma-li Wong conceived, designed, and supervised the study. Material preparation, data collection and analysis were performed by Cuihua Xia and Ma-li Wong. The manuscript was prepared by Chunyu Liu, Ma-li Wong and Cuihua Xia and all authors contributed to the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Brain Transcriptome Data (including sample sizes, disorders, and brain regions)

Disorder	Brain region	# of Samples in Microarray		# of Samples in RNAseq	
		Cases	Controls	Cases	Controls
AD	Entorhinal cortex	14	37		
	Frontal cortex	19	22	9	7
	Hippocampus	50	82		
	Neocortex	7	8		
	Post central gyrus	24	41		
	Superior frontal gyrus	21	46		
	Temporal cortex	7	18	12	17
ASD	Cerebellum	21	22	32	44
	Frontal cortex	16	15	55	81
	Occipital cortex	4	6		
	Temporal cortex	12	13	47	50
BD	Striatum	16	16	18	17
	Cerebellum	34	49		
	Frontal cortex	137	159	68	240
	Hippocampus	18	18	15	29
	Parietal cortex	39	45		
MDD	Anterior cingulate cortex	37	36		
	Cerebellum	13	49		
	Frontal cortex	72	94	80	71
	Hippocampus	17	18	24	18
	Parietal cortex	12	45		
	Striatum	15	16	25	21
PD	Cerebellum	16	15		
	Frontal cortex	11	15	27	42
	Medulla	14	14		
	Striatum	30	32	3	3
	Substantia nigra	41	25	4	5
	Superior frontal gyrus	4	3		
SCZ	Cerebellum	54	63		
	Frontal cortex	193	207	91	239
	Hippocampus	15	18	16	29
	Parietal cortex	51	45		
	Striatum	13	16		
	Temporal cortex	21	17		

Table 2. Summary statistics of the ten dSGs shared by 4 disorders in specific brain regions.

Gene	Disorder	Brain region	logFC (case/control)	P_Value	adj_P_Val
FAM50A	ASD	Cerebellum	-0.0823	0.0103	0.0476
	BD	Frontal cortex	-0.0763	0.000000611	0.0000382
	MDD	Frontal cortex	-0.0490	0.0020542	0.0241
	PD	Substantia nigra	-0.0770	0.0000537	0.00119
HNRNPAB	AD	Hippocampus	0.0732	0.00217	0.0269
	ASD	Cerebellum	-0.106	0.00393	0.0228
	BD	Frontal cortex	-0.0504	0.0000783	0.00137
	MDD	Frontal cortex	-0.0473	0.000213	0.00560
LSM5	ASD	Cerebellum	-0.0951	0.00758	0.0378
	BD	Frontal cortex	-0.0469	0.000902	0.00843
	MDD	Frontal cortex	-0.045	0.00261	0.0283
	PD	Cerebellum	-0.233	0.000103	0.00935
	PD	Striatum	-0.140	0.000275	0.00709
LSM7	ASD	Cerebellum	-0.201	0.0000606	0.000960
	BD	Frontal cortex	-0.0553	0.00325	0.0210
	PD	Substantia nigra	-0.105	0.00155	0.0129
	SCZ	Frontal cortex	0.0368	0.00321	0.0261
	SCZ	Hippocampus	0.140	0.000403	0.0262
PPWD1	ASD	Cerebellum	-0.0710	0.00493	0.0273
	MDD	Anterior cingulate cortex	-0.0611	0.000615	0.0263
	PD	Substantia nigra	-0.0857	0.00503	0.0295
	SCZ	Frontal cortex	0.0561	0.0000165	0.000565
SF3A1	AD	Hippocampus	-0.0540	0.00346	0.0367
	ASD	Frontal cortex	-0.150	0.000264	0.0194
	BD	Frontal cortex	0.0540	0.000000157	0.0000134
	SCZ	Frontal cortex	0.0278	0.00231	0.0208
SF3B5	AD	Neocortex	-0.302	0.000558	0.0143
	AD	Hippocampus	-0.0676	0.00275	0.0317
	ASD	Cerebellum	-0.196	0.000135	0.00174
	BD	Frontal cortex	-0.0395	0.000282	0.00355
	MDD	Frontal cortex	-0.0406	0.00177	0.0219
SNRPB	AD	Frontal cortex	0.0779	0.00105	0.0458
	ASD	Temporal cortex	-0.194	0.0000370	0.00708
	ASD	Cerebellum	-0.321	0.000000675	0.0000434
	BD	Frontal cortex	0.0510	0.0000361	0.000767
	PD	Substantia nigra	0.124	0.0000904	0.00171
	PD	Striatum	-0.129	0.00232	0.0262
	PD	Cerebellum	-0.216	0.00264	0.0499
SNRPD1	AD	Entorhinal cortex	-0.163	0.00000283	0.0320
	ASD	Cerebellum	-0.177	0.00000152	0.0000716
	MDD	Frontal cortex	-0.0505	0.00169	0.0211
	PD	Striatum	-0.149	0.000000630	0.000153

YBX1	AD	Neocortex	0.957	0.00450	0.0457
	ASD	Frontal cortex	0.139	0.000972	0.0371
	BD	Frontal cortex	-0.0359	0.00193	0.0147
	PD	Substantia nigra	0.144	0.00000791	0.000320

Table 3. Significantly enriched (FDR < 0.05) GO terms (18) shared by all 6 disorders (AD, ASD, BD, MDD, PD, and SCZ)

Term ID	Term description
GO:0005681	Spliceosomal complex
GO:1990904	Ribonucleoprotein complex
GO:0005654	Nucleoplasm
GO:0005684	U2-type spliceosomal complex
GO:0071013	Catalytic step 2 spliceosome
GO:0071005	U2-type precatalytic spliceosome
GO:0097525	Spliceosomal snrnp complex
GO:0005689	U12-type spliceosomal complex
GO:0046540	U4/U6 x U5 tri-snRNP complex
GO:0032991	Protein-containing complex
GO:0016607	Nuclear speck
GO:0005686	U2 snRNP
GO:0071007	U2-type catalytic step 2 spliceosome
GO:0003723	RNA binding
GO:0000398	mRNA splicing, via spliceosome
GO:0043484	Regulation of rna splicing
GO:0022618	Ribonucleoprotein complex assembly
GO:0000245	Spliceosomal complex assembly

Table 4. Summary of dSG-related pathways enriched in each disorder identified by the Reactome analyses

Pathway identifier	Pathway name	Disorders
R-HSA-190236	Signaling by FGFR	AD
R-HSA-381033	ATF6 (ATF6-alpha) activates chaperones	AD
R-HSA-381183	ATF6 (ATF6-alpha) activates chaperone genes	AD
R-HSA-5654738	Signaling by FGFR2	AD
R-HSA-8866906	TFAP2 (AP-2) family regulates transcription of other transcription factors	AD
R-HSA-6782210	Gap-filling DNA repair synthesis and ligation in TC-NER	ASD
R-HSA-159227	Transport of the SLBP independent Mature mRNA	MDD
R-HSA-159231	Transport of Mature mRNA Derived from an Intronless Transcript	MDD
R-HSA-8950505	Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	PD
R-HSA-9022692	Regulation of MECP2 expression and activity	PD
R-HSA-2173793	Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	SCZ
R-HSA-2173796	SMAD2/SMAD3:SMAD4 heterotrimer regulates transcription	SCZ
R-HSA-350054	Notch-HLH transcription pathway	SCZ
R-HSA-450408	AUF1 (hnRNP D0) binds and destabilizes mRNA	SCZ
R-HSA-450531	Regulation of mRNA stability by proteins that bind AU-rich elements	SCZ
R-HSA-8864260	Transcriptional regulation by the AP-2 (TFAP2) family of transcription factors	SCZ
R-HSA-8866910	TFAP2 (AP-2) family regulates transcription of growth factors and their receptors	SCZ
R-HSA-6803529	FGFR2 alternative splicing	AD, SCZ
R-HSA-8986944	Transcriptional Regulation by MECP2	PD, SCZ
R-HSA-159236	Transport of Mature mRNA derived from an Intron-Containing Transcript	AD, PD, SCZ
R-HSA-72202	Transport of Mature Transcript to Cytoplasm	AD, PD, SCZ
R-HSA-73856	RNA Polymerase II Transcription Termination	BP, MDD, PD, SCZ
R-HSA-77588	SLBP Dependent Processing of Replication-Dependent Histone Pre-mRNAs	BP, MDD, PD, SCZ
R-HSA-72187	mRNA 3'-end processing	AD, ASD, BP, MDD, SCZ
R-HSA-191859	snRNP Assembly	AD, ASD, MDD, PD, SCZ
R-HSA-194441	Metabolism of non-coding RNA	AD, ASD, MDD, PD, SCZ
R-HSA-429914	Deadenylation-dependent mRNA decay	ASD, BP, MDD, PD, SCZ
R-HSA-430039	mRNA decay by 5' to 3' exoribonuclease	ASD, BP, MDD, PD, SCZ
R-HSA-75067	Processing of Capped Intronless Pre-mRNA	ASD, BP, MDD, PD, SCZ
R-HSA-111367	SLBP independent Processing of Histone Pre-mRNAs	AD, ASD, BP, MDD, PD, SCZ
R-HSA-72163	mRNA Splicing - Major Pathway	AD, ASD, BP, MDD, PD, SCZ
R-HSA-72165	mRNA Splicing - Minor Pathway	AD, ASD, BP, MDD, PD, SCZ
R-HSA-72172	mRNA Splicing	AD, ASD, BP, MDD, PD, SCZ
R-HSA-72203	Processing of Capped Intron-Containing Pre-mRNA	AD, ASD, BP, MDD, PD, SCZ
R-HSA-8953854	Metabolism of RNA	AD, ASD, BP, MDD, PD, SCZ

Table 5. Summary of PPI network analyses of the dSGs.

Term ID	Term description	Disorders
CL:1446	mRNA processing, and primary miRNA binding	SCZ
CL:1448	Mixed, incl. mrna processing, and primary mirna binding	SCZ
CL:1519	Mixed, incl. negative regulation of mrna splicing, via spliceosome, and u2af complex	SCZ
CL:1451	Mixed, incl. mphf zinc finger, and negative regulation of mrna splicing, via spliceosome	SCZ
CL:1789	U1 snRNP	SCZ
CL:1441	mRNA 3-end processing, and RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	BD
CL:1818	U2-type catalytic step 2 spliceosome, and post-spliceosomal complex	ASD
CL:1820	U2-type catalytic step 2 spliceosome	ASD
CL:1822	U2-type catalytic step 2 spliceosome	ASD
CL:1698	U6 snRNP	ASD
CL:1824	Mixed, incl. proct domain, and cwf11 family	ASD
CL:1745	U2 snRNP, and SF3B6, RNA recognition motif	ASD
CL:1693	U4/U6 x U5 tri-snRNP complex	PD, BD
CL:1443	mRNA processing, and K Homology domain, type 1	SCZ, PD, BD
CL:1744	U2 snRNP, and U2-type precatalytic spliceosome	SCZ, MDD, ASD
CL:1696	U6 snRNP, and U4 snRNP	PD, BD, ASD
CL:1694	U4/U6 x U5 tri-snRNP complex	SCZ, PD, BD, ASD
CL:1684	U2-type spliceosomal complex, and U12-type spliceosomal complex	SCZ, PD, MDD, BD, ASD
CL:1690	U2-type precatalytic spliceosome, and U1 snRNP	SCZ, PD, MDD, BD, ASD
CL:1686	U2-type spliceosomal complex, and Spliceosome	SCZ, PD, BD, ASD, AD
CL:1688	U2-type spliceosomal complex, and mRNA cis splicing, via spliceosome	SCZ, PD, MDD, BD, ASD, AD
CL:1692	U2-type precatalytic spliceosome	SCZ, PD, MDD, BD, ASD, AD

Table 6. Overlapped SNPs (18) between the significant SCZ GWAS signals and significant eQTL signals

gene_id	gene_name	snp_chr	snp_pos (hg19)	snp_id (hg19)	nominal_pval in eQTL	regression_slope in eQTL	FDR in eQTL	OR in GWAS	P in GWAS
ENSG00000113141	IK	chr5	140036681	rs778595	7.86509E-16	0.10134	3.14002E-13	0.95849	3.99E-08
ENSG00000175324	LSM1	chr8	38020408	rs55736052	7.20108E-21	-0.111463	4.2296E-18	1.05169	1.72E-08
ENSG00000175324	LSM1	chr8	38021982	rs76873509	4.49567E-19	-0.107262	2.32429E-16	1.05085	3.74E-08
ENSG00000175324	LSM1	chr8	38025511	rs145151767	3.49431E-19	-0.108499	1.82005E-16	1.05159	2.85E-08
ENSG00000175324	LSM1	chr8	38025512	rs138824104	3.51438E-19	-0.108482	1.83016E-16	1.05169	2.63E-08
ENSG00000175324	LSM1	chr8	38032158	rs59911155	8.53479E-15	-0.090546	3.11472E-12	1.05012	4.02E-08
ENSG00000115524	SF3B1	chr2	198260098	rs2564389	1.4984E-11	-0.0748057	3.96889E-09	1.06279	7.15E-14
ENSG00000115524	SF3B1	chr2	198261596	rs56718086	0.00070388	-0.0342933	0.043654927	1.05327	3.93E-11
ENSG00000115524	SF3B1	chr2	198277498	rs35157131	6.59874E-12	-0.0760869	1.81792E-09	1.06269	6.76E-14
ENSG00000115524	SF3B1	chr2	198277551	rs6710530	8.362E-12	-0.0759067	2.27843E-09	1.06269	7.03E-14
ENSG00000115524	SF3B1	chr2	198278571	rs55658871	0.000803814	-0.0338436	0.048579432	1.05285	4.73E-11
ENSG00000115524	SF3B1	chr2	198278834	rs3097384	6.71266E-12	-0.0760603	1.84784E-09	1.06269	6.93E-14
ENSG00000115524	SF3B1	chr2	198280586	rs788022	6.84719E-12	-0.0760286	1.88297E-09	1.06269	6.77E-14
ENSG00000115524	SF3B1	chr2	198281743	rs699318	1.80631E-11	-0.0744033	4.74275E-09	1.06279	6.02E-14
ENSG00000115524	SF3B1	chr2	198285554	rs2565161	9.94044E-12	-0.075609	2.68581E-09	1.06237	9.40E-14
ENSG00000115524	SF3B1	chr2	198286474	rs2565160	7.38492E-12	-0.0759986	2.02396E-09	1.06269	7.31E-14
ENSG00000115524	SF3B1	chr2	198286581	rs2244271	1.93153E-11	-0.0743825	5.05566E-09	1.06237	9.39E-14
ENSG00000115524	SF3B1	chr2	198293691	rs788021	1.1881E-11	-0.0753957	3.18347E-09	1.0629	5.92E-14

Table 7. Significant signals in the TWAS analyses

disorder	ensembl_id	gene_name	zscore	effect_size	pvalue	p.adjust
AD	ENSG00000113141	IK	3.84912755	0.04285998	0.000118539	0.020789242
BD	ENSG00000100023	PPIL2	-3.2590861	-0.1704422	0.001117718	0.026320262
BD	ENSG00000115524	SF3B1	4.6738025	0.17804275	2.96E-06	0.000304807
BD	ENSG00000175324	LSM1	3.73279611	0.16532333	0.000189366	0.007594827
BD	ENSG00000185324	CDK10	3.16849391	0.20750865	0.00153231	0.032635303
MDD	ENSG00000101161	PRPF6	3.89441459	0.11326433	9.84E-05	0.008700002
MDD	ENSG00000113141	IK	-3.2844288	-0.0723026	0.001021893	0.037705023
MDD	ENSG00000115524	SF3B1	3.21955186	0.05673114	0.001283911	0.043989022
MDD	ENSG00000183011	NAA38	3.54189582	0.30829831	0.000397262	0.022138201
SCZ	ENSG00000003756	RBM5	-4.2873749	-17.328226	1.81E-05	0.000960432
SCZ	ENSG00000113141	IK	-5.4341721	-6.5956714	5.51E-08	7.09E-06
SCZ	ENSG00000115524	SF3B1	6.62076154	3.67708879	3.57E-11	1.18E-08
SCZ	ENSG00000175324	LSM1	5.69875569	4.48385814	1.21E-08	1.98E-06
SCZ	ENSG00000183258	DDX41	-4.238048	-20.095715	2.25E-05	0.001136998

Figures

Significant dSGs in the 6 neuropsychiatric disorders. a. Venn diagram and b. UpSet plot show the disease-specific and shared dSGs.

Figure 2

Six-way Venn diagram showing the number of GO terms over-represented in 6 brain disorders using a list of significantly ($FDR < 0.05$) differentially expressed SGs from microarray data of postmortem brain tissues. **a.** Venn diagram shows the numbers of shared GO terms. **b.** UpSet plot shows the numbers of GO terms shared across disorders.

Figure 3

Six-way Venn diagram and UpSet plot showing the numbers of Reactome pathways significantly over-represented in 6 disorders (FDR<0.05) using a list of dSGs and interactors. **a.** Venn diagram shows the numbers of shared Reactome pathways. **b.** UpSet plot shows the numbers of Reactome pathways shared across disorders.

Figure 4

Venn diagram shows the number of PPI terms over-represented in the 6 disorders using a list of dSGs (FDR<0.05). **a.** Venn diagram shows the numbers of shared PPI terms. **b.** UpSet plot shows the numbers of PPI terms shared across disorders.

Supplementary Files

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- [OnlineResource1.xlsx](#)
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