



Neuro-transcriptomic signatures for mood disorder morbidity and suicide mortality



Mbemba Jabbi^{a,b,c,d,*}, Dhivya Arasappan^e, Simon B. Eickhoff^{f,g}, Stephen M. Strakowski^{a,b}, Charles B. Nemeroff^{a,b,h}, Hans A. Hofmann^{c,i}

^a Department of Psychiatry, Dell Medical School, University of Texas at Austin, United States

^b The Mulva Clinic for Neurosciences, Dell Medical School, University of Texas at Austin, United States

^c Institute of Neuroscience, University of Texas at Austin, United States

^d Department of Psychology, University of Texas at Austin, United States

^e Center for Biomedical Research Support, University of Texas at Austin, United States

^f Institute of Systems Neuroscience, Heinrich Heine University Düsseldorf, Germany

^g Institute of Neuroscience and Medicine (INM-7), Research Centre Jülich, Germany

^h Institute of Early Life Adversity Research, United States

ⁱ Department of Integrative Biology, The University of Texas at Austin, United States

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ABSTRACT

Suicidal behaviors are strongly linked with mood disorders, but the specific neurobiological and functional gene-expression correlates for this linkage remain elusive. We performed neuroimaging-guided RNA-sequencing in two studies to test the hypothesis that imaging-localized gray matter volume (GMV) loss in mood disorders, harbors gene-expression changes associated with disease morbidity and related suicide mortality in an independent postmortem cohort. To do so, first, we conducted study 1 using an anatomical likelihood estimation (ALE) MRI meta-analysis including a total of 47 voxel-based morphometry (VBM) publications (i.e. 26 control versus (vs) major depressive disorder (MDD) studies, and 21 control vs bipolar disorder (BD) studies) in 2387 (living) participants. Study 1 meta-analysis identified a selective anterior insula cortex (AIC) GMV loss in mood disorders. We then used this results to guide study 2 *postmortem* tissue dissection and RNA-Sequencing of 100 independent donor brain samples with a life-time history of MDD (N = 30), BD (N = 37) and control (N = 33). In study 2, exploratory factor-analysis identified a higher-order factor representing number of Axis-1 diagnoses (e.g. substance use disorders/psychosis/anxiety, etc.), referred to here as *morbidity* and suicide-completion referred to as *mortality*. Comparisons of case-vs-control, and factor-analysis defined higher-order-factor contrast variables revealed that the imaging-identified AIC GMV loss sub-region harbors differential gene-expression changes in high morbidity-&-mortality versus low morbidity-&-mortality cohorts in immune, inflammasome, and neurodevelopmental pathways. Weighted gene co-expression network analysis further identified co-activated gene modules for psychiatric morbidity and mortality outcomes. These results provide evidence that AIC anatomical signature for mood disorders are possible correlates for gene-expression abnormalities in mood morbidity and suicide mortality.

1. Introduction

Major depressive disorder and bipolar disorder - here together referred to as mood disorders, are the third leading cause of the global disease burden (Collins et al., 2011; Murray et al., 2012). Mood disorders account for the majority of completed suicides (Waern et al., 2002; Marangell et al., 2006) and they were linked to ~48,000 suicides in the United States in 2018 alone (American Foundation for Suicide

Prevention, 2019). However, the convergent neurobiological basis for mood symptoms/syndromes and suicide is unknown, limiting advances in developing novel interventions.

Neuroimaging studies have identified reduction in gray matter volume (GMV) in the anterior insular cortex (AIC) and anterior cingulate cortex (ACC) in association with diagnosis of psychiatric disorders in general (Goodkind et al., 2015), and the regional GMV volume reductions in these AIC and ACC network have been especially implicated in

* Corresponding author. Department of Psychiatry, Dell Medical School, University of Texas at Austin, United States.

E-mail address: mbemba.jabbi@austin.utexas.edu (M. Jabbi).

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mood disorder diagnoses in particular (Goodkind et al., 2015; Wise et al., 2017). Neurobiological integrity of the right AIC is shown to (a) predict mood diagnostic severity (Hatton et al., 2012), (b) modulate subjective responses to distress, pain, and psychosocial adversity (Wager et al., 2013; Eisenberger, 2015), (c) regulate affective interoception (Craig, 2009; Khalsa et al., 2018), (d) associate with stress-related inflammatory markers (Slavich et al., 2010), and (e) predict psycho- and pharmaco-therapeutic efficacy in mood disorders (McGrath et al., 2013). AIC-ACC functional connectivity during affective processing differentiated mood disorder suicide-attempters from non-attempters (Pan et al., 2013). Furthermore, abnormalities in AIC volume and synaptic abnormalities are linked to suicidal-behavior in mood disorder (Wagner et al., 2012; Mathews et al., 2013). AIC response to stress is shown to impact hypothalamic-pituitary-adrenal (HPA) axis-driven inflammatory responses (Khalsa et al., 2013), which may serve to exacerbate mood disorder associated psychiatric morbidity and suicidal-behavior (Oquendo et al., 2014; Wohleb et al., 2016). Although a preponderance of evidence supports abnormal AIC integrity in psychosocial distress (Shneidman, 1998; Mee et al., 2011; Wager et al., 2013) and mood/comorbid psychiatric symptoms, an underlying functional genetic contribution in terms of functional gene-expression changes for these abnormalities remains largely unknown.

The lack of a well-defined relationship between aberrant brain structure and function with underlying molecular changes within these abnormal brain regions is an impediment to understanding pathophysiology. Moreover, evidence for shared genetic mechanisms underlying psychiatric diagnoses (Brainstorm consortium, Anttila et al., 2018) is not well-integrated with brain imaging correlates of psychiatric disease-morbidity and specific behaviors, in this case, suicide. In the present study, MRI meta-analysis was used to test the hypothesis that reduced AIC volume will be the most prominent neuroanatomical signature for mood disorder diagnoses. We confirmed this hypothesis with our meta-analytic findings in study 1 and then used this anatomical hallmark to guide dissection of *postmortem* brain tissue for analyses of molecular/gene-expression signatures that could pave the way for precision profiling of gene functions underlying mood symptoms across diagnoses in clinically-relevant brain sub-regions in study 2. This approach enabled us to further test the hypothesis that the voxel-based morphometry (VBM) imaging meta-analysis defined in study 1 will harbor *postmortem* gene-expression signatures for psychiatric disease morbidity and related suicide-mortality in *postmortem* mood disorder brains, thereby providing a neurobiological framework for characterizing convergent neural-and-gene expression signatures for behavioral brain diseases.

2. Methods

2.1. Participants & Samples

The imaging meta-analysis provided a consolidation of the current mood disorder VBM work by quantitatively integrating published results of volumetric comparisons of interest between controls vs mood disorder participants, or correlations of volumetric measures with mood disorder symptom-specific measures. In total, 47 previous VBM publications consisting of volumetric comparisons or experimental contrasts assessing GMV reductions in healthy controls vs major depression from 26 independent publications (demographics in Table 1A); and volumetric comparisons or experimental contrasts assessing GMV reductions in healthy controls vs bipolar disorder from 21 independent publications (demographics in Table 1A). Only publications with more than 20 subjects per comparison (i.e. publications with more than 10 subjects per each comparison group of major depressive disorder vs controls, or bipolar disorder vs controls) were selected based on the rationale that comparison group averages of less than 10 subjects per diagnostic or control group is likely underpowered in VBM studies. Together, the meta-analysis included 2387 imaging participants.

Table 1A

Demographics and composite sample sizes of included publications in the Meta-Analysis. Table 1A, shows the number of subjects for the collective meta-analytic samples of the 26 publications including the MDD and normal controls in the first column from left. The second, third and fourth columns depicts the number of subjects per samples (and percentage of females in diagnostic sample), mean age at the time of scanning (standard deviation), and age ranges respectively. The second panel (below) shows the number of subjects for the collective meta-analytic samples of the 21 publications that included the bipolar disorder and normal controls in the first column from left; with the second, third and fourth columns depicting number of subjects per samples, (and percentage of females in diagnostic sample), mean age at the time of scanning (standard deviation), and age ranges respectively.

26 publications with 43 experiments (major depressive disorder 'MDD' vs Control whole-brain GMV changes)			
	N (% Females)	Mean Age (years)	Age Range (years)
Major Depressive Disorder	831 (56.6%)	41 ± 13.6	15–80
Normal Controls	783 (59.4%)	39 ± 11.9	15–80
21 publications with 29 experiments (bipolar disorder 'BD' vs Control whole-brain GMV changes)			
	N (% Females)	Mean Age (years)	Age Range (years)
Bipolar Disorder	439 (49.7%)	31 ± 10.9	13–46
Normal Controls	331 (54.4%)	32 ± 9	14–44

The 47 publications included in our meta-analysis, as well as relevant publications that ended up not being included based on the above-mentioned inclusion criterion, are listed in [Supplementary Table 1](#). While 3 of the 72 experimental contrasts included in the meta-analysis assessed suicidal behavior in relation to volumetric changes in mood disorder, suicidal phenotypes was not a specific selection criteria for study inclusion as there were very few studies in the BrainMap database that specifically assessed the relationship between suicidal phenotypes and brain volume. In study 2, RNA samples were extracted from the anatomical likelihood estimation (ALE) defined AIC sub-regional (identified in study 1) *postmortem* tissue of 100 donors from NIMH brain bank (*postmortem* technical/qualitative variables in [Table 1B](#)).

2.2. Design

Based on the principle that neural structure underlies functional control of complex behavioral repertoires (Koechlin, 2016), we localized the structural brain signature for mood disorders across samples and methods in study 1. Experiments of GMV changes associated with mood disorder diagnoses in defined stereotaxic space were included for analysis of localized GMV changes across studies in major depressive disorder, bipolar disorder, and major depressive disorder and bipolar disorder versus (vs) controls using the well-established ALE algorithm (Eickhoff et al., 2009). This signature guided localized anatomical-dissection of *postmortem* tissue for whole-transcriptome characterization of differential gene-expression and weighted gene co-expression network analysis (WGCNA).

2.3. Neuroimaging VBM meta-analysis

For the VBM meta-analysis, results of anatomical changes reported in coordinate space using the Montreal Neurological Institute (MNI) or Talairach coordinates of 3-dimensional brain space were included from 26 publications examining *major depressive disorder* < *controls* whole-brain GMV changes; 21 publications examining *bipolar disorder* < *controls* GMV changes; and we combined the coordinates for the 26 publications examining *major depressive disorder* < *controls* and the 21 publications examining *bipolar disorder* < *controls* GMV changes for

Table 1B
Demographics of postmortem diagnostic and control samples showing number of subjects for each sample, percentage of females per sample, number of suicides and other manners of death information, mean age at death, and postmortem qualitative data including mean postmortem interval, PMI, mean Ph; and mean RNA integrity number, RIN.

RNA-seq of AIC study sample demographics and postmortem quality data						
	N (# of Females)	Manner of Death (# of Females)	Age (in Mean, SD & Range in years)	Postmortem Quality Measures: PMI; Ph; RIN (in Mean & Range)		
Major Depressive Disorder	30 (11)	24 Suicides (10 Female Suicides), 6 Natural (1 Female)	47 ± 16.8; 13 - 75	PMI (28.86; 15–52.5) Ph (6.47; 5.98–6.77) RIN (6.82; 6–7.9)		
Bipolar Disorder	37 (12)	28 Suicides (8 Female suicides), 6 Natural (3 Female), 3 Accidental (1 Female)	43 ± 14.78; 18–76	PMI (31.05; 15–84.5) Ph (6.37; 6–6.86) RIN (6.96; 6–8.2)		
Normal Controls	33 (10)	0 Suicide, 28 Natural (9 Female), 2 Accidental, 3 Homicides (1 Female)	46 ± 15; 17–74	PMI (30.15; 15–60.5) Ph (6.55; 6.25–6.92) RIN (7.37; 6.3–8.3)		

the pooled *mood disorder < controls* comparison. In a nutshell, the ALE approach applied here and utilized in the VBM meta-analysis modeled the spatial uncertainty associated with each reported 3-dimensional brain location of significant between-group differences in GMV changes (Eickhoff et al., 2009). The meta-analysis therefore performed a rigorous ALE by comparatively assessing GMV changes in (i) *major depressive disorder < controls*, (ii) *bipolar disorder < controls*, and (iii) *mood disorders < controls* (i.e., pooled across major depressive disorder and bipolar disorder) vs controls.

2.4. BRAIN DISSECTION, RNA-EXTRACTION and SEQUENCING

Brain Dissection: The NIMH Human Brain Collection Core (HBCC) provided the *Postmortem* samples for which informed consents are acquired according to NIH IRB guidelines. Clinical characterization, neuropathology screening, and toxicology analyses followed previous protocols (Martin et al., 2006). The region of interest targeted for dissection was defined as portion of right AIC encompassing the identified reduced GMV in the completed meta-analysis (Fig. 1A) by the authors. Electronic image slices of the imaging-defined GMV loss volumes are shared with the HBCC neuropathologist who used these images to guide dissection of coronal tissue slabs of each postmortem donor brain at the NIH clinical center. The dissected regional volume corresponded to the anterior portion of the insula where the caudate and putamen are approximately equal in size and tissue was dissected from this section for each brain for use in study 2 RNA-sequencing (Fig. 1B and C).

RNA-Extraction: As additional service, the HBCC further pulverized all dissected tissues separately and aliquoted 50 mg from each sample for standardized total RNA processing. Specifically, RNeasy Lipid Tissue Mini Kit (50) was used for RNA purification using the 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers kit from Qiagen. DNase treatment was applied to the purified RNA using Qiagen RNase-Free DNase Set (50) kit consisting of 1500 Kunitz units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps. After DNase treatment, the purified RNA from the pulverized AIC tissue sections were used to determine RNA quality as measured in RNA integrity number (RIN) values using Agilent 6000 RNA Nano Kit consisting of the microfluidic chips, Agilent 6000 RNA Nano ladder and reagents on Agilent 2100 Bioanalyzer. Samples with RIN < 6 were excluded and the 100 samples meeting inclusion were shipped directly from the NIMH HBCC core to the Genome Sequencing and Analysis Facility (GSAF: <https://wikis.utexas.edu/display/GSAF/Home+Page>) at the University of Texas, Austin, USA for RNA-sequencing.

Illumina-Sequencing, Read-Mapping and Gene-Quantification: Total RNA was extracted and only samples with RNA integrity numbers (RIN values) greater than 6 as confirmed using the Agilent Bioanalyzer were used for library preparation. First, Ribosomal RNA was depleted using RiboMinus Eukaryote kit from Life Technologies (Foster City, CA, USA) for RNA-Seq and confirmed using an Agilent Technologies' Bioanalyzer (Santa Clara, CA, USA). mRNA selection was completed using the Poly(A) purist kit from Thermo Fisher and paired-end libraries with average insert sizes of 200bp were obtained using NEBNext Ultra II Directional RNAs Library Prep kit from New England Biolabs. All 100 samples were processed and then sequenced on the Illumina HiSeq 4000, at the Genome Sequencing and Analysis Facility (GSAF: <https://wikis.utexas.edu/display/GSAF/Home+Page>) at UT Austin, USA.

30 million paired-end reads per sample (150 base pairs in length) were generated by sequencing runs of 4 samples per lane of the sequencer. Sequenced reads were assessed for quality with Fastqc to specifically assess sequencing reads for median base quality, average base quality, sequence duplication, over-represented sequences and adapter contamination (Andrews, 2010). The reads were pseudo-aligned to the human reference transcriptome (GRCh38-gencode) using kallisto (Bray et al., 2016), and gene-level abundances were obtained.

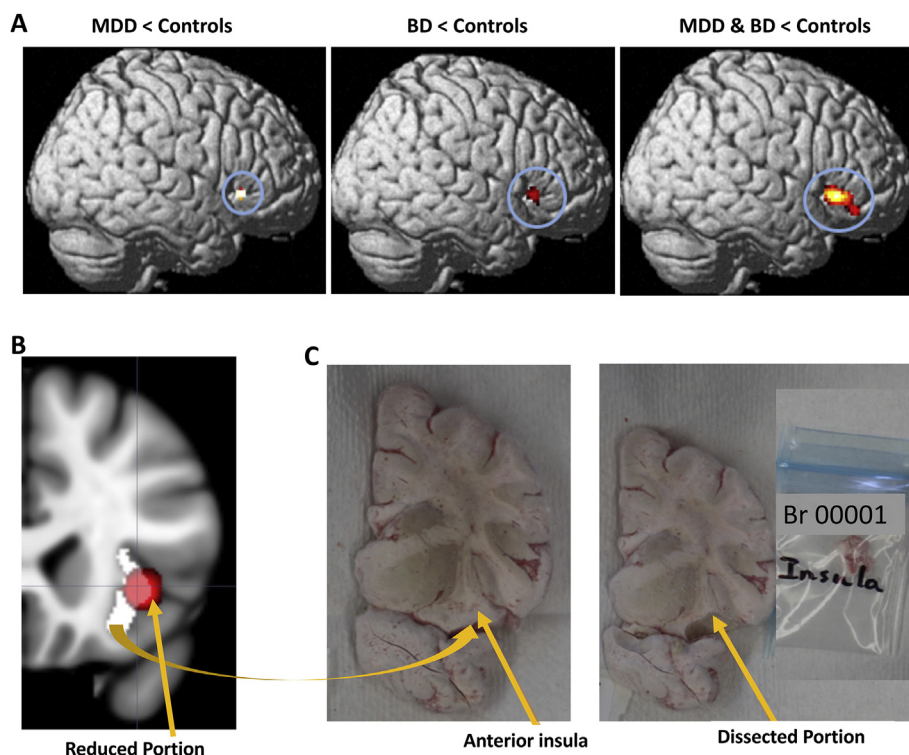


Fig. 1. Mood disorder brain structural signature derived sub-regional dissection. A) Right AIC gray matter volume is reduced (in living) major depressive disorder vs. controls, bipolar disorder vs. controls, and the pooled mood disorder (major depressive disorder and bipolar disorder) vs. controls. B) The anatomical space demarcation of the entire right AIC (white region) and the reduced mood disorder signature (red region) are indicated along with the estimated overlap (red overlapping white) between the reduced region and the AIC proper region that was targeted/dissected in the *postmortem* sample. C) *Postmortem* targeted region and the dissected portion, and dissected tissue (in labeled package). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Any genes with expression of 0 in 80% or more samples were filtered out to remove low count genes from further analysis. The abundances were normalized using DESeq2, and transformed with variance stabilizing transformation (a transformation to yield counts that are approximately homoscedastic, having a constant variance regardless of the mean expression value). Principal Component Analysis was performed using 25% of the highest variance genes in order to look at the underlying structure of the data and to identify the largest sources of variance.

For the DESeq2 analysis, we looked at median base quality, average base quality, sequence duplication, over-represented sequences and adaptor contamination. Median quality at every base was > 30 for all samples, and more than 90% of the reads had average base quality > 30 and as such, no trimming or filtering of reads was done. Because all samples had sequence duplication $< 60\%$ which is typical for high coverage RNA-Seq data, no samples were removed. Adaptor contamination percentages were $< 5\%$, so no adaptor trimming was performed. Lastly, genes with expression value of 0 in 80% of samples or more were removed from further analysis to correct for sporadic large fold-change outliers.

3. Statistical analysis

3.1. VBM meta-analysis

Convergence across the findings reported in previous VBM studies was assessed using ALE, which in brief consists of first modelling the spatial uncertainty associated with each reported location for significant between-group differences (Eickhoff et al., 2009; Turkeltaub et al., 2012). The ALE method therefore computes the convergence across experiments by the union of the ensuing probabilistic model relative to a null-distribution reflecting a random spatial association between the findings of different experiments (Eickhoff et al., 2012). Finally, statistical inference for a whole-brain corrected significance level of $p < 0.001$ used the threshold-free cluster enhancement method (Smith and Nichols, 2009). We performed ALE separately focusing on GMV changes in controls vs major depressive disorder,

controls vs bipolar disorder, and controls vs mood disorders in general (i.e., pooled across major depressive disorder and bipolar disorder).

3.2. Postmortem variable factor-analysis

The *postmortem* variables included *mood disorder-diagnoses*; # of *lifetime-Axis-I diagnostic-occurrences* (e.g. Axis-I-loading of the number of comorbid disorders such as (poly)-substance use disorders, psychosis, anxiety, eating disorders, etc.); # of *lifetime-Axis-III diagnoses* (e.g. medical conditions such as diabetes, cancer, cardiovascular disease, etc.); *manner of death* (e.g. natural, suicides, homicides or accidents) and *cause of death* as specified by the medical examiner reports (e.g. blunt force trauma to the chest, gunshot, motor vehicle accident, drowning, hanging, etc.); *demographics* (race, age at death, sex, years of education, number of children/fecundity, and marital records); *technical variables* (brain-weight, postmortem interval, pH, and RIN-values); and *toxicology* (blood alcohol/blood narcotics levels).

We applied Principal Axis Factoring using the Oblimin Rotation with Kaiser Normalization (Costello and Osborne, 2005) to identify higher-order factors explaining the differences in *postmortem* variables and included those variables with communalities of ≥ 0.5 .

3.3. Differential Gene Expression Analysis

Just like in the imaging meta-analytic ALE, we compared AIC gene expression profiles by first conducting simple comparisons across controls vs major depressive disorder, and controls vs bipolar disorder.

Our exploratory factor analysis assessed the relationship between all the *postmortem* variables to determine the existence of higher-order factor loadings that better explains *postmortem* variance than the original variables. This factor analysis revealed that cumulatively, variables such as 1) *mood disorder-diagnoses*, 2) *Axis I diagnostic-load*, and 3) *manner of death* (i.e. by suicide or other causes) together explains variability in cumulative measures of *psychiatric disease morbidity and suicide mortality* as a higher order factor. Note that data on any originally provided variables such as Axis-III/medical morbidity like cardiovascular disorders or cancer have not been accounted for in our

differential gene expression analyses. To identify gene expression signatures related to differences in our exploratory factor analysis derived higher order factor explaining variance in *psychiatric morbidity and suicide mortality*, we compared high scores on *psychiatric morbidity and suicide mortality* vs low scores of this factor. Specifically, we binned the factor scores of all samples into two groups of high *psychiatric morbidity-&suicide mortality* group with factor scores ≥ 0.82 vs low *psychiatric morbidity-&suicide mortality* group with factor scores of ≤ 0.82 using a split-half method of dividing the maximum *psychiatric morbidity-&suicide mortality* factor score across groups by 2 (i.e. the highest score of 1.64/2). Specifically, we applied a T-test by binning samples into two comparison groups for profiling gene-expression between 1) high vs low *psychiatric morbidity-&suicide mortality* across major depressed and control groups (i.e. major depressed with high scores vs major depressed and all controls with low scores); 2) high vs low *psychiatric morbidity-&suicide mortality* scores across bipolar and control groups (i.e. bipolar with high scores vs bipolar and all controls with low scores); 3) high vs low *psychiatric morbidity-&suicide mortality* scores across all groups (i.e. bipolar and major depressed with high scores vs bipolar, major depressed and all controls with low scores). Differential gene-expression between samples differing in *psychiatric morbidity-&suicide mortality* status was assessed based on the negative binomial distribution for modeled gene counts using DESeq2 (Anders and Huber, 2010). RIN-values were included in the DESeq2 design matrix as a covariate to control for its potentially confounding effects.

Our factor-analysis yielded a higher-order factor representing *psychiatric morbidity-&suicide mortality* including measures of suicide completion. In addition, our mood disorder samples have a substantially high incidence of completed suicides (i.e. 24 suicides vs 6 non-suicides out of 30 MDD and 28 suicides vs 9 non-suicides out of 37 bipolar samples). As such, we specifically examined the relationship between suicide completion and differential gene expression profiles by pooling non-suicide cases with controls. To do this, we applied a T-test to first compare a) gene expression difference in major depressive disorder death by suicides ($n = 24$) vs. major depressive disorder non-suicidal deaths ($n = 6$) & controls ($n = 33$); and b) gene expression differences in bipolar disorder death by suicides ($n = 28$) vs. bipolar disorder non-suicidal deaths ($n = 9$) & controls ($n = 33$); and finally compared c) the pooled mood disorder suicide vs. non-suicide (i.e. major depressive disorder and bipolar disorder death by suicides ($n = 52$) vs. major depressive disorder and bipolar disorder non-suicidal deaths ($n = 15$) without including the controls. Comparison c is in line with Pantazatos et al., 2017, whose results reported comparisons of major depressive disorder suicide 'i.e. MDD-S' vs. major depressive disorder non-suicides and controls 'i.e. MDD + CON' to estimate the effects of suicide. Controls were omitted in our last comparison (i.e. comparison c) to examine the existence of gene expression profiles that might be specifically linked to suicide completion vs non-suicide deaths in persons diagnosed with mood and comorbid psychiatric disorders. Only genes with corrected p-value (after Benjamini-Hochberg multiple testing correction) ≤ 0.05 and absolute fold changes ≥ 1.5 are reported as significantly differentially expressed. Pathways and gene-ontology (GO) terms enriched in these genes were identified using Enrichr (Chen et al., 2013; Kuleshov et al., 2016).

3.4. Weighted Gene Co-Expression Network Analysis (WGCNA)

Scale-free co-expression networks were constructed with gene-abundances using the WGCNA package in R (Langfelder and Horvath, 2008). WGCNA provides a global perspective and allows identification of co-expressed gene-modules. It avoids relying on arbitrary-cutoffs involved in selecting differentially-expressed genes and instead identifies a group of genes that are changing in the same direction and magnitude, even if these changes are smaller in magnitude. WGCNA identifies modules of genes that are co-expressed thereby identifying genes that are likely co-regulated or may belong to the same functional

pathway, using a dynamic tree-cutting algorithm based on hierarchical clustering (i.e. minimum module size = 30). A given module's eigen-gene, which is defined as the first principal component of the expression matrix of the corresponding module, can be correlated to sample variables to identify modules of interest. We correlated the module eigengenes to different postmortem sample characteristics and selected the two modules that showed significant correlation to variables of interest such as *diagnostic, and suicide-linked variables*. Driver genes (i.e. genes within co-expressed gene modules whose singular expression patterns are similar to the overall expression profile of the entire co-expressed modules) within these modules were used to identify pathobiological functions associated with each module.

4. Results

4.1. Identification of a mood disorder neuroanatomical signature in living brains

The study 1 VBM meta-analysis ($N = 2387$) revealed reduced GMV in the right AIC in mood disorders ($p < 0.0001$ corrected) (Fig. 1A) consistent across both major depressive disorder and bipolar disorder, since major depressive disorder and bipolar disorder groups did not differ significantly (Fig. 1A). The localized reduced AIC neuroanatomical-signature for mood disorders was manually segmented in ITKSNAP (<http://www.itksnap.org/pmwiki/pmwiki.php>) (Fig. 1B) and the segmented volume guided *postmortem* dissection of tissue used in RNA-seq characterization of gene-expression (Fig. 1C).

4.2. Postmortem group differences and factor analysis

Using principal axis factoring of the postmortem variables in study 2, our explorative factor analysis revealed three higher-order factors that cumulative explained 42.22% of the total variance of the postmortem variables. Specifically, 17.052% of the total variance was explained by a higher-order factor we called *demographics and medical diagnostic status* [with high communalities loading with the following original postmortem variable: 1) *number of children* (0.643), 2) *marital status* (0.834), 3) *lifetime axis-III medical diagnostic status* (0.635), and 4) *age at death* (0.584)]. Furthermore, 16.27% of the variance was explained by a higher-order factor we called here *psychiatric disease morbidity and mortality* [with high communalities loading with the following original postmortem variables: 1) *diagnosis* (0.897), 2) *life-time axis-I psychiatric diagnostic status* (0.695), and 3) *suicide completion status* (0.670) respectively]. Finally, 8.89% of the total variance was explained by a higher-order factor we called *RIN-scores* [with high communalities loading more prominently with the following original postmortem variables: 1) *RIN-value* (0.801), and showing albeit weaker negative communality loadings with both 2) *sex* (-0.494) and 3) *postmortem interval* (PMI) (-0.357)].

Post-hoc multiple comparisons of the high-order factor-analytic variables yielded group-differences in *psychiatric morbidity-&suicide mortality*: using Bonferroni correction, *psychiatric morbidity-&suicide mortality* was highest in bipolar disorder vs controls ($p < 0.0001$, Supplementary Fig. 1); bipolar disorder vs major depressive disorder ($p < 0.0001$, Supplementary Fig. 1), and major depressive disorder vs controls ($p < 0.0001$, Supplementary Fig. 1). Linear regression revealed that *psychiatric morbidity-&suicide mortality* negatively predicted (a) the higher-order factor termed here as *higher order RIN-scores* (which is an aggregate measure of RIN-values as the variable loading highest on the factor, and negative loadings of sex and PMI which loaded weakly on this factor) at ($B = -2.1$, $t = -3.3$, $p = 0.001$) across groups; (b) fecundity at ($B = -3.17$, $t = -2$, $p = 0.041$, $d = 1.79$) across groups; and (c) age at death at ($B = -8.7$, $t = -0.27$, $p = 0.025$, $d = 2.1$) only across major depressive disorder and bipolar disorder. These findings prescribed our subsequent analytical focus on *psychiatric morbidity-&suicide mortality* and RIN-values were included as covariates for all

differential gene expression analyses. Including RIN-values as covariate was necessary especially given the negative relationship between this important measure of RNA integrity/quality (as reflected in the higher-order factor we are calling *higher-order RIN-score*) and the degree of psychiatric morbidity-&-mortality across the postmortem samples.

4.3. Differential-expression identified enriched Postmortem Anterior Insula Gene-Expression Signatures for Mood Disorders

Differential gene-expression analyses assessed transcriptomic profiles associated with variability between cases vs controls, between high vs low *psychiatric morbidity-&-mortality*, and between *suicides vs non-suicides*. For the case-control analysis, we compared gene expression between controls vs MDD and found 351 differentially expressed genes that are mainly associated with cellular homeostatic, regulation of neurogenesis, and hormonal signaling pathways (see [Supplementary Table 2](#)). We further compared controls vs bipolar disorder and found 23 differentially expressed genes in mainly cellular, metabolic, hormonal signaling and neurodevelopmental pathways (see [Supplementary Table 3](#)).

For comparison of gene expression between high vs low morbidity and mortality, we binned mood disorder associated higher-order factor scores for *psychiatric morbidity-&-suicide mortality* for each sample into ≤ 0.82 for low vs ≥ 0.82 for high scoring individual donors using a split-half method of dividing the maximum score across groups by 2 (i.e. $1.64/2$) and found differentially-expressed immune and inflammatory-pathways, toll-like receptor-signaling, chemokine-signaling, cytokine-cytokine receptor interactive pathway genes, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb-signaling), TNF signaling pathway and immune response signaling ([Fig. 2A–D](#); [Supplementary Fig. 2-3](#)).

Specifically, within major depressive disorder and controls (i.e. major depressive disorder cases scoring high on *psychiatric morbidity-&-mortality* vs low scores and controls), we found 8 under-expressed inflammatory cytokine and AKT-signaling (*CCL3* & *CCL4*); mRNA splicing and enzyme-binding (*HSPA6*); cellular development and homeostasis (*HSPA7*, *PCSK5*, & *SERPINH1*) genes; and 1 mitochondrial cytochrome-C-oxidase lncRNA-pseudogene (*MTCO1P12*); and an over-expressed lncRNA-pseudogene (*MTCO2P12*) ([Fig. 2A–D](#); [Table 2A](#); [Supplementary Fig. 2](#)).

A similar differential gene-expression analysis in bipolar disorder individuals with higher *psychiatric morbidity-&-mortality* scores vs those with lower scores and controls was associated with 3 under-expressed neuroprotection, neurodevelopment and CNS-diseases including major depressive disorder (*CXCL11* & *SELE*) pathway genes and inflammatory cytokine and AKT-signaling (*CCL4*) ([Fig. 2A–D](#); [Table 2B](#); [Supplementary Fig. 3](#)). Analysis of high *psychiatric morbidity-&-suicide-mortality* scores in the pooled major depressive disorder and bipolar disorder (i.e. mood disorders) vs low *psychiatric morbidity-&-mortality* scores in the pooled mood disorders and controls yielded 8 under-expressed inflammatory cytokine and AKT-signaling (*CCL4*); cell-neurodevelopment and CNS diseases (*CXCL11*, *SELE*, *PCSK5*, & *HSPA7*); and transcriptional regulatory-RNA (*MIR5190*); but also 2 over-expressed innate immunity (RP11-566K19.8) pathway genes; and a lncRNA-pseudogene (*MTCO2P12*) ([Fig. 2A–D](#); [Table 2C](#); [Supplementary Fig. 4](#)).

We then examined gene-expression profiles related specifically to suicide by comparing suicide completers vs non-suicide completers first in major depressive disorder and control donors (i.e. comparing gene-expression in major depressive disorder suicides vs major depressive disorder non-suicide and controls), and found 3 over-expressed WNT-signaling (*FZD8*); transcriptional regulation of adaptive responses to oxygen tension/hypoxia, DNA-binding transcriptional activity/co-activation (*HIF3A*); and dioxygenase activity (*PHYHD*) pathway genes ([Table 3A](#)). Comparing gene expression profiles for suicide completion in bipolar disorder-suicides vs non-suicidal bipolar disorder and controls, we found only 1 under-expressed CNS-disease (*SORD2P*) pathway

gene ([Table 3B](#)).

Assessing gene-expression profiles associated with suicide-completion in the pooled major depressive disorder and bipolar disorder completed-suicides vs major depressive disorder and bipolar disorder non-suicidal deaths yielded 20 under-expressed innate immune and inflammatory-cytokine (*CRISPLD1*, *CHI3L1*, *P2RY6*, & *SECTM1*); protein-protein interaction regulatory (*MT1A*, *HILPDA*, *HELZ2*, *FOSB*, *FAM198A*, *SOCS3*, & *TPST1*); neurodegeneration (*RP11-155G14.6*, *SLC39A14*, & *SERPINA3*); cellular-neurodevelopmental and transcriptional (*LIMK2*, *SFN*, & *EDN3*) pathway genes ([Table 3C](#)); as well as uncharacterized genes/pseudogenes (*MTND2P28*, *BAALC-AS1*, *RP11.420L9.5*, & *RP11.435J9.2*). We also found 4 over-expressed intracellular protein transport (*TBC1D3E*); inflammatory (*RP11.1100L3.8*); cell fate and apoptosis regulation (*GZMA*); and transcriptional, embryonic/forebrain cell development and defect (*CTD-2207O23.3*, & *TDGFI*); and neurodevelopmental (*EDN3*) pathway genes ([Table 3C](#)).

WGCNA Identified co-regulated gene modules for *Postmortem Anterior Insula Gene-Expression Signatures for Mood Disorders*.

WGCNA characterized the potential co-regulated gene-modules involved in mood disorder *morbidity and suicide-mortality*. We found 2 co-expression gene modules (coded as blue and black [Figs. 3–4](#); [Supplementary Fig. 5](#)) whose expression strongly correlated with *psychiatric morbidity-&-mortality* variables. Using the 30 most highly connected genes (i.e. hub genes) in these two modules, we identified enriched KEGG pathways to elucidate the biological significance of each set of genes. The Blue module was significantly enriched in biological pathways involved in fundamental cellular signaling processes such as ATPase activity, cAMP signaling, sodium and calcium channel functions, transcriptional regulation, and neuronal development/proliferation and differentiation ([Fig. 3A–C](#); [Supplementary Fig. 5](#)). The Black module was significantly enriched in biological pathways involved in innate immune functions, cellular homeostasis and metabolic regulations, dopamine DARPP32 feedback onto cAMP functions in the regulation of dopaminergic synapse, glial cell development, addiction and major depressive disorder risk ([Fig. 3A–C](#) and [Fig. 4A](#) and [B](#); [Supplementary Fig. 5](#)). The top pathway enriched in the blue module being *Vibrio cholera* infection indicates that the co-expressed genes in this module relate to activating an immune response.

5. Discussion

Using a neuroimaging meta-analysis to refine a structurally reduced AIC brain region of interest in major depression and bipolar disorder, we identified a *postmortem* cross-diagnostic mood disorder linked *psychiatric morbidity-&-mortality* associated gene-expression signature within this neuroimaging meta-analysis identified reduced AIC gray matter signature. Given this AIC sub-region's documented role in regulating affective and physical pain/distress, general bodily homeostatic and interoceptive salience, our convergent structural neurobiological and functional gene-expression findings of (a) a reduced right AIC gray matter signature in living mood disorder patients, coupled with (b) a preponderance of under-expressed, but also albeit to a lesser extent, over-expressed gene-expression signatures within the identified AIC-locale in mood disorder *postmortem* brains, identified an anatomically-precise functional gene-expression basis for mood pathologies. Of note, we found a negative relationship between measures of AIC tissue RNA integrity/RIN and mood disorder morbidity, which could have implications for our results even though we corrected for RIN as a covariate. Whether our observed negative correlations between RIN measures of AIC tissue and mood disorder morbidity and suicide mortality factors is regionally dependent needs to be assessed in future studies.

In light of the right AIC's role in sensing emotional/physical pain associated with social isolation and disconnection ([Eisenberg, 2015](#)), these results provides a potential gene-regulatory window into the neuropathological ramifications for increased mood disorder associated

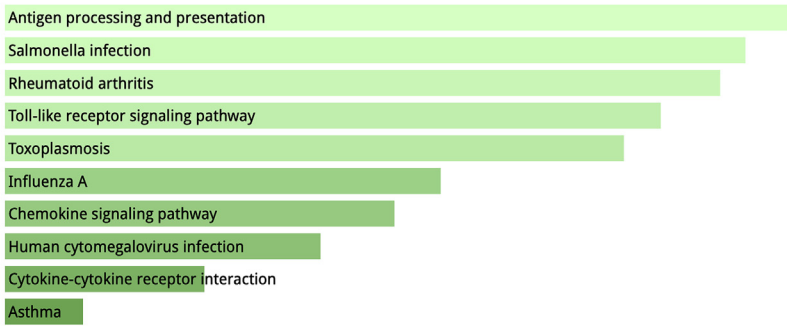
A High vs Low psychiatric morbidity-&-suicide-mortality

DEGS in MDD & Controls			DEGS in Bipolar & Controls		
Gene Name	T-Statistics	P-Adjusted	Gene Name	T-Statistics	P-Adjusted
HSPA6	-7.6509335	9.58E-11	SELE	-5.39264	0.00033
PCSK5	-6.2292396	1.69E-06	CXCL11	-4.50036	0.0244
HSPA7	-5.9769629	5.70E-06	CCL4	-4.26264	0.05
MTCO2P12	5.9698974	5.70E-06			
MTCO1P12	-5.9227774	6.51E-06			
CCL3	-5.391272	0.0001259			
CCL4	-5.2268724	0.0002758			
SERPINH1	-4.7816991	0.0025027			

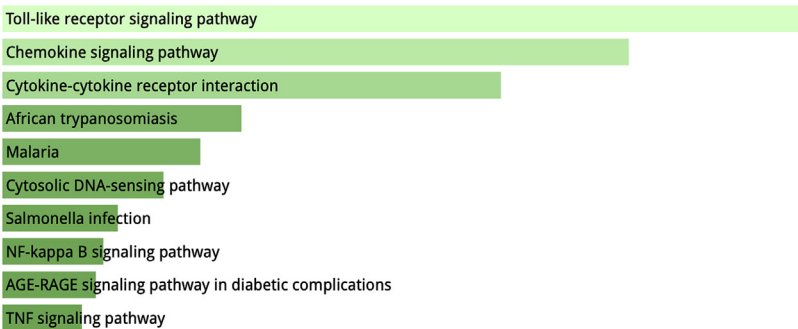
DEGS in MDD & BD		
Gene Name	T-Statistics	P-Adjusted
PCSK5	-6.8492581	3.57E-08
HSPA7	-6.3807189	6.35E-07
MTCO2P12	6.16700751	2.01E-06
CCL4	-5.2725169	0.00032318
SELE	-4.337169	0.0297113
CXCL11	-4.2945224	0.03153445
MIR5190	-4.2341123	0.03306465
RP11-566K19.8	4.2574146	0.03306465

Fig. 2. Differentially-expressed genes associated with high vs low psychiatric morbidity-&-suicide mortality scores. A) Significantly differentially expressed genes identified for MDD&Controls; BD&Controls and all Mood Disorder& Controls relating to high vs low higher-order factor scores on psychiatric morbidity-&-suicide mortality. The gene names are listed along with the log2fold change and adjusted p-value. Genes with absolute fold change ≥ 1.5 (here log2-fold changes are provided) and adjusted p-values ≤ 0.05 were selected as significantly differentially expressed. B-D) shows Kyoto Encyclopedia of Genes and Genome (KEGG) pathways reflecting the list of enriched pathways relating to the genes that were differentially-expressed between high vs low psychiatric morbidity-&-suicide mortality in MDD and controls, BD and controls and in all mood disorder and controls. The pathways are ranked by a combined score of p-value and rank based score. B) MDD and control samples only; C) Bipolar disorder and control samples only; D) major depressive disorder and bipolar samples and controls.

B MDD & Controls – Enriched KEGG Pathways



C Bipolar & Controls – Enriched KEGG Pathways



D Bipolar & MDD – Enriched KEGG Pathways

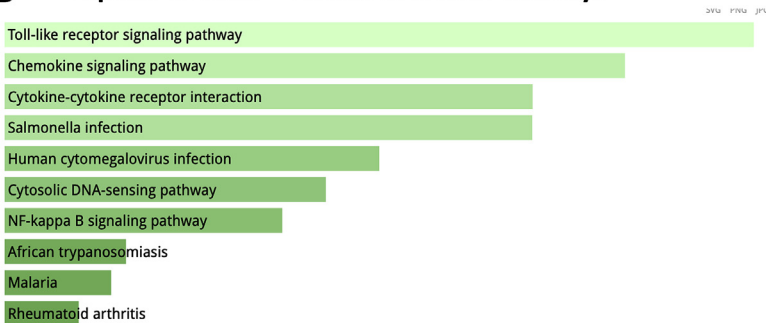


Table 2A
Significantly differentially expressed genes among MDD & Control samples, comparing high vs low psychiatric morbidity-&-suicide mortality.

Ensemble ID	Gene Name	Base Mean	Log2 Fold Change	Log2 Fold Change SE	T statistic	P value	P-Adjusted
ENSG00000173110.7	HSPA6	673.589883	-5.2416381	0.68509784	-7.6509335	2.00E-14	9.58E-11
ENSG00000099139.13	PCSK5	13671.7273	-4.1030604	0.65867757	-6.2292396	4.69E-10	1.69E-06
ENSG00000225217.1	HSPA7	97.7742788	-3.3412255	0.55901728	-5.9769629	2.27E-09	5.70E-06
ENSG00000229344.1	MTCO2P12	17.5004855	2.73252208	0.45771676	5.9698974	2.37E-09	5.70E-06
ENSG00000237973.1	MTCO1P12	1258.78699	-2.9417956	0.49669191	-5.9227774	3.17E-09	6.51E-06
ENSG00000277632.1	CCL3	37.4322345	-3.0366661	0.56325596	-5.391272	7.00E-08	0.00012591
ENSG00000275302.1	CCL4	38.2199233	-2.3413235	0.44793967	-5.2268724	1.72E-07	0.0002758
ENSG00000149257.13	SERPINH1	482.52589	-2.0122431	0.42082178	-4.7816991	1.74E-06	0.00250266

psychiatric morbidity (O'Connor and Nock, 2014; Nock et al., 2018), which could compound suicidal outcomes (Nock et al., 2018). The right AIC's role in coding affective salience and psychological pain (Shneidman, 1998), and the possible collective modulatory impact of these affective states on maladaptive impulses such as the urge to escape unbearable misery via suicide, provides a putative anatomical framework for mood disorder associated psychiatric morbidity-&-mortality (Shneidman, 1998; Craig, 2009; Slavich et al., 2010; Mee et al., 2011; Hatton et al., 2012; McGrath et al., 2013; Wager et al., 2013; Eisenberg, 2015; Goodkind et al., 2015; Koechlin, 2016; Wise et al., 2017; Khalsa et al., 2018). The identified association between mood and associated psychiatric morbidity-&-mortality scores with fecundity, and with predominant under-expressed gene-expressive functions suggests an evolutionary significance of the current results. For instance, genetic abnormalities governing aberrant AIC-mediated social deficits (Jabbi et al., 2012) and severe mood disorders could attenuate reproductive prowess (Mullins et al., 2017).

Within the identified reduced AIC signature, our differential gene-expression analyses identified predominant down-regulations in innate immune functions, inflammation pathways, and AKT-signaling related to mood-associated psychiatric morbidity and suicidal-mortality. Further differential-expressions involving cellular-homeostatic, neurodevelopmental, and transcriptional pathways in the AIC were found to be associated with psychiatric morbidity and suicidal-mortality in less directionally-specific (i.e. up/down-regulatory) patterns. While the cellular-origins of our findings of predominantly downregulated immune-related and neurodevelopmental gene-expression changes cannot be specified with our bulk tissue RNA-seq methods, astrocyte-derived cytokine functions have been documented to induce synapse engulfment/elimination and thereby influence synaptic pruning (Vainchtein et al., 2018; Bennett and Molofsky, 2019). Furthermore, immune pathway mediation of mood dysfunctions and related psychiatric diagnoses are proposed to be likely multifaceted as part of the brain's immune-related response repertoire such as toll-like receptor signaling, can be influenced by both *a*) pathogen-associated molecular patterns (Kawai and Akira, 2007) and *b*) danger-associated molecular patterns (Klune et al., 2008; Piccinini et al., 2010). This perspective on the multifaceted nature of brain immune signaling in relation to behavioral dysfunctions like mood disorders deserves further analysis especially in light of 1) the strong link between endogenous danger-associated immune/inflammatory cellular functions in promoting homeostasis (Klune et al., 2008) and 2) the potential impacts of environmental stress experiences on endogenous cellular stress as well as inflammatory responses (Slavich et al., 2010).

Existing findings of both over-expressed immune-related signals

(Pandey GN, 2017), and our current imaging-guided AIC postmortem findings that clearly replicated previous results of predominantly under-expressed immune/inflammatory function (e.g. chemokine ligand 2 CCL4) and genes/pseudogenes implicated in regulatory cellular functions (e.g. a serpin peptidase inhibitor & HSPA7) in postmortem dorsolateral prefrontal cortex BA9 brains of mood disorder individuals with and without suicide completion (Pantazatos et al., 2017), needs to be better contextualized. For instance whether these differences in the directionality of regulatory patterns of gene-expression findings (i.e. over-expressed vs under-expressed immune expressive genes) in mood disorder postmortem studies is somewhat related to methodological differences in terms of targeted micro-RNA assays vs whole transcriptome sequencing approaches, or differences in sample sizes, sample selection criteria, or qualitative postmortem material differences between studies, needs to be examined more carefully.

While we cannot fully disentangle medication effects from diagnosis effects in our current preliminary analysis, it is worth noting that our observations suggest that diagnostic effects relating to both our suicide related and non-suicide related gene expression results are likely manifold, beyond mood disorders. This is relevant especially in light of our sample characteristics showing that suicide completion is collinear with mood disorder diagnostic severity, and that suicide completers are about 80% of the included cases. Our findings of consistently similar gene expression patterns across the various morbidity & mortality comparisons further support the thesis that suicide completion and mood disorder morbidity are likely more inter-related at the biological level than previously appreciated. Together, our current findings are consistent with data on the role of immune dysfunctions in CNS diseases (Oquendo et al., 2014; Wohleb et al., 2016; Pantazatos et al., 2017; Butovsky and Weiner, 2018), and inflammasome functional prediction of major depressive disorder treatment outcomes (Syed et al., 2018). The results further lend credence to the hypothesis that neurodevelopmental and transcription-factor genes are critical mediators of complex adaptive brain functions (Changeux, 2017); especially within the context of the AIC's integration of affective and physiological feeling states (Craig, 2009; Slavich et al., 2010; Kurth et al., 2010; Eisenberger, 2015; Khalsa et al., 2018) 'including homeostatic maintenances in sickness and health' (Craig, 2009; Khalsa et al., 2018), that are likely not entirely independent of both pathogen-associated molecular patterns (Kawai and Akira, 2007) and danger-associated molecular patterns (Klune et al., 2008; Pantazatos et al., 2017) known to induce brain immune signaling.

At the systems level, the toll-like receptor (TLR) pathway genes found to be under-expressed here are documented to recognize conserved motifs in microorganisms (Akira, 2003) and stimulation of TLRs

Table 2B
Significantly differentially expressed genes among BD & Control samples, comparing high vs low psychiatric morbidity-&-suicide mortality.

Ensemble ID	Gene Name	Base Mean	Log2 Fold Change	Log2 Fold Change SE	T statistic	P value	P-Adjusted
ENSG00000007908.15	SELE	77.6309031	-2.7215607	0.50468091	-5.3926365	6.94E-08	0.00033295
ENSG00000169248.12	CXCL11	17.7305295	-2.2991855	0.51088929	-4.5003596	6.78E-06	0.02439815
ENSG00000275302.1	CCL4	33.6351692	-1.7331318	0.40658683	-4.2626364	2.02E-05	0.05

Table 2C

Significantly differentially expressed genes among disorder (BD & MDD) samples, comparing high vs low psychiatric morbidity-&suicide mortality.

Ensemble ID	Gene Name	Base Mean	Log2 Fold Change	Log2 Fold Change SE	T statistic	P value	P-Adjusted
ENSG00000099139.13	PCSK5	8665.693903	-3.426020	0.500203143	-6.8492581	7.42E-12	3.57E-08
ENSG00000225217.1	HSPA7	74.90215768	-2.6942071	0.422241949	-6.3807188	1.76E-10	6.35E-07
ENSG00000229344.1	MTCO2P12	12.98074852	2.10792157	0.341806227	6.1670075	6.96E-10	2.01E-06
ENSG00000275302.1	CCL4	32.42544283	-1.8328167	0.347617047	-5.2725168	1.35E-07	0.000323181
ENSG0000007908.15	SELE	61.92229332	-1.8647268	0.429941028	-4.3371689	1.44E-05	0.029711296
ENSG00000169248.12	CXCL11	16.72696047	-1.7935016	0.41762541	-4.2945223	1.75E-05	0.031534454
ENSG00000266146.1	MIR5190	2.231468246	-1.4867954	0.351146909	-4.2341122	2.29E-05	0.033064652
ENSG00000273679.1	RP11-566K19.8	3.175462468	1.18656433	0.278705375	4.25741460	2.07E-05	0.033064652

Abbreviations: baseMean, normalized read counts of all samples; log2Foldchange, effect size estimate; lfcSE, standard error of the log2foldchange; stat, Wald statistical test values; pvalue, uncorrected p-value; p-adj, corrected p-value. Fold change is calculated as the ratio of mean expression in high psychiatric morbidity & suicide mortality score to the mean expression in low psychiatric morbidity & suicide mortality score.

are shown to mediate acute-immune defense and cytokine production/release (Perkins, 2007). First, the observed under-expression/suppression of toll-like receptors and immune pathway genes in higher morbidity and suicide mortality afflicted mood disorder donors point to this pathway's possible dysregulation in depressive illness. This is especially in light of evidence that depression was the most recent diagnostic mood episode in 21 of the 37 bipolar donors coupled with data that 26 of the 30 MDD donors have a lifetime history of recurrent depressive episodes in the current samples.

Second, our identified under-expressed NF- κ B pathway genes are implicated in controlling DNA transcription, cytokine production and cell survival (Meffert et al., 2003), and are essential for cellular-immune response to infection, stress-related shocks (Van Amerongen and Nusse, 2009), and synaptic plasticity and memory (Meffert et al., 2003; Van Amerongen and Nusse, 2009). Of interest, NF- κ B pathway genes are also identified as a critical mediator of stress-impaired neurogenesis and depressive-like behavior caused by exposure to chronic stress in mice (Koo et al., 2010). Furthermore, recent studies identified an NF- κ B pathway involvement in the pathophysiology of depressive illness, especially relating to neurogenesis, synaptic transmission and plasticity (Caviedes et al., 2017). NF- κ B related mechanisms have also been shown to have antidepressive therapeutic effects in general (Wu et al., 2018) and in inflammatory disease comorbidity with depression-like symptoms in mice (Su et al., 2017).

Third, the identified under-expressed chemokine-signaling pathways govern critical spatiotemporal cell-positioning during developmental coordination and translational guidance of cell-locomotion and migration (Turner et al., 2014). Fourth, the identified under-expressed cytokines are implicated in cell-specific innate and adaptive inflammatory host defenses, cellular-development, cell-death, angiogenesis, and maintenance of cellular homeostasis (Syed et al., 2018). Conversely, the Wnt- β -catenin signaling pathway found to be over-expressed in major depressive disorder suicides is an evolutionarily conserved inter-cellular communication system that mediates stem cell renewal, cell-proliferation and differentiation during embryogenesis and adulthood (Meffert et al., 2003). Taken together, our observations of convergent under-expressed TLRs, NF- κ B, chemokine, and cytokine-cytokine interactive pathways transcriptomic signatures for psychiatric morbidity-&mortality; and suicide-mortality-specific over-expressed Wnt signaling pathway, suggest that cellular processes may be dysregulated already very early in development. These processes may negatively shape adaptive immune, inflammasome and chemokine-

cytokine responses to adverse socio-emotional and environmental distress, with a prolonged experience of these adverse circumstances likely leading to compromised AIC anatomical and physiological integrity, and associated maladaptive rupture in regulatory mood states.

Of relevance, our AIC postmortem WGCNA results which reflects a global perspective whole transcriptome gene expression by identifying co-expressed gene-modules for a) lifetime mood disorder-diagnoses, b) lifetime Axis-I diagnoses, and c) suicide-completion status as well as the lethality of the committed suicide methods in genetic pathways involved in fundamental cellular signaling processes like transcriptional regulation, ATPase and cAMP signaling, sodium and calcium channel functions, neuronal developmental processes (Blue module); as well as capturing co-expressed gene-modules a) lifetime mood disorder-diagnoses, b) lifetime Axis-I diagnoses, and c) suicide-completion in genetic pathways involved in innate immune functions, cellular homeostasis and metabolic regulations, regulation of dopaminergic synapse, glial cell development, addiction and major depressive disorder risks. While the regional specificity of these findings needs further analysis, the WGCNA results recapitulate the differential gene expression findings and further underscore the complex interactive gene-functions implicated in brain mediation of mood disorder morbidity and related suicidal risk tendencies.

In line with our differential gene expression and WGCNA findings of neurodevelopmental, immune, inflammatory, transcriptional, ATPase and cellular and hormonal signaling pathway alterations in mood disorder morbidity and suicides, previous gene expression studies of the postmortem prefrontal brain system of completed suicides found altered ATPase signaling in cases (Pantazatos et al., 2017). Further studies of suicide victims with and without major depression found extensively altered limbic and hippocampal gene expression where processes linked to major depression were associated with abnormal transcription and cellular metabolic processes (Sequeira et al., 2007). At the synaptic level, altered gene expression related to intracellular signaling in GABAergic (Sequeira et al., 2007; Sequeira et al., 2009) and glutamatergic transmission pathways (Sequeira et al., 2009) have also been reported. Gene set enrichment analysis and expression pattern exploration in postmortem brains across the lifespan in bipolar disorder has recently implicate enrichments of neurodevelopment processes in bipolar illness (Mühleisen et al., 2018). Combining differential expression and WGCNA, a recent postmortem study identified cross-species major rearrangement of transcriptional patterns in the ERK signaling and pyramidal neuron excitability in major depressive illness,

Table 3A

Significantly differentially expressed genes among MDD & Control samples, comparing across suicide completion vs non-suicide deaths.

Ensemble ID	Gene Name	Base Mean	Log2 Fold Change	Log2 Fold Change SE	T statistic	P value	P-Adjusted
ENSG00000124440.15	HIF3A	606.769243	0.876374	0.212153605	4.13085	3.61E-05	0.0368
ENSG00000177283.6	FZD8	300.131493	0.680022	0.164073892	4.14461	3.40E-05	0.0368
ENSG00000175287.18	PHYHD1	256.20944	0.743648	0.183138111	4.06059	4.89E-05	0.0399

Table 3BSignificantly differentially expressed genes among **BD & Control** samples, comparing across suicide completion vs non-suicide deaths.

Ensemble ID	Gene Name	Base Mean	Log2 Fold Change	Log2 Fold Change SE	T statistic	P value	P-Adjusted
ENSG00000259479.6	SORD2P	39.3112	-2.083173	0.437129322	-4.76558	1.88E-06	0.00451

Table 3CSignificantly differentially expressed genes among mood disorder (**BD & MDD**) samples, comparing across suicide completion vs non-suicide deaths.

Ensemble ID	Gene Name	Base Mean	Log2 Fold Change	Log2 Fold Change SE	T statistic	P value	P-Adjusted
ENSG00000278599.5	TBC1D3E	25.4554567	23.06765	1.964082558	11.7447	7.51E-32	1.09E-27
ENSG00000247081.7	BAALC-AS1	89.3999992	-1.401354	0.251885981	-5.56345	2.64E-08	0.000191
ENSG00000125740.13	FOSB	549.939163	-2.064541	0.416963317	-4.95137	7.37E-07	0.002132
ENSG00000133048.12	CHI3L1	673.769904	-1.855486	0.373494995	-4.9679	6.77E-07	0.002132
ENSG00000240758.2	RP11-155G14.6	17.3179072	-1.974256	0.435400142	-4.53435	5.78E-06	0.013931
ENSG00000196136.16	SERPINA3	185.210936	-3.238714	0.732431027	-4.42187	9.79E-06	0.017695
ENSG00000104635.13	SLC39A14	706.876471	-0.896615	0.20633378	-4.34546	1.39E-05	0.020762
ENSG00000175793.11	SFN	19.9333245	-2.489873	0.573913242	-4.33841	1.44E-05	0.020762
ENSG00000135245.9	HILPDA	355.391455	-1.717122	0.404534173	-4.24469	2.19E-05	0.024359
ENSG00000171631.14	P2RY6	45.696962	-0.836171	0.196366906	-4.25821	2.06E-05	0.024359
ENSG00000274340.1	RP11-435J9.2	8.2713634	-1.290148	0.307060726	-4.20161	2.65E-05	0.027386
ENSG00000141574.7	SECTM1	24.3660716	-1.631158	0.393087882	-4.1496	3.33E-05	0.028342
ENSG00000182541.17	LIMK2	562.154237	-0.687634	0.165430434	-4.15664	3.23E-05	0.028342
ENSG00000259884.1	RP11-1100L3.8	30.1302833	1.838831	0.442582349	4.15478	3.26E-05	0.028342
ENSG00000169902.13	TPST1	450.331126	-0.587784	0.146007754	-4.02571	5.68E-05	0.041089
ENSG00000241186.8	TGDF1	25.9563298	1.061656	0.263613427	4.02732	5.64E-05	0.041089
ENSG00000270504.1	RP11-420L9.5	68.8031378	-0.750647	0.188675626	-3.97851	6.93E-05	0.047775
ENSG00000130589.16	HELZ2	172.965424	-0.942267	0.238112529	-3.95724	7.58E-05	0.048403
ENSG00000205362.11	MT1A	35.4404617	-2.163200	0.547133516	-3.9537	7.70E-05	0.048403
ENSG00000121005.8	CRISPLD1	156.537535	-0.70863	0.181614685	-3.90185	9.55E-05	0.049967
ENSG00000124205.15	EDN3	123.582054	0.725545	0.186106883	3.89854	9.68E-05	0.049967
ENSG00000144649.8	FAM198A	135.992749	-0.742381	0.190833801	-3.8902	0.0001	0.049967
ENSG00000145649.7	GZMA	2.36492564	2.726390	0.697779595	3.90724	9.34E-05	0.049967
ENSG00000184557.4	SOCS3	279.864712	-2.207043	0.56519875	-3.9049	9.43E-05	0.049967
ENSG00000225630.1	MTND2P28	2528.60696	-2.346222	0.598989373	-3.91697	8.97E-05	0.049967

Abbreviations: baseMean, normalized read counts of all samples; log2Foldchange, effect size estimate; lfcSE, standard error of the log2foldchange; stat, Wald statistical test values; pvalue, uncorrected p-value; p-adj, corrected p-value. Fold change is calculated as the ratio of mean expression in high psychiatric morbidity & suicide mortality score to the mean expression in low psychiatric morbidity & suicide mortality score.

with limited overlap in males and females in both depressed humans and chronic stressed mice (Labonté et al., 2017). Taken together, substantive evidence of ATPase, cellular processes and signaling, and neurodevelopmental pathway gene expression abnormalities found in limbic and prefrontal brain regions in previous studies are in line with our current findings of AIC postmortem gene expression profiles associated with mood disorder morbidity and mortality. Future studies including multiple brain regions and sex-specific analyses will be needed to provide in-depth mechanistic understanding of the underlying transcriptional landscapes associated with diagnostic and sex-specific aspects of mood illnesses and related suicide mortality.

Unlike cardiovascular disease and cancer research, where pathobiological measures are causally linked to disease morbidity and endpoint mortality, the causal neurobiological root causes of mental illnesses and their associated mortality endpoints such as suicides are unknown, limiting measurable biological predictability of suicidal-mortality. Our findings of convergent structural neuroanatomically defined functional gene-expression signatures for mood disorder associated psychiatric morbidity-&-mortality across major depressive disorder and bipolar disorder supports shared heritable neurogenetic pathologies underlying comorbid neuropsychiatric symptoms (Anttila et al., 2018; Gandal et al., 2018). While the cell-type specific aberrations and their relationship with differential gene expression profiles needs to be studied to better understand the molecular mechanisms underlying abnormal neuroanatomical signatures for mood symptoms, especially in-terms of diagnostic specificity between major depressive disorder and bipolar disorder, our results represent a step towards developing brain region-specific functional gene-expression blueprints for therapeutic targeting of broad/specified molecular pathways. Furthermore, while we are not able to disentangle medication effects in our current findings, the effects of medication on gross neuroanatomical

measures and gene-expression profiles also needs to be assessed in future studies. Our analysis of suicide vs non-suicide mood disorders (excluding controls) did compare similarly medicated diagnostic cohorts and as such, our preliminary results showing similarities in gene expression profiles between suicide comparisons with and without controls suggests that our findings overall are not attributable to medication effects alone. In sum, our findings bridging convergent neuroanatomical and gene-expression signatures for measures of the degree of comorbid psychiatric symptoms in mood disorders and suicides, represents a framework for discoveries of novel biomarkers for brain diseases.

CRediT authorship contribution statement

Mbemba Jabbi conceived and designed the studies, and acquired postmortem material from the NIMH HBCC. Mbemba Jabbi, Dhivya Arasappan, Simon B. Eickhoff, and Hans A. Hofmann performed the experiments and analyzed the data and results. Mbemba Jabbi drafted the manuscript and Dhivya Arasappan, Simon B. Eickhoff, Stephen M. Strakowski, Charles B. Nemeroff, and Hans A. Hofmann contributed critically to the original drafting, interpretation of the findings, and writing of the paper.

Declaration of competing interest

Mbemba Jabbi, none.

Dhivya Arasappan, none.

Simon Eickhoff, none.

Hans Hofmann, none.

Stephen Strakowski: chairs DSMBs for Sunovion as a Consultant, and has research grants with Janssen pharmaceuticals and Alkermes.

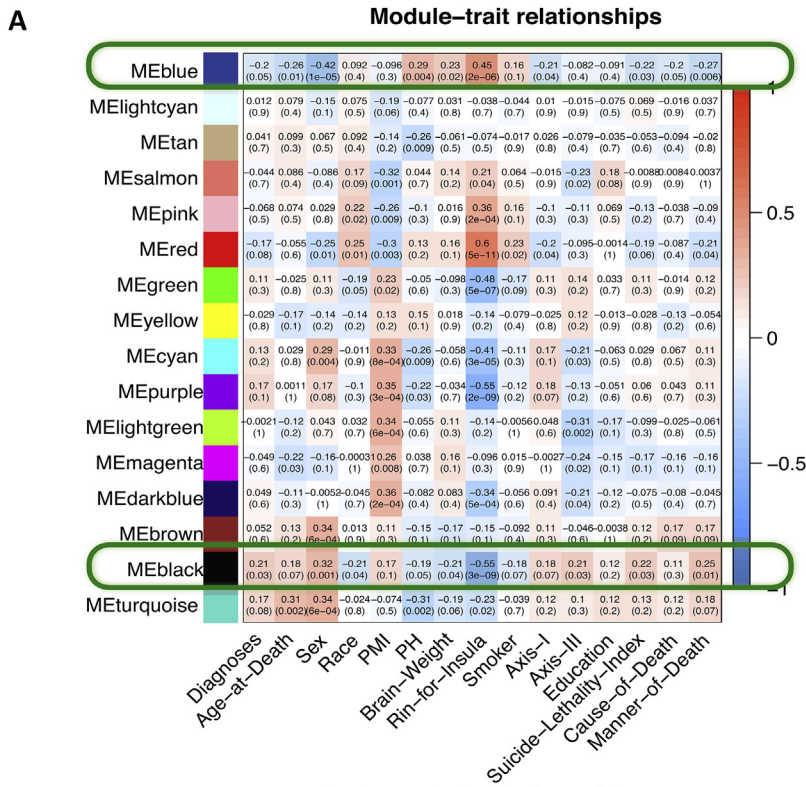
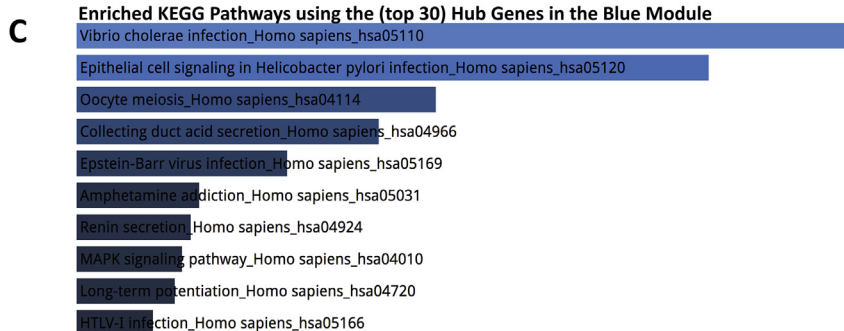
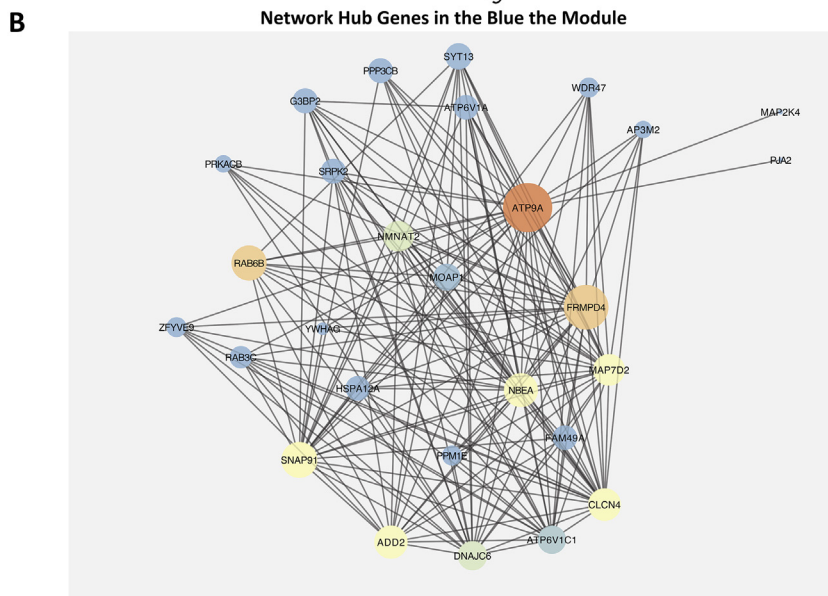


Fig. 3. A). Module-trait relationship: Heat map showing the correlation (relationship) of all WGCNA modules (black and blue modules highlighted) to sample traits (shown in the x-axis). Color scale (red-blue) represents the strength of the correlation between the module and the trait. Correlation values (R^2) followed by corresponding p-values (in parenthesis) are listed within the heat map. Other modules shown in A have greater negative correlation with biological covariates (i.e. RIN, sex and race, & technical covariates such as pH). B) Hub Genes from WGCNA Blue Module: Genes with the highest connectivity (correlation) to other genes in the blue module were identified as hub genes. Gene-Gene network was constructed using the hub genes with the size of nodes scaled by degree (number of connections) and color of nodes scaled by betweenness centrality (darker colors for lower values). C) Enriched KEGG pathways for the 30 hub genes for the Blue module were identified in order to give biological significance to the blue module. The pathways are ranked by a combined score of p-value and rank based score. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



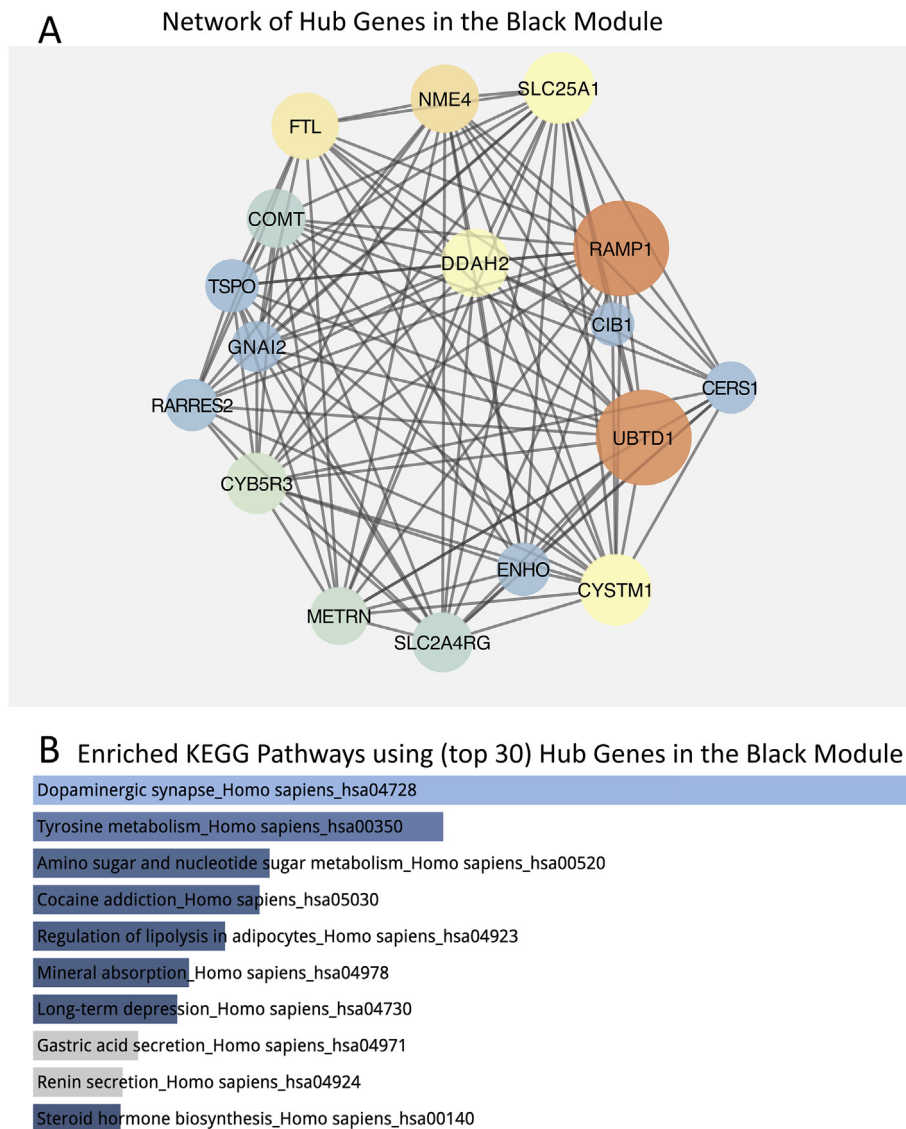


Fig. 4. A) Gene-Gene network constructed using the hub genes with the size of nodes scaled by degree and color of nodes scaled by betweenness centrality (darker colors for lower values). B) Enriched KEGG pathways for the 30 hub genes for the black module. The pathways are ranked by a combined score of p-value and rank-based score. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Charles Nemeroff: 1) Consulting (*last three years*) for Xhale, Takeda, Taisho Pharmaceutical Inc., Bracket (Clintara), Fortress Biotech, Sunovion Pharmaceuticals Inc., Sumitomo Dainippon Pharma, Janssen Research & Development LLC, Magstim, Inc., Navitor Pharmaceuticals, Inc., TC MSO, Inc., Intra-Cellular Therapies, Inc; 2) Stockholder for Xhale, Celgene, Seattle Genetics, Abbvie, OPKO Health, Inc., Antares, BI Gen Holdings, Inc., Corcept Therapeutics Pharmaceuticals Company, TC MSO, Inc., Trends in Pharma Development, LLC; 3) Scientific Advisory Board member for American Foundation for Suicide Prevention (AFSP), Brain and Behavior Research Foundation (BBRF), Xhale, Anxiety Disorders Association of America (ADAA), Skyland Trail, Bracket (Clintara), Laureate Institute for Brain Research (LIBR), Inc; 4) Board of Directors member for AFSP, Gratitude America, ADAA; 5) Income sources or equity of \$10,000 or more from American Psychiatric Publishing, Xhale, Bracket (Clintara), CME Outfitters, Takeda, Intra-Cellular Therapies, Inc., Magstim, EMA Wellness; 6) Patents: Method and devices for transdermal delivery of lithium (*US 6,375,990B1*); Method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by ex vivo assay (*US 7,148,027B2*); Compounds, Compositions, Methods of Synthesis, and Methods of Treatment (CRF Receptor Binding Ligand) (*US 8,551,*

996 B2).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jpsychires.2020.05.013>.

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