Archival Report

Biological Psychiatry

Major Depressive Disorder Is Associated With Differential Expression of Innate Immune and Neutrophil-Related Gene Networks in Peripheral Blood: A Quantitative Review of Whole-Genome Transcriptional Data From Case-Control Studies

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ABSTRACT

BACKGROUND: Whole-genome transcription has been measured in peripheral blood samples as a candidate biomarker of inflammation associated with major depressive disorder.

METHODS: We searched for all case-control studies on major depressive disorder that reported microarray or RNA sequencing measurements on whole blood or peripheral blood mononuclear cells. Primary datasets were reanalyzed, when openly accessible, to estimate case-control differences and to evaluate the functional roles of differentially expressed gene lists by technically harmonized methods.

RESULTS: We found 10 eligible studies (N = 1754 depressed cases and N = 1145 healthy controls). Fifty-two genes were called significant by 2 of the primary studies (published overlap list). After harmonization of analysis across 8 accessible datasets (n = 1706 cases, n = 1098 controls), 272 genes were coincidentally listed in the top 3% most differentially expressed genes in 2 or more studies of whole blood or peripheral blood mononuclear cells with concordant direction of effect (harmonized overlap list). By meta-analysis of standardized mean difference across 4 studies of whole-blood samples (n = 1567 cases, n = 954 controls), 343 genes were found with false discovery rate $<$5% (standardized mean difference meta-analysis list). These 3 lists intersected significantly. Genes abnormally expressed in major depressive disorder were enriched for innate immune-related functions, coded for nonrandom protein-protein interaction networks, and coexpressed in the normative transcriptome module specialized for innate immune and neutrophil functions.

CONCLUSIONS: Quantitative review of existing case-control data provided robust evidence for abnormal expression of gene networks important for the regulation and implementation of innate immune response. Further development of white blood cell transcriptional biomarkers for inflamed depression seems warranted.

Keywords: Eigengene, FDR, Gene ontology, Metafor, Protein-protein interaction networks, Reactome, Weighted gene coexpression network analysis, WGCNA

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Major depressive disorder (MDD) is a syndromal diagnosis, based entirely on self-reported symptoms and behavioral signs. No laboratory-based biomarkers are required for a DSM-5 diagnosis of MDD (1). Indeed, biomarker evidence for inflammatory disease would conventionally be regarded as prohibiting a diagnosis of MDD, instead implying an alternative diagnosis of secondary or comorbid depression. However, there is increasing interest in the concept that depressive symptoms, whether diagnosed as MDD or comorbid with bodily disorders, can be caused, in at least a proportion of cases, by inflammatory mechanisms (2,3). The evidence supporting this hypothesis has motivated a search for biomarkers of immune status that could be used to stratify MDD cases and to predict therapeutic response to anti-inflammatory drugs (2,4). Anti-inflammatory drugs have been associated with antidepressant effects in clinical trials for depression (5), in planned post hoc analysis of a subgroup of patients with MDD defined by a blood protein biomarker (6), and in clinical trials for arthritis and other systemic inflammatory or autoimmune disorders often associated with comorbid depression (7–9). It is already clear that to optimize the potential of anti-inflammatory interventions for depressive symptoms, new therapeutics must be precisely guided by development of immune biomarkers and companion diagnostics (4).

Blood is one of the most clinically convenient tissues to sample for immune biomarkers, whereas the brain is arguably the least convenient. For blood biomarkers to be relevant to the inflammatory pathogenesis of depression, it is assumed

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that the immune status of the central nervous system is correlated with, or caused by, the immune status of the periphery. Experimental work in animal models has demonstrated causal mechanisms that link peripheral inflammation to central inflammation, altered neuronal function, and quasi-depressive behaviors (10–13). There is growing evidence that gene transcription measured in human blood is correlated with transcripts measured in many other body systems including the central nervous system (14,15).

In the early 1990s, the search for inflammatory blood biomarkers of depression initially focused on acute phase proteins, such as C-reactive protein (CRP), and proinflammatory cytokines, such as interleukin-6 (16,17). Meta-analyses have demonstrated that CRP, interleukin-6, and some other cytokines are moderately but robustly increased on average in MDD cases versus controls (standardized mean difference [SMD] approximately 0.1–0.5) (18–21). However, the case-control difference in mean CRP concentration should not obscure the fact that only a minority of MDD cases (<30%) will have CRP greater than the upper limit of the normal range (3 mg/L) (22). Moreover, compared with the elevated proinflammatory cytokine levels observed in patients with autoimmune disorders, the levels found in MDD are at lower (pg/mL) concentrations, often below the lower limits of quantification or detectability for standard assays. Cytokine levels are responsive to exercise, diet, stress, time of day, annual season, and other potentially confounding factors. Most fundamentally, modestly increased blood levels of a few proteins do not specify causal pathways with cellular or subcellular precision (16).

In this context, gene expression in white blood cells has been increasingly investigated as an alternative class of immune biomarkers. Case-control studies have measured expression of a subset of preselected candidate genes in whole-blood, peripheral blood mononuclear cell (PBMC), and monocyte-sorted samples (23–25). Transcriptional differences have been repeatedly reported for candidate genes with innate inflammatory, glucocorticoid, and neuroplasticity-related functions (25). Recent technical advances have enabled transcriptional measurement of the whole genome, by microarray or RNA sequencing (RNA-seq) methods, in blood samples and postmortem brain tissue samples from MDD case-control studies (26,27).

Here we endeavored to quantitatively review all published whole-genome transcriptional datasets from peripheral blood samples in case-control studies of MDD (Table 1). First, we simply compiled the published overlap list of genes that were called significant by 2 or more of 10 methodologically heterogeneous studies. Second, to mitigate statistical heterogeneity between studies, we reanalyzed the subject-level statistics from 8 openly accessible datasets by technically harmonized standards so we could compile the harmonized overlap list of genes that ranked in the top 3% of differentially expressed genes that were concordantly overexpressed or underexpressed in MDD for 2 or more primary studies. Third, we meta-analytically estimated the SMD between cases and controls for each gene on average over 4 harmonized datasets from whole-blood samples to compile the SMD meta-analysis list of genes that were concordantly and differentially expressed at a false discovery rate (FDR) of 5%. We used ontology enrichment analysis, protein-protein interaction (PPI) networks, and gene coexpression networks to explore functional specialization and network roles of these MDD case-control gene lists.

METHODS AND MATERIALS

Primary Studies

Eligible studies were identified using PubMed, Google Scholar, and the Gene Expression Omnibus to search for peer-reviewed publications indexed by the terms “major depressive disorder,” “human,” “blood,” and “gene expression.” We found 28 studies reporting genome-wide transcriptional data, 18 of which used a case-control design. Some of these studies were subsequently excluded if 1) the transcriptional measurement was of microRNA, long noncoding RNA, or circular RNA (n = 3);) the study sample was composed solely of participants with geriatric onset depression (n = 2); 3) multiple patient samples were pooled before transcriptional measurement (n = 1); or 4) only candidate genes were reported (n = 2). One study (28) included data from cells at baseline and after lipopolysaccharide stimulation ex vivo; the lipopolysaccharide-stimulated data were excluded from further analysis, while the baseline data (i.e., before lipopolysaccharide stimulation) were included. This process resulted in 10 eligible datasets, originally published in 9 articles (Table 1), of which 8 datasets were accessible for harmonized reanalysis of subject-level data.

Harmonized Differential Expression Analysis

The harmonized workflow included 1) filtering out, rather than imputing, missing data or samples with missing metadata; 2) normalization of expression statistics between samples in the same dataset, either by quantile normalization for microarray data or by the DEseq2 median of ratios method for RNA-seq data (29); and 3) univariate statistical analysis of differential expression using a linear model that included the same covariates (age, gender, and batch, where available) for each study (see Supplement 1 for study-specific nuances of this procedure). A total of 24,976 genes were measured in at least 2 studies, which was the minimum criterion for a gene to be included in harmonized analysis.

Compilation of Harmonized Overlap List

For quantitative review of the 8 openly accessible datasets, we did not immediately discount smaller studies but leveraged consensus across studies to filter false positives in a way that is robust to outliers. We ranked genes within each study by their p value for MDD case versus control differential expression and thus defined the top 3% most differentially expressed genes, with the smallest p values, for each primary dataset. While any cutoff is somewhat arbitrary, the top 3% threshold includes approximately 500 genes per study, which is comparable to the median number of genes defined as statistically significant across all primary studies by multiple univariate analyses with $p < .01$ per gene. The harmonized overlap list comprised genes present in the top 3% list of 2 or more of the primary studies that were concordant in direction-of-effect or sign of differential (over- or under-) expression.

Table 1. Primary Studies of Genome-wide Blood Gene Expression in MDD Cases Compared With Healthy Controls

Publication	Tissue Type	Published Cohort	Platform	Covariate Adjustments	Reported Findings	Data Access/Samples in Reanalysis
Spijker *et al.*, 2010 (28)	Whole blood	21 MDD 21 CTL	Agilent 44K Human whole-genome arrays	None reported Age-/gender-matched	Genes: Report on 12-gene expression signature stimulated by LPS. 5 transcripts had directional mismatch on qPCR	GSE19738 LPS stimulated data not included in re-analysis Baseline data available on 33 MDD and 34 CTL
Yi *et al.*, 2012 (44)	Lymphocytes	8 SSD 8 MDD 8 CTL	Affymetrix Human Genome U133 plus 2.0 array	None reported Age-/gender-matched	Genes: Topline results for 48 genes that classified SSD+MDD vs. CTL	GSE32280
Savitz *et al.*, 2013 (45)	PBMCs	8 bipolar 21 MDD 24 CTL	Illumina Human HT-12 v4 Expression BeadChip	None reported Age-matched	Genes: 12 protein-coding and 14 non–protein-coding transcripts identified. 3 transcripts had directional mismatch on qPCR Enrichments: 2 pathways highlighted: inflammation; cell cycle and kinase signaling	GSE39653 21 MDD 24 CTL
Mostafavi *et al.*, 2014 (33)	Whole blood	463 MDD 459 CTL	Illumina HiSeq 2000	Adjusted for 39 covariates including BMI, smoking, medication, etc.	Genes: 29 genes selected at FDR ≤ .25. 14 additional genes included at $p_{uncorrected} < .05$ as members of the significant interferon α/β signaling pathway Enrichments: Biological functions including innate immune processes, vesicle trafficking, cell cycle regulation, and splicing Pathway enrichment of interferon α/β signaling pathway	Data available from Depression Genes and Networks study (PI, DF Levinsin) through application to: https://nimhgenetics.org 462 MDD 458 CTL
Guilloux *et al.*, 2015 (46)	Whole blood	34 MDD 33 CTL	Illumina HT12-v4.0 gene array	Age-/gender-adjusted (random intercept model)	Genes: 256 genes reported as significant at $p < .01$	Data not available
Jansen *et al.*, 2016 (31)	Whole blood	882 MDD 635 rMDD 331 CTL	Affymetrix U219 array	Age, gender, BMI, smoking, red blood cell count	Genes: 130 genes at FDR ≤ .1 for MDD vs. CTL Enrichments: Upregulated genes: enriched for IL-6 signaling; downregulated genes: enriched for natural killer cell pathways	dbGaP Study Accession: phs000486.v1.p1
Hori *et al.*, 2016 (47)	Whole blood	14 MDD 14 CTL	Agilent Whole Human Genome 4×44K array	None reported Age-/gender-matched	Genes: 230 genes identified by $p < .01$and fold-change >1.5	Data not available
Leday *et al.*, 2018 (32) (GSK-HiTDIP)	Whole blood	113 MDD 57 CTL	Affymetrix Human Genome U133 plus 2.0 array	Age, gender, batch, anxiety	Genes: 130 genes identified in HiTDIP dataset at FDR ≤ .1. Bayesian consensus analysis of HiTDIP and BRC datasets identified 165 genes Enrichments: Upregulated genes: innate immune system; downregulated genes: adaptive immune system	GSE98793 128 MDD 64 CTL
Leday *et al.*, 2018 (32) (Janssen-BRC)	Whole blood	94 MDD 100 CTL	Affymetrix Human Genome U133 plus 2.0 array	Age, gender, batch, anxiety	Genes: 12 genes identified in BRC dataset at FDR ≤ .1. Bayesian consensus analysis of HiTDIP and BRC datasets identified 165 genes	Request through https://yoda.yale.edu/unipolar-depression-blood-gene-expression-study 94 MDD 100 CTL
Le *et al.*, 2018 (48)	PBMC	78 MDD 79 CTL	Illumina HiSeq 3000	WGCNA: batch effect removed Module-phenotype associations corrected for age, gender, BMI, and batch	Report on 2 transcriptional network modules associated with MADRS score 1) 291 genes enriched for apoptosis, B-cell receptor signaling 2) 109 genes enriched for interactions of VPR with host proteins	https://github.com/insilico/DepressionGeneModules Counts for analyzing antisense strand were published, and counts from the sense strand have since been deposited. Both sense and antisense data were used for quantitative review. 78 MDD 79 CTL

Gene lists called significant for each study are provided in Table S1 in Supplement 1.

BMI, body mass index; CTL, healthy controls; dbGaP, the database of Genotypes and Phenotypes; FDR, false discovery rate; IL, interleukin; LPS, lipopolysaccharide; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; PBMC, peripheral blood mononuclear cell; PI, principal investigator; qPCR, quantitative polymerase chain reaction; rMDD, remitted MDD; SSD, subsyndromal depression; VPR, viral protein R; WGCNA, whole-genome coexpression network analysis.

We assessed the similarity between studies in terms of the pairwise concordance of the sign of case-control differential expression for each gene. For each primary study, we estimated a direction-of-effect vector, with +1 coding overexpressed genes and −1 coding underexpressed genes. Then the pairwise concordance between studies was defined as the cosine similarity between their 2 direction-of-effect vectors.

Compilation of SMD Meta-analysis List

We estimated the SMD, or Cohen's *d*, fitting a random-effects model using the R package metafor (30) to combine differential expression statistics across primary studies. This is technically appropriate for the meta-analysis of homogeneous datasets without obvious outliers. We therefore restricted this meta-analysis to the 4 large independent case-control studies of whole-blood samples (31–33) and to 16,302 genes measured in at least 3 of the studies. The meta-analytic effect size for each gene was tested under the null hypothesis of zero case-control difference in gene expression, with per-gene *p* values corrected for multiple comparisons using the Benjamini-Hochberg method (34), to compile the SMD meta-analysis list with FDR of 5% and low heterogeneity ($\tau^2 < .01$).

Enrichment Analysis

We used the enricher and cnetplot functions from the R package ClusterProfiler. A universe of background genes was defined by the set of 24,976 genes measured in 2 or more studies for the harmonized overlap analysis or the 16,302 genes measured in 3 or more studies in the SMD meta-analysis. Significant enrichment was defined probabilistically, controlling FDR < 5%, for GO Biological Processes (35), Reactome (36), and KEGG (37) pathways and visualized as gene-pathway association networks. We used Fisher's exact test to assess the enrichment of harmonized overlap or SMD meta-analysis gene lists for previously reported gene lists associated with obesity (38), smoking (39), and biological age (40) (see Supplement 1).

Protein-Protein Interaction Networks

PPI networks were visualized using the STRINGdb v11.0 (41) application within Cytoscape v3.7.2 (cytoscape.org). The probability of the number of edges in each PPI network, under the null hypothesis that the proteins were coded by a random set of genes, was calculated by permutation testing.

Weighted Gene Coexpression Network Analysis

To provide some independent, normative context for the MDD case-control gene lists, we accessed a public database of whole-genome transcription measured by RNA-seq in whole-blood samples from 755 healthy participants in the GTEx (Genotype-Tissue Expression) project (42) (see Supplement 1).

Then we used weighted gene coexpression network analysis (WGCNA) (43) to represent the pattern of correlations between all possible pairs of transcripts as a signed network or transcriptome. In this graph, each node is a gene, and each edge represents the coexpression or correlation between a pair of gene transcripts. Using WGCNA parameters of soft power = 14 and deep split = 4, the normative gene coexpression network was divided into 17 modules or communities (Tables S7 and S8 in Supplement 2).

We summarized each primary study by a set of 17 eigengenes, calculated by the WGCNA software function multiSetMEs, which represented the weighted average expression in each primary study of all genes affiliated to each of the normative network modules. The similarity of each pair of studies was then quantifiable by the plotEigengeneNetworks function in WGCNA, which calculates the preservation of normative network community structure between all pairs of modular eigengenes for each pair of primary datasets (see Supplement 1).

RESULTS

Primary Studies

Ten studies, originally published in 9 articles (28,31–33,44–48), satisfied eligibility criteria for quantitative review (Table 1). For 7 studies (28,32,33,44,45,48), the raw, patient-level data for all participants were openly accessible, and for 1 study (31), genome-wide differential expression statistics were available for all participants. Thus, 8 out of 10 eligible studies could be reanalyzed or meta-analyzed by technically harmonized methods (Table 2).

Sample size varied from approximately 1500 to approximately 10 MDD cases across studies. The 3 earliest studies (published 2010–2013) had small samples (≤ 20 cases) (28,44,45); later studies (published since 2014) had larger samples (approximately 100–1000 cases) (31,33). Some studies included subgroups of MDD cases, i.e., MDD with high anxiety (32) or remitted MDD (31).

Seven studies measured transcription in whole-blood samples, including all cell types; 2 studies measured transcription in PBMCs; and 1 study measured transcription in

Table 2. Summary of Key Parameters and Results of 3 Analyses Used for Quantitative Review

Analysis	Primary Studies	MDD Cases	Healthy Controls	Transcripts Measured	Differentially Expressed Genes	Overexpressed Genes in MDD Cases	Underexpressed Genes in MDD Cases
Published Overlap List	10	1754	1145	Variable between primary studies	52 (45 concordant)	19	26
Harmonized Overlap List	8	1706	1098	24,976 in at least 2 studies	272, concordant	139	133
SMD Meta-analysis List	4	1567	954	16,302 in at least 3 studies	343, concordant	229	114

MDD, major depressive disorder; SMD, standardized mean difference.

lymphocytes. Eight studies used one of a variety of microarray platforms to measure messenger RNA (6 in whole blood, 1 in PBMCs, and 1 in lymphocytes); 2 studies used RNA-seq (1 in whole blood and 1 in PBMCs) (Table 1).

Case-control differences in gene expression were statistically controlled for different sets of confounding factors (28,44,45) across studies. Age, gender, and messenger RNA assay batch were most frequently included as covariates in the linear model used for estimation of differential gene expression; however, some studies included zero covariates, whereas one study included 39 potential confounds (body mass index, tobacco smoking, medication, etc.) (33). Two studies used multivariate methods, such as WGCNA, but most studies reported only mass or multiple univariate analysis of between-study differences with a genewise threshold for statistical significance. Three studies used the FDR to account for the large number of tests (approximately 10,000) required to survey the whole genome and to control the number of false-positive tests expected by chance (31–33).

Published Overlap Gene List

Reading across all 10 studies, 1455 genes were called significant by at least one study (Table S1 in Supplement 2). Fifty-two genes were called significant by more than one study (Figure 1; Table S2 in Supplement 2) and therefore constituted the published overlap list. Of these genes, 45 had concordant sign of case-control differential expression: 19 genes were overexpressed and 26 were underexpressed in MDD cases. Only one gene, *SETD6*, was called significant by 3 studies (32,46,48). SETD6 is a protein that methylates the genes for nuclear factor-κB (49) and *PAK4*, promoting activation of the WNT/β-catenin pathway (50). The published overlap list was significantly enriched for functional terms "cell activation," "immune system processes," and "defense response to other organisms" (Figure 1; Table S3 in Supplement 2). However, the number of edges in the PPI network ($n = 11$) was not significantly greater than expected by chance ($n = 9$; $p > .05$) (Figure 1).

Harmonized Overlap List

A total of 543 genes were ranked in the top 3% most differentially expressed genes for at least 2 studies; 52 genes were common to at least 3 studies; and 3 genes, *LPCAT1*, *MS4A7*, and *TROVE2*, were among the top 3% in 4 studies (Figure 2; Tables S4 and S5 in Supplement 2). The 543 genes included 133 genes that were concordantly underexpressed and 139 that were concordantly overexpressed in MDD cases, out of 24,976 gene transcripts measured in at least 2 studies. These 272 concordant genes constituted the harmonized overlap list, which included significantly more ($n = 10$) of the 52 genes in the published overlap list than expected by chance ($p < 10^{-9}$, Fisher's exact test).

The harmonized overlap list was significantly enriched for the functional terms "cell activation," "endocytosis," "granulocyte-activation," "leukocyte-activation," "neutrophil-activation," and "degranulation" (Figure 2; Table S6 in Supplement 2). Genes in the harmonized overlap list were significantly overrepresented in the module of the normative transcriptional network specialized for neutrophil- and granulocyte-mediated immunity (Figure 3), and they coded for a PPI network comprising more known biochemical interactions ($n = 259$) than expected for a set of proteins coded by 272 randomly sampled genes ($n = 179$; $p < 10^{-7}$) (Figure 2).

Between-Study Similarity of Harmonized Transcriptional Datasets

By pairwise concordance analysis of the sign of differential expression, we found the most similar datasets were based on the largest whole-blood samples (31–33). Studies based on PBMC samples were more similar to each other than to studies of whole-blood samples (Figure 3). By preservation analysis of normative transcriptome community structure, we again found that the whole-blood studies (28,32,44) were more similar to each other than to the PBMC-based studies (Figure 3).

SMD Meta-analysis List

To identify a set of genes that were significantly differentially expressed across a homogeneous subset of studies, we estimated the SMD for each gene on average over the 4 independent studies of whole-blood samples that were most similar to each other by concordance and preservation analysis (31–33). This analysis revealed 343 genes (229 overexpressed and 114 underexpressed in cases) for which the SMD between cases and controls was significant at FDR $< .05$ and the heterogeneity was low, with $\tau < .01$ (SMD meta-analysis list) (Figure 4; Table S9 in Supplement 2). Of these genes, 21 were also included in the 272 genes of the harmonized overlap list, which was significantly more than expected by chance ($p < 10^{-7}$, Fisher's exact test).

The SMD meta-analysis gene list was highly enriched for similar pathways to those enriched in the harmonized overlap list: "cell activation involved immune response," "granulocyte-activation," "leukocyte-activation," "neutrophil-activation," and "degranulation." Additional enrichments were seen for functional terms "exocytosis," "cell death," and "apoptotic processes" (Figure 4; Table S10 in Supplement 2). Collectively these genes coded for a PPI network comprising more known biochemical interactions ($n = 362$) than expected for a set of proteins coded by 343 randomly sampled genes ($n = 296$; $p < .0002$) (Figure 4). Overexpressed genes in the SMD meta-analysis list were significantly overrepresented only in the module of the normative network specialized for neutrophil- and granulocyte-mediated immunity; underexpressed genes were significantly overrepresented in the normative network modules specialized for RNA processing and other functions (Figure 5).

Assessment of Potentially Confounding Factors

MDD case-control differences in gene expression could be confounded by many factors that were not consistently measured in all primary studies, e.g., obesity and smoking, and can therefore not be statistically controlled as covariates in the linear model used for harmonized analysis. However, whole-blood gene expression studies of obesity (38), smoking (39), and biological age (40) have previously published lists of genes associated with these potential confounds (Table S11 in Supplement 2). We tested the 272 genes in the harmonized

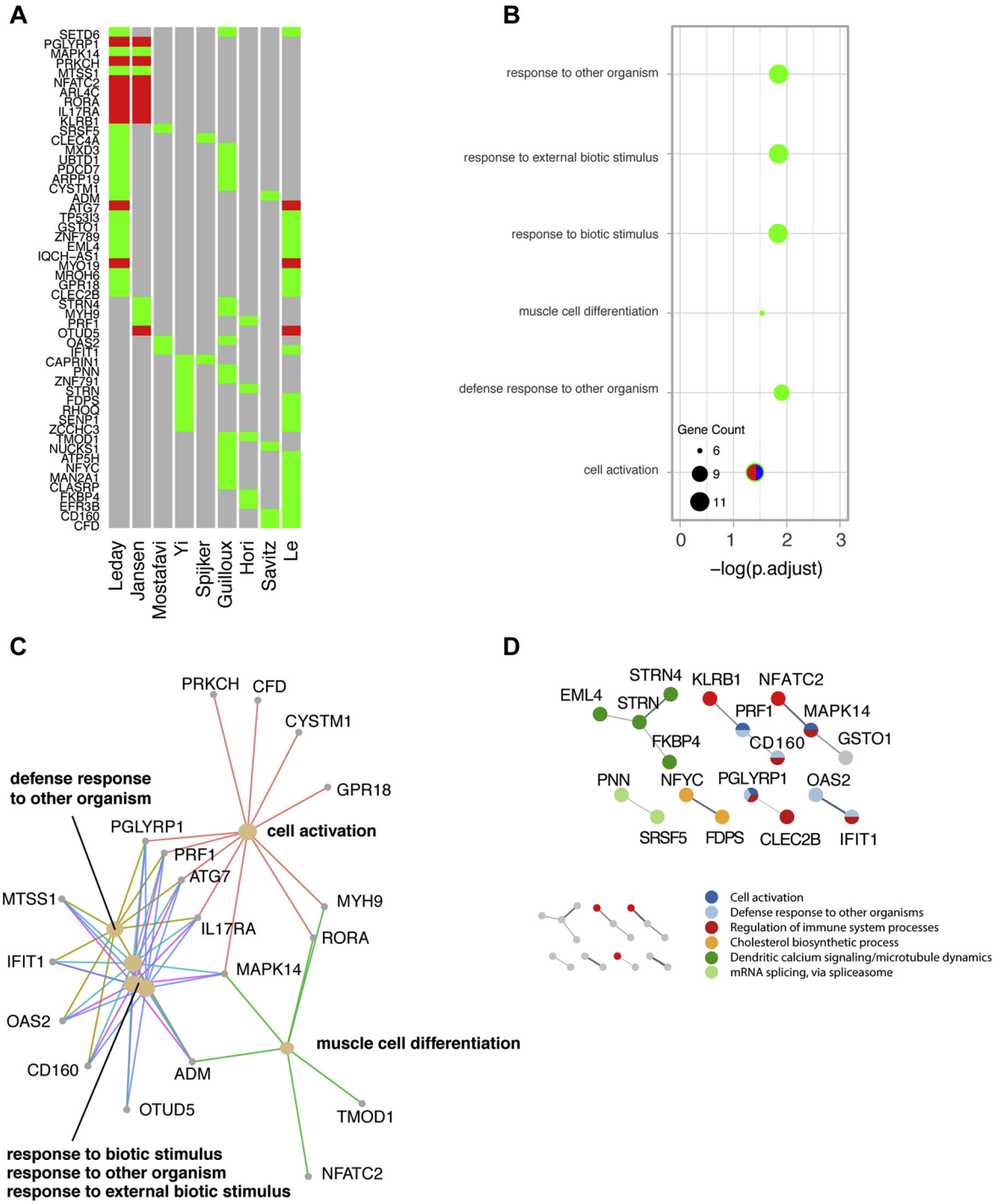

Figure 1. Published overlap gene list. **(A)** Fifty-two genes were called significant by 2 or more of 10 studies in 9 peer-reviewed publications (highlighted in green or red). Red genes were also included in the harmonized overlap list (Figure 2). **(B)** Functional enrichment analysis of the published overlap list. Red and blue highlights indicate a function (cell activation) that was also significantly enriched in the harmonized overlap list and in the SMD meta-analysis list, respectively. **(C)** Gene-pathway association network indicating genes affiliated to significantly enriched pathways. **(D)** Protein-protein interaction networks. Given the small number of genes, a medium level of confidence, 0.4, was selected to represent interaction edges between protein nodes; disconnected nodes are not shown. The 4-protein network, comprising EML4, STRN, STRN4, and FKBP4, was not immune related; these proteins are typically found in neuronal dendrites, where they are involved in calcium signaling and microtubule dynamics, and FKBP4 has been implicated in tau protein pathogenesis (58). Color indicates functional enrichment. (Inset) Red nodes indicate proteins that were also coded by genes in the harmonized overlap list. mRNA, messenger RNA; SMD, standardized mean difference.

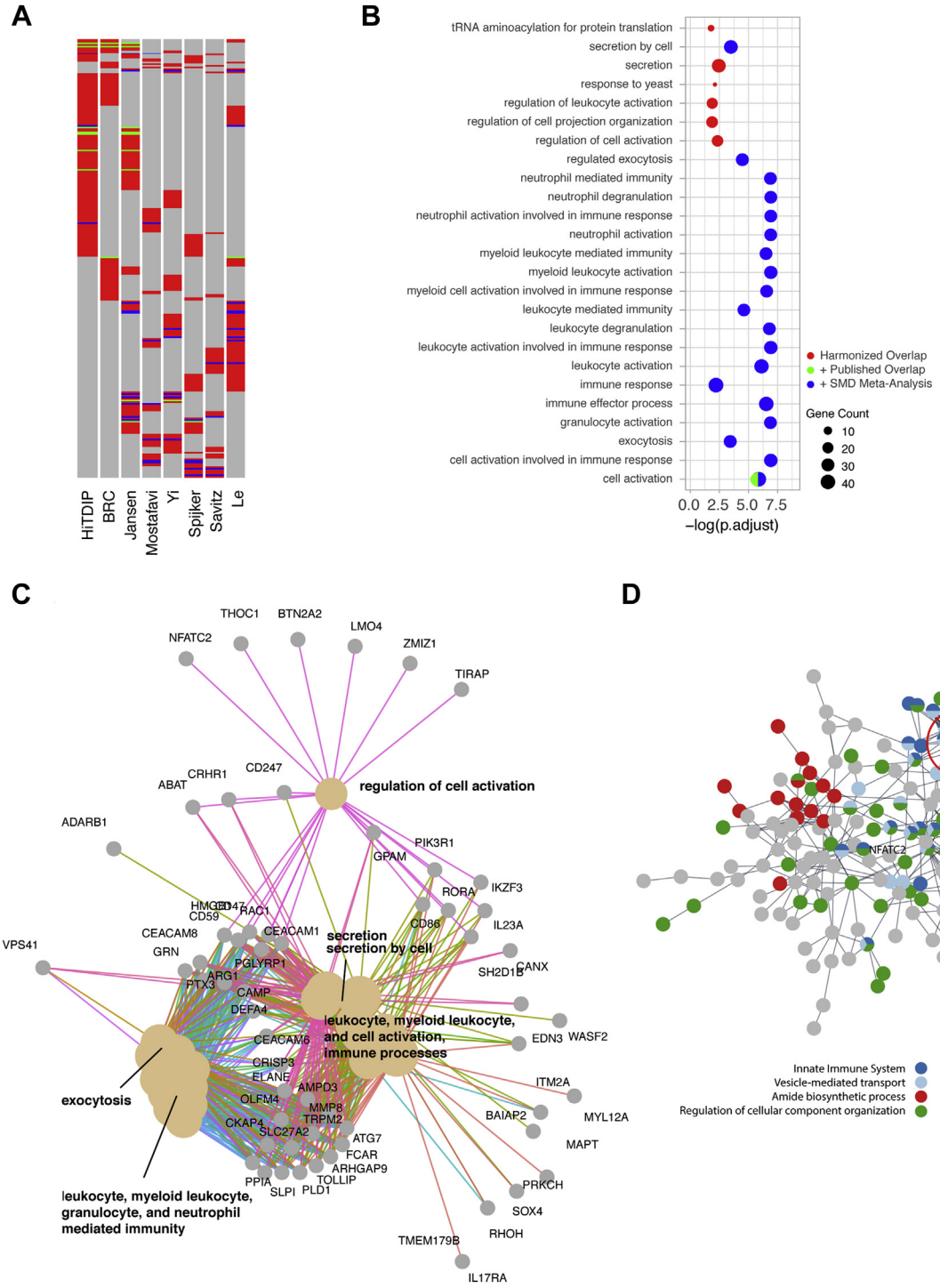

Figure 2. Harmonized overlap gene list. **(A)** A total of 272 genes were ranked in the top 3% most differentially expressed genes with concordant sign in 2 or more of 8 primary studies (highlighted in red). Green genes were also included in the published overlap gene list, and blue genes were also included in the SMD meta-analysis list. **(B)** Functional enrichment analysis of the harmonized overlap list. Green indicates a function that was also enriched in the published overlap list, and blue indicates a function that was also enriched in the SMD meta-analysis list. **(C)** Gene-pathway association network indicating genes affiliated to significantly enriched pathways. **(D)** Protein-protein interaction network. Given the larger set of genes in the harmonized overlap list, we used a higher level of confidence, 0.7, to represent edges, and neither disconnected nodes nor pairs of connected nodes were shown. Labeled genes circled in red are involved in neutrophil granule formation; other representative genes are labeled in black. Color indicates functional enrichment. (Inset) Green nodes indicate proteins that were also coded by genes in the published overlap list, and blue nodes indicate proteins that were also coded by genes in the SMD meta-analysis list. SMD, standardized mean difference; tRNA, transfer RNA.

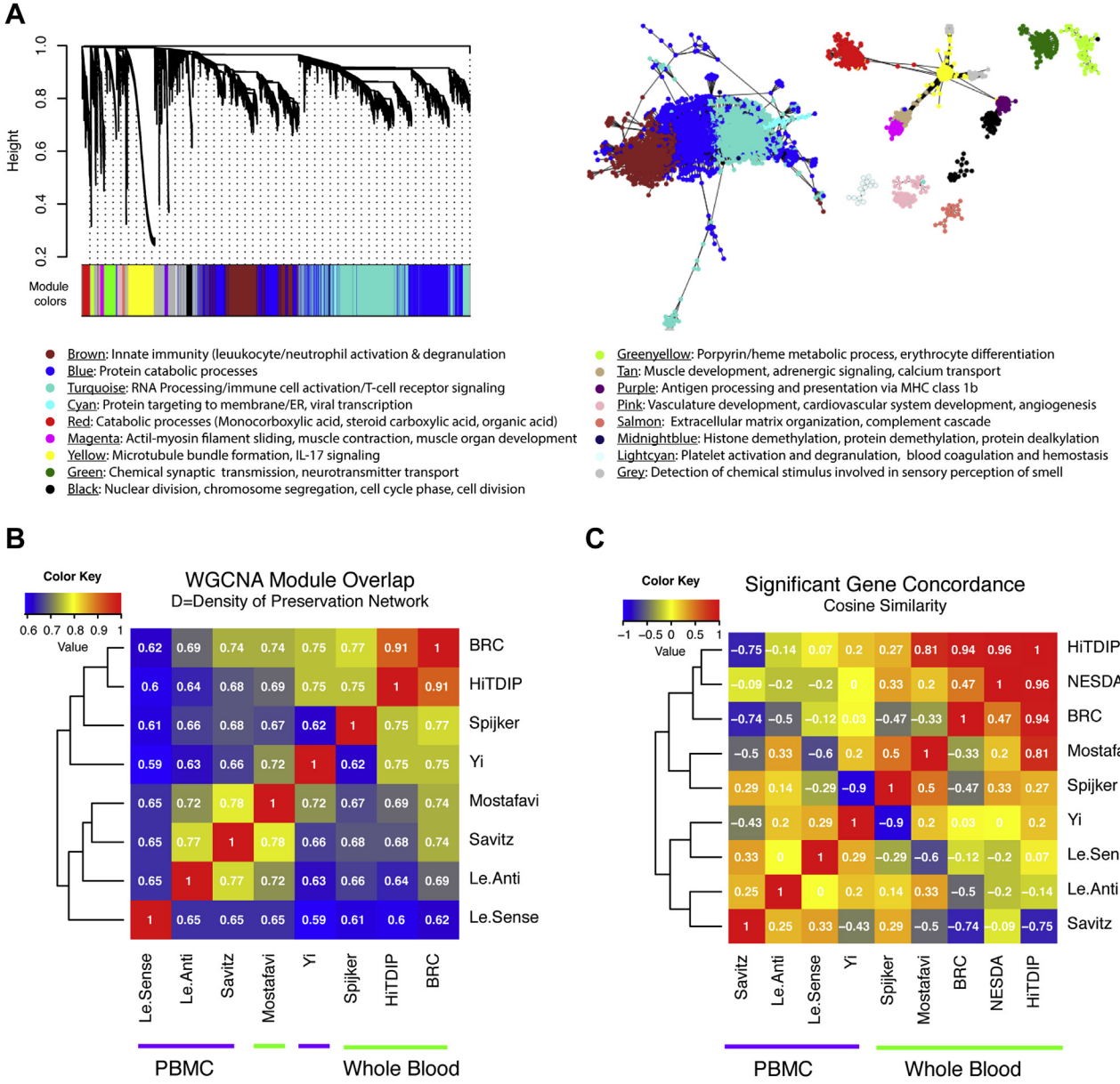

Figure 3. Normative whole-blood transcriptional network and similarity of primary studies. **(A)** WGCNA of whole-genome transcripts measured by RNA sequencing in whole-blood samples from 755 healthy controls in the independent GTEx cohort (42). The dendrogram (left panel) and topological overlap network (right panel) illustrate a community structure of 17 modules distributed across multiple connected components. Topologically, genes affiliated to the same module are strongly coexpressed with each other and weakly coexpressed with genes in different modules. Biologically, genes affiliated to the same module were enriched for proteins that shared the same biochemical or cellular functions, as indicated by color coding of nodes (bottom). **(B)** Harmonized control data from each study were used to calculate 17 eigengenes corresponding to the modules defined in the independent normative transcriptome. The similarity of each pair of primary studies was then quantified in terms of preservation of the normative network community structure (white text and color code in panel **A**). Hierarchical clustering on the pairwise preservation density scores highlights the greater similarity between whole-blood studies. **(C)** Pairwise concordance of the differential expression direction-of-effect vectors was estimated for each pair of studies. Hierarchical clustering on the pairwise concordance scores highlights the relatively high similarity between studies of whole-blood samples and the low similarity between studies of whole-blood and PBMC samples. ER, endoplasmic reticulum; IL-17, interleukin-17; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; WGCNA, weighted gene coexpression network analysis.

overlap list and the 343 genes in the SMD meta-analysis list for intersection with these prior gene sets. There were no significant intersections between either of the 2 MDD-related lists and the smoking- or age-related gene sets; there were 2 obesity-related genes (*RPS7* and *RPS3A*) in the harmonized overlap list (p = .03).

DISCUSSION

We reviewed 10 whole-genome transcriptional case-control studies of MDD, collectively including data on 1754 MDD cases and 1145 healthy controls (Table 1). We have reported 3 principal analyses (Table 2), of which only the first is descriptive: we described the set of genes reported as significant by

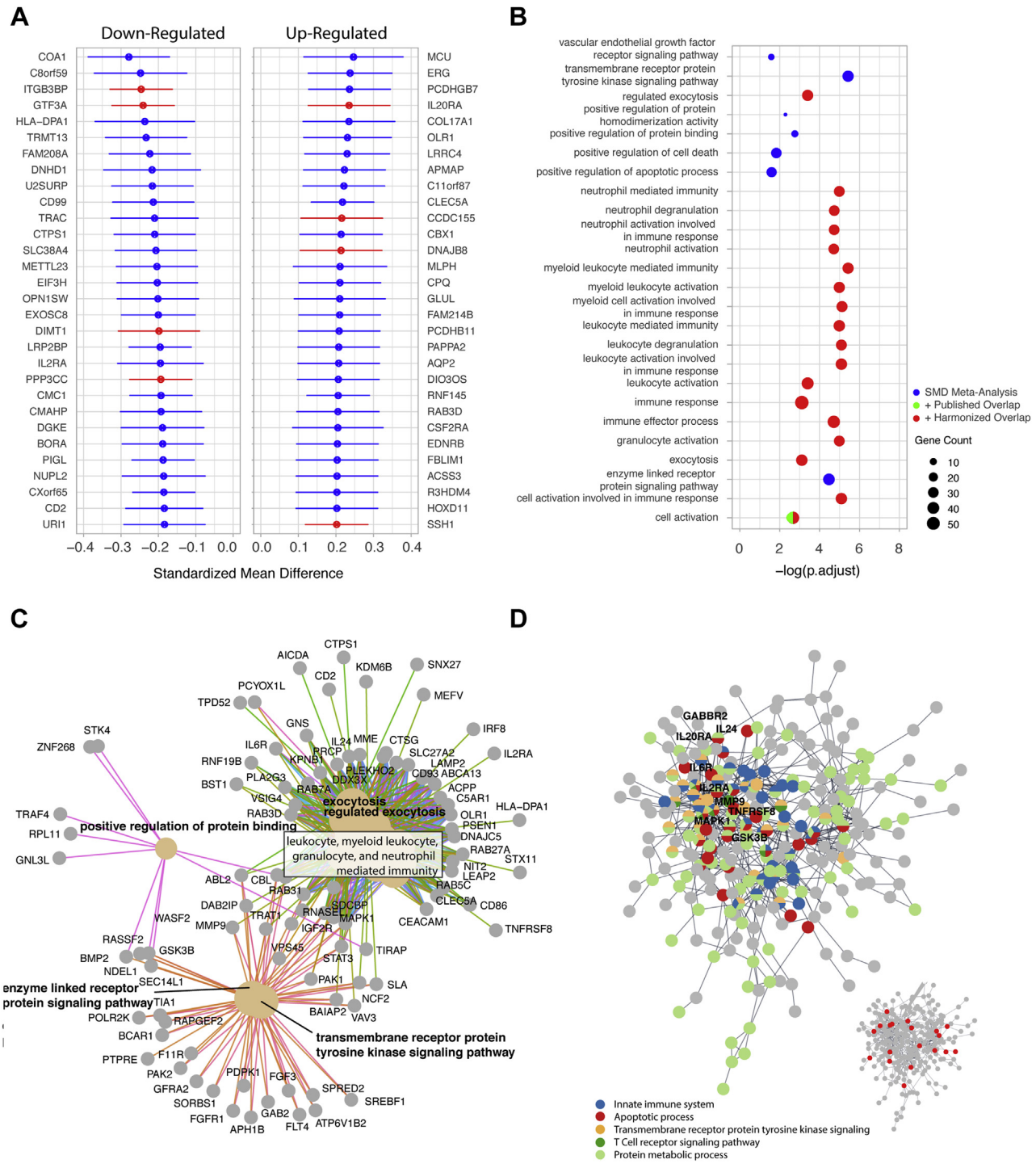

Figure 4. SMD meta-analysis gene list. **(A)** A total of 343 genes were identified with significant case-control difference in expression at FDR < .05, and the top 30 overexpressed and underexpressed genes, each with |SMD| ≥0.2, are shown here. Red genes were also included in the harmonized overlap gene list. **(B)** Results of functional enrichment analysis of the 343 genes in the SMD meta-analysis list. Red indicates functional terms that were also significantly enriched in the harmonized overlap list. **(C)** Gene-pathway association network indicating genes affiliated to the most significantly enriched pathways. **(D)** Protein-protein interaction network. Color of each node indicates functional enrichment of corresponding proteins. (Inset) Red nodes indicate proteins that were also included in the harmonized overlap list. FDR, false discovery rate; SMD, standardized mean difference.

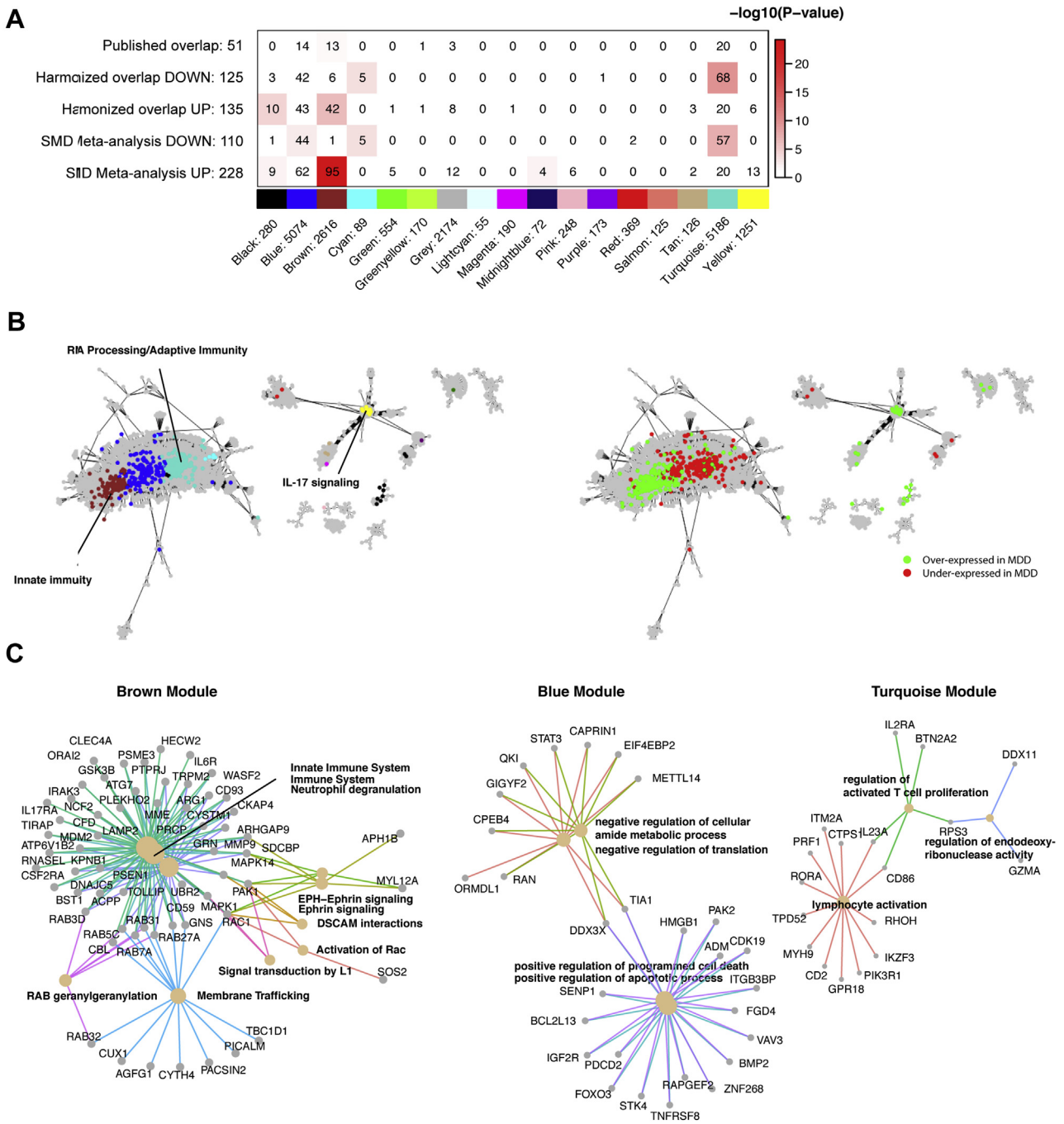

Figure 5. MDD-related differential gene expression in the context of the normative transcriptome. **(A)** Table summarizing overlap between MDD case-control gene lists (rows) and modules of the normative gene coexpression network. Significant *p* values, by Fisher's exact test, are highlighted in shades of red. For this analysis, the SMD meta-analysis list was subdivided into genes that were significantly overexpressed ($n = 229$) or underexpressed ($n = 114$) in MDD cases with FDR ≤ .05. **(B)** The omnibus list of 660 genes that were included in at least 1 of the 3 lists, with concordant sign of differential expression, were projected onto the normative transcriptome (see also Figure 3). (Left panel) Nodes corresponding to differentially expressed genes are colored according to their normative modular affiliation. (Right panel) Nodes corresponding to differentially expressed genes are colored green if they were overexpressed in MDD cases and red if they were underexpressed in MDD cases. **(C)** Gene-pathway association networks for the 3 normative modules that were most significantly enriched for genes differentially expressed in MDD. FDR, false discovery rate; IL-17, interleukin-17; MDD, major depressive disorder; SMD, standardized mean difference.

one or more of the primary studies. This preliminary effort to "read across" primary studies highlighted the high degree of heterogeneity between studies on many important factors, including the blood tissue type (whole blood or PBMCs), the microarray or RNA-seq platforms used to measure whole-genome transcription, the diagnostic and eligibility criteria used to define depressed cases, and the methods used for statistical analysis. To assess and mitigate the statistical contribution to heterogeneity, we accessed individual whole-genome transcripts or differential expression statistics deposited in open repositories by 8 studies and completely reanalyzed these primary data using identical models and standards for statistical significance. Finally, to address the cellular contribution to heterogeneity, we restricted attention to 4 studies of whole-blood samples, which represented the majority of the available case-control data (Table 2).

We first compiled the published overlap list of 52 genes that were called significant by at least 2 primary studies. This list was enriched for cell activation and defense response to other organisms, but it did not code for a densely connected PPI network (Figure 1). It is a signal, but not a strong one, probably owing to high methodological heterogeneity. We therefore estimated differential expression statistics by technically harmonized methods and then compiled the harmonized overlap list of genes that were ranked in the top 3% of the most differentially expressed genes, with concordant sign of overexpression or underexpression, in at least 2 studies. This list comprised 272 genes that were enriched for neutrophil and other innate immune-related functions and coded a set of proteins with more known biochemical interactions between them than expected by chance (Figure 2).

However, harmonized data analysis disclosed another important cellular source of heterogeneity between studies: the use of whole-blood versus PBMC samples. Primary studies measuring differential expression in whole-blood samples were more consistent with each other and with the modular community structure of the normative transcriptome (42) than with the results of primary studies based on PBMCs (Figure 3). This is not surprising, as PBMC samples by definition exclude platelets and neutrophils (the largest single class of peripheral immune cells), so PBMC transcripts are expected to have lower levels of neutrophil-related gene expression than whole-blood transcripts.

To control cellular heterogeneity, we focused on 4 primary studies that had measured gene expression in whole blood. We meta-analytically estimated the SMD between MDD cases and healthy controls in expression of each of 16,302 genes measured in at least 3 studies and probabilistically thresholded these statistics with an FDR of 5%. The SMD meta-analysis list comprised 343 genes that were differentially expressed with concordant sign in depressed cases compared with healthy controls (Figure 4). There was a significant degree of convergence between this list and the harmonized overlap list. The SMD meta-analysis list was also significantly enriched for neutrophil activation and degranulation, apoptosis, and transmembrane signaling, and it coded a PPI network that was significantly more densely connected than expected by chance. The 229 genes that were concordantly overexpressed in the SMD meta-analysis list were significantly affiliated to the module of the normative transcriptome specialized for neutrophil functions (Figure 5).

These methodologically harmonized results more convincingly indicate that MDD is robustly associated with increases in expression of neutrophil-related and innate immune genes. It is a stronger signal, but what does it mean biologically and in relation to the pathogenesis of depression?

Biologically, this transcriptional signal from whole blood could represent either an MDD-related increase in the number of neutrophils, or relative overexpression of inflammatory genes by circulating neutrophils, or both an increased number and activation status of neutrophils owing to expansion of more developmentally immature and hypersegmented subclasses of neutrophils (51). We were unable to explore this issue any further immediately owing to lack of cell count data provided by the primary studies. There is prior evidence that MDD is associated with increased numbers of neutrophils (52) and increased neutrophil/lymphocyte ratio (53–55). Flow cytometry and transcriptional analysis of sorted cell classes or single cells could be used in the future to resolve the immune cellular phenotype and its relationship to transcriptional changes more precisely.

In relation to pathogenesis of MDD, these case-control differences in innate immune gene expression (SMD approximately 0.2–0.5) (Figure 4) are of the same order of magnitude as previously reported case-control differences in CRP and inflammatory cytokines (SMD approximately 0.1–0.5). There is some prior evidence that increased neutrophil counts are positively correlated with increased inflammatory proteins in MDD (52), and neutrophils are known to produce many cytokines and chemokines (56). Thus, neutrophil expansion and/or activation may constitute at least one of the cellular sources of peripherally increased proinflammatory cytokines in MDD, which in turn could communicate across the blood-brain barrier to cause central immune state changes and depressive behaviors (10–13). We expect that these small- to moderate-sized case-control group mean differences are representative of one or more subgroups of inflamed cases, e.g., with hyperphagia and higher BMI (57), included within the broad clinical syndrome of MDD.

The main technical focus of our review has been to mitigate statistical and cellular sources of heterogeneity when comparing or aggregating data between primary studies. We have been able to do this post hoc, from openly accessible or published data, but only to some extent. There are some aspects of methodological heterogeneity, such as microarray versus RNA-seq assays or differences in diagnostic or eligibility criteria for caseness, that we have not addressed. There are also many potential confounding factors, e.g., comorbid medical disorder, that were not consistently controlled a priori or easily controllable post hoc across this set of studies. We benchmarked the MDD-harmonized gene lists against prior lists of genes differentially expressed in association with age, smoking, and obesity and found little evidence for confounding effects on blood transcription. However, future biomarker studies of depression might endeavor to go beyond case-control binarization and collect richer clinical data to explore the relationships between immune profiles and subsyndromes of MDD and comorbid depression.

Current best practice in the bioinformatics community includes depositing genome-wide expression data in an accessible repository, such as the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) or the database of Genotypes and Phenotypes (https://www.ncbi.nlm.nih.gov/gap/). Our results clearly demonstrate the value added to primary publications if the raw data on individual participants are openly accessible for harmonized reanalysis and meta-analysis. It is hoped that future studies will share measurement, analytic, and open science protocols to minimize unnecessary heterogeneity between studies and to accelerate collective convergence on optimal standards.

Overall, we consider that this quantitative review provides encouraging evidence of consistent and significant blood transcriptional changes, especially in neutrophil and other myeloid cell–related genes, which merit further investigation as candidate biomarkers of depression associated with inflammation.

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