Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways

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Genome-wide association studies (GWAS) of psychiatric disorders have identified multiple genetic associations with such disorders, but better methods are needed to derive the underlying biological mechanisms that these signals indicate. We sought to identify biological pathways in GWAS data from over 60,000 participants from the Psychiatric Genomics Consortium. We developed an analysis framework to rank pathways that requires only summary statistics. We combined this score across disorders to find common pathways across three adult psychiatric disorders: schizophrenia, major depression and bipolar disorder. Histone methylation processes showed the strongest association, and we also found statistically significant evidence for associations with multiple immune and neuronal signaling pathways and with the postsynaptic density. Our study indicates that risk variants for psychiatric disorders aggregate in particular biological pathways and that these pathways are frequently shared between disorders. Our results confirm known mechanisms and suggest several novel insights into the etiology of psychiatric disorders.

Psychiatric disorders account for a large proportion of global disease burden[1]. They are clinical syndromes with largely unknown etiology whose classification has been developed on the basis of their observable symptomatology and course of illness. However, there is considerable evidence for strong heritability of these disorders, and recent work by the Psychiatric Genomics Consortium (PGC) using genome-wide association study (GWAS) data has demonstrated that a considerable proportion of this heritability is attributable to common genetic variants[2] and has also shown clear evidence of shared genetic risk at individual loci[3].

To make further progress in the treatment and prevention of these disorders, there is an urgent need to clearly identify the biological mechanisms and pathways underlying risk. However, the analyses available to date have focused primarily on single disorders and on gene-expression approaches (for example, in schizophrenia[4]) and, although interesting, such approaches are subject to potential confounding by the downstream effects of disorders and their treatment. Genetic pathway analysis methods for GWAS data have been developed[5,6] and aim to identify which biological pathways show an excess of etiological association. Though the power to detect pathway associations can be limited by lack of power in the original GWAS data, genome-wide 'chip-heritability' estimates[3] demonstrate that the loci showing nominal significance, but with values below genome-wide significance cutoffs, contribute to a significant proportion of disease liability. Pathway analysis provides a way to separate the true signals among these loci from the noise. Furthermore, pathway analysis can translate GWAS signals into a level of understanding that is biochemical and/or system-wide and can provide successful replication in the presence of allelic and locus heterogeneity[7,8].

In psychiatric genetics, several reports have found significant association with biological processes using GWAS. Analyses of bipolar disorder provided evidence of association with hormone action and adherens junctions[7,9,10]. Activity of voltage-gated calcium ions was also implicated in a pathway analysis of a bipolar disorder GWAS data set[11]. We hypothesized that combining pathway-based GWAS signals across multiple related disorders could be a powerful approach to identify pathways susceptible to genetic risk in neuropsychiatric disorders.

The PGC was established in 2007 (http://pgc.unc.edu)[12] and has been conducting field-wide mega-analyses of genomic data for common and severe psychiatric disorders[2,3,11,13–15]. Summary data are now available for PGC phase 1 studies that comprise >60,000 study participants representing schizophrenia (SCZ), major depressive disorder (MDD), bipolar disorder (BIP), autism-spectrum disorder (ASD) and attention deficit–hyperactivity disorder (ADHD). We have previously reported cross-disorder analyses via a single-nucleotide polymorphism (SNP)/association-based approach[3] and as estimated "chip-heritability" and genetic covariance via the genome-wide evidence[2]. We now extend this work, seeking to statistically identify the molecular pathways implicated by variants underlying genetic risk, the identification of which may have major impact on the understanding and future treatment of psychiatric disorders.

RESULTS

We summarize pathway data sets and provide gene membership in **Supplementary Tables 1** and **2**, respectively. From an initial compiled set of 19,752 pathways across five gene set databases (GO, KEGG, Panther, Reactome, TargetScan), we restricted downstream analyses to the 4,949 pathways of size 10–200 genes.

Comparisons among methods

We first obtained pathway-level *P* values for each pathway for the five disorders (SCZ, MDD, BIP, ASD and ADHD) across the five methods

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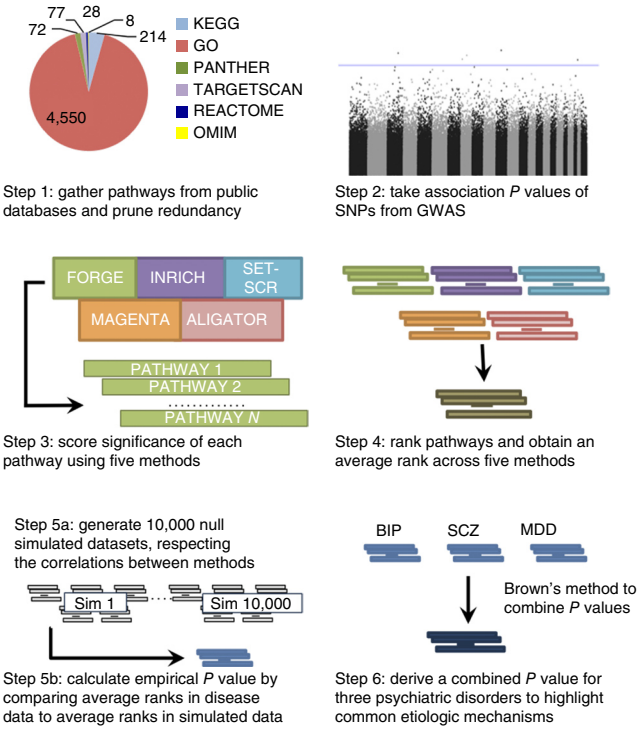


Figure 1 Overview of statistical approach for integrative pathway analysis of GWAS data. A summary of an analysis of one disorder is shown. Simulated data were generated by drawing from a null pathway *P*-value distribution for each method and for each disease that accounted for correlations between methods. Pathway results from all disorders were subsequently combined using Fisher's method.

(SETSCREEN, MAGENTA, INRICH, FORGE and ALIGATOR). Overall, methods were significantly correlated with each other (see **Supplementary Fig. 1** and **Supplementary Table 3**). Using both disorder data (SCZ) and null data (permuted phenotypes), we noted a statistically significant degree of overlap among methods (**Supplementary Fig. 1**).

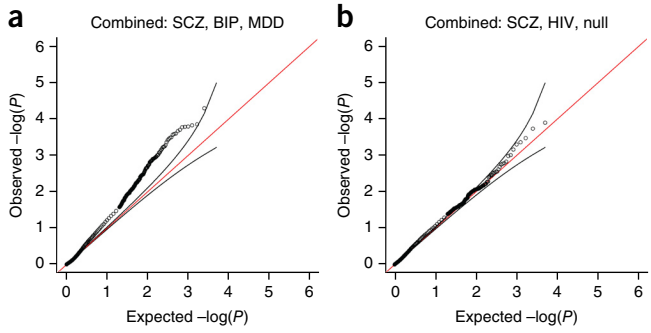
Deriving a method-wise and disorder-wise joint statistic

Pathways that achieve strong association using all five methodologies would be expected to be more robustly associated with disorder, owing to the differences between methods. We estimated a combined *P* value for each pathway (within each disorder) by calculating the average rank of each pathway within each method (ranks were used to ensure comparability between methods) and then comparing to a null distribution of expected ranks, built by drawing from the uniform distribution, accounting for intermethod correlation (**Fig. 1**; see Online Methods).

There was a significant degree of correlation of pathway-specific enrichment *P* values between disorders (Pearson correlation 0.2–0.3, **Supplementary Table 4**). Looking for pathways common to the adult disorders (SCZ, BIP, MDD), we then derived a combined statistic across disorders using Brown's extension of Fisher's combined *P* value[16] (see Online Methods). **Figure 2** shows quantile-quantile plots of *P* values from combining all methods across disorders. **Supplementary Figure 2** shows quantile-quantile plots of combined *P* values for SCZ, BIP, MDD, ASD, ADHD and, for contrast, null and HIV acquisition data. The former plots show a marked enrichment of significant *P* values, indicative of shared disease biology captured by the pathways tested. The null and HIV plots shows no enrichment of *P* values, indicating both that there is a lack of shared biology (as expected) and that our analysis does not cause a systematic inflation of significance. Similarly, the HIV data set showed no inflation.

Top pathways in individual adult-onset psychiatric disorders

We present the results for the different psychiatric disorder data sets in **Supplementary Table 5**, obtained using our approach. We identified 10, 1 and 4 pathway(s) that are suggestively enriched (at an FDR *q*-value <0.1) with BIP, SCZ and MDD susceptibility alleles, respectively (**Table 1**). Note that the pathways showing enrichment may change as sample sizes increase.



Top pathways shared across adult psychiatric disorders

The degree of rank correlation between pathways across pairs of disorders was significant (**Supplementary Tables 4** and **6**), with 49 pathways with combined *q*-value <0.1 spanning the three adult disorders (**Table 2**, **Supplementary Table 7**). Of these, 16 are significant at $q < 0.05$, with the top pathway (GO:51568: histone H3-K4 methylation) having a $q = 0.0003$ (**Table 2**). These results are more significant than those observed in any of the disorders analyzed alone. We then use multidimensional scaling (MDS) to cluster these sets in terms of shared genes. **Figure 3** shows a plot of every pathway with suggestive $q < 0.1$ on the first two MDS axes derived from the shared genes between these gene sets. The pathways separate in multidimensional space and reveal two distinct branches for neuronal synapse– and histone methylation–related gene sets, with a third branch containing pathways with genes that share membership in pathways with immune or neurotrophic functional annotation (**Fig. 3**). Although not a main focus of our analysis, the most significant pathway (GO:0005262, calcium channel activity) from the previous PGC Cross Disorder group SNP-based meta-analysis GWAS[3] across the five disorders was found to show nominally significant association in our pathway level meta-analysis across all five disorders ($P = 3.13 \times 10^{-3}$) and also in SCZ, MDD and BIP ($P = 8.07 \times 10^{-3}$).

Analysis of null and control disorder data

The enrichment *P* values of the pathways in **Table 2** (and **Supplementary Table 7**), combined across SCZ, BIP and MDD, indicated that there are multiple significant pathways. To confirm that this was due to shared disorder biology, we repeated the rank-combining analysis on two further data sets as controls: a GWAS of HIV[17], chosen because it is not thought to share significant disorder

Figure 2 Quantile-quantile plot showing *P*-value distribution for a combined analysis combining results from five pathway analysis methods and six pathway databases. (**a**,**b**) Data are shown for schizophrenia (SCZ), bipolar disorder (BIP) and major depressive disorder (MDD; **a**) and SCZ, HIV acquisition and a null simulated data set (**b**).

Table 1 Top pathways in schizophrenia, bipolar disorder and major depressive disorder[a]

No. methods	Avg. rank	*P* rank	*q*-value	Pathway ID	Description
BIP					
5	17	1.01×10^{-6}	0.005	GO:51568	Histone H3-K4 methylation
5	50.4	3.82×10^{-5}	0.093	path:hsa05218	Melanoma
5	79.2	1.16×10^{-4}	0.093	GO:7129	(Chromosomal) synapsis
5	81.8	1.27×10^{-4}	0.093	path:hsa05213	Endometrial cancer
5	83.3	1.34×10^{-4}	0.093	P00003	Alzheimer disease–amyloid secretase pathway
5	83.4	1.35×10^{-4}	0.093	path:hsa05215	Prostate cancer
5	87	1.50×10^{-4}	0.093	path:hsa05216	Thyroid cancer
4	89.5	1.59×10^{-4}	0.093	GO:90066	Regulation of anatomical structure size
5	95.6	1.81×10^{-4}	0.093	path:hsa05214	Glioma
5	96.9	1.87×10^{-4}	0.093	GO:70192	Chromosome organization involved in meiosis
SCZ					
5	38.4	1.58×10^{-5}	0.078	GO:14069	Postsynaptic density
5	68.6	7.15×10^{-5}	0.160	GO:45211	Postsynaptic membrane
5	76.8	9.67×10^{-5}	0.160	GO:43197	Dendritic spine
5	85.4	1.36×10^{-4}	0.168	GO:51568	Histone H3-K4 methylation
5	95.8	1.74×10^{-4}	0.173	GO:33267	Axon part
MDD					
5	25.4	2.63×10^{-6}	0.012	GO:8601	Protein phosphatase type 2A regulator activity
5	54.6	3.88×10^{-5}	0.092	GO:34330	Cell junction organization
5	68.8	7.70×10^{-5}	0.094	GO:43297	Apical junction assembly
5	70	7.92×10^{-5}	0.094	GO:45216	Cell-cell junction organization
5	99.8	1.97×10^{-4}	0.186	GO:31056	Regulation of histone modification

[a]Top pathways with $q < 0.1$, for the Schizophrenia (SCZ), Bipolar Disorder (BIP) and Major Depressive Disorder (MDD) data sets. For disorders with fewer than five pathways with $q < 0.1$, the top five pathways are listed.

etiology with psychiatric disorders but might instead share real technical artifacts, and a null GWAS, simulated using the same SNPs as the psychiatric GWAS but with no significant loci. The most significant pathways for the HIV and null data sets analyzed separately are shown in **Supplementary Table 4**. As expected, no pathways showed significant enrichment in the null data set or HIV data set, and the SCZ, HIV and null data sets combined analysis (**Supplementary Table 8**) gave no significant pathways after multiple-testing correction (minimum *q*-value = 0.454), in contrast to the results for SCZ, MDD and BIP shown in **Table 2**. This gives further evidence that the significant pathway enrichments in **Table 2** are due to shared biology across all three disorders, rather than being driven by enrichments in any single disorder.

Follow-up of brain gene expression of significant pathways

To place these pathways in a more specific neurobiological context, we analyzed coexpression relationships between the genes in pathways identified here at $q < 0.1$ using gene-expression data spanning brain regions and developmental time points[18]. Out of 797 genes assessed, 294 (32–37% of each branch from **Fig. 3**) were coexpressed in 7 modules. **Figure 4a** provides a network plot showing intramodular and intermodular connections between the top 10 hub genes in each module. **Figure 4b** summarizes the average expression level of genes in each module in different brain regions and temporal periods. These expression patterns are predominantly driven by temporal regulation (four modules have greater than twofold temporal change, while only one module, green, shows a twofold change between regions, **Supplementary Table 9**), suggesting that genetic risk across neuropsychiatric disorders affects neurodevelopmentally regulated pathways.

Of note, the yellow module is similarly expressed across regions and contains half (19/37) of the coexpressed histone methylation genes. Genes in this module exhibit over threefold higher average expression during early prenatal development (postconception week (PCW) 13–24) than during postnatal development or later aging, consistent with histone genes primarily functioning during neuronal differentiation and cell-fate commitment. Complete module membership and network details are available in **Supplementary Table 9**, which contains complete information about module-region associations and module-stage associations. Three other modules predominantly contain immunological and neuronal signaling and synapse genes whose expression increases in childhood and plateaus around adolescence to late adulthood (blue, brown, turquoise); three other modules are relatively highly expressed throughout life with ~75% maximum difference in expression between regions (green, black and red modules).

We then asked whether these modules exhibited a cell type–specific pattern by using gene lists from 35 genetically tagged and translationally profiled cell types in mouse[19–21]. Two of the modules containing immune-neuronal signaling and synapse genes that plateau in expression in maturing brain also exhibited cell type enrichment, with the brown module enriched for striatal neurons, particularly $Drd1^+$ medium spiny neurons, and the turquoise module enriched for Cnp^+ myelinating oligodendrocytes, suggesting its involvement in white matter maturation. Other modules did not exhibit strong cell type specificity, suggesting that either they affect multiple cell lineages (for example, the yellow module) or the matching cell type profile has yet to be defined.

Table 2 Top results from integrative pathway analysis of three adult disorders

Rank	BIP	MDD	SCZ	Combined *P*	*q*-value	Pathway ID	Description
1	0.0000	0.0592	0.0001	5.75×10^{-8}	0.0003	GO:51568	Histone H3-K4 methylation
2	0.0004	0.0500	0.0006	1.46×10^{-5}	0.0362	GO:16571	Histone methylation
3	0.0004	0.1462	0.0011	4.73×10^{-5}	0.0414	GO:43414	Macromolecule methylation
4	0.0008	0.0630	0.0014	5.10×10^{-5}	0.0414	GO:34968	Histone lysine methylation
5	0.4200	0.0001	0.0023	5.58×10^{-5}	0.0414	GO:45216	Cell-cell junction organization
6	0.0001	0.0910	0.0064	5.69×10^{-5}	0.0414	P00003	Alzheimer disease–amyloid secretase pathway
7	0.0007	0.0495	0.0024	5.86×10^{-5}	0.0414	P04393	Ras pathway
8	0.3120	0.0000	0.1286	7.12×10^{-5}	0.0422	GO:8601	Protein phosphatase type 2A regulator activity
9	0.8980	0.0001	0.0017	7.83×10^{-5}	0.0422	GO:43297	Apical junction assembly
10	0.0013	0.0207	0.0055	9.25×10^{-5}	0.0422	P00052	TGF-β signaling pathway
11	0.4890	0.0203	0.0000	9.53×10^{-5}	0.0422	GO:14069	Postsynaptic density
12	0.0085	0.0009	0.0239	0.0001	0.0422	GO:32869	Cellular response to insulin stimulus
13	0.0188	0.0054	0.0022	0.0001	0.0450	P00010	B cell activation
14	0.0023	0.2988	0.0003	0.0001	0.0450	GO:8757	S-adenosylmethionine–dependent methyltransferase activity
15	0.0073	0.0080	0.0044	0.0001	0.0454	GO:23061	Signal release
16	0.4590	0.0000	0.0168	0.0002	0.0473	GO:34330	Cell junction organization

Shown are the top 16 pathways with combined $q < 0.05$ spanning the three adult disorders. Full results for the three adult disorders are given in **Supplementary Table 7** and for the five disorders in **Supplementary Table 8**. **Supplementary Table 10** lists all pathways with $q < 0.1$ and **Supplementary Table 11** the underlying SNP *P* values in all genes with these gene sets.

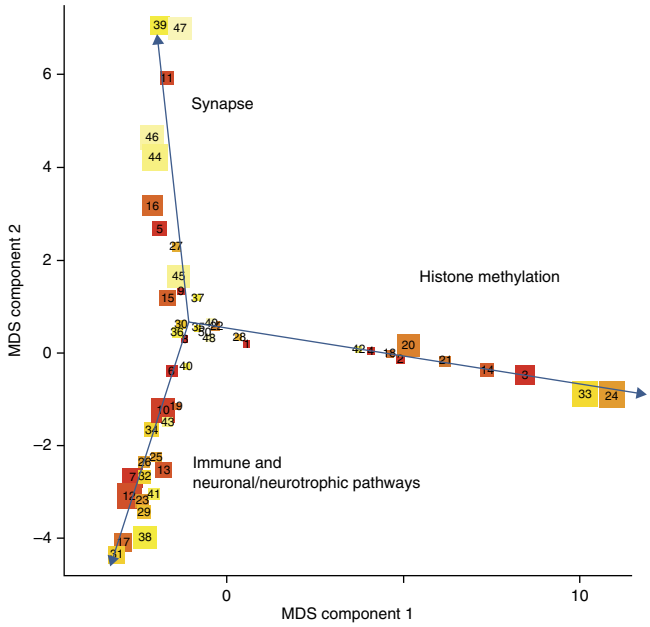


Figure 3 Multidimensional scaling plot of top 50 pathways with suggestive (<0.1) *q*-values ranked across five methods and three disorders (schizophrenia, bipolar disorder and major depressive disorder). The number of genes in each pathway is listed in **Table 2**. Color reflects rank (red represents top-ranking sets with lowest *P* values). Sizes reflect the number of genes in the set (maximum of 200, minimum of 11). See **Supplementary Data** for source data.

DISCUSSION

Major advances have occurred in psychiatric genetics over recent years, largely driven by an order-of-magnitude increase in sample sizes. While the identification of specific loci is critical to moving the field forward, so too is developing an understanding of the underlying biology. In this study we address the latter, integrating data from the largest reported psychiatric genetics data sets with well-established tools for interrogating such data sets. We developed a novel rank-based method to combine pathway enrichment results across analysis methods and disorders in a manner that is not confounded by the biases or shortcomings of the methods, to maximize the informativeness of the results. This contrasts with the approach of the previous PGC Cross Disorder group GWAS[4], which used a SNP-based meta-analysis. Pathway analyses based on such results will be powerful if the same SNP is implicated across disorders. Importantly, the pathway analyses described here do not require the same SNP (or, indeed, gene) within a pathway to be implicated across disorders, so they will be more robust to allelic heterogeneity within genes and within pathways across disorders, thereby providing a conceptually more powerful framework for conducting analyses, while losing

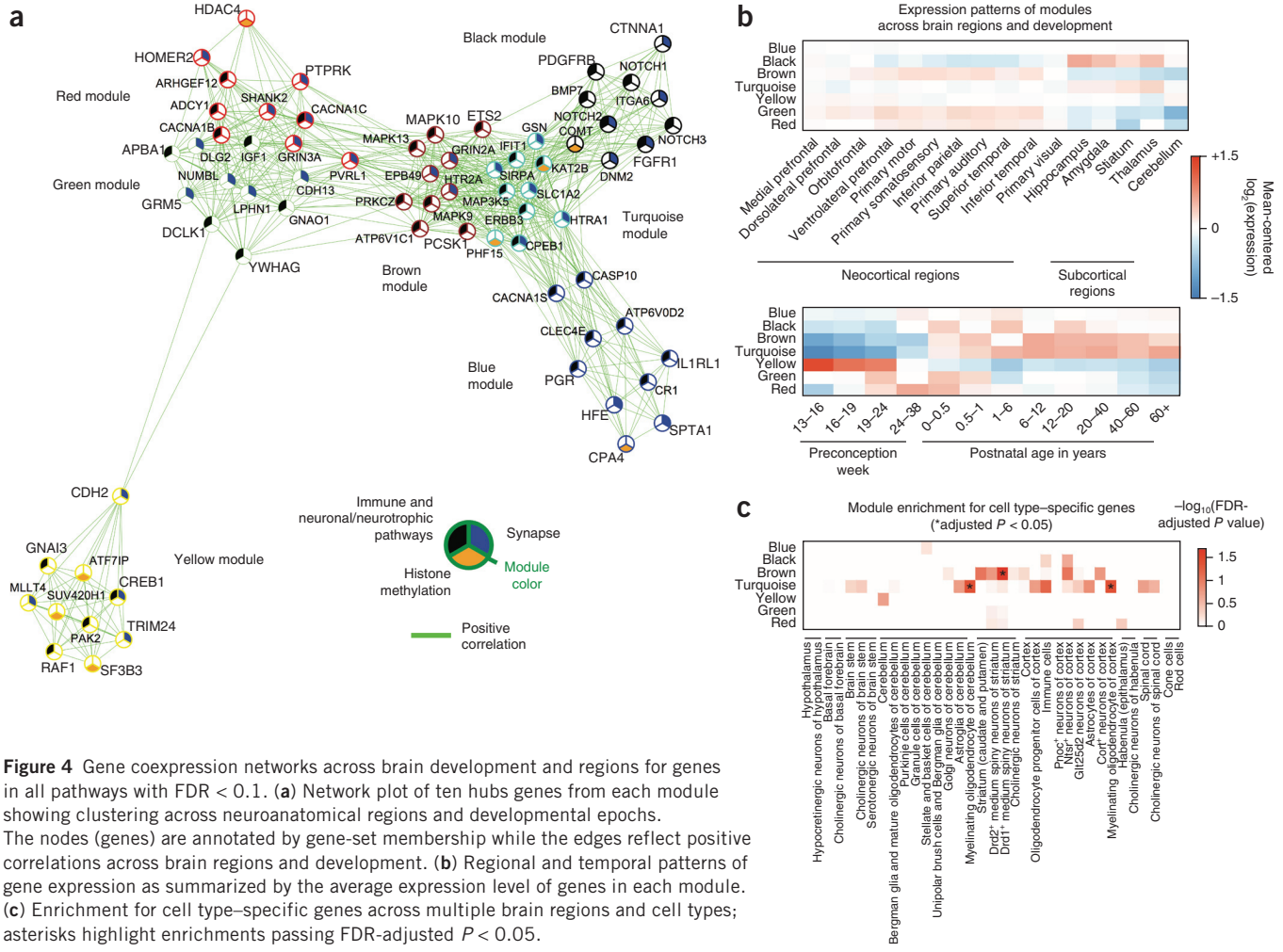


Figure 4 Gene coexpression networks across brain development and regions for genes in all pathways with FDR < 0.1. (**a**) Network plot of ten hubs genes from each module showing clustering across neuroanatomical regions and developmental epochs. The nodes (genes) are annotated by gene-set membership while the edges reflect positive correlations across brain regions and development. (**b**) Regional and temporal patterns of gene expression as summarized by the average expression level of genes in each module. (**c**) Enrichment for cell type–specific genes across multiple brain regions and cell types; asterisks highlight enrichments passing FDR-adjusted *P* < 0.05.

power compared to single-SNP-level analyses if the same SNPs are driving the association across related disorders. We note that the most significant pathway identified from the PGC cross-disorder analysis[3] (GO:0005262, calcium channel activity) also showed nominally significant enrichment in our analysis, confirming a role for calcium channel activity in these disorders.

Our analysis method uses GWAS summary statistics rather than using phenotype permutations on individual genotype data for two reasons. First, the PGC data sets are large and highly complex (>50 separate data sets) with a mixture of study designs and covariates (for example, those modeling ethnic stratification). For example, the PGC autism and ADHD genotype data are mixtures between trio and case-control studies. All of these complexities greatly increase the computational time necessary, so we implemented a more efficient method. For example, using a permutation-based approach that performed sufficient permutations across all the different data sets to generate disorder-level *P* values would have been computationally prohibitive. In addition, it is not always possible to obtain individual genotype data, particularly for large meta-analyses. It is therefore important that a pathway analysis method is applicable in such situations.

The primary strength of our integrated GWAS pathway analysis approach is the use of multiple analysis methods that differ in their assumptions and individual strengths. Methods that combine individual SNP *P* values across genes and pathways (FORGE, SETSCREEN) will pick up pathways containing genes with multiple, perhaps weak, independent association signals. Conversely, methods such as ALIGATOR or INRICH assign significance to genes based on the single most significant SNP in that gene, and will thus detect enrichment to pathways containing genes with individually, stronger associations. Notably, despite these differences, all of these methods yielded pathway rankings that were correlated with each other (**Supplementary Table 5**). As expected, the strongest correlations were observed between the most similar methods: FORGE and SETSCREEN, and ALIGATOR and INRICH.

Biological themes within and across disorders

Our primary aim was to combine pathway associations across disorders, as we hypothesized that this would be a more powerful approach, and we show a large increase in the evidence arising after meta-analyzing across disorders. The correlation of pathway-specific enrichment *P* values between SCZ and BIP was the highest among all pairs of disorders (0.29, **Supplementary Table 5**), consistent with the reported genetic correlation using common SNPs for these disorders[4]. Notably, combining the pathway-specific *P* values across the three 'adult' disorders in our primary meta-analysis (SCZ, BIP and MDD) resulted in greatly increased significance compared to the analyses of the separate disorders. This allowed us to identify biological themes spanning these disorders.

A secondary aim was to examine pathway themes within individual disorders, in order to show which disorders were contributing most to the cross-disorder findings. Among individual disorders, BIP, SCZ, MDD and ADHD gave significant results at FDR $q < 10\%$, but ASD and HIV did not (**Table 1** and **Supplementary Table 4**). Our results suggest that histone methylation appears to play a more prominent role in bipolar disorder and that synapse- and postsynapse-related processes are more strongly implicated in the etiology of schizophrenia (**Table 1**). We note that pathways involved in the methylation of other molecules, such as DNAs, were not highly enriched in this study, suggesting a degree of specificity in the nature of methylation gene sets implicated here. For schizophrenia, we note that "KEGG_DOPAMINERGIC_SYNAPSE" was reported as the third-highest-ranked pathway (of 9,016) in the latest GWAS data (analysis by ALIGATOR)[22]. Our top SCZ pathway—postsynaptic density—did not rank highly in either the ALIGATOR or INRICH analysis reported in that paper, but those pathway analyses were applied to a heavily restricted set of genes (those containing a SNP with $P < 5 \times 10^{-8}$) and used only two of our five analysis methods (ALIGATOR and INRICH), making that analysis not directly comparable with ours. We also include methods (FORGE, MAGENTA, SETSCREEN) that may favor more polygenic, complex patterns of association. Analyses presented here use a more relaxed significance criterion, thus picking up signals that do not reach genome-wide significance. We note that strong, independent support of our findings comes from the observation that analyses of rare variants have also implicated postsynaptic pathways in SCZ etiology[23,24], suggesting that both rare and common variants are relevant.

Emerging landscape of psychiatric pathways

We show that synapse-related as well as newly implicated histone methylation and immune and neuronal signaling pathways are statistically significantly associated within and across SCZ, BIP and MDD. These pathways are core molecular processes, disruption of which may increase risk for multiple psychiatric disorders. Especially interesting in this regard is the identification of synaptic and immune dysfunction as the major pathways altered in postmortem brain in ASD[25], as well as of emerging genetic overlap across many neurodevelopmental conditions. Our top results for schizophrenia, postsynaptic density, has been previously suggested by CNV findings and convergent lines of evidence[23,26], as well as recent exome sequencing data[27,28], suggesting a role for both rare and common variants in affecting SCZ-relevant changes in the postsynaptic membrane proteins. "Histone H3-K4 methylation" featured among top bipolar hits, achieving study-wide significance ($q = 0.005$). Variable H3-K4 methylation of synapsin genes has been shown to give rise to altered expression patterns in bipolar disorder and major depression[29], which may suggest a role for epigenetic regulatory mechanisms in the etiology of mood disorders. Histone methylation mechanisms have roles in the coordination of complex cognitive processes such as long-term memory and roles in conditions from addiction to schizophrenia to neurodegeneration[30].

Recent studies of *de novo* mutations have also implicated this pathway (often referred to by a broader term, chromatin regulation/modification or transcriptional regulation) in ASD[31–33]. However, our ASD data set, based on GWAS, did not have sufficient power (owing to both the smaller sample size available and the combination of case-control and trio data sets) and was excluded from the primary pathway analysis. For MDD alone the top pathway was "protein phosphatase type 2A regulator activity," which achieved a *q*-value of 0.012. Prior studies have implicated this pathway in serotonergic neurotransmission and in the mechanism of response to antidepressants[34].

We excluded the schizophrenia-associated MHC region of 6p21–22 in our analysis to avoid confounding of methods by the very high levels of linkage disequilibrium at this locus. Despite this, key immune processes such as TGF-beta_signaling (P00052), B_cell_activation (P00010) and T_cell_activation (P00053) feature highly in our significant pathways. KEGG infectious disease pathways also featured at suggestive significance. Although these pathways contain immune processes, such as the Tuberculosis (hsa05152) and Hepatitis C (hsa05160) pathways, their role in the brain has been highlighted by, for example, studies finding that both hepatitis C infection and

interferon alpha treatment for hepatitis C are associated with a range of additional neuropsychiatric symptoms[35].

We also found pathway genome-wide association across SCZ, BIP and MDD with histone methylation (rather than DNA methylation) processes via Histone H3-K4 methylation (GO:51568), Histone methylation (GO:16571), Histone lysine methylation (GO:34968) and Macromolecule methylation (GO:43414). Replicated environmental risks for schizophrenia occur at critical periods early in development, for example, in the Dutch Hunger Winter and Chinese famine studies[36], when the epigenome is known to be particularly labile. At this time rapid cell replication is occurring and the standard epigenetic signals, including histone H3-K4 and lysine methylation and other related processes, are driving development and tissue differentiation[37]. Given the role of this process in establishing active promoters[38], it appears likely that the dysregulation of histone methylation may have downstream effects with the potential to disrupt neurodevelopment and coordinated gene expression, as animal studies have demonstrated[39]. Our results suggest that dysregulation of the genes in histone methylation pathways is a common etiological mechanism for adult psychiatric disorders.

Our gene network analysis of how the identified pathways are expressed in brain revealed modules of coexpressed genes that identify developmental time points and brain regions that may inform future experiments aimed at manipulating the identified pathways (**Fig. 4**). Additionally, enrichment for cell type–specific transcriptomic signatures identifies which cellular subtypes may be affected upon manipulation of specific genes. For example, the temporal trajectory of modules suggests which pathways may not be amenable to pharmacologic manipulation as a result of their prominence in early development (for example, the yellow module which contains histone methylation genes), but also pathways whose activity is potentially modifiable in adults (for example, the brown and turquoise modules, which were enriched for genes specific to striatal neurons and white matter, respectively). Additionally, the blue module's expression pattern is similar across regions, and the hub genes suggest both intracellular and intercellular signaling processes. It shows an increase during late prenatal and early postnatal development, peaking before age 6, suggesting the genes in this module may be related to postnatal synaptic pruning, another potential target for developing interventions. We emphasize that these results should be considered preliminary, as they are a first-pass analysis using the results of a novel approach. Nevertheless, they do point a clear way forward for unbiased assessment of pathways affected by common genetic variation, which is expected to explain a majority of the genetic architecture of neuropsychiatric diseases. They suggest that the integration of gene expression with pathway-level analyses on larger GWAS can identify even greater specificity. Furthermore, future analyses can increase neurobiological specificity by searching across denser time point data or using cell type–specific transcriptomes acquired from single-cell sequencing in human brain, as they become available.

Our analyses have shown the general ability of pathway analyses to discover novel biology underlying complex human disorders. The immune-neuronal signaling and histone methylation findings illustrate how genetic risk aggregation in pathways may underlie vulnerability to environmental risk factors in the prenatal environmental, while strengthening the evidence for the role of synaptic pathways. Our results shed light on the biology underlying GWAS of psychiatric disorder and could suggest novel functional and drug discovery studies, as pathways make far larger and better drug targets than individual genes[40]. Our observation that the degree of correlation between pathways across disorders is higher than expected by chance builds on the observation of shared genetics between these disorders[4] and, importantly, indicates that polygenic overlap is nonrandom at a molecular or pathway level.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

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ONLINE METHODS

Method rationale. The PGC (Psychiatric Genomics Consortium) has applied SNP association data[3] and estimated "chip-heritability" estimate approaches[2] to compare and contrast psychiatric disorders. Here we study the molecular pathways in which genetic risk for psychiatric disorders aggregate and examine whether or not these pathways are shared between related psychiatric disorders. The multiplicity of methods and parameters that can be used for such an undertaking led to the formation of a subgroup of the Psychiatric Genomics Consortium (http://pgc.unc.edu/) to develop a protocol and pipeline for five published methods of GWAS pathway analysis along with a methodology to combine results from different analytical methods to show the most robust pathway signals arising from GWAS data.

Samples and genotypes. The samples for these analyses (total N = 61,220) included cases, controls and family-based samples assembled for published genome-wide mega-analyses of individual-level data conducted by the PGC (see refs. 2,3,11,13–15 for details). To ensure comparability across samples, raw genotype and phenotype data for each study were uploaded to a central server and processed through the same quality control, imputation and analysis pipeline. This approach is detailed elsewhere[11], but we describe it briefly here: to ensure independence of individual disorder analyses, only one of pair of related or duplicate individuals were retained, and in only one disorder case or control set, resulting in 61,220 cases and controls in total. Stringent and standardized quality control procedures were applied as previously described[3]. For the family-based samples, alleles transmitted to affected offspring ("trio cases") were matched with untransmitted alleles ("pseudo-controls"). The disorder samples comprised ASD (4,788 trio cases, 4,788 trio pseudo-controls, 161 cases, 526 controls), ADHD (1,947 trio cases, 1,947 trio pseudo-controls, 840 cases, 688 controls,), BPD (6,990 cases, 4,820 controls), major depressive disorder (9,227 cases, 7,383 controls) and SCZ (9,379 cases, 7,736 controls). Identity-by-descent relationships were estimated for all pairs of individuals to identify any duplicate individuals across the component data sets. When duplicates were detected, one member of each set was retained. These individuals were then randomly apportioned to a single disorder case-control data set. All subjects were of European ancestry and met DSM-III-R or DSM-IV criteria for the primary disorder of interest.

Study sample numbers for individual disorders were: ASD (n = 4,949 affected/5,314 unaffected), ADHD (n = 2,787/2,635), BIP (n = 6,990/4,820), MDD (n = 9,227/7,383) and SCZ (n = 9,379/7,736). Imputation was conducted using HapMap III data as references, resulting in over 1.2M SNPs. SNPs with imputation quality scores less than 0.8 were filtered out. Single SNP-based association analyses were conducted using logistic regression on individual disorders with ancestry covariates and GC-corrected. The MHC region on chromosome 6 (25–35 Mbp) was excluded from further analyses to prevent potential impact of extensive linkage disequilibrium (LD) in the region.

For all analyses, five P-value sets were used: schizophrenia (SCZ), 1,227,336 SNPs; bipolar disorder (BIP), 1,223,695 SNPs; major depressive disorder (MDD), 1,220,925 SNPs; ADHD, 1,219,982 SNPs; autism (ASD), 1,232,050 SNPs. Our primary analysis included SCZ, BIP and MDD.

We also analyzed a null GWAS sample, to assess the degree of dependence between methods, as well as to ensure that our analysis did not generate an excesive amount of false positives. This was generated from unrelated CEU+TSI Hapmap3 data sets via random assignment of case/control phenotypes (100 cases and 100 controls). PGC1 data involves only European subjects (imputed using Hapmap3 CEU+TSI panels) and thus the use of Hapmap3 European data most closely matches the LD structure of the PGC1 disease samples. Since the null sample by definition contains no true effects, power is not an issue. Therefore, the relatively small sample size is not important.

Gene and pathway data. We used Ensembl[41] gene definitions as the reference gene annotation and map. We combined gene set data from six sources: KEGG, GO, PANTHER, TARGETSCAN, REACTOME and OMIM. An overview of our methodology is shown in **Figure 1**. All gene sets were downloaded from their respective sources (11 August 2011). The parsing of these sets is summarized in **Supplementary Table 1** and summary statistics are given in **Supplementary Table 2**.

To ensure the specificity of gene set association findings, further analyses were restricted to 4,949 gene sets of at most 200 genes and at least 10 genes (we limited our analysis to pathways containing 10–200 genes because statistics for smaller gene sets were over-dispersed and the few outlier sets with >200 genes were computationally inefficient to analyze and largely nonspecific due to the large number of genes (data not shown)). Identical pathways were removed. Overlap, measured by correlation between gene content across pathways, between the six gene set resources were minimal (R^2 < 0.12). The different pathway sets were combined into one database and identical pathways merged.

Gene definitions. For all analyses, we used Ensembl identifiers as the master gene set, with −35 kb upstream and +10 kb downstream to define the gene boundaries, since transcriptional regulatory elements are likely to be contained within these intervals and that there is thus merit in capturing the variation within these regions[42]. Analysis was run both with and without the MHC region (chromosome 6, 25–35 Mb).

Standardization of pathway inputs. SNPs were assigned to genes based on human genome build 37 positions if they lay within 35 kb upstream or 10 kb downstream of the gene. In total, 739,373 SNPs were assigned to 18,689 genes. Note that if SNPs mapped within more than one gene, they were assigned to all such genes. SNPs were also filtered by imputation quality (INFO > 0.8), which resulted in 477,792–543,578 SNPs being assigned to 16,334–17,352 genes (these numbers vary slightly between disorders). We used a standardized framework of data input for our analyses. Empirical results (P values for individual SNPs) from PGC GWAS were gathered for each disorder, and all P values were GC-corrected. For all analyses, we used pathways with a minimum of 10 genes and a maximum of 200 genes.

Five published pathway analysis methods were used. These fell into two classes with differing approaches and assumptions regarding genomic architecture of risk variants in pathways as well as different methods for the correction of LD and gene size effects. FORGE[43] and SET SCREEN TEST[44] are meta-analysis methods that combine P values across all the SNPs in genes or pathways while adjusting for the confound of LD. INRICH[45], ALIGATOR[9] and MAGENTA[46] are "best SNP per gene" methods that count the number of genes in a pathway where a number of independent SNPs exceed a predefined significance, and adjust for LD and genomic structure with corrected statistics derived by Monte-Carlo simulation. We describe these methods below.

FORGE method. FORGE is a software suite that implements a range of methods for the combination of P values for the individual genetic variants within a gene or genomic region while adjusting for linkage disequilibrium–induced correlations[43]. The software can be used with summary statistics (marker ids and P values) and accepts as input the result file formats of commonly used genetic association software. In addition, several utility programs are distributed with FORGE allowing users to (i) map SNP to genes using the Ensembl human genome annotation, (ii) parse different gene-set files, and (iii) calculate meta-analysis statistics for gene and gene-set analysis results when studies are carried out on multiple data sets. For each pathway, a nonparametric test yields a P value for enrichment of genes in a pathway given the entire set of pathways analyzed. FORGE can be freely accessed at https://github.com/inti/FORGE.

MAGENTA method. MAGENTA is an acronym for Meta-Analysis Gene-set Enrichment of variaNT Associations and is a program that takes as input summary P values from GWAS[46]. Testing for statistical significance of pathways using MAGENTA is a 3-step process. First, every gene is assigned the best GWAS P value that falls within that gene or the user-defined upstream and downstream regions of that gene. These P values are corrected, using multivariate linear regression, for known confounders of P values including gene size and linkage disequilibrium properties. Finally, for each pathway, the observed number of gene P values surpassing a certain user-specified threshold for P values (here 95%) is compared against expected number of gene P values surpassing that threshold for a given pathway size (i.e., number of genes). For each pathway, a nonparametric test yields a P value for enrichment of genes above the predetermined threshold.

Set screen test method. The set screen test is based on theoretical approximation of Fisher's statistics such that the combination of P values at a gene or across a pathway is carried out in a manner that accounts for the correlation structure, or

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linkage disequilibrium, between single nucleotide polymorphisms. The approach is similar to that applied in FORGE (LD)[43]. The test is implemented in PLINK[47]. We applied this method to the PGC data, corrected for GC inflation, using CEU founders from HapMap to describe the LD structure. We used the same gene sets as described above which were filtered to contain no less than 2 and no more than 200 genes. For a given pathway or set, we assigned a *P* value to the set when at least one SNP was present. Where more than one SNP was present, the combined *P* value (accounting for LD) was given.

ALIGATOR method. ALIGATOR converts a list of significant and nominally significant SNPs into a list of significant genes, and, for each predefined gene set, tests whether this gene list contains more genes from the gene set than would be expected by chance[9]. This is done by comparing the gene list to 100,000 random gene lists of the same length generated by sampling SNPs (not genes) at random, correcting for variable numbers of SNPs per gene and variable gene size. Correction for the multiple testing of non-independent gene sets is performed using a bootstrap method repeated 5,000 times. Gene sets require at least two signals to be counted as enriched to remove the possibility of a small gene set being deemed significantly enriched based on one signal. An important modification to the original ALIGATOR method is that significant genes in the same gene set that mapped less than 1 Mb apart (and thus could be explained by the same association signal) are counted as a single signal. In this analysis, SNP-wise *P* value criteria for defining "significant" SNPs, and thus "significant" genes, were chosen so that the resulting list of significant genes contained the top 5% of all genes. When no filtering was performed, *P* value criteria varied from 8.4×10^{-4} to 3.31×10^{-4} when the −35 kb/+10 kb gene window was used. When SNPs were filtered by information score, the *P* values increased slightly, from 1.64×10^{-3} to 5.18×10^{-3}.

INRICH method. INRICH[45] takes a set of independent, nominally associated genomic *intervals* and then tests for the enrichment of predefined gene sets. An interval will typically correspond to a genomic region of SNP association defined by LD from a genome-wide scan, although intervals could also represent regions identified as homozygous-by-descent, for example, deletion or duplication events observed in cases. The INRICH analysis procedure comprises three major steps: (i) linkage disequilibrium (LD)-based interval data generation to identify unique regions of association; (ii) empirical enrichment calculation using an interval-based permutation strategy; and (iii) second-step permutation for multiple testing correction at the gene set level. INRICH also presents global enrichment statistics G_p, and the empirical significance of G_p is evaluated within a permutation procedure.

Pathway analysis strategy. Given that the results of the five analysis methods are correlated but not identical, pathways genuinely involved in disease susceptibility would be expected to show consistent enrichment for association signal across several methods. Therefore, we ranked the pathways in ascending order of enrichment *P* value for each method and calculated the average rank of each pathway across all five methods. This analysis was carried out for each disease separately. The lower the average rank of a pathway, the more consistent its evidence for enrichment of association signal across the methods, and thus the greater the likelihood of involvement in disease susceptibility. Ranks were used to control for differing power of each method.

Our general approach is outlined in **Figure 1**. Our primary analysis sets consisted of the samples for the adult disorders of schizophrenia (SCZ), bipolar disorder (BIP) and major depressive disorder (MDD) as these three have the highest genetic relationship in the recent pair-wise analysis of the five psychiatric disorders using GWAS data[2]. Supplemental analyses were performed on attention deficit hyperactivity disorder (ADHD) and autism (ASD) data sets[3,13]. We used a Monte-Carlo simulation approach, modeling the dependence between methods in terms of the observed pairwise correlations of pathway enrichment *P* values to calculate the average rank and significance of a pathway in a disorder across all methods (**Supplementary Analysis** and **Supplementary Tables 12–15**). However, the analysis method and results are robust to variation in these correlations (**Supplementary Tables 15** and **16**). The motivation for using ranks rather than *P* values was to ensure that all methods were treated equally. Specifically, FORGE and SET-SCREEN combine the *P* values of the SNPs, and it is therefore possible for them to achieve very small enrichment *P* values if the pathway contains strongly associated SNPs. This is not the case for ALIGATOR, INRICH and MAGENTA, which use simulation to give enrichment *P* values. Note also that INRICH and ALIGATOR require a pathway to contain two significant genes for an enrichment *P* value to be calculated. Thus, missing enrichment *P* values count as evidence against enrichment for these methods, and such pathways are assigned the joint bottom rank. It is possible that a pathway may rank relatively poorly on one method compared to the others, thus reducing the power of the average rank to detect enrichment. We therefore performed a secondary analysis based on the average of the best four ranks. However, this had little effect on the results (see **Supplementary Analysis** and **Supplementary Tables 12** and **13**).

We calculated observed and expected overlaps between all combinations of methods to assess the extent of concordance between methods. For the top 10% ranked pathways in each disorder, we then compared observed and expected overlaps between all combinations of the three adult disorders.

Combination of pathway ranks across methods. Finally, we combined pathway *P* values across disorders using Brown's method (an extension of Fisher's method for correlated data). To ensure that the observed pathway enrichments across the disorders were a result of shared biology, rather than artifacts of the analysis method, we also applied our rank-based method to a null data set (simulated to have no phenotype effects) and a GWAS of HIV-1 acquisition[17]. HIV infection acquisition can be assumed to share little biology with the psychiatric disorders studied here because, although two MHC associations have been discovered, we excluded the entire extended MHC region from our analyses. The null and HIV data sets were tested individually, as well as in combination with the SCZ data set.

The following procedure yields a single combined *P* value for each pathway in a given disease data set by merging results across the 5 methods, accounting for correlation. For each disease, do the following:

1. Determine average rank per pathway within each method. After ranking *P* values (ties receive the average rank), average the ranks across the five methods to yield 1 rank per pathway.

2. Determine the expected distribution of averaged ranks under the null. (a) Calculate the Pearson correlation statistics between pairs of methods. Null data (generated from unrelated European CEU+TSI Hapmap3 GWAS data set via random phenotype assignment (100 cases, 100 controls)) from each method was used for these calculations. (b) Generate 5 sets of null *P* values (1 for each pathway drawn without replacement from the uniform distribution [0,1]) such that the intercorrelation among methods is preserved, using the method described at http://comisef.wikidot.com/tutorial:correlateduniformvariates. Do this 10,000 times. (c) Transform *P* values into ranks for each permuted *P* value distribution. Note that in the permuted data, there will be few ties, if at all. Therefore, introduce ties by replacing the ranks for those pathways that tied in the real data with their average rank in the permuted data. (d) For each set of 5 permuted and ranked distributions, determine the average rank per pathway (as in Step 1).

3. Assign empirical *P* values to each pathway. $P_{\text{final}} = \frac{\sum_{i=1}^{N} i}{10{,}000}$, where $i = 0$ if the permuted rank is greater than the real rank, and $i = 1$ if the permuted rank is less than or equal to the real rank. Note that this procedure does not allow for dependence between pathways, so it cannot be used to test whether there is an excess of pathways with average rank achieving a given level of significance. However, it does give a valid test of significance for each pathway separately. To correct for multiple testing of pathways, *q*-values were calculated[48]. For each pathway, the *q*-value corresponds to the minimum value of the FDR at which that pathway would be declared significant.

Comparisons between methods: method overlap. To robustly test the significance of overlap in enriched pathways between methods, it is necessary to restrict the analysis to a set of independent pathways (by gene membership). This was done by pruning by Jaccard distance (see below). Note that such a restriction is not necessary and was not used for the pathway analysis combining methods, which uses the full set of pathways. To facilitate comparisons between methods, we use quantiles, not *P* values. Specifically, we focus on pathways that are in the top 25% for a particular method within a disease or null data set. This is because it is otherwise difficult to compare pathway *P* values from methods that have

different statistical power. We use the following procedure to determine the extent to which methods overlap:

1. For a given data set, reduce the data set down to only pathways that are in the top 25% in ≥1 of the methods;

2. Reduce redundancies in this data set by removing smaller pathways for which there exists a larger pathway whose Jaccard distance (intersection divided by the union) is >0.2;

3. Using this reduced data set, which represents a set of independent pathways that are in the top 25% of ≥1 of the methods, calculate all overlaps among five methods (5-way, 4-way, 3-way and 2-way);

4. Calculate the expected overlap between pathways in the top 25% assuming top 25% of pathways for each method is random.

Testing pathway overlap between diseases. In order to enable testing to be carried out for correlation in pathway enrichment *P* values and overlap in top pathways among diseases, a subset of 1,918 pathways was selected such that no two pathways had a Jaccard similarity measure >0.2. Initially, Pearson correlation coefficients were calculated between the pathway-specific enrichment *P* values of the null data set and each of the five disease data sets in turn. These correlations lay between 0.111 and 0.156, with a mean of 0.132. This indicates that ranking pathways within methods and calculating the average rank across methods induces some correlation between pathway ranks between data sets. For example: ALIGATOR and INRICH only return a *P* value if a pathway contains at least two significant genes, otherwise the pathway was assigned equal bottom rank. Thus, small pathways are likely to have low ranks for these methods in most data sets. However, correlations between the diseases with respect to the pathway-specific enrichment *P* values were higher than those with the null or HIV (see **Supplementary Tables 5** and **14**), suggesting that the interdisease correlation is not simply a function of methodological correlation.

To test for significant correlation and overlap between the five disease data sets while allowing for correlations induced by the ranking method (as described above), we generated, for each pair of diseases, 1,000 random sets of 1,918 bivariate uniform variables with correlation of 0.132. The Pearson correlation of the two variables within each replicate data set was calculated and compared to that observed in the actual data. A similar method was used to compare the overlap in the top 10% of pathways between the two variables to that observed in the actual data. *P* values for these comparisons are shown in **Supplementary Table 6**. Correlation coefficients in the actual data were nominally significant for all pairs of the five diseases of interest (ADHD, ASD, BIP, MDD, SCZ), but not between these diseases and HIV or the null data set (with the exception of the HIV-MDD correlation, which was nominally significant but is likely to be an artifact of multiple testing).

Combining pathway enrichments across diseases. For each pathway, *P* values were combined across diseases using Brown's method (an extension of Fisher's method that accounts for correlation between data sets). This method is described[49], but a brief description is provided here. To attain a joint test statistic for *N* tests that are not independent, the statistic has a mean $m = 2N$ and a variance (σ^2) where

$$\sigma^2 = 4N + 2\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} \mathrm{cov}(-2\log_\theta p_i, -2\log_\theta p_j)$$

and where p_i and p_j ($i, j = 1,\ldots, N$) are the *P* values for each test and covariance (cov) is calculated as

$$\mathrm{cov}(-2\log_\theta p_{i,} -2\log_\theta p_j) = \rho_{ii}(3.25 + 0.75\rho_{ii})$$

for non-negative correlation coefficients ρ_{ij} between the two *P* value distributions (here, set to be 0.132, which is the average correlation in *P* values between the disease data sets and the null, calculated in the previous section). Finally the overall significance of a set of non-independent tests is calculated using the statistic *T* which under the null hypothesis follows the central chi-square distribution $T = T_0/c$, with $2N/c$ degrees of freedom, where

$$c = \frac{\sigma^2}{4N}$$

and T_0 is the sum of $-2\ln(p)$, as used in Fisher's method.

This analysis was primarily performed on the three adult diseases (SCZ, BIP, MDD), which have larger and more powerful samples than the other diseases (ADHD, ASD). A secondary analysis of all five diseases was also performed. Finally, an analysis was performed combining SCZ, HIV and the null data set to confirm that pathway enrichments observed in the analysis of SCZ+BIP+MDD were due to shared biology rather than artifacts of the analysis method.

When the diseases were tested separately, one pathway was significant after correction for multiple testing in BIP (GO:51568: histone H3-K4 methylation, $q = 0.005$) and MDD (GO:8601: protein phosphatase Type 2A regulator activity, $q = 0.012$). No pathway achieved $q < 0.05$ in the other diseases (or the null data set). When the three adult diseases were combined, 15 pathways were significant at $q < 0.05$, with the top pathway (GO:51568: histone H3-K4 methylation) having a *q*-value of 0.0005. These results are more significant than those observed in any of the diseases analyzed separately, and illustrate the power to be gained by combining pathway analyses across diseases with shared biology. When all five diseases were combined, GO:51568 was still significant ($q = 0.045$), although its significance was reduced due to lack of enrichment in ADHD or ASD. Finally, when SCZ was combined with HIV (not expected to share common biology with SCZ) and the null data set, no pathway was significant after multiple testing correction, as expected.

Supervised weighted coexpression network analysis. We performed a secondary analysis of 797 genes comprising all pathways with $q < 0.1$ from the primary cross disorder pathway enrichment analysis to explore how pathways relate to processes of *in vivo* brain development and aging. We asked how the genes cluster across brain regions and developmental time points using the BrainSpan exon array data[18]. We applied weighted gene coexpression network analysis[50] to group genes into modules of coexpression. Modules were characterized for regional and temporal patterns as well as cell type specificity[21].

Network analysis was performed using the WGCNA package in R. Gene-expression data were obtained from GSE25219 for 16,874 genes across 1,340 samples (75 individuals spanning 15 developmental stages with up to 16 brain regions per individual). Only regions with at least 10 samples were used, leaving 1,281 samples. After intersecting genes in the immune, synapse and methylation pathways, 797 genes were left for the network analysis, whose module parameters and membership are outlined in **Supplementary Table 17**. The specific parameters are made available in the supplemental R script for **Figure 4** (**Supplementary Software**). Similar modules were found with variations on these parameters.

The top 10 genes in each module are plotted in **Figure 4a** using the igraph package in R. Only correlations with $r > 0.2$ are shown, and the Fruchterman-Reingold force-directed algorithm as implemented in igraph was used to layout nodes using default parameters. Module expression profiles were summarized by taking the mean expression level of all genes in each module for each different brain region (across all time points) and each neurodevelopmental epoch (across all regions). We focused on relatively large changes between regions or time points (>75%), though statistical analysis of spatial and temporal patterns with Kruskal-Wallis tests and pairwise Mann-Whitney *U*-tests demonstrates many smaller differences are significant (due to the large sample size). In general, alternative methods for evaluating regional and temporal differences (for example, correlations with regional indicator variables, ANOVA with regions as factors) yielded similar patterns as those seen in **Figure 4b**. For simplicity of interpretation and discussion, we therefore chose to focus on these larger fold changes in order to highlight the most salient neurodevelopmental changes at the pathway level.

Cell type–specific enrichment for modules was performed by using the cell specific expression analysis (CSEA) tool (http://genetics.wustl.edu/jdlab/csea-tool-2/). This tool contains cell type–specific genes that are derived from a translational profiling approach that isolates transcriptomes in mouse from specific, marker-defined cellular subpopulations[21]. We assessed each module for enrichment of lists for 35 broad and specific cell type gene sets (CSEA specificity threshold set to 0.05) across multiple brain regions in mouse, and corrected for multiple comparisons across 7 modules and the 35 cell type lists. We report enrichments at Benjamini-Hochberg corrected $P < 0.05$ in **Figure 4c**. The code underlying this analysis is included as **Supplementary Software**.

A **Supplementary Methods Checklist** is available.

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