Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap

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Abstract

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The predisposition to neuropsychiatric disease involves a complex, polygenic, and pleiotropic genetic architecture. However, little is known about how genetic variants impart brain dysfunction or pathology. We use transcriptomic profiling as a quantitative readout of molecular brain-based phenotypes across 5 major psychiatric disorders, including autism (ASD), schizophrenia (SCZ), bipolar disorder (BD), depression (MDD), and alcoholism (AAD), compared with matched controls. We identify patterns of shared and distinct gene-expression perturbations across these conditions. Notably, the degree of sharing of transcriptional dysregulation is related to polygenic (SNP-based) overlap across disorders, suggesting a significant causal genetic component. This comprehensive systems-level view of the neurobiological architecture of major neuropsychiatric illness demonstrates pathways of molecular convergence and specificity.

Despite remarkable success identifying genetic risk factors for major psychiatric disorders, it remains unknown how genetic variants interact with environmental and epigenetic risk factors in the brain to impart risk for clinically distinct disorders (1, 2). We reasoned that brain transcriptomes, a quantitative, genome-wide molecular phenotype (3), would allow us to determine whether disease-related signatures are shared across major neuropsychiatric disorders with distinct symptoms and whether these patterns reflect genetic risk.

We first analyzed published gene-expression microarray studies of cerebral cortex across five major neuropsychiatric disorders (3–11) using 700 cerebral cortical samples from subjects with ASD (n=50 samples), SCZ (n=159), BD (n= 94), MDD (n=87), AAD (n=17), and matched controls (n=293) (12). These disorders are prevalent and disabling, contributing substantially to global disease burden. Inflammatory bowel disease (IBD, n=197) was included as a non-neural comparison.

Individual datasets underwent stringent quality control and normalization (Fig. 1; (12)), including re-balancing to alleviate confounding between diagnosis and biological (e.g., age, sex) or technical (e.g., post-mortem interval, pH, RIN, batch, $3'$ bias) covariates (Figs. S1, S2). Transcriptome summary statistics for each disorder were computed with a linear mixed-effects model to account for any sample overlap across studies (12). Comparison of differential gene expression (DGE) $\log_2$ fold change ($\log_2$FC) signatures revealed a significant overlap among ASD, SCZ, and BD and SCZ, BD, and MDD (Fig. 2A; all Spearman's ρ 0.23, $P < 0.05$, 40,000 permutations). The regression slopes between ASD, BD, and MDD $\log_2$-FC effect sizes compared to SCZ (5.08, 0.99, and 0.37) indicate a gradient of transcriptomic severity with ASD > SCZ ≈ BD > MDD (Fig. 2B). To ensure robustness, we compared multiple methods for batch correction, probe summarization, and feature selection, including use of integrative correlations, none of which changed the qualitative observations (Fig. S3; (12)). Results were also unaltered after first regressing gene-level RNA degradation metrics, suggesting that systematic sample quality issues were unlikely to drive these correlations (Fig. S3). Further, the lack of (or negative) overlap between AAD and other disorders suggests that similarities are less likely due to comorbid substance abuse, poor overall general health, or general brain-related post-mortem artefacts.

Disease-specific DGE summary statistics (Data Table S1) provide human *in vivo* benchmarks for determining the relevance of model organisms, *in vitro* systems, or drug effects (13, 14). We identified a set of concordantly down- and upregulated genes across

disorders (Fig. S4), as well as those with more specific effects. Complement component 4A (*C4A*), the top GWAS-implicated SCZ disease gene (15), was significantly upregulated in SCZ (log$_2$FC=0.23, P=6.9×10^{-6}) and in ASD (RNAseq; log$_2$FC=0.91, P=0.014; Data Table S1) but not BD, MDD, or AAD. To investigate potential confounding by psychiatric medications, we compared disease signatures with those from non-human primates treated with acute or chronic dosing of antipsychotic medications. Significant negative overlap (Fig. S5; (12)) was observed, indicating that antipsychotics are unlikely to drive, but rather may partially normalize, these transcriptomic alterations, whereas the psychotomimetic PCP partially recapitulates disease signatures.

To validate that these transcriptomic relationships are generalizable, we generated independent RNAseq datasets for replication for 3 out of the 5 disorders (Fig. S6; (12)). We identify 1099 genes whose DGE is replicated in ASD (OR 6.4, P=3.3×10^{-236}, Fisher's exact test; Table S2), 890 genes for SCZ (BrainGVEX; OR 4.5, P=7.6×10^{-155}), and 112 genes for BD (BrainGVEX; OR 3.9, P=4.6×10^{-26}), which is likely due to the relatively smaller RNAseq sample size for BD (12). We observed similarly high levels of transcriptomic overlap among ASD, SCZ, and BD, and a similar gradient of transcriptomic severity (Figs. 2C; S7). The SCZ and BD patterns were further replicated in the CommonMind dataset, although gene-level overlap was lower (12, 16) (Fig. S7). The ASD signature was largely consistent across the four major cortical lobules, indicating that this pattern is not caused by regional differences (Fig. 2D).

To more specifically characterize the biological pathways involved, we performed robust weighted gene co-expression network analysis (rWGCNA; (12, 17)), identifying several shared and disorder-specific co-expression modules (Fig. 3). Modules were stable (Fig. S8), showed greater association with disease than other biological or technical covariates (Fig. S9), and were not dependent on corrections for covariates or batch effects (Fig. S10). Moreover, each module was enriched for protein-protein interactions (Fig. S8) and brain enhancer-RNA co-regulation (Fig. S11) derived from independent data, which provides anchors for dissecting protein complexes and regulatory relationships.

An astrocyte-specific module (CD4, hubs *GJA1, SOX9*) was broadly upregulated in ASD, BD, and SCZ (FDR-corrected P's < 0.05, Fig. 3C, Data Table S2; (12)) and enriched for glial cell differentiation and fatty-acid metabolism pathways. In contrast, a module strongly enriched for microglial markers (CD11) was upregulated specifically in ASD (two-sided t-test, FDR-corrected P=4×10^{-9}). Hubs include canonical microglial markers (*HLA-DRA, AIF1*), major components of the complement system (*C1QA, C1QB*) and *TYROBP*, a microglial signalling adapter protein (18). Results fit with convergent evidence for microglial upregulation in ASD and an emerging understanding that microglia play a critical role regulating synaptic function during neurodevelopment (19).

One module was upregulated specifically in MDD (CD2, FDR-corrected P=0.009; Data Table S2) and was enriched for G-protein coupled receptors, cytokine-cytokine interactions, and hormone activity pathways, suggesting a link between inflammation and dysregulation of the hypothalamic-pituitary (HPA) axis, consistent with current models of MDD pathophysiology (20). Several modules annotated as neuronal/mitochondrial were

downregulated across ASD, SCZ, and BD (CD1, CD10, CD13; Fig. 3C, Data Table S2; (*12*)). The overlap of CD10 with a mitochondrial gene-enriched module previously associated with neuronal firing rate (21) links energetic balance, synaptic transmission, and psychiatric disease (Data Table S2).

The transcriptome may reflect the cause or the consequence of a disorder. To refine potential causal links, we compared SNP-based genetic correlations between disease pairs (22) with their corresponding transcriptome overlap. SNP co-heritability was significantly correlated with transcriptome overlap across the same disease pairs (Fig. 2C, Spearman's ρ =0.79, 95% confidence interval [0.43–0.93], P=0.0013), suggesting that a major component of these gene-expression patterns reflects biological processes coupled to underlying genetic variation.

To determine how disease-associated variants may influence specific biological processes, we investigated whether any modules harbor genetic susceptibility for specific disorders or for relevant cognitive or behavioral traits (12). We identified significant enrichment among several of the downregulated, neuronal co-expression modules (CD1, CD10, CD13) for GWAS signal from SCZ and BD, as well as for educational attainment and neuroticism (Fig. 4A; FDR-corrected P's < 0.05, Spearman; (12)). We also observe enrichment for the three downregulated neuronal co-expression modules in the iPSYCH Consortium (23) ASD GWAS cohort (Fig 4A; Table S3; (12)). In contrast, these modules showed no enrichment for MDD, AAD, or IBD. Further, none of the microglial- or astrocyte-specific modules showed psychiatric GWAS enrichment. Extending this analysis to disease-associated rare variants (Data Table S3; (2, 12)), we found that the CD1 neuronal module was enriched for genes harbouring rare, non-synonymous *de novo* mutations identified in ASD (OR 1.36, FDR-corrected P=0.03, logistic regression) and SCZ cases (OR 1.82, FDR-corrected P=0.014) but not unaffected controls (Fig. 4B). A similar CD1-enrichment was observed for genes affected by rare, recurrent copy-number variation (CNV) in ASD (OR 2.52, FDR-corrected P=0.008) and SCZ (OR 2.46, FDR-corrected P=0.014). These results suggest convergence of common and rare genetic variation acting to downregulate synaptic function in ASD and SCZ.

We next used LD score regression (24) to partition GWAS heritability (Fig. 4C; Data Table S4) into the contribution from SNPs located within genes from each module ((12); Fig. 4D). CD1 again showed significant enrichment for SCZ (2.5 fold, FDR-corrected P=8.9×10^{-11}), BD (3.9 fold, FDR-corrected P<0.014), and educational attainment (1.9 fold, FDR-corrected P<0.0008; χ^2) GWAS, accounting for ~10% of SNP-based heritability within each dataset, despite containing only 3% of the SNPs. This illustrates how gene network analysis can begin to parse complex patterns of common variants, each of small effect size, to implicate specific biological roles for common variant risk across neuropsychiatric disorders.

These data provide a quantitative, genome-wide characterization of the cortical pathology across five major neuropsychiatric disorders, providing a framework for identifying the responsible molecular signalling pathways and interpreting genetic variants implicated in neuropsychiatric disease risk. We observe a gradient of synaptic gene down-regulation, with ASD > SZ ≈ BD. BD and SCZ appear most similar in terms of synaptic dysfunction and

astroglial gene up-regulation, which may represent astrocytosis, activation, or both. ASD, an early-onset disorder, shows a distinct upregulated microglial signature, which may reflect the role for microglia in regulation of synaptic connectivity during neurodevelopment (19). MDD shows neither the synaptic nor astroglial pathology, but does exhibit dysregulation of HPA-axis and hormonal signalling not observed in the other disorders.

Our data suggest that shared genetic factors underlie a substantial proportion of cross disorder expression overlap. Given that a minority of these relationships represent eQTL (Fig. S12), most of the genetic effects are likely acting indirectly, through a cascade of developmental and cell-cell signalling events rooted in genetic risk. Genetic variation is also not the only driver of expression variation; there is undoubtedly a contribution from environmental effects. Hidden confounders could introduce a correlation structure that matches SNP-level genetic correlations, but parsimony and hidden covariate correction suggests that this is unlikely. Diagnostic misclassification could artificially elevate shared signals, but the results are robust to disorder removal (Fig. S13), and misclassification would not account for the substantial overlap we observe with ASD, which has a highly distinct phenotypic trajectory from later onset disorders. Finally, we have replicated broad transcriptomic and cell-type specific patterns independently for ASD, SCZ and BD, providing an organizing pathological framework for future investigation of the mechanisms underlying specific gene and isoform-level transcriptomic alterations in psychiatric disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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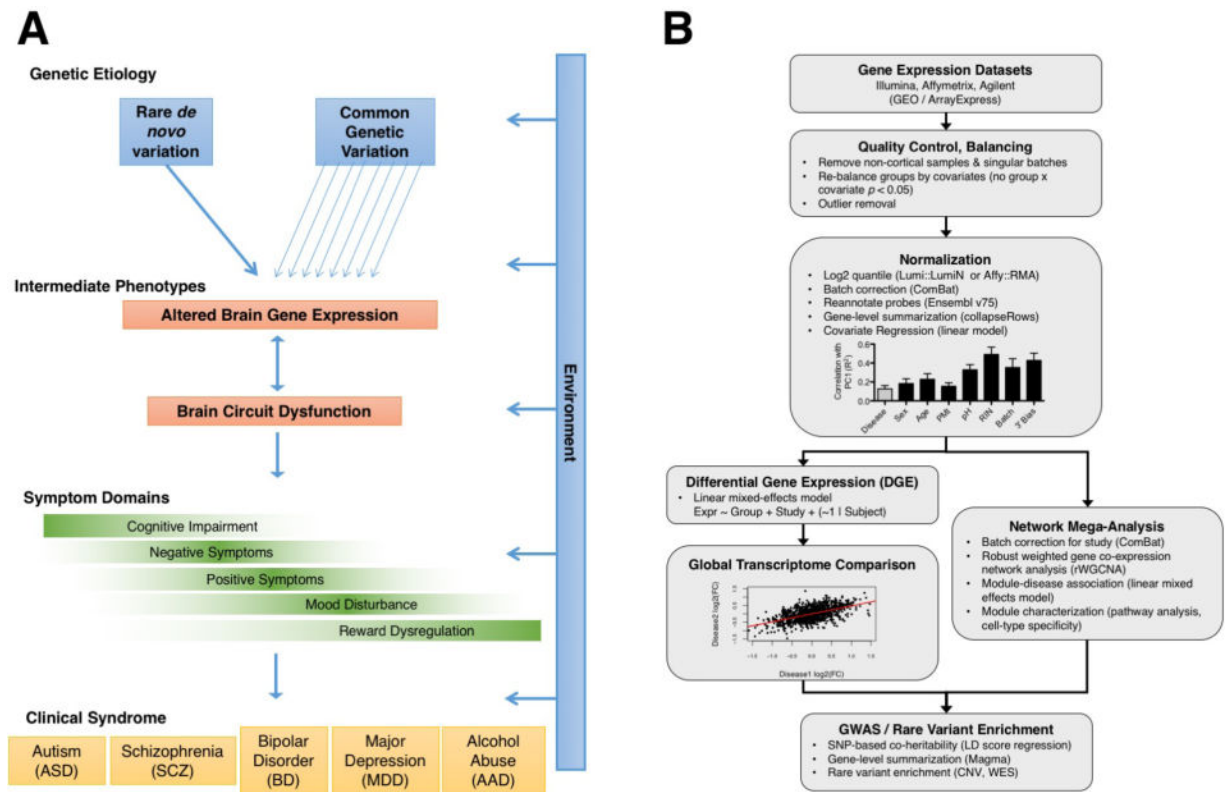
One Sentence Summary

Autism, schizophrenia, and bipolar disorder share specific global gene expression patterns, characterized by astrocyte activation and disrupted synaptic processes.
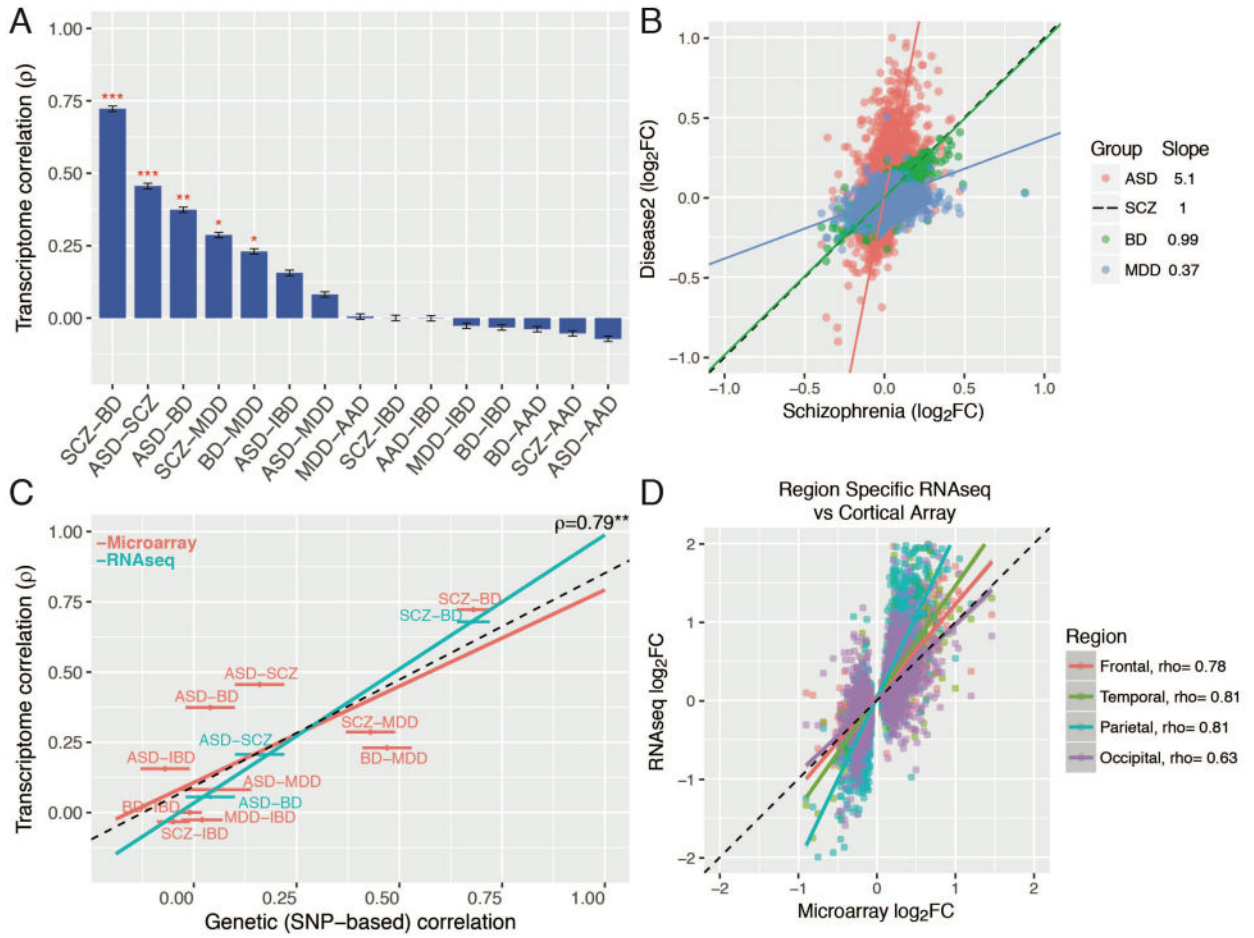
Fig. 1.

(**A**) Model of psychiatric disease pathogenesis. (**B**) Flowchart of the cross-disorder transcriptome analysis pipeline (12). Cortical gene expression datasets were compiled from cases of ASD (n=50 samples), SCZ (n=159), BD (n=94), MDD (n=87), AAD (n=17), and matched non-psychiatric controls (n=293) (12) (see Table S1).

Fig. 2.
Cortical gene expression patterns overlap. (**A**) Rank order of microarray transcriptome similarity for all disease pairs, as measured by Spearman's correlation of differential expression $\log_2$FC values. (**B**) Comparison of the slopes among significantly associated disease pairs indicates a gradient of transcriptomic severity, with ASD > SCZ ~ BD > MDD. (**C**) Overlapping gene expression patterns across diseases are correlated with shared common genetic variation, as measured by SNP co-heritability (22). The Y-axis shows transcriptome correlations using microarray-based (discovery, red) and RNAseq (replication, blue) datasets. (**D**) RNAseq across all cortical lobes in ASD replicates microarray results and demonstrates a consistent transcriptomic pattern. Spearman's ρ is shown for comparison between microarray and region-specific RNAseq replication datasets (all P's < 10^{-14}). Plots show mean +/− SEM. *P < 0.05, **P < 0.01, ***P < 0.001.
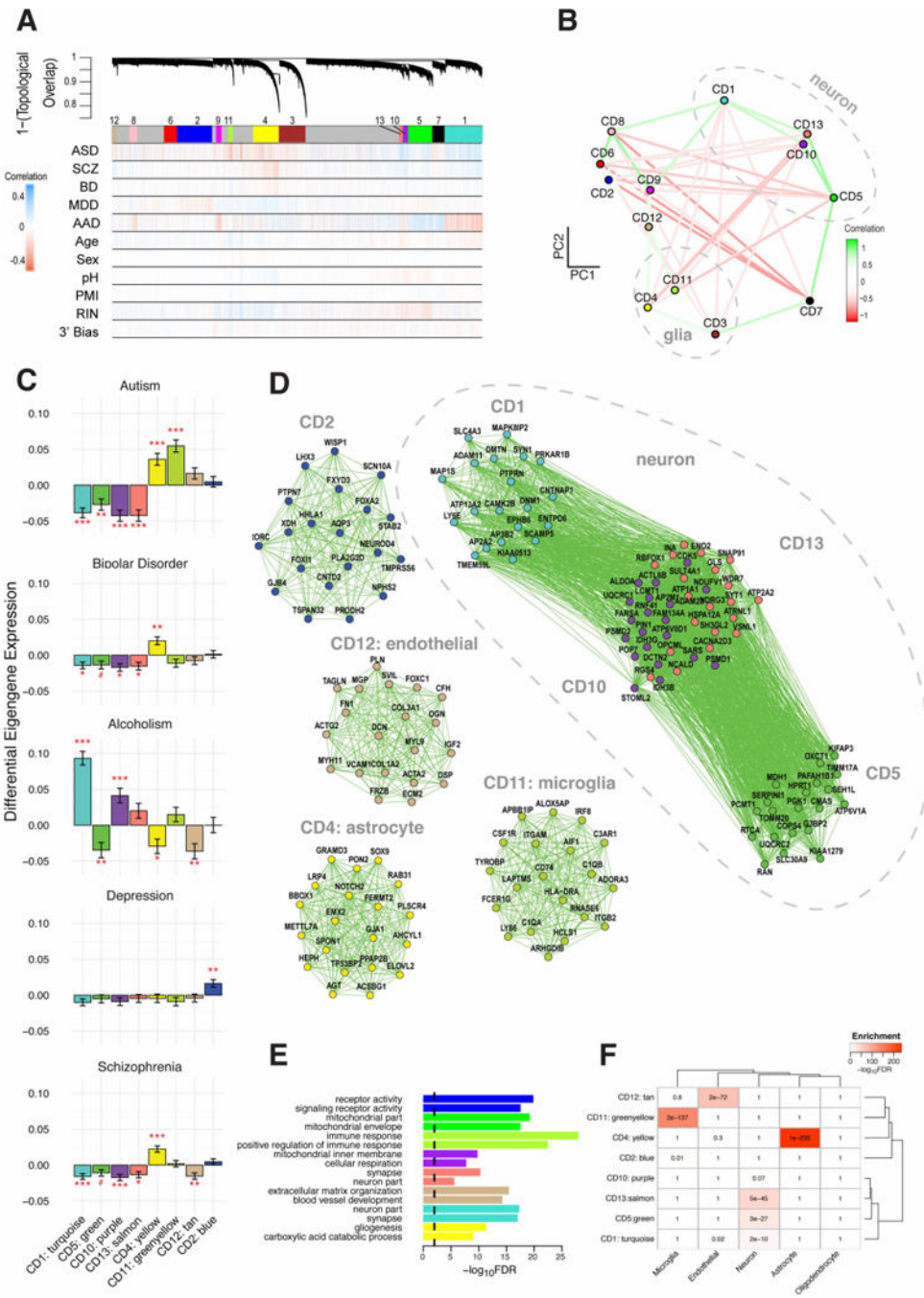
Fig. 3. Network analysis identifies modules of co-expressed genes across disease
(**A**) Network dendrogram from co-expression topological overlap of genes across disorders. Color bars show correlation of gene expression with disease status, biological, and technical covariates. (**B**) Multidimensional scaling plot demonstrates relationship between modules and clustering by cell-type relationship. (**C**) Module-level differential expression is perturbed across disease states. Plots show beta values from linear mixed-effect model of module eigengene association with disease status (FDR-corrected #P<0.1, *P<0.05, **P<0.01, ***P<0.001). **D)** The top twenty hub genes are plotted for modules most

disrupted in disease. See Data Table S2 for a complete list of genes' module membership (kME). Edges are weighted by the strength of correlation between genes. Modules are characterized by (**E**) Gene Ontology enrichment (top two pathways shown for each module) and (**F**) cell-type specificity, on the basis of RNAseq of purified cell populations from healthy human brain samples (25).
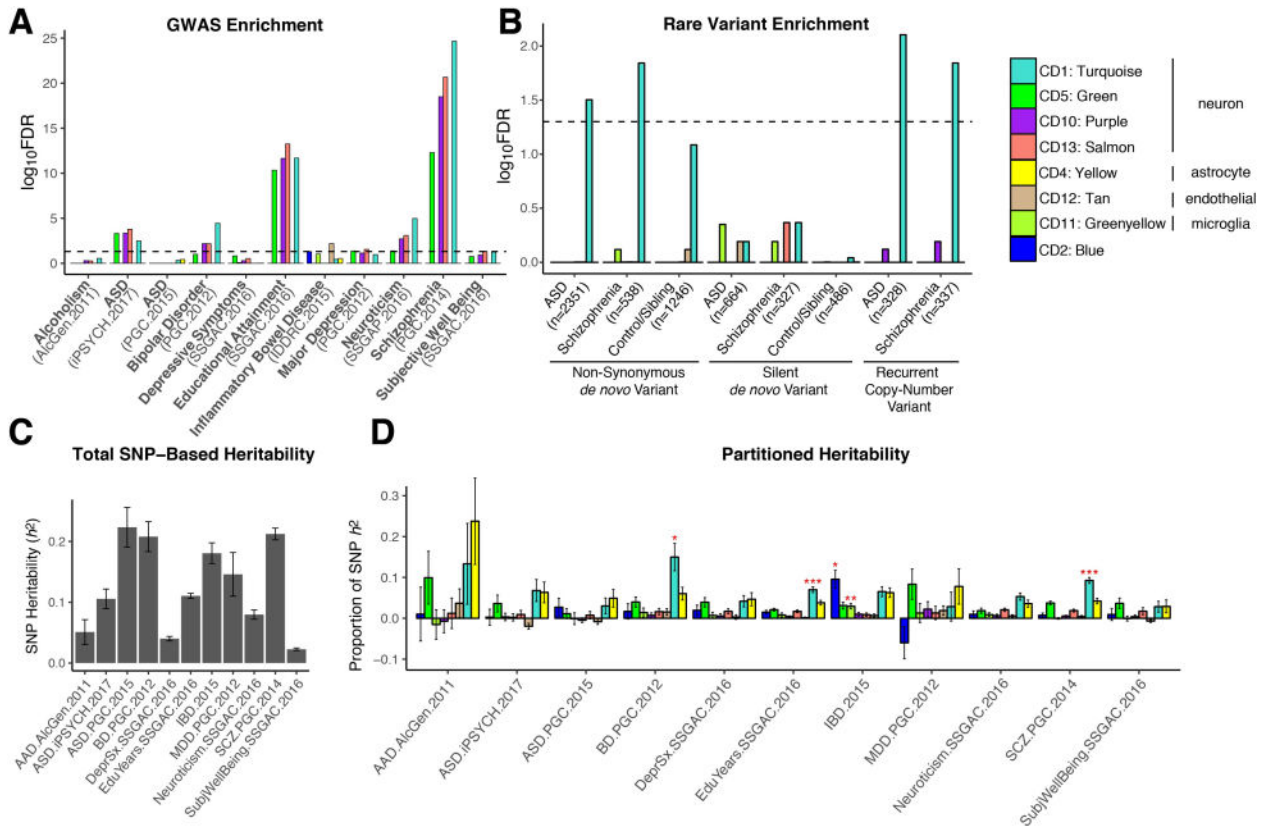
Fig. 4. Downregulated neuronal modules are enriched for common and rare genetic risk factors
(**A**) Significant enrichment is observed for SCZ-, ASD-, and BD-associated common variants from GWAS among neuron/synapse & mitochondrial modules (12). GWAS datasets are listed in Table S3. (**B**) The CD1 neuronal module shows significant enrichment for ASD- and SCZ-associated non-synonymous *de novo* variants from whole exome sequencing. The number of genes affected by different classes of rare variants is shown in parentheses. Significance was calculated using logistic regression, correcting for gene length. P-values are FDR corrected. (**C**) Total SNP-based heritability (liability scale for psychiatric diagnoses) calculated from GWAS using LD-score regression. (**D**) Proportion of heritability for each disorder or trait that can be attributed to individual co-expression modules. Significance (FDR-corrected *P<0.05, **P<0.01, ***P<0.001) is from enrichment statistics comparing the proportion of SNP heritability within the module divided by the proportion of total SNPs represented. The CD1 module shows significant enrichment in SCZ, BD, and educational attainment.