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

Cuihua Xia  
Central South University

Rujia Dai  
SUNY Upstate Medical University

Jing Yu  
Central South University

Chunling Zhang  
SUNY Upstate Medical University

Ma-li Wong  
SUNY Upstate Medical University

Chunyu Liu  
liuch@upstate.edu  
SUNY Upstate Medical University

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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 mammalians 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 $\text{adj}_P\text{Val} < 0.05$. 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.

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 (WGCNA) 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 signals 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 ($P = 8.52E-23$) 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 cingulated 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 (HNRNPA2B1, LSM5, LSM7, SF3A1, SF3B5, SNRPB, 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 (SNRPB and YBX1) (Online Resource 5) shared by the 6 disorders in the 6 pathways were also part of the 10 dSGs.

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 precatalytic 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). LSM7 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 (RNP)s, 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-called 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 (LMS5 and LMS7), 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].

HNRNPA/B (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].

LMS5 (U6 snRNA-associated Sm-like protein LSm5; Chr7p14.3) and LMS7 (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, LMS7, 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_Value |
|--------|----------|----------------|----------------------|-----------|-------------|
| 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      | Frontal 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               | 0.00000157 | 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               | 0.00000675 | 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               | 0.000264  | 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               | 0.00000630 | 0.00153    |
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 mRNA 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-HE 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, BR MDD, PD, 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, BR MDD, PD, SCZ |
| R-HSA-72163        | mRNA Splicing - Major Pathway                                                 | AD, ASD, BR MDD, PD, SCZ |
| R-HSA-72165        | mRNA Splicing - Minor Pathway                                                 | AD, ASD, BR MDD, PD, SCZ |
| R-HSA-72172        | mRNA Splicing                                                                 | AD, ASD, BR MDD, PD, SCZ |
| R-HSA-72203        | Processing of Capped Intron-Containing Pre-mRNA                              | AD, ASD, BR MDD, PD, SCZ |
| R-HSA-8953854      | Metabolism of RNA                                                             | AD, ASD, BR 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. mmr processing, and primary mima binding                            | SCZ                |
| CL:1519 | Mixed, incl. negative regulation of mmr splicing, via spliceosome, and u2af complex | SCZ                |
| CL:1451 | Mixed, incl. mphf zinc finger, and negative regulation of mmr 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 | regression_slope | 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  |
| 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  | -0.076069        | 1.81792E-09 | 1.06269    | 6.76E-14  |
| ENSG00000115524  | SF3B1     | chr2    | 198277551      | rs6710530     | 8.362E-12    | -0.075907        | 2.27843E-09 | 1.06269    | 7.03E-14  |
| ENSG00000115524  | SF3B1     | chr2    | 198278571      | rs55658871    | 0.000803814  | -0.0338436       | 0.048579432 | 1.05285    | 4.72E-11  |
| ENSG00000115524  | SF3B1     | chr2    | 198278834      | rs3097384     | 6.71266E-12  | -0.076063        | 1.84784E-09 | 1.06269    | 6.93E-14  |
| ENSG00000115524  | SF3B1     | chr2    | 198280586      | rs788022      | 6.84719E-12  | -0.076028        | 1.88297E-09 | 1.06269    | 6.77E-14  |
| ENSG00000115524  | SF3B1     | chr2    | 198281743      | rs699318      | 1.80631E-11  | -0.0744034       | 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**

**Figure 1**

*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

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

- OnlineResource1.xlsx
- OnlineResource2.xlsx
- OnlineResource3.xlsx
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