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A Transcriptomic Signature of Depressive Symptoms in Late Life

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ABSTRACT

BACKGROUND: Depressive symptoms in late life can impair daily function and accompany cognitive decline. However, the molecular mechanisms that underlie these changes in the brain remain poorly understood.

METHODS: Differential expression analysis was performed on bulk-tissue RNA sequencing data generated from dorsolateral prefrontal cortex samples of elderly participants in ROS/MAP (Religious Orders Study and Memory and Aging Project; N = 998, mean age at death = 89.7 years). Bulk tissue RNA sequencing was analyzed against depressive symptoms measured prior to death, controlling for Alzheimer's disease neuropathology, medication status, and lifestyle factors. Sex-stratified models were also tested.

RESULTS: Increased abundance of the Prader-Willi syndrome–associated gene *PWAR1* (corrected $p = 5.47 \times 10^{-3}$) and *CTDSPL2* (corrected p = .03) were associated with a higher burden of depressive symptoms in the combined sample. An additional 14 genes showed suggestive associations, including several with known links to neuropsychiatric illness (e.g., *ACVR2B-AS1*, *COL19A1*). Functional enrichment analysis revealed downregulation of aerobic metabolism and upregulation of both amino acid catabolism and DNA modification processes. Differential expression signatures were poorly correlated between males and females (Pearson r = 0.12; 95% CI, 0.10 to 0.13), and only the male group showed independently significant differential expression. Little overlap was found with previously published analyses of major depressive disorder.

CONCLUSIONS: Building on recently published single-nucleus profiling, we present the largest-ever study of transcriptomic correlates of depressive symptoms in late life, revealing new insights into sex-specific regulators. *PWAR1* and *CTDSPL2* were identified as putative markers of late-life depression in the dorsolateral prefrontal cortex and warrant further study.

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Depressive symptoms are commonly observed in late life and are often associated with neurodegenerative conditions such as Alzheimer's disease (AD) and other dementias (1,2). Importantly, the causal factors and neurobiology of late-life depression (LLD) may differ from those of early- and midlife depressive syndromes, which are commonly diagnosed as major depressive disorder (MDD) (3). To improve our understanding of these mechanisms, genomic technologies offer the opportunity to describe molecular signatures of LLD.

In populations of older adults, dozens of candidate gene studies, as well as one genome-wide association study (4), have been performed to identify genetic variants associated with depression compared with age-matched controls (5). This has led to the identification of several genetic variants, including those in *APOE*, *BDNF*, and *SLC6A4*, as being potentially important in LLD. Work in animal models has provided a deeper understanding of the specific roles played by each of these loci in promoting LLD; for example, transgenic mouse models with 2 copies of the human AD risk allele *APOE* ε4 (*Apoe4/Apoe4*) exhibit significantly more depressive-like behaviors than *Apoe3/Apoe3* mice both when unperturbed and when subjected to stress (6). BDNF (brain-derived neurotrophic factor) reduction may also be involved in the loss of hearing and memory in late life in the context of depression (7), and methylation of serotonin transporter *SLC6A4* may promote LLD (8).

Compared to studies of genetic variation, transcriptomic investigations of depressive symptoms in late life are less common. One study used microarray analysis in peripheral blood of 36 participants to identify over 3000 genes differentially expressed in clinical LLD and found 14 genes with convergent evidence in a mouse model (9). In another investigation, gene coexpression network analysis was performed on the same dataset, and evidence was found for perturbations in immune pathways in LLD (10). Several studies have examined differential gene expression in brain tissue in the context of MDD. Labonté *et al.* (11) measured gene expression in postmortem brain samples of 48 individuals of

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French-Canadian origin (26 MDD, 22 control), with equal numbers of males and females. Labonté *et al.* (11) determined that MDD-specific transcriptional signatures differed greatly between males and females (at most 15% overlap) and closely resembled signatures in male and female mice subject to chronic variable stress. Seney *et al.* (12) replicated these findings and also demonstrated strong sex-dependent expression differences in depression. Most recently, transcriptome-wide association analysis and single-nucleus RNA sequencing (RNA-seq) differential expression analysis of postmortem brain cells from 424 elderly donors were performed, identifying 76 genes associated with depressive symptoms (13). Many of these genes localized to neurons and astrocytes, and fewer than 20% of these associations had been found previously. These important studies have informed our molecular understanding of depression in midlife and late life.

To build on these findings, we have conducted transcriptome-wide differential expression analyses using the largest-ever postmortem bulk-brain dataset from prefrontal cortex tissue, over an order of magnitude larger than the previous largest postmortem study of midlife depression (12) and over twice the size of the recent single-cell effort by Zeng *et al.* (13). This dataset includes antemortem depressive symptom assessments and full autopsy-based neuropathological measures. We tested a series of differential expression models to provide insight into the molecular correlates of LLD symptom burden, independent of pathological AD diagnosis or medication status and stratified by biological sex.

METHODS AND MATERIALS

Study Participants

We analyzed data from up to 998 older adults (mean age at death: 89.7 years [range: 67–108]) from ROS/MAP (Religious Orders Study and Memory and Aging Project) (14). ROS and MAP are ongoing longitudinal studies of older persons that include comprehensive annual assessments of cognition, daily function, and brain donation at the time of death. Postmortem, brains were assessed for neuropathology, and brain tissue from the dorsolateral prefrontal cortex (DLPFC) was profiled for gene expression using RNA-seq. All participants provided informed written consent and signed an Anatomic Gift Act and repository consent. Both studies were approved by the Institutional Review Board at Rush University Medical Center.

Depressive Symptoms and Diagnosis of AD

Our primary outcome, depressive symptom burden, was calculated as the sum of responses on the 10-item version of the Center for Epidemiologic Studies Depression (CES-D) scale (15). This measurement was made at the last study visit prior to death for all participants. To account for potentially unreliable subjective assessments of depressive symptoms in participants diagnosed with dementia, we calculated a second score as the averages of CES-D measurements from study baseline prior to a probable clinical diagnosis of mild cognitive impairment. For CES-D measured at the last visit, 382/998 participants (142 male and 240 female) had scores of 0 (no symptoms).

Neuropathologic diagnosis of AD was assigned as a binary variable according to National Institute on Aging-Reagan criteria, assessed postmortem, namely the presence of neurofibrillary tangles (as assessed by Braak scoring) and neuritic plaques (as assessed by CERAD [Consortium to Establish a Registry for Alzheimer's Disease] scoring) (16,17). AD was defined as high or intermediate likelihood of AD, and no AD was defined as low likelihood or no AD.

RNA-Seq Data

Bulk tissue RNA-seq was performed on postmortem tissue samples from the DLPFC in 13 batches. Additional quality control steps were performed for RSEM-generated expected counts, which were aggregated from the 13 initial batches to form a single dataset. This resulted in a final dataset of 998 participants with nonmissing outcome and covariate data. Additional sample and RNA-seq processing information can be found in the Supplement.

Estimation of Cell-Type Proportions

An expression matrix corrected for estimated DLPFC cell-type proportions (CTPs) was generated prior to all differential expression analyses to remove potential confounding. CTPs were estimated using BRETIGEA (18) in R. From a set of 50 human marker genes from Darmanis *et al.* (19), 46 genes were used in the application of BRETIGEA's singular value decomposition method (the adjustBrainCells function). For secondary analyses, we used the MarkerGeneProfile R package (20) and single-nucleus RNA-seq–derived marker gene lists to estimate 18 CTPs in bulk RNA-seq data, as described previously (21).

Modeling of Differential Gene Expression

The Limma (22) lmFit function was used to fit robust linear regression models to expression data. The eBayes function was used to assess effect significance. Covariates included pathological diagnosis of AD, biological sex, postmortem interval, age at death, years of education, *APOE* ε4 carrier status, AD medication status at last visit, depression medication status at last visit, RNA sequencing batch, and a set of technical covariates (Table S1). Models were also tested with smoking status and average reported daily alcohol consumption at study entry as additional covariates.

Additional analyses were performed to test sensitivity to our modeling assumptions, namely that 1) the measurement of depressive symptoms at the last study visit was not confounded by concomitant dementia and 2) different latencies between last depressive symptom measurement and participant age of death did not significantly impact bulk expression signatures. To do this, analyses were repeated as before, first modeling the outcome of the average CES-D sum from baseline up to the year of first AD diagnosis rather than just the participant's final observation. Scores for depressive burden at the last visit were correlated with averages of scores taken from baseline to first AD diagnosis. As an additional check against the possibility of severe cognitive impairment confounding our findings, we also performed stratified analyses only in participants without a clinical diagnosis of dementia at the time of death (n = 562 total; 359 female, 203 male). Second, models were fit including a covariate

representing the latency (in years) between a participant's last study visit and their postmortem evaluation.

To account for the potential impact of cerebrovascular disease, models were also generated with cerebral atherosclerosis and arteriosclerosis included as covariates; the variable cvda_4gp2 rates large vessel atherosclerosis from none or possible (0) to severe (3) based on visual inspection, while arteriol_scler rates histological changes in small blood vessels from none (0) to severe (3) through imaging.

To assess the associations of estimated CTPs for brain-cell subtypes derived from MarkerGeneProfile analysis, we performed linear regressions for each cell-type estimate against depressive symptoms, with covariates as specified above for differential expression analysis. Two-sided p values were corrected using the false discovery rate (FDR) method.

Gene Set Enrichment Analysis

Lists of genes ranked by effect (moderated t statistic) served as input for gene set enrichment analysis (GSEA) using the gseGO function in the R package clusterProfiler (23). GSEA aims to determine whether specific sets of genes are enriched across a ranked list of association statistics. Genes with similarly high or low ranks that participate in shared pathways may provide evidence for the aggregate involvement of the pathway as a whole. Using GSEA, we analyzed the entire set of genes reported, ranked by effect statistic, as specified for each contrast. Lists of enriched pathways were visualized using the gseaplot2 function of the enrichplot R package (24). Significant gene ontology groups ($q_{FDR} < .05$) were consolidated to minimize semantic redundancy using the REVIGO tool (25), with the dispensability parameter set to 0.

Comparison With Published Data

To explore whether genes identified in our analyses overlapped with those associated with MDD in earlier studies by Labonté *et al.* (11) and Seney *et al.* (12), we extracted lists of genes nominally associated with depressive symptoms (uncorrected $p < .05$). Shared gene candidates were defined as genes with $p < .05$ in each of the 3 studies. Given the strong sex specificity of differential expression patterns observed across studies, this procedure was followed independently for male and female results. In addition, we compared association statistics for 11 genes identified as being significantly associated with depressive symptoms by Zeng *et al.* (26) in individual cell types (6 for depressive symptoms at the last study visit and 5 for average depressive symptoms over study period): *ADHFE1*, *DCUN1D2*, *NTPCR*, *AC005225.2*, *KCNN2*, *SCAI*, *WASF3*, *TBC1D5*, *PSMG1*, *SOCS6*, and *SAP130*. Notably, in our bulk analyses, *AC005225.2* was not detected.

RESULTS

Genes Associated With LLD Symptoms

In total, 625/998 participants (62.6%) had a neuropathologic diagnosis of AD at autopsy (435/663 females [65.6%] and 190/335 males [56.7%]). Differential expression analysis of the full dataset revealed significant associations ($q_{FDR} < .05$) for 2 genes: *PWAR1* ($q = 5.47 \times 10^{-3}$, $t = 5.15$) and *CTDSPL2* ($q = .03$, $t = 4.68$) (Figure 1A and Table S2). Twelve additional genes passed a more liberal threshold of $q < .1$: *AP006623.1*, *ACVR2B-AS1*, *LINC00865*, *CCDC177*, *STEAP4*, *MRM1*, *ARHGAP22*, *ADGRE2*, *IRF8*, *KCNH7*, *PDYN*, and *COL19A1*.

Depressive burden measured at the last visit and average overall depressive burden prior to first AD diagnosis were moderately correlated across all participants (Pearson $r = 0.70$; 95% CI, 0.67 to 0.74) (Figure S2A). Sensitivity analyses of this phenotype indicated that no genes were significantly associated with average CES-D sum values calculated from baseline to first AD diagnosis (Figure S1A), with transcriptome-wide effects showing a moderate correlation (Pearson $r = 0.43$; 95% CI, 0.42 to 0.44) (Figure S2D). In stratified analysis of the non-AD subset ($n = 562$), *PWAR1* and *CTDSPL2* were no longer transcriptome-wide significant ($q = .16$ and $q = .38$, respectively); however, they remained among the top genes associated with depressive symptoms (*PWAR1* uncorrected $p = 2.9 \times 10^{-3}$; *CTDSPL2* uncorrected $p = 2.2 \times 10^{-4}$). Full association statistics for the non-AD subset analysis, including sex-stratified models, are presented in Tables S3–S5. Models were not affected by the inclusion of a variable representing latency between last depressive symptom measurement and postmortem evaluation (Figure S3A). Correcting for cerebrovascular disease covariates revealed 2 additional genes significantly associated with depressive burden: *ACVR2B-AS1* ($q = .048$, $t = 4.48$) and *AP006623.1* ($q = .049$, $t = -4.41$). We found no significant associations between estimated CTPs and depressive symptomatology after correction for multiple testing (Table S6).

Rank-based enrichment testing with GSEA revealed 34 significantly enriched biological processes. When consolidating gene ontology terms based on semantic similarity, general functional themes emerged (Figure 1B); pathways most enriched included downregulated pathways of aerobic respiration, such as proton motive force–driven ATP (adenosine triphosphate) synthesis ($q = 1.05 \times 10^{-4}$, normalized enrichment score [NES] = −2.32), aerobic electron transport chain ($q = 1.44 \times 10^{-3}$, NES = −2.06), and mitochondrial ATP synthesis coupled electron transport ($q = 1.44 \times 10^{-3}$, NES = −2.05). Upregulation of pathways related to DNA modification and mitosis/meiosis was also observed, specifically chromatin remodeling ($q = 1.14 \times 10^{-3}$, NES = 1.74), DNA geometric change ($q = 1.51 \times 10^{-3}$, NES = 2.00), and nuclear chromosome segregation ($q = 9.52 \times 10^{-3}$, NES = 1.61). In contrast to the results of sensitivity analyses at the gene level, at the pathway level, aerobic respiratory pathways were also significantly enriched in association with average depressive symptom burden prior to first AD diagnosis (Figure S1B). Again, the incorporation of measurement latency as a covariate did not impact pathways linked to depressive symptoms in late life (Figure S3B).

Sex-Specific Differential Expression Analysis

Previous work by Labonté *et al.* (11) and Seney *et al.* (12) demonstrated strong sex-specific associations of gene expression with MDD (27). Therefore, next we performed sex-stratified differential expression analysis. In female participants ($n = 663$), no genes were significantly differentially expressed at $q < .05$ or $q < .1$ (Figure 2B). However, in males ($n = 335$), we observed significant associations for *FCGR2A* ($q = 4.83 \times$

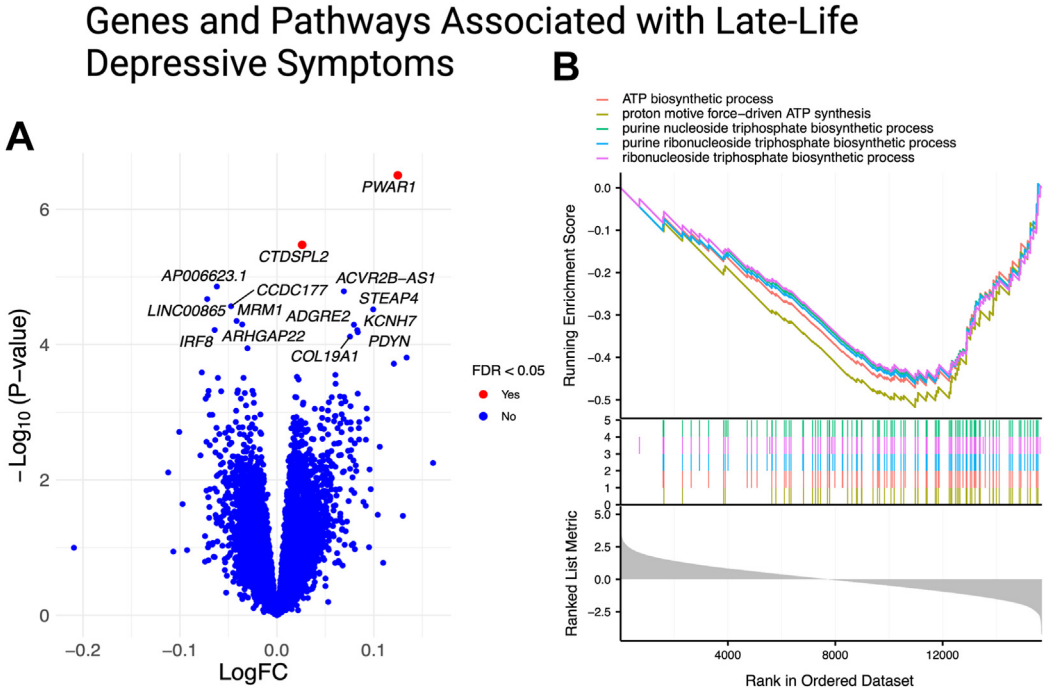

Figure 1. Differential expression and pathway analyses identified genes and pathways significantly associated with antemortem clinical depression during late life. **(A)** Volcano plot depicting gene associations with late-life depressive symptoms. Red, FDR < .05; blue, FDR > .05. Labeled genes, FDR < .1. **(B)** Gene set enrichment analysis plot showing the top 5 pathways most significantly enriched across depression-associated genes. ATP, adenosine triphosphate; FC, fold change; FDR, false discovery rate.

10^{-2}, $t = 4.63$), *ENPEP* ($q = 4.83 \times 10^{-2}$, $t = 4.59$), *FAM189A2* ($q = 4.83 \times 10^{-2}$, $t = 4.49$), *CH25H* ($q = 4.83 \times 10^{-2}$, $t = 4.42$), *HACL1* ($q = 4.83 \times 10^{-2}$, $t = 4.34$), *AC124312.3* ($q = 4.83 \times 10^{-2}$, $t = 4.37$), and *NOS3* ($q = 4.83 \times 10^{-2}$, $t = -4.40$) (Figure 2A and Table S7). At $q < .1$, we also observed differential expression of *GK* ($q = .09$, $t = 4.11$), *VMP1* ($q = .09$, $t = 4.09$), *LINC00865* ($q = .07$, $t = -4.22$), and *AC083843.3* ($q = .09$, $t = 4.11$). We note that there was no overlap between the genes differentially expressed in males and those differentially expressed in unstratified analysis. Association of effects between sexes, determined by correlation of moderated t statistics, was small (Pearson $r = 0.12$, 95% CI, 0.10 to 0.13) (Figure 2C), confirming a high degree of sex specificity in observed signatures.

In contrast to analysis in the combined sample, sensitivity analyses of the dataset stratified by sex revealed suggestive associations ($q < .1$) with the average burden of depressive symptoms prior to first AD diagnosis. These included all genes that were significantly associated ($q < .05$) with depressive burden at the last visit (Figure S4A). In females, we identified an association of the average burden of depressive symptoms prior to AD diagnosis with just 1 gene, *AL136531.3* ($q = 5.0 \times 10^{-2}$, $t = 4.74$) (Figure S4B). Concordance of effects between sexes for this average measure was again small and, in this case, inverse (Pearson r −0.20; 95% CI, −0.22 to −0.19) (Figure S4C). As in the complete dataset, overall depressive burden measured at the last visit and average overall depressive burden prior to first AD diagnosis were correlated for males (Pearson $r = 0.72$, 95% CI, 0.65 to 0.77) and females (Pearson $r = 0.70$; 95% CI, 0.65 to 0.74) (Figure 2B, C). In addition, correspondence of differential expression effects was again moderate within males (Pearson $r = 0.55$; 95% CI, 0.54 to 0.56) and females (Pearson $r = 0.58$; 95% CI, 0.57 to 0.59) (Figure 2E, F).

In the subset of male participants without a clinical diagnosis of dementia at the time of death, *PWAR1* was significantly associated with depressive symptoms ($t = 4.25$, $q = .02$). In addition, similar to the full sample analysis, no genes were significantly associated with depressive symptoms in females. In males, 4/7 genes significantly associated with depressive symptoms in the full analysis remained transcriptome-wide significant in the nondementia subset (*ENPEP*, *CH25H*, *AC124312.3*, and *HACL1*), and an additional 56 significant genes were identified. The Pearson correlation of transcriptome-wide moderated t statistics was $r = 0.71$ (95% CI, 0.7 to 0.71) in males and $r = 0.35$ (95% CI, 0.33 to 0.36) in females. Again, the addition of latency as a covariate in male-specific models had minimal impact on the results (Figure S5A).

Sex-Stratified GSEA

In males, upregulated biological processes were related to metabolic pathways for the breakdown of amino acids, a known alternative energy source to carbohydrates (Figure 2D). These included branched chain amino acid catabolic process ($q = 5.77 \times 10^{-3}$, NES = 2.19) and cellular amino acid metabolic process ($q = 7.12 \times 10^{-3}$, NES = 1.71). In contrast, females showed downregulation of pathways related to aerobic

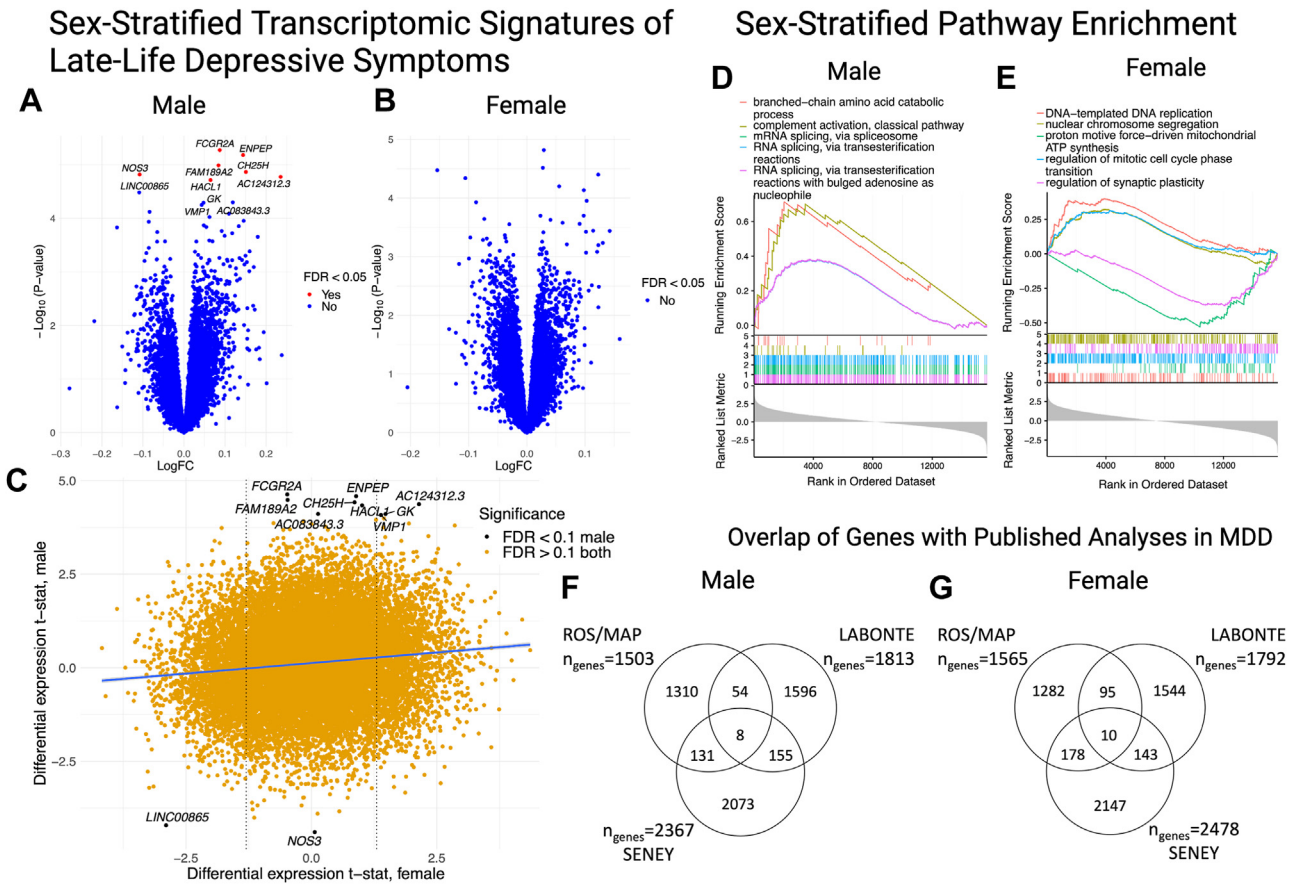

Figure 2. Sex-stratified differential expression for late-life depressive symptoms. **(A, B)** Volcano plots of differential expression analyses in males and females separately. **(C)** Scatterplot showing the correlation of differential expression effects (*t* statistics) for all genes in males (y-axis) and females (x-axis) (Pearson $r = 0.12$; 95% CI, 0.10 to 0.13). **(D, E)**. Gene set enrichment analysis plots of pathways enriched in males and females. **(F, G)** Venn diagrams showing overlap of genes significantly associated with depressive symptoms in late-life in the current study (ROS/MAP), Labonté *et al.* (11) (LABONTE), and Seney *et al.* (12) (SENEY) in males and females at a significance threshold of uncorrected $p < .05$. ATP, adenosine triphosphate; FC, fold change; FDR, false discovery rate; MDD, major depressive disorder; mRNA, messenger RNA; ROS/MAP, Religious Orders Study and Memory and Aging Project.

respiration, similar to the unstratified analysis (Figure 2E). The same was also true for NADH dehydrogenase complex assembly ($q = 2.73 \times 10^{-3}$, NES = −2.07) and mitochondrial respiratory chain complex I assembly ($q = 2.73 \times 10^{-3}$, NES = −2.07). This overlap extended beyond metabolism to pathways of mitosis and meiosis (e.g., chromatid segregation [$q = 2.03 \times 10^{-3}$, NES = 1.80], chromatin remodeling [$q = 1.71 \times 10^{-3}$, NES = 1.72], regulation of mitotic cell cycle phase transition [$q = 1.40 \times 10^{-3}$, NES = 1.74], and DNA recombination [$q = 3.66 \times 10^{-3}$, NES = 1.69]).

In males, enrichments for association with average depressive burden prior to first AD diagnosis also included pathways related to aerobic respiration of alternative energy sources, such as alcohols and sterols, which showed significant enrichment (Figure S4D). Pathways mediating the structural organization of the extracellular matrix were also enriched (Figure S4D). In females, pathways of aerobic respiration were again significantly enriched but not mitotic or meiotic pathways (Figure S4E). Inclusion of the latency covariate did not impact enrichment (Figure S5B).

Comparison With Existing Transcriptomic Studies

Finally, we compared our results with those from sex-specific studies of MDD in midlife by Labonté *et al.* (11) and Seney *et al.* (12). Of the genes that we identified as differentially expressed in males, none were significantly associated with MDD in either previous study. At an uncorrected $p < .05$, only 8/1503 genes identified in our male subset were shared with the genes identified by Labonté *et al.* (11) and Seney *et al.* (12). In females, 10/1565 genes were identified by all 3 studies at this same threshold (Figure 2F, G and Table S8). This suggests unique transcriptomic signatures for LLD symptoms compared to a clinical diagnosis of MDD earlier in life.

Comparing directly to cell-specific differential expression results reported by Zeng *et al.* (26), we found that none were significantly associated with depressive symptoms at $q < .05$. However, correcting for 10 comparisons (threshold $\alpha < .005$), we found that *DCUN1D2* was associated with depressive symptoms in a subset of participants who did not have a clinical diagnosis of dementia at the time of their death

(t = 3.22, p = .0014) (Figure S6). In the full sample including participants with clinical diagnoses of dementia—but still correcting for neuropathology and other covariates—we observed nominal associations of *DCUN1D2* (t = 1.78, uncorrected p = .076) and *SOCS6* (t = −2.05, uncorrected p = .041) with depressive symptoms at the last study visit. While not significant, these associations were also directionally consistent with those reported by Zeng *et al.* (26).

DISCUSSION

Our analyses represent the largest transcriptomic study of LLD symptoms reported to date, and we identified 2 genes and multiple biological pathways significantly associated with depressive symptoms measured proximal to death. Most of these signals were sex specific and not shared with genes that had previously been identified in smaller case-control studies of brain tissues from individuals diagnosed with MDD.

At the gene level, *PWAR1* and *CTDSPL2* were significantly associated with depressive symptoms when controlling for important confounders. *PWAR1* is a known paternally expressed non–protein-coding gene, which is found within a region of chromosome 15, specifically 15q11.2–q13, that is moved to chromosome 19 in a known reciprocal translocation (t[15;19][q11.2;q13.3]) (28). This translocation has been identified in a patient with Prader-Willi syndrome (28), a disorder that results from dysfunction of the hippocampal-pituitary-adrenal axis and is characterized by sleep disturbance. Hippocampal-pituitary-adrenal dysregulation is also known to occur in individuals with depression, especially during late life (29). Complicating this finding, *PWAR1* is an exon within the gene encoding *SNHG14*, which is adjacent to several other genes (including *IPW* [also known as *Lnc-SNRPN-8*], *DMAC1P1* [also known as *TMEM261P1*], *NPAP1*, *PWAR4*, *PWAR5*, *PWAR6*, *SNRPN*, and *UBE3A*), and surrounded by 2 gene clusters encoding *SNORD115* and *SNORD116*. Previous work has shown that serum *SNHG14* is significantly associated with depression (30), although notably these transcripts were either not detected in our sample (e.g., *SNORD115*, *SNORD116*, *IPW*, *DMAC1P1*) or showed no evidence for association with depressive symptoms (e.g., *SNHG14, PWAR5, PWAR6, UBE3A*). Comparatively, *CTDSPL2* encodes a nuclear phosphatase, CTDSPL2p, that promotes gluconeogenesis and restricts mitosis (31). *CTDSPL2* has previously been linked to bipolar disorder (32) and schizophrenia (33) and may impact the risk of depression and other neuropsychiatric disorders through the modulation of energy metabolism (34). At a more lenient significance threshold of $q_{FDR} < .1$, 14 additional genes were identified. Among these were *ACVR2B-AS1*, which harbors a single nucleotide polymorphism, rs73063271, that was associated with unipolar depression in a genome-wide association study (35). We also identified *STEAP4*, a metalloreductase critical for the maintenance of iron and copper homeostasis in the body (36). These metals interfere with the proper functioning of enzymes and when out of balance lead to neuropsychiatric illness (37). Thus, *STEAP4* may contribute to depressive symptoms by creating an imbalance in iron and copper levels in the brain (37).

At the pathway level, impaired metabolism has been linked to MDD (34). Patients exhibit reduced blood flow and glucose metabolism in the brain and a tricarboxylic cycle that is dysfunctional (34). Reflecting a similar pattern, we observed downregulation of pathways of aerobic respiration. Consistent with gene expression results, pathway analysis signatures using models with and without accounting for latency did not show significant differences.

When stratifying by sex, we observed significant associations of several genes with depressive symptoms in males but not in females. It is unlikely that this observation was influenced by statistical power because we analyzed a larger sample of females than males. This is consistent with previous observations of highly sex-specific transcriptomic signatures of depression (11,12). Top genes associated with depressive symptoms in males included *ENPEP* and *ACVR2B-AS1*, RNA transcripts encoded by genes that contain a single nucleotide polymorphism significantly associated with MDD (38). We also identified genes without prior associations with depression, including *FAM189A2*, a gene encoding a transmembrane activator of ubiquitin ligase ITCHp and a known breast cancer biomarker (39). We also identified the gene *FCGR2A*, which encodes a cytokine and is a member of the FC-gamma receptor gene family. *FCGR2A* has been linked to immune-related aspects of several illnesses, including squamous cell carcinoma (40), immune thrombocytopenic purpura (41), severe pneumonia comorbid with A/H1N1 influenza (42), and COVID-19 (43). Notably, when we analyzed only the subset of participants without clinical dementia at the time of death, the lower correlation of association statistics in females than males suggests the possibility of sex-specific differences in the impact of dementia symptoms on the molecular manifestation of depressive symptoms in the prefrontal cortex.

At the pathway level, gene sets related to the use of amino acids as an alternative energy source were upregulated in males. In females, both the downregulation of aerobic respiration and the upregulation of mitotic pathway components were observed. Using amino acids as an alternative energy source to glucose could present an alternative strategy to compensate for dysfunction in a specific part of the aerobic respiratory pathway that relies on glucose as its primary energy source. This is supported by the gluconeogenesis gene *CTDSPL2*, which was significantly associated with depressive symptoms; gluconeogenesis is one of the pathways that is used to convert amino acids into a respiratory intermediate that can be aerobically metabolized (44).

We found little overlap between our results and those from previously published bulk expression studies (11,12). This may reflect the fact that mechanisms that drive depression in late life are thought to differ from those that drive depression earlier in life: LLD arises due to dysfunction in the reward, salience, and cognitive control networks of the brain (45). In contrast, MDD may arise as a result of any of dysfunction in the hypothalamic-pituitary-adrenal axis; functional deficiency in serotonin, dopamine, and norepinephrine; and neuroinflammation induced by the presence of reactive oxygen species, inflammatory cytokines, activated inflammasomes; or the misactivation of specific genes (46). Discrepancies in study design might also have played a role; ROS and MAP participants ranged in age from 67 to 108, whereas participants in the study by Labonté *et al.* (11) were members of a younger founder French-Canadian population (ages 41–78) and those

in the study by Seney *et al.* (12) were individuals aged 16 to 74 who were autopsied at the Medical Examiner's Offices of Allegheny County (Pittsburgh, Pennsylvania; n = 95) and Davidson County (Nashville, Tennessee; n = 5) (12). Additionally, important differences exist in sample sizes and tissue sources; our sample included 998 participants with tissue samples from the DLPFC. In contrast, the study by Labonté *et al.* (11) included only 36 participants and used DLPFC tissue (only 2 participants over the age of 65). Similarly, of 100 participants from Seney *et al.* (12), only 9 were 65 years of age or older, and tissues were meta-analyzed from 8 microarray studies from the DLPFC, anterior cingulate cortex, and amygdala. Nonetheless, the lack of overlap between studies is notable, illuminating an emerging challenge in the study of LLD that may be due to both disease- and study design-related factors.

While our study enhances the understanding of sex-specific transcriptomic markers of LLD symptoms, previous research has identified a broad spectrum of other biomarker types. For example, variants in the aromatase enzyme gene *CYP19A1* have been associated with LLD in women but not in men (47). Additionally, a sex-stratified genome-wide association study of MDD identified 11 loci associated with depression in females but only 1 in males (48). Epigenetic studies have also shown increased methylation associated with LLD in males compared with females (49), and, at the protein level, numerous plasma proteins have been associated with LLD in both females and males (50). Sex-specific markers of LLD also include the inflammation marker NGAL and measures of metabolic dysregulation (51,52).

Our study has several limitations. First, the full study sample is not reflective of the general population. ROS enrolls members of the religious community (nuns, priests, and brothers) who are enriched for resilient lifestyle factors as previously noted (14). Second, our results are limited to a single brain region (DLPFC). Although dysfunction in MDD has often been associated with the subgenual cingulate cortex (53), additional brain regions including the DLPFC (41), the hippocampus (42), and the dentate gyrus specifically (43) have been implicated in imaging studies and molecular investigations. Moreover, MDD has been linked to broader brainwide changes, such as alterations in the balance between neural excitation and inhibition across all brain regions (54). Third, our measure of depressive symptoms, derived from the sum of responses on the CES-D, is a basic measurement and does not provide insights into the heterogeneity of symptom dimensions that underlie depression (55). Finally, despite comprehensive statistical controlling and sensitivity analyses, the presence of individuals with AD neuropathological changes may contaminate the sample, and the results may still reflect transcriptomic changes of depression in AD. In the future, the use of in vivo biomarkers of neurodegeneration for sample stratification may help disentangle effects that are due to pathological brain changes before a clinical diagnosis is reached and prior to autopsy.

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ROS/MAP resources can be requested at http://www.radc.rush.edu and http://www.synapse.org.

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