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Shared and unique transcriptional changes in the orbitofrontal cortex in psychiatric disorders and suicide

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Abstract

Psychiatric disorders like major depressive disorder (MDD), schizophrenia (SCZ), and bipolar disorder (BPD) represent a significant global public health concern. Sex differences in the prevalence and presentation of psychiatric disorders and the association of a psychiatric diagnosis with an increased risk of suicide are well-established. However, the neurobiology underlying the features of these diseases is not well understood. Dysfunction of the orbitofrontal cortex (OFC), a brain region responsible for decision-making and sensory processing, has been implicated in psychiatric disorders but remains understudied compared to other frontocortical brain regions.

In this study, we studied transcriptional changes in psychiatric illnesses by analyzing publicly available OFC transcriptional profiles (RNAseq data obtained from the Stanley Neuropathology Consortium) from male and female individuals with SCZ, BPD, MDD, and non-psychiatric controls (n=15/group). We also conducted a transnosological analysis of male and female subjects who died by suicide compared to those who died of other causes. Gene set enrichment analysis (GSEA) revealed significant differences in immune-related processes in male and female comparisons in psychiatric disorder subjects relative to controls.

Immune-related pathways were also significantly enriched in studies of suicide. Findings from analysis of top differentially expressed genes include significant changes in the microglia marker gene P2RY12 in males and females who died by suicide. Our results further our understanding of the shared and unique molecular pathways underlying psychiatric disorders and suicide in male and female subjects.

Keywords: Immune Response, Sexual Dimorphism, Psychiatric Disorders, Suicide, Orbitofrontal Cortex

1. Introduction

Mental health disorders like major depressive disorder (MDD), schizophrenia (SCZ) and bipolar disorder (BPD) affect millions of people globally (1, 2). The most prevalent of these disorders is MDD, which affects over 280 million individuals around the world (1); schizophrenia affects approximately 24 million individuals (3). In the United States alone, over 57 million people are thought to grapple with some form of mental illness (4). These disorders significantly impact the quality of life of patients and their families. Symptoms of these conditions include, but are not limited to, changes in mood, psychosis, delusions, cognition, low energy, and countless other symptoms (1).

Sex differences have been found in the prevalence and severity of psychiatric disorders (5). Recent studies support sex differences in the neurobiology of disorders like MDD. A study of the transcriptome of six different brain regions found that as few as 5-10% of the differentially expressed genes in MDD were common to male and female subjects (6). Historically, females have been understudied in the neurosciences; there are 4 times as many exclusive studies of males than females indicating a lack of focus on the female brain (5, 7). Thus, it is vital to include both male and female subjects in studies of the brain and psychiatric disorders.

Psychiatric disorders like BPD, MDD and SCZ are major risk factors for suicide (8). Suicide is a global public health concern, claiming over 700,000 lives annually (9). In the US alone, around 50,000 people died by suicide in 2021 (10). Another risk factor for dying by suicide is biological sex, with males comprising 80% of suicide cases (10). The neurobiology underlying sex differences in suicide are poorly understood. Recent research found significant differences in the prefrontal cortex transcriptome among individuals who died by suicide (11). Understanding suicide in the context of risk factors like psychiatric diagnosis and sex can inform proactive measures and prevention strategies.

The orbitofrontal cortex (OFC) is located on the ventral surface of the frontal lobe. It plays a crucial role in decision-making and sensory processing (12). Recent large-scale voxel-level neuroimaging studies have shown functional connectivity changes in the OFC in MDD, suggesting this is an important brain region in the study of psychiatric disorders (13). Despite its role in important functions that are relevant to psychiatric disorders, the OFC remains relatively understudied in comparison to other brain regions such as the prefrontal cortex. Therefore, focusing on the OFC may uncover vital information about the mechanisms underlying these disorders and lead to the development of more effective treatments and interventions for mental disorders.

Here, we investigate transcriptional profiles and apply complementary pathway analyses to the study of publicly available datasets generated from the OFC of SCZ, MDD and BPD subjects. We study differences in gene expression in male and female subjects in each disorder. We also look at the differences in the transcriptional profiles of psychiatric disorder subjects who died by suicide compared to those who did not die by suicide.

Diagnosis	**BPD**	**SCZ**	**MDD**	**CTL**	**BPD**	**SCZ**	**MDD**	**CTL**
Sex	**Male**				**Female**			
N	7	9	9	9	6	5	6	6
Race	7W/0 NW	8W/1 NW	9W/0 NW	8W/1 NW	5W/1 NW	3W/2 NW	6W/0 NW	6W/0 NW
Age	38.4±10.4	39.2±11.5	49±8.0	50.3±6.6	45.2±12.5	51.4±13.0	42.8±10.6	44.7±15.0
PMI	31.8±14.3	33.7±14.4	23.0±10.2	20.8±8.6	35.8±19.8	35.2±17.8	34.2±8.1	28.2±11.0
pH	6.1±0.2	6.2±0.3	6.2±0.2	6.3±0.2	6.3±0.3	6.2±0.1	6.1±0.2	6.2±0.3
Antipsychotics	5on/2off	9on/0 off	0on/9 off	0on/9 off	5on/1 off	4on/1off	0on/6 off	0on/6 off
Suicide	4Y/2N/1U	2Y/7 N	4Y/5 N	0Y/9 N	3Y/3 N	2Y/2N/1U	3Y/3 N	0Y/6 N

Table 1: Subject Demographics

2. Methods

2.1 Postmortem Brain samples

The Stanley Medical Research Institute supports research on psychiatric disorders (14). The Neuropathology Consortium of the Stanley Medical Research Institute holds RNAseq data from 60 subjects (n=15 per group) diagnosed with schizophrenia (SCZ), bipolar disorder (BPD), major depressive disorder (MDD) and non-psychiatrically ill controls (CTL) (14). Postmortem brain samples were collected with permission from the families and according to institutional approval (14). Diagnosis was confirmed by two senior psychiatrists using medical records, DSM-IV criteria and, when necessary, telephone interviews with family members (Neuropathology Consortium, Stanley Medical Research Institute). The diagnoses of the unaffected controls stemmed from structured interviews with family member(s) conducted by a senior psychiatrist to rule out Axis I diagnoses. Subjects were screened for the presence of cardiovascular disease, hemorrhage, trauma, tumors, and other pathology

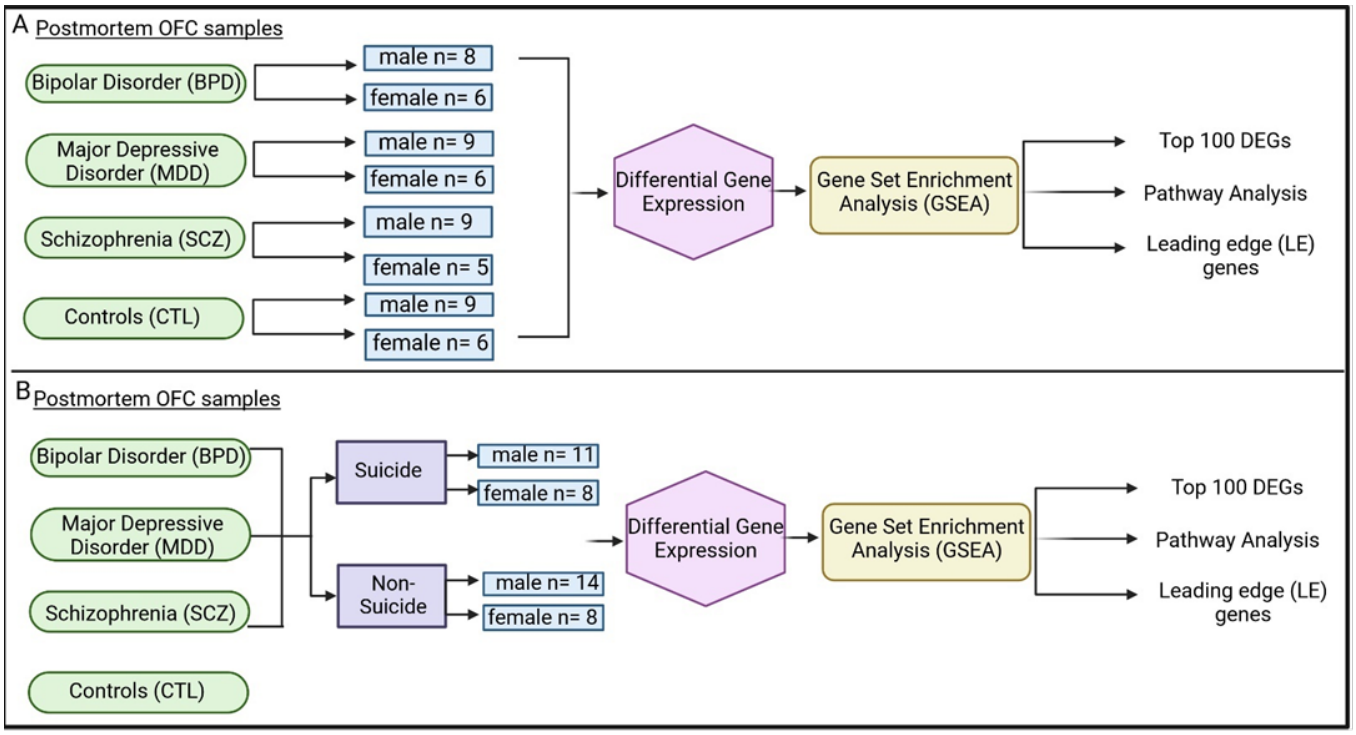

Figure 1 Study workflow. A) Postmortem orbitofrontal cortex (OFC) brain samples from bipolar disorder (BPD), Major Depressive Disorder (MDD), Schizophrenia (SCZ), and a control group (CTL) were analyzed. Male disease vs matched male CTL and female disorder vs matched female CTL were analyzed for all disorders. Following generation of differential gene expression for each comparison, data is analyzed by gene set enrichment analysis (GSEA). B) Workflow for a transnosological analysis of male and female subjects diagnosed with a psychiatric disorder who died by suicide compared to those who did not die by suicide (non-suicide). The differential gene expression data for each comparison is analyzed by gene set enrichment analysis (GSEA). Created by Biorender

(https://www.stanleyresearch.org/brain-research/neuropathology-consortium/). The total lifetime antipsychotic medication (in fluphenazine milligram equivalents) is 52,267±62,062 (SCZ) and 20,827±24,016 (BPD) as estimated by (14).

For this study, RNAseq data generated from fresh frozen brain samples from the orbitofrontal cortex (Brodmann areas 10, 11, and 47) of postmortem brains was used (Neuropathology Consortium, Stanley Medical Research Institute). The samples were collected, stored, and processed as previously described (14). The subjects were grouped based on sex and diagnosis. For studies of suicide, RNAseq data from all subjects diagnosed with SCZ, MDD, and BPD who died by suicide (n=9 male, n=8 female) were compared to SCZ, MDD, and BPD subjects who died by other causes (n=14 male, n=8 female). Subject demographics, including age, postmortem interval (PMI), sex, antipsychotic medication status, brain tissue pH, and cause of death, are provided in Table 1. The raw RNAseq data files were downloaded from the Neuropathology Consortium. Figure 1 summarizes the workflow used to organize and analyze our data.

2.2 *Differential gene expression*

The FASTQ files were aligned to the Ensembl human genome assembly GRCh38.107 using the STAR aligner v2.7.9a (15). The alignment was performed with the following arguments: --outSAMtype BAM SortedByCoordinate –outSAMunmapped Within –outSAMattributes Standard. Next, the reads mapped to each gene (feature) were counted using the featureCounts function from the Rsubread R package (16). Multi-mapped reads were disabled (countMultiMappingReads = FALSE), and the strandedness was specified as unstranded (strandSpecific = 0) after conducting tests for both forward and reverse strandedness to ensure accuracy. Differential gene expression analysis was performed in R using the count data and the edgeR R package (17). Volcano plots of differentially expressed genes (DEGs) for all comparisons are in Supplementary Figure 1.

2.3 *Pathway analysis*

DEGs were analyzed by Gene Set Enrichment Analysis (GSEA) for each comparison. GSEA performs pathway analyses using the full transcriptome. GSEA determined the top upregulated and downregulated pathways from an input of

ranked gene lists by adjusted p-value and log2FC. "Top" pathways are those with the highest log2FC or lowest negative log2FC in expression and the lowest adjusted p-value. GSEA's full transcriptome analysis is completed by analyzing the dataset with gene sets instead of singular genes, and top pathways are determined by p-value and enrichment scores (18, 19). The gene sets were defined by the Gene Ontology pathway package (20, 21).

GSEA provides a leading-edge (LE) gene analysis in which the genes that are most influential for the enrichment of significant pathways are identified (22). LE genes are a subset of genes contributing to the enrichment score in a single pathway gene set. Therefore, these LE genes can be interpreted as the core genes within a pathway gene set that account for the majority of the pathway gene set's enrichment signal. A gene that is in many of the leading-edge subsets is more likely to be of interest than a gene that is in only a few of the leading-edge subsets. "Top" LE genes are those genes identified in the greatest number of biological pathways. Thus, we analyze the overlap between multiple leading-edge subsets.

3. Results

3.1 Top 100 DEGs in the OFC in psychiatric disorders

The top DEGs, i.e., statistically significantly altered ($p<0.05$) genes with the greatest log2FC difference in expression in the disease group compared to the corresponding control, in each comparison, are shown in Figure 2 and Supplementary Table 2. Interestingly, the top DEGs in BPD are predominately downregulated (- (negative) log2FC), but in MDD and SCZ comparisons, top DEGs are upregulated (+ (positive) log2FC) and downregulated, relative to corresponding controls. The heatmaps also show distinct differences in gene expression in male and female subjects in each comparison. Unique clusters of DEGs are identified for each sex in each disorder. For example, clusters of genes (black boxes) are downregulated in female MDD (Figure 2B) and SCZ (Figure 2C) but not in the male comparisons, suggesting sex-dependent differential gene expression in each disorder.

3.2 Top 100 DEGs in the OFC following transnosological analysis of suicide

As seen in MDD and SCZ analyses, the top 100 DEGs comprise both upregulated and downregulated genes in suicide comparisons (subjects diagnosed with a psychiatric disorder who died by suicide compared to subjects diagnosed with a psychiatric disorder who did not die by suicide). There are also prevalent differences in the patterns of gene expression in male and female subjects who died by suicide e.g. a cluster of genes that are downregulated in female suicides (Figure 2D, black box) but not in the male suicide comparison. Differences in gene expression may contribute to the pathophysiological mechanisms underlying sex differences in the rates of death by suicide. Venn diagrams of the top 100 DEGs show that most of these are unique to each comparison. BPD female and suicide female comparisons share 26 of their top 100 DEGs (Figure 2E), the highest overlap of any groups, suggesting common changes in gene expression in females with BPD and those who die by suicide. MDD male and female comparisons (Figure 2F) and SCZ male and female comparisons (Figure 2G) each share 13 top DEGs, but few of the top 100 DEGs are shared with the suicide comparisons. These results show that there are significant gene expression differences in males and females with the same disorder and that suicide is driven by unique gene expression changes compared to psychiatric disorders.

3.3 Pathway analysis

Following GSEA analysis of all psychiatric disorder and suicide comparisons, pathways were clustered using the PAVER (23) approach and presented in Figure 3. PAVER analysis identified 54 different pathway clusters (y-axis). The clusters and individual pathways that comprise the clusters are listed in Supplementary Table 3. Pathway clusters related to the immune system were enriched across all disorders and suicide comparisons (Supplementary Table 3). Protein metabolism, mitochondria-related, and synaptic signaling-related pathway clusters were also enriched across all comparisons. GSEA analysis also supports the enrichment of mostly downregulated pathways in BPD but upregulated pathways in other comparisons, which was also seen with the top 100 DEGs.

Although overall patterns of enriched pathway clusters are similar within disorders (Figure 3), pathway analysis also identified unique pathway enrichment in male and female comparisons. Greater pathway enrichment is seen in male MDD comparisons compared to females in clusters related to synaptic signaling and metabolism (e.g., "ion channel activity" and "respiratory electron transport chain"). In SCZ comparisons, the pathway cluster "fatty acid metabolism" is enriched in females but "regulation of cell death" is enriched only in males. Unlike the other disorders, pathway analysis of BPD found downregulation of pathways in male and female subjects. Pathways involved in "tyrosine receptor kinase activity" and "cell-cell adhesion" were enriched in male but not female BPD subjects.

Specific sex differences were also identified in pathways associated with male and female suicide analyses. Energy metabolism pathway clusters "oxidative phosphorylation" and "respiratory complex" (see arrow, Figure 3) are downregulated in female but not male suicide vs non-suicide comparisons.

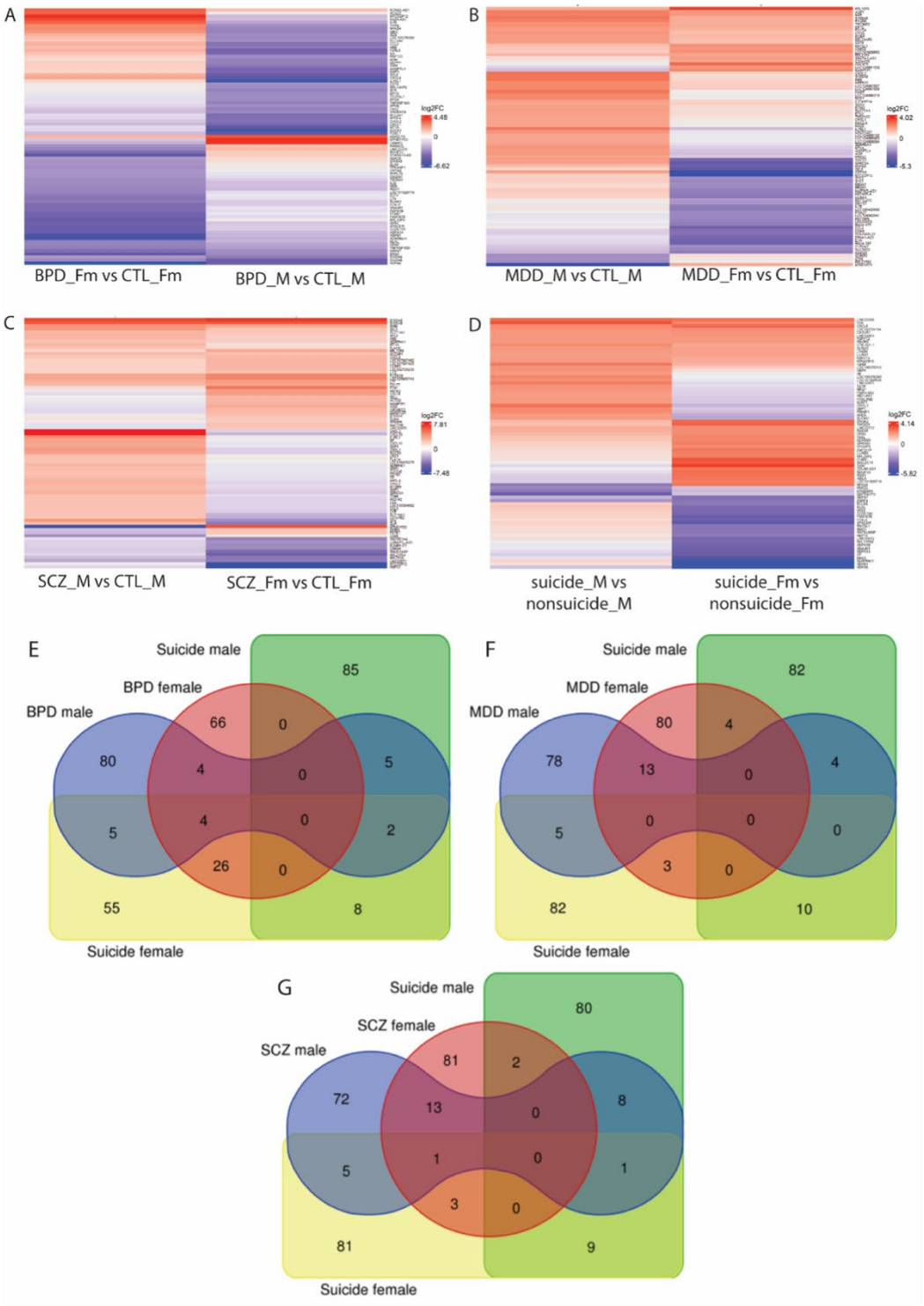

Figure 2 *Heatmap of the top 100 differentially expressed genes in males and females in BPD (A), MDD (B), and SCZ (C) compared to controls (CTL) and in suicide (BPD + MDD + SCZ subjects who died by suicide) compared to non-suicide (BPD + MDD + SCZ subjects who did not die by suicide) (D). Upregulated genes are red and downregulated genes are blue. Black boxes represent clusters of top (most) differentially upregulated or downregulated genes in male and female comparisons. Venn diagram summarizing the top 100 DEGs that are shared between male and female BPD (Figure 2E), MDD (Figure 2F) and SCZ (Figure 2G) groups and male and female suicide comparisons.*

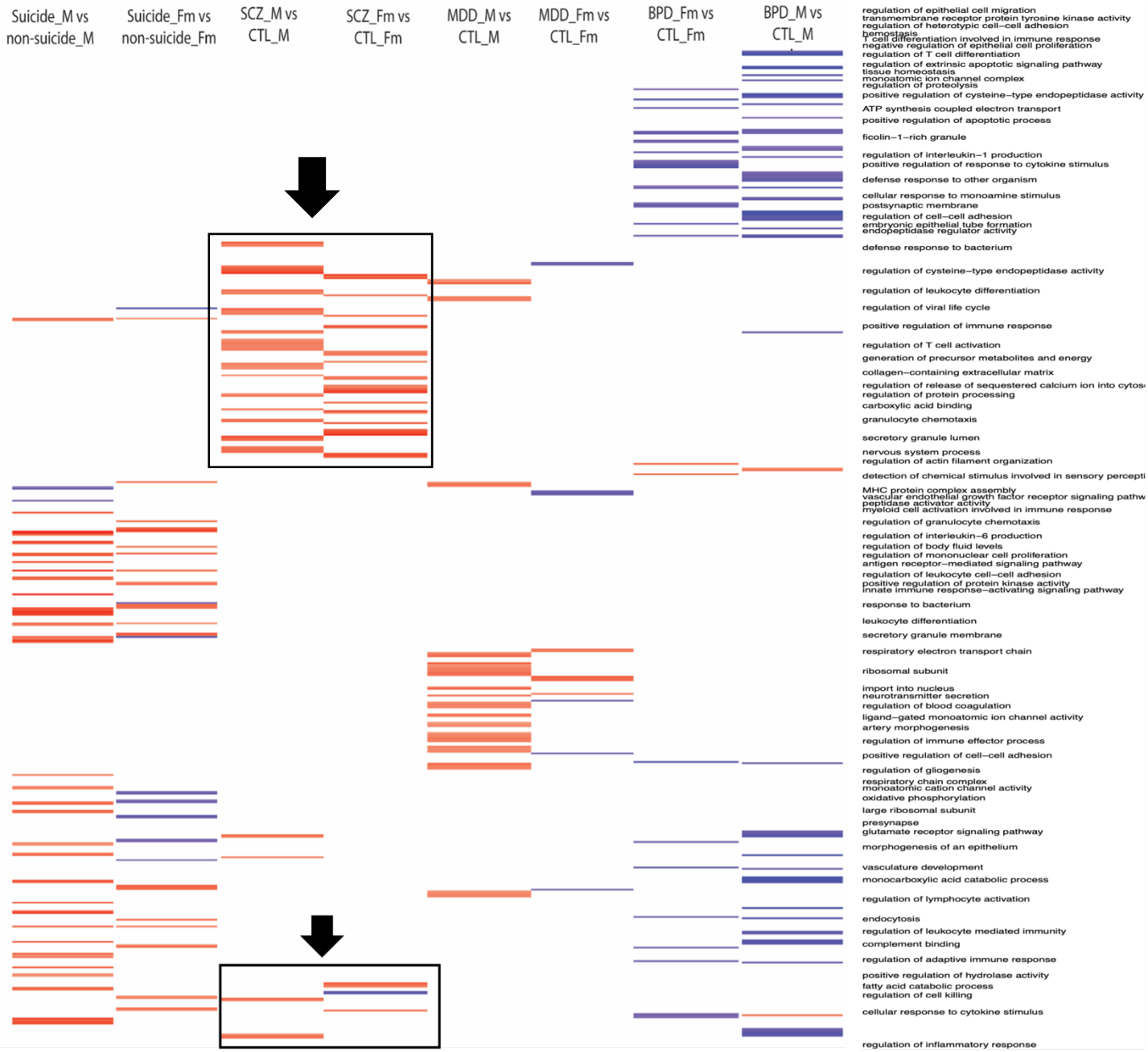

Figure 3 *Paver Heatmap. Pathway analysis heatmap clusters similar pathways. Pathway clusters are listed on the y-axis. Red pathways are upregulated, and blue pathways are downregulated based on normalized enrichment score. Box and arrows indicate pathways differentially enriched in males and females.*

Overall, similar clusters of biological pathways are altered in BPD, MDD, SCZ and suicide but the individual pathways that comprise these clusters are different, potentially reflecting sex differences in the pathophysiological mechanisms underlying these psychiatric disorders and suicide.

3.4 Top 10 upregulated and downregulated GSEA pathways

Immune-related pathways were significantly enriched in the upregulated gene set for both male and female suicide cohorts, the upregulated gene set for males in SCZ and MDD cohorts, and the downregulated gene sets for males with BPD and females with MDD.

Other significant enriched pathways included Synaptic Signaling, which was enriched in upregulated gene sets of both males and females in the BPD cohort, downregulated gene sets for both males and females in the MDD cohorts, down-regulated gene set for females in the SCZ cohort, and downregulated gene set of males in the suicide cohort; and Ribosomes and Protein Metabolic Processes, enriched in the upregulated gene sets in both males and females in the MDD cohorts and downregulated gene sets for females in the BPD and Suicide cohorts and males in the SCZ cohort.

Comparing all the groups with each other, it can be seen that in males, both SCZ and MDD exhibit an upregulation of immune responses, which is not observed in BPD. Additionally, signaling processes are highly upregulated in BPD but show the opposite pattern in MDD. In females, MDD is associated with a downregulation of immune response, whereas BPD and SCZ do not display this immune response change. Furthermore, protein metabolism is downregulated in BPD but upregulated in MDD among females. As with psychiatric disorder analyses, immune pathways are also the top upregulated pathways in male and female suicide comparisons. Neurotransmitter signaling-related pathways and protein metabolism pathways are the most downregulated in male and female suicide analyses, respectively.

3.5 LE Genes

In Figure 4, representative top 10 LE genes are shown. The top LE genes for other comparisons are shown in Supplementary Figure 2. From Figure 4, several interesting associations can be found between gene expression patterns in different psychiatric disorders among males and females. The most common LE genes identified in different groups include cytokines IL6, TGFB1, IL1B, and CXCL8, which are involved in the proinflammatory immune response. These complement pathway findings related to the immune system in males and females in all psychiatric disorders and suicide. In suicide comparisons, the microglial marker gene P2RY12 (Supplementary Figure 2) is a top upregulated LE gene in male and female subjects. However, while immune-related LE genes are upregulated in male and female suicide and SCZ comparisons in our study, they are downregulated in BPD (Figure 4A) and female MDD comparisons. Signaling-related genes including glutamate receptor genes GRIN2A and GRIN2B are upregulated in male and female BPD (Figure 4B) but downregulated in male MDD and male suicide. Mitochondrial genes like MT-ND2-6, which encodes for the mitochondrial complex I enzyme NADH ubiquinone, are downregulated in female MDD and female suicide (Figure 4C). Changes in mitochondrial function may be involved in MDD and suicide in females (24). The only comparison that identified the top 10 LE genes involved in olfactory receptors OR3A2, OR2T10, OR2L3) was female SCZ vs CTL (Figure 4D). This is interesting because olfactory dysfunction is found in patients with SCZ (25). Sex differences in the correlations of odor acuity and cognition are also reported. Smell identification is significantly associated with better cognitive performance in the Trails Making Test in female but not male SCZ patients (26). The OFC is an olfactory center, and females have more OFC grey matter (27), which may contribute to sex differences in olfactory processing deficits in this disorder.

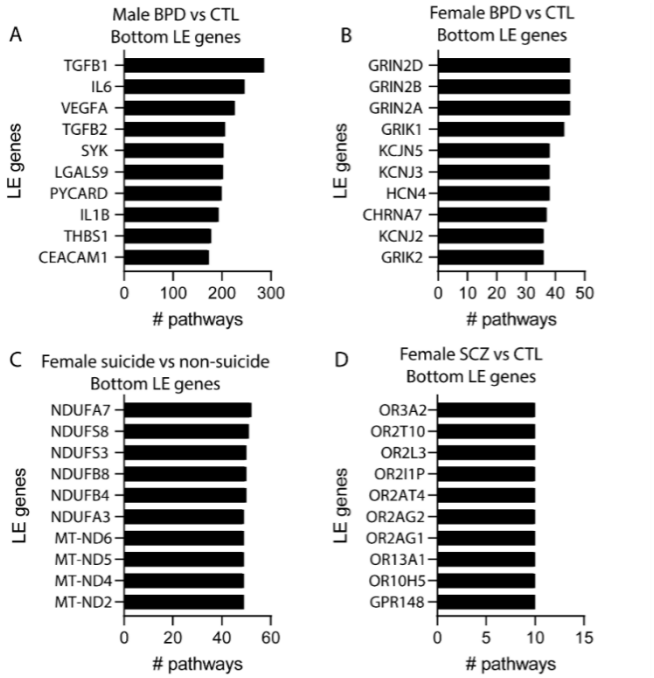

Figure 4 *Leading Edge (LE) genes. Representative charts of the top or bottom 10 LE genes for BPD_M (A), BPD_Fm (B), suicide_Fm (C) and SCZ_Fm (D) comparisons. The number of times a LE gene was present in a pathway is on the x-axis. BPD bipolar disorder, SCZ schizophrenia.*

Overall, gene expression analysis identified changes in gene expression and biological pathways in BPD, SCZ, MDD and suicide compared to controls. Individual immune gene expression is upregulated or downregulated in different disorders and suicide, but immune-related pathway

expression is typically upregulated in psychiatric disorders except BPD. Other processes that are different in psychiatric disorders are metabolic and signaling-related processes but the specific pathways and most differentially expressed genes that are altered are often unique to males and female groups in different comparisons.

4. Discussion

We analyzed transcriptional datasets from the OFC and found significant differences in biological pathways and LE genes in male and female subjects with different psychiatric disorders. The OFC is an important brain region for emotion regulation and cognition (12); however, it is relatively understudied compared to the dorsolateral prefrontal cortex in psychiatric disorders. Our results are an important addition to the field as we identify changes in gene expression in the OFC in BPD, MDD, and SCZ. We also found sex differences in subjects diagnosed with a psychiatric disorder who died by suicide compared to those who died by other causes.

Pathways related to immune-system function had the greatest enrichment across all psychiatric disorders and were also found in male and female comparisons. Neuroinflammation is often implicated in psychiatric disorders (28). Our results also found upregulated immune pathways and LE genes, including proinflammatory cytokines in MDD, SCZ, and suicide comparisons. Overall, our study supports altered immune function in the brain in severe mental illness. Surprisingly, immune pathways and LE genes were downregulated in BPD in our study. Others report increased immune activation in BPD in the brain and periphery and anti-inflammatory medication to treat BPD but there are also conflicting reports that do not report such changes in BPD (29). The downregulation of immune pathways and LE genes may be due to the brain region studied here, the OFC. Others report that immune dysfunction may play a role in a subset of BPD patients (30), so our findings may reflect heterogeneity in the BPD population being studied here.

GSEA analysis of transnosological suicide comparisons identified upregulated immune-related pathways in both males and females including pathways such as "positive regulation of immune response" and "leukocyte activation." The upregulation of immune pathways is not surprising, as increased expression of neuroinflammatory markers is reported in the brain and periphery in suicide (31, 32). As all subjects in this analysis were diagnosed with a psychiatric disorder, dysregulated immune activation may be a common mechanism associated with suicide in male and female subjects. Furthermore, when looking at the LE genes in suicide, the microglial marker gene P2RY12 was found in analyses of both sexes. Microglia serve as the brain's immune cells and microgliosis is reported in the frontal cortex of subjects who died by suicide (33). This gene was also associated with different biological pathways such as "cell activation" in females and "positive regulation of response to external stimulus" in males, suggesting it may also play different roles in males and females who die by suicide. P2RY12 gene expression was also significantly altered in the frontal cortex in a transnosological comparison of violent suicide and non-suicide subjects (11) and a study of SCZ subjects who died by suicide (34). The data from our study is further evidence of the role of this gene in suicide, although the functional consequences of its altered expression have yet to be elucidated.

In contrast to the similarity in upregulated pathways, the downregulated pathways in males and females in suicide differed greatly. The male downregulated pathways were mostly related to signaling, with pathways such as "Anterograde trans-synaptic signaling" and "Chemical synaptic transmission" appearing in our analysis. However, for female comparisons, the data showed a consistent downregulation of protein metabolism processes, with pathways such as "Response to unfolded protein" and "Chaperone-mediated protein folding" being significantly downregulated.

Our analysis also identified the downregulation of genes involved in the "protein folding processes" pathway in female suicide subjects but not male suicide subjects. Significant changes in the expression of genes involved in unfolded protein response pathways were reported in MDD patients who died by suicide (35). Dysregulation of protein metabolism processes like protein folding is associated with inflammation and depressive-like behavior (36, 37). Our analysis suggests that this mechanism may be associated with suicide in female subjects, independent of the underlying psychiatric disorder state, as we identified genes and pathways related to protein folding, such as HSPA6, in our transnosological analysis.

A limitation of this study is the lack of data available on patient medication history. No data is available describing whether patients were treated with mood stabilizers or antidepressants. As almost all SCZ subjects were on antipsychotics at the time of death, it was not possible to compare the effects of medication in this analysis. Future studies examining the effects of psychotropic medications on gene expression will be necessary. Another limitation is the transnosological approach to the suicide analysis. This approach allows us to identify changes in gene expression that are specific to suicide, independent of any single psychiatric diagnosis. Subjects were screened for potential confounders, including the history of trauma and neuropathology associated with neurodegenerative disorders, which are associated with risk for suicide (38, 39). However, we cannot exclude that the underlying psychiatric disorder may be driving specific transcriptional changes associated with suicide that cannot be captured by our analysis (diagnosis x suicide gene interactions).

In summary, our study of the top DEGs, pathway analysis and LE gene analysis support changes in immune dysregulation as the most prevalent and shared feature of SCZ, MDD, BPD and suicide in males and females in the OFC. Although immune dysregulation is found across all comparisons, there

are many unique genes and pathways that contribute to these changes in each comparison and may explain heterogeneity in males and females affected by these conditions.

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