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ALTERED EXPRESSION OF GENES INVOLVED IN INFLAMMATION AND APOPTOSIS IN FRONTAL CORTEX IN MAJOR DEPRESSION

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Abstract

The etiology of major depression (MDD), a common and complex disorder, remains obscure. Gene expression profiling was conducted on post-mortem brain tissue samples from Brodmann Area 10 (BA10) in the prefrontal cortex from psychotropic drug-free persons with a history of MDD and age, gender, and post-mortem interval matched normal controls (n=14 pairs of subjects). Microarray analysis was conducted using the Affymetrix Exon 1.0 ST arrays. A list of differential expression changes were determined by dual fold change-probability criteria ($|ALR| > 0.585$ [equivalent to a 1.5-fold difference in either direction], $p < 0.01$), while molecular pathways of interest were evaluated using Gene Set Enrichment Analysis (GSEA) software. The results strongly implicate increased apoptotic stress in the samples from the MDD group. Three anti-apoptotic factors, Y-box binding protein 1 (YBX1), Caspase-1 dominant-negative inhibitor

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pseudo-ICE (COP1), and the putative apoptosis inhibitor FKGS2 were over-expressed. Gene set analysis suggested up-regulation of a variety of pro- and anti-inflammatory cytokines, including interleukin 1 α (IL1 α), IL2, IL3, IL5, IL8, IL9, IL10, IL12A, IL13, IL15, IL18, interferon gamma (IFN γ), and lymphotoxin alpha (LTA; TNF super family member 1). The genes showing reduced expression included metallothionein 1M (MT1M), a zinc binding protein with a significant role in the modulation of oxidative stress. The results of this study suggest that post-mortem brain tissue samples from BA10, a region which is involved in reward-related behavior, show evidence of local inflammatory, apoptotic, and oxidative stress in MDD.

Keywords

Major depression; microarray; transcriptome profiling; frontal cortex; gene expression; inflammation; apoptosis; cytokines; oxidative stress

INTRODUCTION

A variety of brain imaging, post-mortem morphometric, and other studies suggest that major depression involves abnormalities of cortico-limbic structures, including prefrontal cortex, anterior cingulate, amygdala, and hippocampus.¹ In spite of the fact that there is considerable evidence suggesting an underlying neurobiology for major depression (MDD), the molecular basis has been elusive. A number of gene expression profiling studies in post-mortem brain tissue have been conducted comparing depressed and control samples, with variable results.²⁻⁹ Transcriptome profiling is a powerful, albeit complex method, which is affected by a number of factors, including accuracy of the pre-mortem diagnosis; peri-mortem characteristics such as cause of death, the presence of prolonged agonal states, the presence of drugs or toxins;^{10,11} postmortem factors such as the post-mortem interval, pH, and RNA integrity; processing methods, and other issues. Even when controlling for all of these variables, contemporary microarrays provide enormous amounts of data, creating challenges in data handling. Further, MDD is not a unitary condition and is likely to comprise a range of clinical phenotypes and endophenotypes, which may affect the results of microarray analysis.

The present study was aimed at conducting gene expression analysis of a brain area, BA10, which was chosen for several reasons. First, BA10 has been shown to be significantly involved in the mediation of reward related behavior, which is central to the clinical phenomenology of depression¹². For example, Rogers et al.¹² showed that a task which involved choices between high probability – low reward versus low probability – high reward options resulted in the activation of three adjacent regions in the orbito-frontal cortex, BA10, BA11, and BA47 using [¹⁵O] positron emission tomography. Cocaine¹³ and amphetamine¹⁴, highly reinforcing substances, have also been shown to produce activation of BA10. Kufahl et al.¹³ showed that cocaine administration resulted in decreased fMRI brain oxygen level dependent (BOLD) signal in ventral tegmental area (VTA), nucleus accumbens, subcallosal cortex, ventral pallidum, amygdala, parahippocampal gyrus, posterior orbital gyrus, inferior and superior temporal gyrus, but marked and sustained increases in BOLD signal in BA10 and 11. Similarly, amphetamine administration increased

BA8 and 10 activities as measured by single photon emission tomography¹⁴. These data suggest that BA10 is involved in reward and reinforcement processing, a core dysfunction in depression^{15,16}.

Only one prior study has evaluated gene expression in post-mortem tissue samples from BA10 in MDD compared with controls.¹⁷ This study found 99 differentially-expressed genes, which including sets of transcripts of genes involved in cell proliferation. Most of the altered genes are of unknown significance with regard to MDD. However, one up-regulated transcript, fibroblast growth factor receptor 1 (*FGFR1*) may be significant; the expression of both *FGFR1*¹⁸ and its endogenous ligands *FGF1* and *FGF2*³ had previously been shown to be altered in MDD samples.

The present study investigated post-mortem brain tissue samples from 14 depressed persons who were psychotropic drug free at the time of death and age and sex -matched normal controls. Eleven of the 14 samples were from persons who met full DSM-IV criteria for MDD, melancholic subtype antemortem. The melancholic subtype is a distinct clinical phenotype, characterized by symptoms of hyperarousal (e.g., insomnia and anorexia) and hypothalamic-pituitary-adrenal axis activation¹⁹ that may persist even after successful treatment.²⁰ The Affymetrix Exon 1.0 ST array was used for assessing expression of gene transcripts using mRNA of high integrity. In addition to the gene expression analysis, molecular pathways of interest were evaluated using Gene Set Enrichment Analysis (GSEA) to determine altered expression of sets of genes implicated in depression. The results showed altered expression of a large number of genes, but specifically demonstrated upregulation of the expression of pro- and anti-inflammatory cytokines and specific anti-apoptotic factors.

MATERIALS AND METHODS

Human Brain Samples and Tissue Preparation

All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research and the Vanderbilt University Health Sciences Institutional Review Board. Fresh-frozen brain specimens were obtained during routine autopsies conducted by a medical examiner after consent for tissue donation was given by the next of kin. Samples were obtained from 14 pairs of persons with major depressive disorder (MDD) and control (CTR) postmortem brains matched for sex and race, and as closely as possible for age (MDD=47.2 [SD=14.0] and CTR=48.9 [SD=12.1] respectively), and postmortem interval (PMI) (17.8 [SD=7.6] hours and 17.8 [SD=6.5] hours respectively) (all values mean \pm SD)(Table 1). Depressed and control subject brain pH (6.59 [SD=0.30] and 6.71 [SD=0.26] respectively) and RNA integrity number (RIN; greater than 7 for all subjects) were also assessed (Table 1). There were no statistically significant differences in age, PMI, pH, or RIN between groups. All samples came from persons free from all known psychotropic agents and recent substance abuse. Blood samples for toxicology were obtained at the time of the brain collection; all participants were confirmed to be free of psychotropic medications except for one control subject who tested positive for temazepam. Toxicology results are listed in Table 1. The brain samples were collected through the local medical examiner's office and

all patients died a non-hospitalized, acute death, with virtually no agonal state that could bias the gene expression measurements.

Field evaluations were conducted by trained and experienced clinicians and consisted of structured interviews with family members of the deceased. Written informed consent was obtained from all participants. After field interviews were completed, a committee of experienced research clinicians performed an independent diagnostic conference and assigned consensus DSM IV diagnoses (including depressive subtypes) for each subject on the basis of medical records and the results of structured interviews conducted with family members of the deceased. Eleven of the 14 depressed samples came from persons with the melancholic subtype.

Upon receipt, the brains were dissected coronally, flash-frozen and stored at -80°C . An approximately $5\text{mm} \times 5\text{mm}$ gray matter cube from BA10 (spanning the cortical gray matter) was carved out from the frozen block using pre-chilled dental tools. This ensured a more homogenous sample, greatly diminishing harvesting bias (variable white matter/gray matter ratio) and reducing experimental noise in our dataset. Furthermore, this approach allowed us to preferentially detect expression changes in neuron- and glia-enriched tissue. The tissue piece was immediately placed in cold Trizol reagent, homogenized and used for RNA extraction.²¹

Design and Processing of RNA Microarrays

RNA concentration was determined with an Agilent BioAnalyzer 2100 system (Agilent, Palo Alto, California)²¹. The RNA integrity number (RIN) was >7 for all subjects. cDNA synthesis, amplification and labeling were performed using the manufacturer's protocol (http://www.affymetrix.com/support/technical/technotes/human_exon_wt_target_technote.pdf). Briefly, the labeling started with a ribosomal RNA reduction step, in which 60–80% of the ribosomal RNAs are eliminated. cDNA is generated by a random priming method. The random primers incorporate a T7 promoter sequence which is subsequently used in an in vitro transcription to produce antisense cRNA fragments, where a modified dUTP is incorporated instead of dTTP. The modified dUTP is subsequently recognized by the enzymes Urasil-DNA glycosylase (UDG) and human apurinic/apyrimidinic endonuclease 1 (APE 1) which will cut the DNA, resulting in fragmentation of the cDNA. Each DNA fragment is end-labeled with biotin using terminal deoxynucleotidyl transferase (TdT) before being hybridized to the arrays.

In this study we used the Human Affymetrix Exon 1.0 ST arrays (Affymetrix, Santa Clara, CA); for this study we will report the analyses for the 60,000 core transcripts. Hybridization to the Human Exon 1.0 ST array, post-hybridization washes, staining, array image generation, segmentation and QC analysis were performed by the Vanderbilt Microarray Shared Resource (VMSR) using the manufacturer's standard protocols.

Identifying differentially expressed genes

After a visual inspection of unsegmented microarray scans, standard image segmentation was performed and DAT files were generated. CEL files produced by the VMSR were imported into Expression Console software, version 1.1 (Affymetrix), and data normalized

using the Robust Multi-Array Averaging (RMA) algorithm. Export of the data from the Expression console using the “Core” data filter collapsed exon-level data onto known transcripts, producing ~60,000 transcript-level data points for each subject. Data were analyzed in Excel (Microsoft, Redmond, WA) in two steps. A Student’s group wise two-tailed t-test compared RMA intensities of MDD and controls samples. Then, signal intensity magnitude difference was calculated as a \log_2 based Average Log Ratios (ALR). ALR is calculated according to the following equation: $ALR = \text{mean}(\log_2\{D_1\} \dots \log_2\{D_n\}) - \text{mean}(\log_2\{C_1\} \dots \log_2\{C_n\})$, where D=depressed and C=control individual gene expression values. As gene expression differences of miniscule magnitude often may have statistical significance (but represent mostly artifacts), we used a conservative, previously established dual-criteria approach^{21–24} to identify the differentially expressed genes in our dataset: a gene was considered differentially expressed if it showed an $|ALR| > 0.585$ (representing a 1.5 fold [50%] difference in either direction) and a group wise $p < 0.01$ between MDD and control subjects.

Clustering

Hierarchical clustering was performed to identify similarities in gene expression change (potentially co-regulated genes) and to assess the capacity of the disease gene “signature” to dichotomize the subject population into disease and control groups (i.e. does the disease gene signature adequately “diagnose” the disease). Differentially expressed genes ($ALR > 0.585$, $p < 0.01$) were subjected to an unsupervised two-way clustering analysis based on Euclidian distance (RMA-normalized expression profile for individual genes \times individually analyzed samples) using GenePattern²⁵ as previously described²¹.

Correlation analysis

Bivariate correlations were determined using the Pearson Product-Moment correlation coefficient (SPSS version 16, SPSS, Inc., Chicago, IL). RMA-normalized ALR values were used as input data.

Gene set analysis

Gene set analysis was performed using Gene Set Enrichment Analysis (GSEA) software as described previously.²⁶ This method determines whether a priori defined set of genes shows statistically significant, concordant differences between two biological states or phenotypes. The Molecular Signatures Database (MSigDB) contains more than 3000 knowledge-based gene sets for use with GSEA, and the dataset is analyzed simultaneously for multiple gene sets.²⁷ To simultaneously analyze for gene set enrichment and control for false discovery, we performed 1,000 permutations per analysis using the MDD versus control phenotypes²⁷. False discovery rate threshold in GSEA was measured by Q-value, a test that measures the proportion of false positives incurred when that particular test is called significant. Q-value was set at $q = 0.05$ ²⁸, identifying differentially expressed gene sets that had $>95\%$ probability to represent true, biological observations. Enrichment sets represent BioCarta-derived (<http://www.biocarta.com/genes>) gene sets (designated as “pathways”), corrected for multiple comparisons. The enrichment score (ES) represents the degree to which a specific set is represented at the extremes (high or low) of the entire list. The enrichment score

corresponds to a weighted Kolmogorov Smirnov-like statistic.²⁷ The statistical significance (nominal p-value) was calculated using permutation testing. The core members of high scoring gene sets that contribute to the *ES* are extracted by defining the subset of genes in the set that appear in the ranked list at or before the point where the running sum reaches its maximum deviation from zero. This is the subset of a gene set that accounts for the enrichment signal.²⁷

Real-Time Quantitative Polymerase Chain Reaction

Ten genes, showing differential expression between MDD and CTR subjects, were selected for quantitative polymerase chain reaction (qPCR) analysis. For each of the selected genes, cDNA was synthesized with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Foster City, California). TaqMan Gene Expression Assays (Applied Biosystems) were performed on 50 ng cDNA/sample for 10 genes: histone cluster 1, H2aj (HIST1H2AJ), Y box binding protein 1 (YBX1), caspase-1 dominant-negative inhibitor pseudo-ICE (COP1), Zinc Finger Protein 117 (ZNF117), Casein Alpha S1 (CSN1S1), Calponin 3, acidic (CNN3), olfactory receptor, family 10, subfamily A, member 5 (OR10A5), vacuolar protein sorting 37 homolog C (*S. cerevisiae*) (VPS37C), Metallothionein 1M (MT1M), and Moesin (MSN). The results were then verified and normalized against endogenously glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which did not display a significant gene variation between MDD and CTR subjects. The qPCR reactions were performed by an ABI Prism 7000 SDS cycler (Applied Biosystems) with the ABI Prism 7000 SDS software. The software was run with the automatic baseline and threshold (Ct) detection options selected. The quantified qPCR data was exported to Microsoft Excel to organize MDD and CTR Ct, determine MDD Ct - CTR Ct (ΔCt).

Data Sharing

Complete microarray data will be made available by request upon publication.

RESULTS

The Affymetrix Human Exon 1.0 array allowed us to analyze 60,000 gene expression products. Samples from MDD and CTR subjects were obtained from Brodman's area 10 in the prefrontal cortex. Our analysis revealed 30 transcripts which displayed significant expression differences between MDD and CTR samples, using a metric of $|ALR| > .585$ (50% change) and $p < 0.01$. Of these genes, 14 genes (46.7%) were found to be in MDD samples compared to CTR samples with a mean ALR of 0.80 (1.7-fold increase; Table 2) and 16 genes (53.3%) were found to be under-expressed in MDD as compared to CTR samples with a mean ALR value of -0.70 (1.6-fold decrease; Table 2).

qPCR Findings

Ten genes were selected to validate our microarray findings. These transcripts were selected for validation due to their putative role in dysregulation of cytokine pathways in MDD as compared to CTR subjects. Five of the gene transcripts were over-expressed (HIST1H2AJ, YBX1, COP1, ZNF117, CSN1S1) and the other five were under-expressed (CNN3, OR10A5, VPS37C, MT1M, MSN) in the MDD samples. In addition, one over expressed

cytokine, IL-9 was also included. For these 11 transcripts, the Ct qPCR findings were highly correlated ($r=0.92$; $p<0.001$) with the microarray data between MDD and CTR subjects (Figure 2).

The effects of age, sex, pH, and PMI on expression levels

Correlations between pH, PMI, age and differentially-expressed genes were performed. A significant correlation was found only between pH and the expression level of ZFN117 ($r=0.411$, $df=28$, $p=0.03$); after correction for multiple comparisons this was no longer significant. All differentially-expressed genes were compared by sex using the Students t-test; all comparisons were not significant.

Correlational analysis

Associations between the selected genes differentially-expressed in MDD were tested using the Pearson Product Moment correlation coefficient. Genes for this analysis were selected based on their effects on transcriptional regulation (HIST1H2AJ, ZNF117, MKRN1, RGS17), apoptosis (YBX1, COP1, FKSG2), structural integrity (MSN), or modulation of oxidative stress (MT1M). Most genes showed significant direct or inverse correlations with other differentially-expressed genes. There were particularly strong correlations ($r>0.600$) found between MT1M, MKRN1, and MSN, all with reduced expression. In addition, the anti-apoptotic factors YBX1, COP1, and FKSG2 were significantly correlated with each other, and with the histone protein HIST1H2AJ.

Gene Set Enrichment Analysis

Of the 8 pathways which demonstrated significant enrichment (Table 3) the most robust finding revealed an up regulation of transcripts pertaining to the cytokine pathway in the MDD group, with a nominal p value=0.0062 and FDR q value=0.03 (Table 7). Of the 18 constituents of this pathway, 13 were significant in the core enrichment of the dataset, including interleukin 1 α (IL1 α), IL2, IL3, IL5, IL8, IL9, IL10, IL12A, IL13, IL15, IL18, interferon gamma (IFN γ), and lymphotoxin alpha (LTA; TNF super family, member 1; TNF β).

DISCUSSION

The microarray mRNA expression analysis in this study from 14 matched depressed and matched control brains tissue samples from BA10 suggest that there may be increased inflammatory or other pro-apoptotic stress in the MDD sample group. For example, there was significant up regulation of Caspase-1 dominant-negative inhibitor, pseudo-ICE (COP1). Caspases are cysteine proteases that are involved in cell death cycles via apoptosis or inflammation. COP1 has significant homology with the caspase recruitment domain (CARD) of Caspase-1 and it acts as an inhibitor of both Caspase-1 and -4,²⁹ both of which are up regulated in face of inflammatory stress.³⁰ Increased expression of COP1 is protective of Caspase-mediated cell death.³¹ Similarly, Both FKSG2, a putative apoptosis inhibitor protein, and Y-box binding protein 1 (YBX1) were significantly upregulated in the MDD sample. Relatively little is known about FKSG2, although it is known to be up regulated by cysteinyl leukotrienes, which are mediators of tissue inflammation.³² YBX1 is

a member of the cold shock domain super family of proteins, a highly evolutionarily conserved transcription factor.³³ YBX1 is involved in a variety of intracellular functions including transcriptional regulation (either activation or repression), DNA repair, and response to stress signals.³³ One role of YBX1 is to bind to free mRNA, which prevents translation (“mRNA masking”) and degradation.³³ YBX1 is up regulated in face of a variety of stressors, including hyperthermia, UV radiation, and viral infection, and it is involved in nuclear viral replication. Upregulation of the expression of specific anti-apoptotic proteins suggests the possibility of apoptotic stress in this region, which has been hypothesized as a causal factor in depression.^{34,35} In fact, a variety of studies have implicated impaired neuroplasticity, reduction in regional brain volumes, and decreased neuronal and glial cell body numbers in depressed patients relative to controls, all of which implicate apoptosis.³⁵

Traditional gene expression analysis has tended to focus on single genes that are differentially expressed between two groups. However, although this is a conservative and highly rigorous approach, it may miss subtle but important dysregulation of genes distributed across networks of related pathways.²⁷ GSEA was developed to analyze gene set data, and has been successfully applied to discover distributed networks of genes in a number of conditions, such as b-cell lymphoma³⁶ and prostate cancer.³⁷ The results of gene set analysis in the present study amplify the evidence of upregulation of the expression of genes involved in response to stress and inflammation. GSEA tests *a priori* gene groupings based on underlying biological similarities. In the present analysis, only the pre-set “cytokine” grouping showed significant up-regulation (Table 3). Although the absolute fold increase for each was modest, the set analysis suggests dysregulation of proinflammatory mechanisms. A number of cytokines were up regulated, including IL1 α , IL2, IL3, IL5, IL8, IL9, IL10, IL12A, IL13, IL15, IL18 (also known as interferon γ [IFN γ] inducing factor), as well as IFN γ itself and lymphotoxin alpha (TNF β), most of which are pro-inflammatory (Table 7). An exception is IL10, which is an anti-inflammatory cytokine that inhibits synthesis of IL2, IL3, and IFN γ , which may be up regulated in response to the increase in these cytokines. Together with the up regulated expression of COP1, YBX1, and FKSG2, these results suggest a local environment experiencing significant inflammatory stress.

Evidence for inflammation as a basis for depression is supported by prior research suggesting a connection between depression and proinflammatory cytokines and related proteins.^{38,39} It has been postulated that certain proinflammatory cytokines act on the central nervous system causing so-called “sickness behavior” in both humans and animals. Symptoms that are common during infection share features with depression, including down mood, loss of interest in usual activities (anhedonia), anorexia, sleep disturbance, and decreased locomotor activity³⁸. In fact, therapeutically administered interferons such as IFN α for hepatitis C can produce a syndrome that is indistinguishable from depression⁴⁰. Moreover, this can be attenuated or prevented by treatment with antidepressant medication^{41,42}. Several antidepressants have been shown to suppress proinflammatory cytokine production and to release endogenous anti-inflammatory cytokines such as IL10.⁴³⁻⁴⁷

A variety of studies have found elevated peripheral cytokines and other proinflammatory mediators in depression, including C-reactive protein, IL-1, IL-6, TNF α , and IFN γ in MDD

(for reviews see Raison et al.³⁸, Dantzer⁴⁸, Garcia-Bueno et al.,⁴⁹ and Miller et al.⁵⁰). To our knowledge, however, the present study is the first demonstration of elevated levels of cytokines in brain in MDD, which suggests that the elevations of cytokines demonstrated in previous studies are actually reflected in the brain itself. This strongly supports inflammation as a mediator of depression in at least some patients with depression.

Although immune activation is associated with peripheral immune cells such as neutrophils, natural killer cells, and macrophages, it is now clear that locally resident cell types including microglia, astrocytes, and neurons mount innate immune responses in the central nervous system.⁵¹ As an example, acute immune activation of microglia, such as that seen with the gram-negative endotoxin lipopolysaccharide, can result in a robust release of TNF- α , IL-1 α , IL-1 β , and IL-6.⁵² Similarly, stress may also activate similar immune responses in brain without the presence of typical pathogen associated molecules.⁵³ However, both chronic immune activation⁵² and chronic stress⁵⁴ leads to a progressive reduction in the release of these molecules and an increase in anti-inflammatory mediators such as IL-10.⁵⁵ Chronic immune activation may account, then, for the apparent discrepancy between the results we have shown in brain and the prior observation in peripheral samples, in which increased IL-6 and TNF α have been most consistently shown.^{38,50}

The actual causal pathway connecting proinflammatory cytokines and depression remains obscure, but there are some mechanisms that have been implicated. For example, inflammatory cytokines including IL1 β , TNF α ⁵⁶ IL3 and IL5⁵⁷ activate p38 mitogen activated protein kinase (p38 MAPK), which has been shown to induce catalytic activation of the serotonin transporter, which increases uptake of serotonin, decreasing synaptic availability^{56,58}. Both IL1 β and TNF α increase serotonin uptake in both a rat embryonic raphe cell line and synaptosomes isolated from mouse brain.^{56,58} In addition, proinflammatory cytokines, including IFN γ , activate the enzyme indoleamine 2,3-dioxygenase (IDO) leading to the synthesis of kynurenine from tryptophan, the precursor to serotonin. This shift to kynurenine synthesis leads to a relative depletion of serotonin which could induce depressive symptoms in vulnerable individuals.⁵⁹ The kynurenine pathway leads to the synthesis of several neuroactive products, including the neuroprotective intermediaries kynurenic acid and picolinic acid and the NMDA receptor agonist neurotoxin quinolinic acid.⁶⁰ Inflammatory cytokines also have significant effects on dopamine and associated reward related behavior. For example, in rats, IL2 (which was elevated in the tissue samples from depressed patients in the present study) has been shown to decrease dopamine efflux in nucleus accumbens and to inhibit self-stimulation via an electrode in the median forebrain bundle, a model of rewarding behavior.

While the present study implicates local inflammatory and apoptotic stress in BA10, the mechanisms mediating these effects are unclear. One clue may be found in one of the genes found to be reduced relative to controls in the present sample, metallothionein 1M (MT1M). Metallothioneins (MTs) represent a family of cysteine-rich proteins that bind heavy metals through the cysteine thiol group.^{61,62} In particular, MT1M is involved in zinc homeostasis via zinc-thiol binding. Under normal conditions, zinc concentrations are tightly regulated by MT1M and related zinc binding (thiolate) proteins.^{61,62} MTs also directly regulate oxidative stress by cysteine capture of free radicals.⁶³ Under conditions of oxidative stress, MTs

scavenge free radicals, releasing zinc into the cytoplasm. Reduced availability of MT1M would be expected to enhance oxidative stress directly by altered regulation of superoxide radicals, and indirectly by effects on zinc metabolism. Low MT1M, then, would create a state of vulnerability to oxidative stress by creating a redox imbalance by a failure to maintain an appropriate reducing environment. In fact, prior research indicates that MTs exert anti-apoptotic functions via a variety of mechanisms, including inflammatory and oxidative stress.⁶¹ Notably, a recent study has shown that exogenous administration of MT markedly attenuated microglia activation and quinolinic acid expression induced by IFN γ .⁶⁰ Clearly, this novel finding needs further investigation to determine what, if any, role MTs may play in depression vulnerability.

The results of this study are at variance with previous post-mortem brain microarray analyses in MDD,^{2,3,5,8,64} which could be related to a variety of factors, including differences in microarray platforms, brain region, and clinical phenotype. BA10 is involved in reward-related cognitive processing¹² and is activated in response to highly reinforcing stimuli.^{13,14} Cellular dysfunction in BA10 may, then, account for some of the deficits in response to reward that are found in depression.^{15,16} Only one previous study investigated BA10, and found a dissimilar set of differentially-expressed genes.¹⁷ A variety of factors may have contributed to these differences. For example, in that study, nine of 11 depressed subjects were on psychotropics, whereas the current set were free of known psychotropic medications. Another possible source of variance with this and other microarray studies may be the clinical phenotype chosen for our study. Most of the patient samples in the current study came from persons with the clinical phenotype of melancholic MDD, which shows particularly severe deficits of reward responding;⁶⁵ the proportion of samples in previous studies from the melancholic subtype is unknown. Melancholic MDD is a distinct clinical phenotype, which tends to be characterized by marked psychomotor disturbances, sleep and appetite changes, and reduced responses to hedonic stimuli^{65,66}. Moreover, the melancholic subtype of depression may be more likely to show dysregulation of peripheral markers of immune function⁵³. For example, one study showed that, whereas non-melancholic depressed patients exhibited increased leukocytes (particularly natural killer cells) but no differences in cytokines, melancholics had elevated IL2, IL10, and IFN γ , which is consistent with the results of the gene set analysis in the present study⁶⁷. Whether melancholia is specifically characterized by local apoptotic and inflammatory stress in brain should be further investigated in future studies.

There are significant limitations to the current analysis. We have shown altered expression of specific genes and gene sets; however, this may or may not be reflected in actual gene products or downstream functions. Further, the results were limited to a single brain area; whether these differences are found in other brain regions is unknown. We also oversampled the melancholic subtype of MDD; the accuracy of this subtype distinction depends on the precision of the diagnosis made via interview with the next-of-kin, which may or may not reflect the actual diagnosis in life.

In summary, the present study suggests that, in BA10, depressed persons show evidence of increased inflammatory and apoptotic stress, including elevations in specific cytokines as well as anti-apoptotic proteins. Although the causal mediators of these abnormalities are not

known, oxidative stress is implicated. Clearly further research is needed to validate these findings and to further investigate causal mechanisms.

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Reference List

1. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct.* 2008; 213:93–118. [PubMed: 18704495]
2. Sibille E, Arango V, Galfalvy HC, Pavlidis P, Erraji-Benchekroun L, Ellis SP, et al. Gene expression profiling of depression and suicide in human prefrontal cortex. *Neuropsychopharmacology.* 2004; 29:351–361. [PubMed: 14603265]
3. Evans SJ, Choudary PV, Neal CR, Li JZ, Vawter MP, Tomita H, et al. Dysregulation of the fibroblast growth factor system in major depression. *Proc Natl Acad Sci U S A.* 2004; 101:15506–15511. [PubMed: 15483108]
4. Sequeira A, Gwadry FG, French-Mullen JM, Canetti L, Gingras Y, Casero RA Jr, et al. Implication of SSAT by gene expression and genetic variation in suicide and major depression. *Arch Gen Psychiatry.* 2006; 63:35–48. [PubMed: 16389195]
5. Klempan TA, Sequeira A, Canetti L, Lalovic A, Ernst C, French-Mullen J, et al. Altered expression of genes involved in ATP biosynthesis and GABAergic neurotransmission in the ventral prefrontal cortex of suicides with and without major depression. *Mol Psychiatry.* 2007; 14:175–189. [PubMed: 17938633]
6. Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP, et al. Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proc Natl Acad Sci U S A.* 2005; 102:15653–15658. [PubMed: 16230605]
7. Aston C, Jiang L, Sokolov BP. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Molecular Psychiatry.* 2005; 10:309–322. [PubMed: 15303102]
8. Kang HJ, Adams DH, Simen A, Simen BB, Rajkowska G, Stockmeier CA, et al. Gene expression profiling in postmortem prefrontal cortex of major depressive disorder. *J Neurosci.* 2007; 27:13329–13340. [PubMed: 18045927]
9. Tochigi M, Iwamoto K, Bundo M, Sasaki T, Kato N, Kato T. Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains. *Neurosci Res.* 2008; 60:184–191. [PubMed: 18068248]
10. Lewis DA. The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology.* 2002; 26:143–154. [PubMed: 11790510]
11. Mirmics K, Levitt P, Lewis DA. DNA microarray analysis of postmortem brain tissue. *International Review of Neurobiology.* 2004; 60:153–181. [PubMed: 15474590]
12. Rogers RD, Owen AM, Middleton HC, Williams EJ, Pickard JD, Sahakian BJ, et al. Choosing between small, likely rewards and large, unlikely rewards activates inferior and orbital prefrontal cortex. *J Neurosci.* 1999; 19:9029–9038. [PubMed: 10516320]
13. Kufahl PR, Li Z, Risinger RC, Rainey CJ, Wu G, Bloom AS, et al. Neural responses to acute cocaine administration in the human brain detected by fMRI. *NeuroImage.* 2005; 28:904–914. [PubMed: 16061398]
14. Devous MD, Trivedi MH, Rush AJ. Regional cerebral blood flow response to oral amphetamine challenge in healthy volunteers. *Journal of Nuclear Medicine.* 2001; 42:535–542. [PubMed: 11337538]

15. Pizzagalli DA, Iosifescu D, Hallett LA, Ratner KG, Fava M. Reduced hedonic capacity in major depressive disorder: Evidence from a probabilistic reward task. *J Psychiatr Res.* 2008; 43:76–87. [PubMed: 18433774]
16. Pizzagalli DA, Jahn AL, O’Shea JP. Toward an objective characterization of an anhedonic phenotype: a signal-detection approach. *Biol Psychiatry.* 2005; 57:319–327. [PubMed: 15705346]
17. Tochigi M, Iwamoto K, Bundo M, Sasaki T, Kato N, Kato T. Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains. *Neuroscience Research.* 2008; 60:184–191. [PubMed: 18068248]
18. Gaughran F, Payne J, Sedgwick PM, Cotter D, Berry M. Hippocampal FGF-2 and FGFR1 mRNA expression in major depression, schizophrenia and bipolar disorder. *Brain Res Bull.* 2006; 70:221–227. [PubMed: 16861106]
19. Carroll BJ, Cassidy F, Naftolowitz D, Tatham NE, Wilson WH, Iranmanesh A, et al. Pathophysiology of hypercortisolism in depression. *Acta Psychiatrica Scandinavica, Supplementum (433):90–103.* 2007:90–103.
20. Pintor L, Torres X, Navarro V, Martinez de Osaba MA, Matrai S, Gasto C. Corticotropin-releasing factor test in melancholic patients in depressed state versus recovery: a comparative study. *Progress in Neuro-Psychopharmacology & Biological Psychiatry.* 2007; 31:1027–1033. [PubMed: 17433515]
21. Garbett K, Ebert PJ, Mitchell A, Lintas C, Manzi B, Mirnics K, et al. Immune transcriptome alterations in the temporal cortex of subjects with autism. *Neurobiol Dis.* 2008; 30:303–311. [PubMed: 18378158]
22. Hashimoto T, Arion D, Unger T, Maldonado-Aviles JG, Morris HM, Volk DW, et al. Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Mol Psychiatry.* 2007; 13:147–161. [PubMed: 17471287]
23. Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnics Z, Lee VM, et al. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell.* 2005; 120:701–713. [PubMed: 15766532]
24. Mirnics K, Pevsner J. Progress in the use of microarray technology to study the neurobiology of disease. *Nat Neurosci.* 2004; 7:434–439. [PubMed: 15114354]
25. Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP. GenePattern 2.0. *Nat Genet.* 2006; 38:500–501. [PubMed: 16642009]
26. Campbell DB, D’Oronzio R, Garbett K, Ebert PJ, Mirnics K, Levitt P, et al. Disruption of cerebral cortex MET signaling in autism spectrum disorder. *Ann Neurol.* 2007; 62:243–250. [PubMed: 17696172]
27. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. From the Cover: Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences.* 2005; 102:15545–15550.
28. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A.* 2003; 100:9440–9445. [PubMed: 12883005]
29. Wang X, Narayanan M, Bruely JM, Rigamonti D, Cattaneo E, Reed JC, et al. Protective role of Cop in Rip2/caspase-1/caspase-4-mediated HeLa cell death. *Biochim Biophys Acta.* 2006; 1762:742–754. [PubMed: 16920334]
30. Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.* 2007; 14:10–22. [PubMed: 16977329]
31. Wang X, Wang H, Figueroa BE, Zhang WH, Huo C, Guan Y, et al. Dysregulation of receptor interacting protein-2 and caspase recruitment domain only protein mediates aberrant caspase-1 activation in Huntington’s disease. *J Neurosci.* 2005; 25:11645–11654. [PubMed: 16354923]
32. Uzonyi B, Lotzer K, Jahn S, Kramer C, Hildner M, Bretschneider E, et al. Cysteinyl leukotriene 2 receptor and protease-activated receptor 1 activate strongly correlated early genes in human endothelial cells. *Proc Natl Acad Sci U S A.* 2006; 103:6326–6331. [PubMed: 16606835]
33. Kohno K, Izumi H, Uchiumi T, Ashizuka M, Kuwano M. The pleiotropic functions of the Y-box-binding protein, YB-1. *Bioessays.* 2003; 25:691–698. [PubMed: 12815724]

34. McKernan DP, Dinan TG, Cryan JF. "Killing the Blues": A role for cellular suicide (apoptosis) in depression and the antidepressant response? *Progress in Neurobiology*. 2009; 88:246–263. [PubMed: 19427352]
35. Dwivedi Y. Brain-derived neurotrophic factor: role in depression and suicide. *Neuropsychiatr Dis Treat*. 2009; 5:433–49. [PubMed: 19721723]
36. Monti S, Savage KJ, Kutok JL, Feuerhake F, Kurtin P, Mihm M, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. *Blood*. 2005; 105:1851–1861. [PubMed: 15550490]
37. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, et al. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med*. 2004; 10:594–601. [PubMed: 15156201]
38. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol*. 2006; 27:24–31. [PubMed: 16316783]
39. Maes M. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. *Minireview. Neuro Endocrinol Lett*. 2008; 29:287–291. [PubMed: 18580840]
40. Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. *Biol Psychiatry*. 2004; 56:819–824. [PubMed: 15576057]
41. Capuron L, Gummnick JF, Musselman DL, Lawson DH, Reemsnyder A, Nemeroff CB, et al. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. *Neuropsychopharmacology*. 2002; 26:643–652. [PubMed: 11927189]
42. Raison CL, Woolwine BJ, Demetrashvili MF, Borisov AS, Weinreb R, Staab JP, et al. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. *Aliment Pharmacol Ther*. 2007; 25:1163–1174. [PubMed: 17451562]
43. Maes M, Song C, Lin AH, Bonaccorso S, Kenis G, De Jongh R, et al. Negative immunoregulatory effects of antidepressants: inhibition of interferon-gamma and stimulation of interleukin-10 secretion. *Neuropsychopharmacology*. 1999; 20:370–379. [PubMed: 10088138]
44. Leonard BE. The immune system, depression and the action of antidepressants. *Prog Neuropsychopharmacol Biol Psychiatry*. 2001; 25:767–780. [PubMed: 11383977]
45. Kubera M, Lin AH, Kenis G, Bosmans E, van Bockstaele D, Maes M. Anti-inflammatory effects of antidepressants through suppression of the interferon-gamma/interleukin-10 production ratio. *J Clin Psychopharmacol*. 2001; 21:199–206. [PubMed: 11270917]
46. Kenis G, Maes M. Effects of antidepressants on the production of cytokines. *Int J Neuropsychopharmacol*. 2002; 5:401–412. [PubMed: 12466038]
47. O'Brien SM, Scott LV, Dinan TG. Cytokines: abnormalities in major depression and implications for pharmacological treatment. *Hum Psychopharmacol*. 2004; 19:397–403. [PubMed: 15303243]
48. Dantzer R. Cytokine, sickness behavior, and depression. *Neurol Clin*. 2006; 24:441–460. [PubMed: 16877117]
49. Garcia-Bueno B, Caso JR, Leza JC. Stress as a neuroinflammatory condition in brain: damaging and protective mechanisms. *Neurosci Biobehav Rev*. 2008; 32:1136–1151. [PubMed: 18468686]
50. Miller AH, Maletic V, Raison CL. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. *Biological Psychiatry*. 2009; 65:732–741. [PubMed: 19150053]
51. Griffiths, M.; Neal, JW.; Gasque, P. Innate Immunity and Protective Neuroinflammation: New Emphasis on the Role of Neuroimmune Regulatory Proteins. In: Giacinto Bagegta, MTCa, editor. *International Review of Neurobiology Neuroinflammation in Neuronal Death and Repair*. Vol. 82. Academic Press; 2007. p. 29-55.
52. Ekdahl CT, Kokaia Z, Lindvall O. Brain inflammation and adult neurogenesis: The dual role of microglia. *Neuroscience*. 2009; 158:1021–1029. [PubMed: 18662748]
53. Hayley S, Poulter MO, Merali Z, Anisman H. The pathogenesis of clinical depression: Stressor- and cytokine-induced alterations of neuroplasticity. *Neuroscience*. 2005; 135:659–678. [PubMed: 16154288]

54. Bartolomucci A, Palanza P, Parmigiani S, Pederzani T, Merlot E, Neveu PJ, et al. Chronic psychosocial stress down-regulates central cytokines mRNA. *Brain Research Bulletin*. 2003; 62:173–178. [PubMed: 14698350]
55. Ekdahl CT, Kokaia Z, Lindvall O. Brain inflammation and adult neurogenesis: The dual role of microglia. *Neuroscience*. 2009; 158:1021–1029. [PubMed: 18662748]
56. Zhu CB, Carneiro AM, Dostmann WR, Hewlett WA, Blakely RD. p38 MAPK activation elevates serotonin transport activity via a trafficking-independent, protein phosphatase 2A-dependent process. *J Biol Chem*. 2005; 280:15649–15658. [PubMed: 15728187]
57. Ip WK, Wong CK, Wang CB, Tian YP, Lam CW. Interleukin-3, -5, and granulocyte macrophage colony-stimulating factor induce adhesion and chemotaxis of human eosinophils via p38 mitogen-activated protein kinase and nuclear factor kappaB. *Immunopharmacol Immunotoxicol*. 2005; 27:371–393. [PubMed: 16237950]
58. Zhu CB, Blakely RD, Hewlett WA. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology*. 2006; 31:2121–2131. [PubMed: 16452991]
59. Delgado PL, Charney DS, Price LH, Aghajanian GK, Landis H, Heninger GR. Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry*. 1990; 47:411–418. [PubMed: 2184795]
60. Chung RS, Leung YK, Butler CW, Chen Y, Eaton ED, Pankhurst MW, et al. Metallothionein treatment attenuates microglial activation and expression of neurotoxic quinolinic acid following traumatic brain injury. *Neurotox Res*. 2009; 15:381–389. [PubMed: 19384571]
61. Formigari A, Irato P, Santon A. Zinc, antioxidant systems and metallothionein in metal mediated-apoptosis: biochemical and cytochemical aspects. *Comp Biochem Physiol C Toxicol Pharmacol*. 2007; 146:443–459. [PubMed: 17716951]
62. Krezel A, Hao Q, Maret W. The zinc/thiolate redox biochemistry of metallothionein and the control of zinc ion fluctuations in cell signaling. *Archives of Biochemistry and Biophysics*. 2007; 463:188–200. [PubMed: 17391643]
63. Kumari MVR, Hiramatsu M, Ebadi M. Free radical scavenging actions of metallothionein isoforms I and II. *Free Radical Research*. 1998; 29:93–101. [PubMed: 9790511]
64. Sequeira A, Klempan T, Canetti L, French-Mullen J, Benkelfat C, Rouleau GA, et al. Patterns of gene expression in the limbic system of suicides with and without major depression. *Mol Psychiatry*. 2007; 12:640–655. [PubMed: 17353912]
65. Leventhal AM, Rehm LP. The empirical status of melancholia: implications for psychology. *Clin Psychol Rev*. 2005; 25:25–44. [PubMed: 15596079]
66. Parker G, Hadzi-Pavlovic D, Austin MP, Mitchell P, Wilhelm K, Hickie I, et al. Subtyping depression. I. Is psychomotor disturbance necessary and sufficient to the definition of melancholia? *Psychol Med*. 1995; 25:815–823. [PubMed: 7480459]
67. Rothermundt M, Arolt V, Fenker J, Gutbrodt H, Peters M, Kirchner H. Different immune patterns in melancholic and non-melancholic major depression. *Eur Arch Psychiatry Clin Neurosci*. 2001; 251:90–97. [PubMed: 11407444]

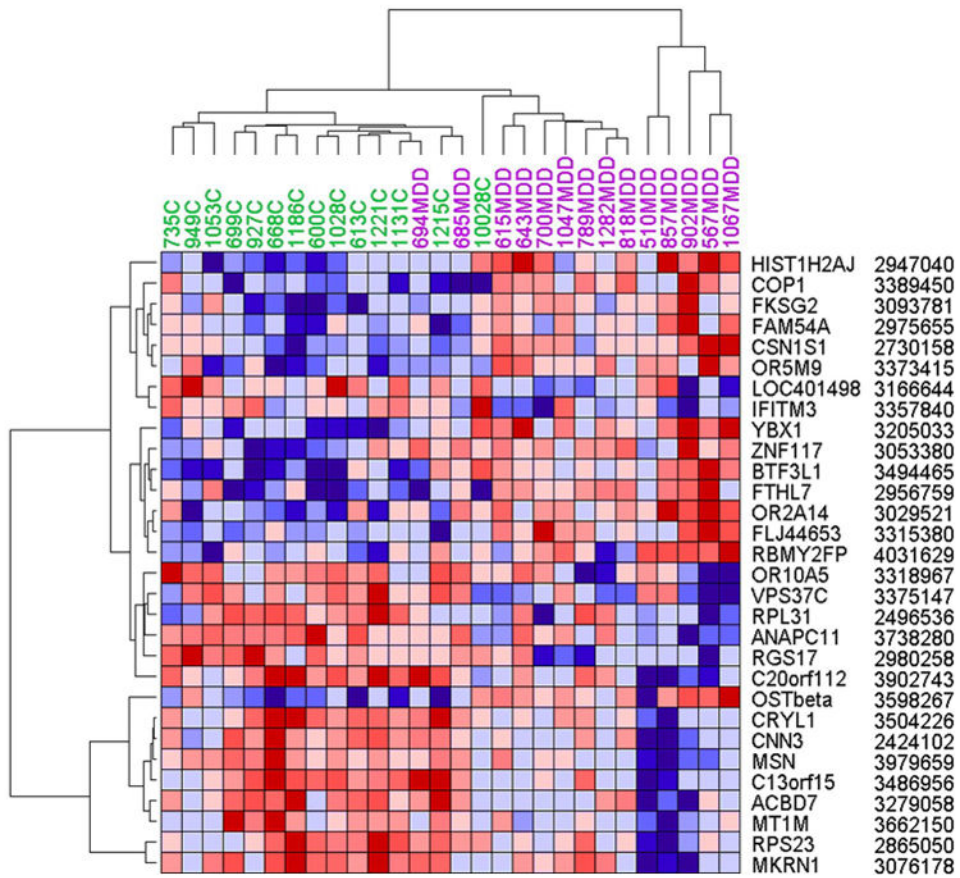


Figure 1. Hierarchical clustering of differentially expressed genes

Normalized log₂ intensities were clustered by GenePattern in two dimensions (horizontal: genes; vertical: samples [purple=MDD, green=controls]) on the basis of Euclidian distance. Each colored pixel represents a single gene expression value in one subject. The color intensity is proportional to its relative expression level (blue: under expressed; red: over expressed). Note that the statistical segregation of depressed and control subjects are almost complete, with an overlap of only two controls. Labels on the right denote gene symbols and probe identifiers. For more data, see Table 2.

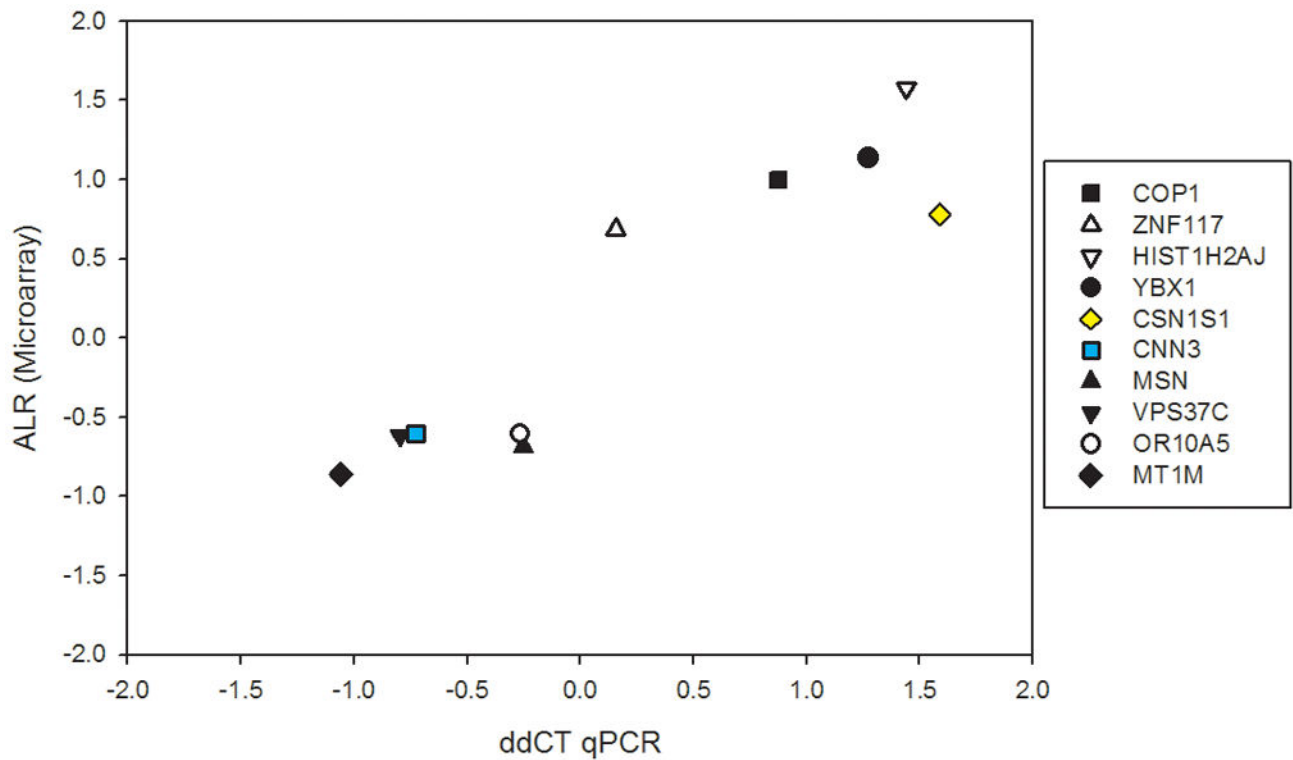


Figure 2. qPCR Analysis of selected cytokine-related mRNAs in MDD subjects and matched controls

X axis denotes microarray-reported expression differences (ALR), while Y axis shows qPCR-obtained differential expression values (ΔCt) for 10 selected transcripts. Each symbol represents a different gene, denoted in the figure legend. Note that all the observations occupy quadrants 2–3 (and no observations are located in quadrants 1 and 4), showing that all microarray-qPCR results were concordant in directionality of change. The ALR- ΔCt results were highly correlated ($r=0.92$; $p < 0.001$).

Table 1

Sample characteristics.

Subject Inumber	Group	Pair	Sex	Race	Age	PMI (hours)	pH	Cause of Death	Manner of Death	Blood Toxicology
600	MDD	1	M	W	63	9.9	6.72	Hanging	Suicide	None
613	MDD	2	M	W	59	15.6	6.95	Gunshot wound	Suicide	None
668	MDD	3	M	W	34	24.3	7.00	Hanging	Suicide	None
699	MDD	4	M	W	65	5.5	6.71	Gunshot wound	Suicide	None
735	MDD	5	F	W	40	14.0	6.84	Pulmonary embolism	Accidental	None
927	MDD	6	M	W	58	24.9	6.11	ASCVD	Natural	None
949	MDD	7	M	W	38	25.0	6.23	Cardiac arrhythmia	Natural	None
1028	MDD	8	M	W	39	14.5	6.18	Gunshot wound	Suicide	None
1053	MDD	9	M	W	47	24.0	6.57	ASCVD	Natural	Lidocaine
1131	MDD	10	M	W	29	26.6	6.92	Gunshot wound	Suicide	None
1186	MDD	11	M	W	45	6.6	6.25	Traumatic asphyxiation	Accidental	None
1215	MDD	12	M	W	44	11.0	6.54	ASCVD	Natural	None
1221	MDD	13	F	B	28	24.8	6.61	Pulmonary embolism	Natural	None
10028	MDD	14	F	W	72	23.1	6.66	Gunshot wound	Suicide	Ibuprofen
MDD Mean (SD):					47.2 (14.0)	17.8 (7.6)	6.59 (6.46)			
510	CONTROL	1	M	W	63	12.4	6.51	GI hemorrhage	Natural	None
685	CONTROL	2	M	W	56	14.5	7.06	Coronary artery disease	Natural	Alcohol (0.01%)
694	CONTROL	3	M	W	38	20.7	6.73	Subarachnoid hemorrhage	Natural	None
615	CONTROL	4	M	W	62	7.2	6.39	Ruptured aortic aneurysm	Natural	None
567	CONTROL	5	F	W	46	15.0	6.77	Mitral valve prolapse	Natural	None
902	CONTROL	6	M	W	60	23.6	6.74	ASCVD	Natural	None
700	CONTROL	7	M	W	42	26.1	6.95	ASCVD	Natural	None
1047	CONTROL	8	M	W	43	13.8	6.63	ASCVD	Natural	Chlorphen-iramine
643	CONTROL	9	M	W	50	24.0	6.23	ASCVD	Natural	None
789	CONTROL	10	M	W	22	20.0	7.04	Asphyxiation	Accidental	None
1067	CONTROL	11	M	W	49	6.5	6.55	Hypertensive heart disease	Natural	Doxylamine, metoprolol, temazepam
857	CONTROL	12	M	W	48	16.6	6.54	ASCVD	Natural	None

Subject number	Group	Pair	Sex	Race	Age	PMI (hours)	pH	Cause of Death	Manner of Death	Blood Toxicology
1282	CONTROL	13	F	W	39	24.5	6.84	Cardiac arrhythmia	Natural	None
818	CONTROL	14	F	W	67	24.0	7.06	Anaphylaxis	Accidental	None
Control Mean (SD):					48.9 (12.1)	17.8 (6.5)	6.71 (0.25)			

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Table 2

Genes with increased and decreased expression in BA10 of subjects with major depressive disorder

A. Increased Expression:					
UniGene	Gene Name	Gene Symbol	Chromosome	ALR*	P***
Hs.406691	Histone cluster 1, H2aj	HIST1H2AJ	6p22-21.3	1.577	<0.0001
Hs.473583	Y box binding protein 1	YBX1	1p34	1.136	0.0014
Hs.348365	Caspase-1 dominant-negative inhibitor pseudo-ICE	COP1	11	0.996	0.0036
Hs.534533	Organic solute transporter beta	OSTbeta	15q22.31	0.819	0.0015
Hs.3155	Casein alpha s1	CSN1S1	4q21.1	0.778	0.0070
Hs.651853	Apoptosis inhibitor	FKSG2	8p11.2	0.741	0.0066
Hs.567241	Basic transcription factor 3, like 1	BTF3L1	13q22	0.688	0.0066
Hs.250693	Zinc finger protein 117	ZNF117	7q11.21	0.684	0.0005
Hs.534547	Olfactory receptor, family 2, subfamily A, member 14	OR2A14	7q35	0.671	<0.0001
Hs.121536	Family with sequence similarity 54, member A	FAM54A	1q32.3	0.664	0.0065
Hs.660426	FLJ44653 protein	FLJ44653	10q26.3	0.653	0.0002
Hs.684794	RNA binding motif protein, Y-linked, family 2, member F pseudogene	RBMV2FP	Yq11.223	0.624	0.0085
Hs.333125	Ferritin, heavy polypeptide-like 7	FTHL17	Xp21	0.622	0.0004
Hs.553749	Olfactory receptor, family 5, subfamily M, member 9	OR5M9	11q11	0.592	0.0007
B. Decreased Expression:					
UniGene	Gene Name	Symbol	Chromosome	ALR*	P***
Hs.166313	Regulator of G-protein signalling 17	RGS17	6q25.3	-0.924	0.0012
Hs.534456	APC11 anaphase promoting complex subunit 11	ANAPC11	17q25.3	-0.912	0.0064
Hs.516978	Chromosome 20 EST	C20orf112	20q11.1-11.23	-0.901	0.0095
Hs.647370	Metallothionein 1M	MT1M	16q13	-0.864	0.0104
Hs.644598	Acyl-coenzyme A binding domain containing 7	ACBD7	10p13	-0.804	0.0056
Hs.646477	interferon-induced transmembrane protein 3	IFITM3	7p11.2	-0.779	0.0049
Hs.490347	Makorin, ring zinc finger protein, 1	MKRN1	7q34	-0.690	0.0092
Hs.507866	Chromosome 13 open reading frame 15	Cl3orf15	13q14.11	-0.672	0.0097
Hs.87752	Moesin	MSN	Xq11.2-q12	-0.686	0.0025
Hs.469473	Ribosomal protein L31	RPL31	2q11.2	-0.630	0.0087
Hs.523715	Vacuolar protein sorting 37 homolog C	VPS37C	11q12.2	-0.620	0.0084

A. Increased Expression:

UniGene	Gene Name	Gene Symbol	Chromosome	ALR*	P**
Hs.447478	Olfactory receptor, family 10, subfamily A, member 5	OR10A5	11p15.4	-0.606	0.0059
Hs.527193	Ribosomal protein s23	RPS23	5q14.2	-0.605	0.0091
Hs.483454	Calponin 3, acidic	CNN3	1p22-p21	-0.603	0.0039
Hs.370703	Crystallin, lambda 1	CRYL1	13q12.11	-0.589	0.0043
Hs.522063	RIKEN A930001M12	LOC401498	9p21.1	-0.588	0.0017

* Mean ALR, depressed minus control

** Groupwise p value

Table 3

Gene set enrichment analysis:

A. BioCarta pathways showing gene enrichment.				
BioCarta pathway name	Size (genes)	Enrichment Score	p-val	Change direction
VEGFPATHWAY	25	0.657942	0.008163	Decreased
CDMACPATHWAY	15	0.699933	0.02268	Decreased
INTEGRINPATHWAY	33	0.549887	0.03527	Decreased
ECMPATHWAY	20	0.629207	0.038855	Decreased
PROTEASOMEPATHWAY	16	0.760643	0.041929	Decreased
ATMPATHWAY	18	0.529419	0.04918	Decreased
MCALPAINPATHWAY	22	0.62423	0.049587	Decreased
<i>CYTOKINEPATHWAY</i>	18	-0.79414	0.006211	<i>Increased</i>

B. GSEA cytokine pathway constituents (listed by Running ES rank).				
Gene Symbol	Gene Name	Rank Metric Score ^a	Running ES ^b	Core Enrichment
IL18	Interleukin 18 (interferon-gamma-inducing factor)	-0.031	-0.7628	Yes
IL5	Interleukin 5 (colony-stimulating factor, eosinophil)	-0.061	-0.7613	Yes
LTA	Lymphotoxin alpha (TNF superfamily, member 1; TNFβ)	-0.075	-0.7345	Yes
IFNG	Interferon, gamma	-0.098	-0.7007	Yes
IL15	Interleukin 15	-0.106	-0.6492	Yes
IL12A	Interleukin 12a (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	-0.113	-0.5942	Yes
IL1A	Interleukin 1, alpha	-0.114	-0.5334	Yes
IL10	Interleukin 10	-0.131	-0.4741	Yes
IL8	Interleukin 8	-0.137	-0.4033	Yes
IL13	Interleukin 13	-0.167	-0.3277	Yes
IL2	Interleukin 2	-0.208	-0.2289	Yes
IL9	Interleukin 9	-0.228	-0.1106	Yes
IL3	Interleukin 3 (colony-stimulating factor, multiple)	-0.245	0.0182	Yes
IL6	Interleukin 6	0.074	-0.6347	No
IL4	Interleukin 4	0.036	-0.6943	No
IL12B	Interleukin 12b (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	0.016	-0.7183	No
IL16	Interleukin 16 (lymphocyte chemoattractant factor)	0.007	-0.7297	No
TNF	Tumor necrosis factor (TNF super family, member 2 [TNFα])	-0.011	-0.7515	No

^aRank metric: GSEA uses the signal-to-noise metric to rank the genes (for details, see “Metrics for Ranking Genes” at http://www.broadinstitute.org/gsea/doc/GSEAUserGuideFrame.html?Run_GSEA_Page). The larger the signal-to-noise ratio, the larger the differences of the means (relative to the standard deviations). A high signal-to-noise metric indicates greater separation between phenotypes.

^bRunning ES: Running enrichment score, which reflects the degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes (for details, see “GSEA Statistics” at http://www.broadinstitute.org/gsea/doc/GSEAUserGuideFrame.html?Run_GSEA_Page).