GENE EXPRESSION DYSREGULATION IN MEDICATION

REFRACTORY DEPRESSION

by

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ABSTRACT

Depressive disorders (DD) are a leading cause of disability worldwide and display diverse symptoms including anhedonia, melancholia, decreased concentration, sleep and appetite disturbances, and suicidal thoughts and acts. Current available medications are still ineffective for more than 30% of patients, suggesting DD are multi-faceted heterogeneous disorders. Despite intense research, as yet there are no widely used biological diagnostic tests for DD. Since DD are likely a manifestation of both genetic and environmental factors, gene expression of peripheral blood leukocyte allows for a non-invasive means to evaluate trait- and state-dependent neurophysiological dysregulation. In this dissertation, we employed real-time quantitative polymerase chain reaction (qPCR) to evaluate differences between healthy controls and patients with medication-refractory depression, for a panel of candidate genes previously implicated in depression as well as novel genes implicated in related neurological disorders. Chapter 1 begins with an overview of the multiple domains involved in depression and of previous literature findings evaluating protein and gene expression. Chapter 2 describes one of our studies of gene expression of a small panel of 14 genes involved in the immune and stress response in 19 patients with medication-refractory depression, before and following symptom improvement, compared to 20 healthy controls. We found that patients displayed trait- and state-dependent dysregulation in immune cytokines IL-10

and IL-6, transcription factor NFkB1, the serotonin receptor HTR1D, $GABA_A$ modulator DBI, and the adrenergic receptors ADR2A and ADRB1. Furthermore, the dopamine receptor DRD4, the glucocorticoid receptor NR3C1, and SULT1A1 displayed acute changes following treatment, though no differences were observed Pretreatment. In Chapter 3, we describe another gene expression study with results using a larger sample size (42 patients and 38 controls) and an expanded gene panel (46 genes) that includes candidate genes from diverse biological pathways. In this study, we found upregulation of IL-10 and IL-6 as well as dysregulation in the amyloid precursor protein, neuregulin-1, and several ion channels that have roles in anxiety, pain, and fatigue. Finally, Chapter 4 summarizes results from both studies and discusses future research into promising biomarkers and novel mechanisms of depression.

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CHAPTER 1

DEPRESSIVE DISORDERS AND POTENTIAL MECHANISMS

EVIDENCE FROM GENE EXPRESSION

Introduction

Depressive disorders (DD) are a rapidly growing burden on society, with up to 20% lifetime prevalence and estimated to become the second leading cause of disability by 2020 (World Health Organization, 2004). Symptoms include low self-esteem, feelings of guilt, anhedonia, decreased motivation and concentration, disruption in eating and sleeping patterns, and importantly, suicidal thoughts and acts. Though diagnosis only requires having some of these symptoms for a period of 2 weeks, DD are often chronic and present with recurring lifetime episodes (Maletic et al., 2007). In other words, this disease pattern suggests chronic imbalances with state-dependent changes due to acute triggers.

For over 50 years, the dominate hypothesis has been that DD result from low synaptic availability of the monoamines serotonin, dopamine, and norepinephrine (Schildkraut, 1965). This "monoamine hypothesis" still guides the development of the majority of currently available antidepressants. However, in a great proportion of cases treatments are ineffective or only partially effective. The STAR*D trial showed only 30% remission rate after the first line of antidepressant using a selective serotonin reuptake inhibitor and marked decreased effectiveness of subsequent similar medications. These data strongly indicate the heterogeneity of disease mechanisms and the inadequacy of the monoamine hypothesis as a model of treatment (Trivedi et al., 2006). Other research to date has implicated diverse contributions to the mechanism of DD beyond low monoamine synaptic availability. These include dysfunction/imbalances in: the stress and immune response, metabolic and mitochondrial function, growth factors,

DNA regulation, and environmental effects (Krishnan and Nestler, 2008, 2010). Depression is thus a multifaceted and dynamic disease and its symptomatic episodes are likely the result of internal and external influences (Maletic, 2007).

Current diagnosis of DD relies solely on clinical assessment. There are many forms of DD and many likely etiologies. Nevertheless, most are treated the same. Drug development as well as patient therapy would benefit from a better understanding of disease mechanisms and if we could evaluate a patient's disease as well as medication response via reliable biological tests (biomarkers). One promising approach that might allow for this better understanding as well as individualized therapy is to examine differences in mRNA gene expression, which can demonstrate the effects of genetic, epigenetic, and environmental influences. mRNA results from transcription of DNA, the genetic code that remains largely invariable throughout life. mRNA translation, in turn, results in the synthesis of proteins which mediate nearly all biological processes. The formation of mRNA depends not only upon our DNA genetic code (which is why DNA approaches to predict or understand DD have been so inadequate), but also upon many factors that affect which sections of DNA are transcribed into mRNA. This is a complex process with multiple regulation steps allowing control of specific transcripts, for example: 1) control of unraveling DNA from histones 2) DNA methylation and hydroxylation that impedes DNA binding of necessary machinery for gene expression, and 3) diverse families of transcription factors that bind to upstream sites allowing for coordinated responses. In short, which mRNA is generated is a result not only of our genetic code, but also of numerous influences upon DNA transcription. These processes

are affected by the physiological environment of the person. So, when we look at mRNA expression, we look at our inborn biology and the effects of our environment on its expression. We hypothesize, then, that mRNA gene expression may be used to better understand physiological states in depressed individuals. Altered expression of some specific genes may be associated with chronic imbalances, while others may change with symptomology.

Interestingly, and important to the practicality of measuring such mRNA gene expression, many of the pathways implicated in DD have not only central (brain) components, but also peripheral (outside the CNS) ones. For example, blood leukocytes mediate the immune and inflammatory response to stress, and display many of the same receptors that are also found in central brain cells (Gladkevich et al., 2004; Sullivan et al., 2006). Additionally, other factors implicated in DD can be measured in peripheral blood cells. This may initially seem surprising, but one must understand that white blood cells (WBCs) are exposed to the influences of both central and peripheral processes and factors. Thus, the use of blood test to evaluate DD would provide an easy and relatively inexpensive means to diagnose disease as well as individually tailor and follow treatment effects. To this aim, in this dissertation, we have examined WBC gene expression in patients with severe medication-refractory depression, as well as changes following successful treatment.

Peripheral gene expression using qPCR to explore molecular biology of depression

While DNA remains largely unchanged across cell types and an organism's life, mRNA expression is labile, being greatly affected by environmental factors and as such, can describe current physiological states. Although postmortem brain tissue has provided interesting observations of the anatomical differences between suicide and nonsuicide deaths, potential confounders such as temperature, tissue pH, agonal factors, and variation in cause of death make these data difficult to interpret (Mehta et al., 2010). Interestingly, Sullivan et al. found that whole blood displays high gene expression correlation with multiple regions of the central nervous system and could therefore be informative as to the state of the brain (Liew et al., 2006; Sullivan et al., 2006). This is not surprising, given that white blood cells or leukocytes (composed of cell types including lymphocytes, monocytes, and macrophages) are exposed to the same chemical milieu as are neurons, including hormones, neurotransmitters, and growth factors (Gladkevich et al., 2004). The development of microarrays has allowed investigation of tens of thousands of genes at a time, providing a global overview of gene expression (Forster et al., 2003). Essentially, known sequences are immobilized on a matrix chip in distinct sections and a sample of mixed transcripts is overlaid. When the immobilized transcripts bind to those in the sample, they emit a measurable fluorescent signal. This allows investigation of novel disease contributors before and after treatments. However, due to their large scope and lack of distinct a priori hypotheses, microarrays are marred by substantial difficulties in data processing, false positives, and

reproducibility across research groups, thus making them a semiquantitative technique (Forster et al., 2003; Walker and Hughes, 2008; Mehta et al., 2010).

Unlike microarrays, real-time quantitative polymerase chain reaction (qPCR) allows measurements of gene transcripts in between rounds of DNA replication. These are later normalized to one or several constitutively expressed internal controls and quantified during stable periods of replication. Most noteworthy, the qPCR technique displays greater sensitivity than microarrays, and given that it has a considerably narrower scope with genes of interest chosen a priori, it has historically been used to validate candidate genes from microarrays, though this technique is not without its own technical challenges (Bustin and Mueller, 2005). Given that WBCs express neurotransmitter receptors and are an integral part of the immune/stress response, biological systems that are found to be dysregulated in depressive disorders, qPCR gene expression from blood using hypothesis-driven target genes could result in diagnostic biomarkers for DD. If mRNA gene expression could represent a temporal snapshot of a person's current physiological state, subjects in different symptomatic states including depressive, manic, euthymic, or remissive periods, may also be distinguished physiologically by gene expression (Bustin and Mueller, 2005; Iga et al., 2008). Therefore, studies that examine blood before and after symptom remission are valuable in understanding potential novel mechanisms of depression as well as in identifying biomarkers to monitor treatment response. Below we will describe some of the proposed mechanisms of depression, specifically in relation to evidence from gene expression.

Roles of serotonin, norepinephrine, and dopamine in depression

The monoamines were implicated in depressive disorders based on some serendipitous observations that drugs modulating levels of monoamines such as the catecholamines norepinephrine and dopamine or the indolamine serotonin could result in depressive symptoms or their alleviation (Schildkraut, 1965). For example, the monoamine oxidase inhibitor (MAOI) iproniazid, which hinders monoamine metabolism, was found to improve mood in patients treated for tuberculosis, while the antihypertensive drug reserpine, which lowers norepinephrine and serotonin synaptic levels by preventing their packaging in to vesicles, worsened depressive symptoms (Schildkraut, 1965; Lopez-Munoz and Alamo, 2009). These early pharmacological observations shifted the viewing of depression as a purely psychological disease to one with a biological basis.

Serotonin

In addition to reserpine's depressive effects, further evidence of a role for serotonin in depression came from observed decreased levels of serotonin in the brain and its metabolite in the cerebral spinal fluid (CSF) of patients with depression (Schildkraut et al., 1965). Furthermore, depletion of tryptophan (a necessary precursor for serotonin synthesis) in patients in remission after administration of selective serotonin reuptake inhibitors (SSRIs), results in rapid emergence of depressive symptoms (Krishnan and Nestler, 2008; Nemeroff and Owens, 2009). More recent work suggests that downstream adaptive changes including receptor expression and gene

expression occur with medication treatment. For example, Belzeaux et al. observed no differences in the serotonin receptors 1B and 2A at baseline, yet a significant increase in both following antidepressant treatment (Belzeaux et al., 2010). Iga et al. found that mRNA levels for the serotonin transporter (5HTT) in leukocytes were elevated at baseline in antidepressant-naïve depressed subjects compared to controls, and then decreased significantly at 8 weeks following treatment with the SSRI paroxetine (Iga et al., 2005). These changes are relevant given that SSRIs and NRIs require several weeks for a clinical effect. It is important to note that mRNA levels and their proteins can have complicated interactions, such that increased mRNA gene expression need not correlate with the increased number of receptor binding sites and in fact could be compensatory. For example, patients with depression have been observed to have *decreased* number of 5HTT binding sites in peripheral blood cells, with that number increasing following antidepressant treatments, seemingly contradicting the Iga et al. study and the monoamine hypothesis (Pena et al., 2005). Finally, a genetic polymorphism in the promoter region of the 5HTT has been found with subjects carrying the short (s) allele being more susceptible to early life stress compared to the long (l) allele (Caspi et al., 2003). Interestingly, PET imaging has shown that the depression-sensitive s allele was accompanied by a decreased number of 5HTT binding sites (Heinz et al., 2000).

Norepinephrine and adrenergic receptors

The catecholamine norepinephrine (NE) also displays imbalances in animal models and human subjects with depression (Ressler and Nemeroff, 2000). NE is

produced by neurons in the Locus Coeruleus and affects numerous brain regions. These include those regions involved in learning, memory, executive function, sleep regulation, and the stress response. It is not surprising then, that NE can play a pivotal role in depression (Goddard et al., 2010). DD are associated with low NE levels in blood and CSF and medications that deplete NE led to symptom resurgence in patients with remission (Ressler and Nemeroff, 2000; Goddard et al., 2010). NE plays an important role in mediating the stress response. In animal models of acute stress, there is an increase in NE release and activity with concomitant decrease in binding sites of the α2A-adrenergic receptor (α2AR), α1R, and β1. However, in chronic stress and postmortem tissue, in general, there are decreased NE activity and turnover, increased density and expression of α2a/c receptors, and increased density of β-adrenergic receptors (Pandey et al., 1987; Ressler and Nemeroff, 2000; Morilak et al., 2005; Goddard et al., 2010). In a study examining platelet protein levels of α2AR receptor, it was found that patients with depression had elevated levels of α2AR, which decreased after treatment with the α2AR antagonist mirtazapine and that decreases correlated to improvement (Garcia-Sevilla et al., 2004). Antidepressants may also act on specific receptor subtypes. For example desipramine, an NE reuptake inhibitor which has antidepressant effects in both mice and rats under both normal conditions and chronic stress, appears to be mediated by functional α2A, but not β1 or β2, adrenergic receptors (Yalcin et al., 2005; Zhang et al., 2009). Also, it was proposed that the α1R has an important role in the mechanism by which desipramine prevents cognitive dysfunction due to chronic stress (Bondi et al., 2010). Thus, acute stress leads to NE hyperactivity and resultant decreased α2AR while

chronic stress results in NE downregulation and increased α2AR (Goddard et al., 2010). This implies that examination of adrenergic gene expression could help to better understand specific and dynamic changes in NE processing in depressed patients.

Dopamine

There is also evidence for the important role of dopamine (DA) in depression. DA plays an important role in reward-seeking and motivation, the disruption of which can lead to anhedonia. Perona et al. found that knock-out (KO) mice lacking the dopamine transporter (DAT), responsible for soaking up free synaptic dopamine, displayed anti-depressive like behavior in three separate tests (Perona et al., 2008). Conversely, decreased DAT binding has been observed in several human studies of depression, perhaps a consequence of chronic dopamine depletion, similar to changes seen with NE (Meyer et al., 2001b; Sarchiapone et al., 2006). Decreased mRNA for the dopamine receptor (DRD4) has been observed in lymphocytes from patients with MDD, followed by normalization after an 8-week treatment with the SSRI paroxetine (Stefanis et al., 1998; Rocc et al., 2002; Xiang et al., 2008). Decreased DRD3 mRNA levels in peripheral cells have been found in patients with schizophrenia and bipolar disorder, and subtype-specific changes in receptor expression have been observed in postmortem tissue in the amygdala (Stefanis et al., 1998; Klimek et al., 2002; Vogel et al., 2004; Xiang et al., 2008).

These results suggest dysregulation of monoamine signaling for serotonin, dopamine, and norepinephrine in people with depressive disorders that may be related to symptoms and physiological states. A test that examines gene expression from

multiple receptors involved in monoamine signaling could help identify population subgroups and the development of patient-specific treatment strategies.

HPA, glucocorticoids, and immune response

An early and reproducible finding in patients with depression is hyperactivity of the hypothalamic pituitary adrenal (HPA)-axis, as evidenced by high levels of the glucocorticoid cortisol in 50% of patients with depression and increased levels of stress hormones and pro-inflammatory cytokines (Nemeroff and Vale, 2005). The glucocorticoid receptor (GR) mediates cortisol negative feedback and regulating immune function following particular environmental stimuli. Normally sequestered in the cytoplasm, upon binding cortisol, the GR dimerizes, enters the nucleus, and binds to glucocorticoid response elements (GRE) to inhibit gene transcription, including acting on transcription factors (TF) such as the nuclear factor kappa B (NFkB1) and the cyclic AMP response element binding protein (CREB1) (Anacker et al., 2011a). These TFs have numerous roles in growth, cell differentiation, and immune activation. Cortisol-induced inhibition of these TFs is beneficial during an acute stress response, but is likely detrimental in chronic situations where it results in heightened immune function and neuronal atrophy. Pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-1, interferon (IFN)-γ, and TNFα, which are all regulated by NFkB-1, have been shown to be elevated in DD in both serum and plasma (Dowlati et al., 2010; Shelton et al., 2011). Furthermore, increased cytokine mRNA is seen in healthy humans during chronic stress (Miller et al., 2008; Miller et al., 2009). One historic finding in humans is that treatment

of cancer using IFN-γ, results in increases in cortisol pathway components and can lead to depressive symptoms comparable to those in subjects with DD that are otherwise medically healthy (Capuron et al., 2003; Capuron et al., 2009). In animal models, increases in pro-inflammatory cytokines are associated with depressive-like behaviors, which can then be ameliorated by antidepressants, receptor KOs, or cytokine-specific antibodies (Zunszain et al., 2012). Changes have also been observed for the glucocorticoid expression in animals and humans. For example, in mutant mice, 50% under-expression was pro-depressive, while over-expression was protective (Ridder et al., 2005). Also, Hageman et al. observed that GR mRNA expression was decreased in subfields of the hippocampus in animals exposed to chronic stress, which also led to depressive symptoms in the forced swim test, a model of learned helplessness. Both of these effects are reversed in stressed rats that receive electric convulsive stimulations (ECS). Interestingly, ECS displayed modest or no effect on nonstressed animals, which seems to indicate that ECS is only beneficial when righting imbalances (Hageman et al., 2009). GR in humans is complicated by the existence of two isoforms: GRα, which is transcriptionally active, and GRβ, which is not. Matsubara et al. found that in patients with DD, levels of the transcriptionally active GRα (but not GRβ) were significantly reduced compared to controls, both during a depressive episode and in remission, which is potentially indicative of imbalance that leads to glucocorticoid dysfunction and elevated cortisol levels (Matsubara et al., 2006). Since cytokines and hormones have reciprocal effects on GRs (Turnbull and Rivier, 1999), simultaneous examination of GR

and cytokine expression could serve as biomarkers for depression and potentially be indicators of treatment response (Miller et al., 2009; Zunszain et al., 2011).

Transcription and growth factors

Transcription factors, which are a necessary component for chromatin remodeling and consequently gene expression regulation, are also observed to display dysregulation in DD. One class of interest is the histone deacetylase class (HDAC), of which sirtuins are a subclass, which function to stabilize histone-DNA binding and are therefore responsible for preventing DNA lengthening and gene transcription. Covington et al. found decreases in postmortem HDAC2 protein as well as in an animal model of chronic stress using social defeat (Covington et al., 2009). Interestingly, treatment with an HDAC inhibitor led to antidepressant effects in the chronic stress model, but not in controls (Covington et al., 2009). Acute, but not chronic, treatment with an HDAC inhibitor can actually cause depressive effects in healthy mice (Gundersen and Blendy, 2009). These results parallel human studies in which patients in a depressive state exhibited differences in factors belonging to the HDAC/SIRT family, differences that were either mitigated or reversed in subjects in remission (Hobara et al., 2010; Abe et al., 2011b). CREB-1 has also garnered attention owing to its important role in a myriad of cellular and physiological functions. Though Iga et al. found higher levels of CREB-1 mRNA in patients, while Lai et al. found no difference compared to controls, both groups found significant decrease in CREB-1 after 8 weeks of administration of an SSRI or SSRI/SNRI, respectively (Lai et al., 2003; Iga et al., 2007a).

Because patients with depression display neuronal atrophy, for instance in the hippocampus, research studies have examined the expression of several growth factors. Among these, Neuropeptide Y (NPY) was found to be increased following ECS, including in the axons and fibers of hippocampal cells (Ma et al., 2002). Roles for the vascular endothelial growth factor (VEGF) in antidepressant actions in animals as well as elevations in VEGF gene expression in depressed patients that normalize following treatment have also been observed (Iga et al., 2007b; Lee et al., 2009; Minelli et al., 2011). NPY is a neuropeptide involved in the stress response that has been suggested to counteract the detrimental effects of CRF and be involved in depression (for a review see (Morales-Medina et al., 2010)). Specifically, NPY has been suggested to possess anxiolytic properties in animals and humans; it is downregulated following acute stress, increased following antidepressant treatment and repeated ECS, and decreased in CSF and brain tissue of individuals with some affective disorders (Kuromitsu et al., 2001; Heilig, 2004). Importantly, Otsuki et al. found that mRNA gene expression for several growth factors were state-dependent, with MDD subjects during a depressive state displaying dysregulation, and those in remission not (Otsuki et al., 2008). This highlights the importance of monitoring both gene expression and treatment response when attempting to develop promising biomarkers.

Mitochondria and metabolism

Because symptoms of depression include loss of energy and decreased motivation, the roles of mitochondria and energy metabolism have attracted attention.

In particular, the numbers of mitochondria and energy metabolites are decreased in brain regions of patients, such as the prefrontal cortex, a region important for executive function, including decision making and social behavior (Scaglia, 2010; Manji et al., 2012). Differences have also been found in blood. For example, Beech et al. found using microarrays, that whole blood from depressed subjects with BPD had an over-representation of electron transport chain (ETC) genes, with many ETC genes displaying increases compared to healthy controls. Conversely, several other studies have observed decreases in multiple genes involved in the ETC, in both blood and tissue from subjects with BPD (Sun et al., 2006; Klempan et al., 2009). These discrepancies may be due to the differences in the psychological state of the subjects. For example, in a study examining platelets from patients with schizophrenia, alterations in ETC complex I were found to be dependent on mental state (Dror et al., 2002). On the other hand, Naydenov et al. found no changes, or even decreases in gene expression involved in the mitochondrial respiratory chain, following glucose deprivation in cultured lymphocytes from BPD subjects in manic, remissive, or depressed states (Naydenov et al., 2007; Beech et al., 2010). It would be interesting to examine gene expression during these multiple psychological states since these likely represent distinct metabolic demands.

Roles of certain ion channel roles in pain, fatigue, and depression

In addition to the many pathways that have been implicated in DD, published results from our research group suggest that pathways that underlie the sensations of pain and fatigue, noted symptoms and comorbidities in DD, display altered gene

expression in neurological disorders, including fibromyalgia (FM) and chronic fatigue syndrome (CFS) (Light et al., 2009; Light et al., 2012a). Implicated metabolite detecting receptors include those of the purinergic families (P2X ionotropic and P2Y metabotropic) that sense ATP metabolites, the transient vanilloid receptor (TRPV) involved in the detection of heat, acid, pressure, and osmolarity, and the acid-sensing ion channels (ASIC), important for sensing changes in concentration of cations. mRNA gene expression of several of these were found to be upregulated in subjects with FM at baseline, or following a moderate exercise task in patients with CFS (Light et al., 2009). Gene expression levels also correlated with sensations of task-induced mental and physical fatigue. Interestingly, numerous animal studies have implicated these receptors as also playing a role in depressive symptomatology. In the case of the ASIC1a receptor, ASIC1a KO mice or WT mice treated with ASIC1a inhibitors show antidepressant and anxiolytic behavior (Coryell et al., 2009; Dwyer et al., 2009). Stimulation of the P2Y1 receptor has been shown to have anxiolytic effects, while P2X7 KO mice displayed antidepressant-like but not anxiolytic behavior (Kittner et al., 2003; Basso et al., 2009). In humans, it has been suggested that polymorphisms in P2X7 may predispose individuals to DD, though these findings have been disputed by some (Lucae et al., 2006; Green et al., 2009; Skaper et al., 2010). Finally, anticonvulsants and antidepressants are often prescribed for the treatment of neuropathic pain with positive results, suggesting common mechanisms. Given the overlap of function in fatigue, pain, anxiety, and depression, we hypothesize that subjects with DD will display abnormalities in gene

expression for some of these receptors that may be dependent upon depressive states and comorbidities.

Conclusion

The etiology of depression is still poorly understood, and given the heterogeneity of symptoms and relatively poor treatment response, depression is not a singular disease but rather a multifaceted group of disorders. It would be of great benefit to physicians and patients to find noninvasive diagnostic tests that would help in the development of patient-specific treatments. We hypothesize that depressive episodes are likely the result of chronic dysfunction with state-dependent imbalances. Since the inflammatory and immune responses are involved in depression, we suggest that peripheral markers in cells that mediate these pathways and are exposed to the same chemical milieu as brain cells can provide insight into the dynamic psycho-physiological state of an individual. Gene expression measurements using qPCR allow for hypothesis-driven studies that can nevertheless include genes representing multiple biological pathways. As yet, there is no widely used diagnostic test, though several are being explored by other groups (Papakostas et al., 2011; Pajer et al., 2012). This dissertation will describe preliminary gene expression studies examining diverse pathways involved in depression, including: adrenergic, monoamine, mitochondrial, metabolic, HPA axis, ion channel, and inflammatory pathways (Table 1.1). Chapter 2 describes a small set of genes before and following treatment in a group of 23 medication-refractory depressed subjects diagnosed with DD versus matched controls. This is part of an add-on study

Table 1.1 Implicated and novel genes for mRNA gene expression in DD

Pathways	Classes	Genes
Monoamine and peptides	Serotonin, dopamine	HTR1d, DRD4
	Adrenergic receptors	ADR2A,**ADR2C**,ADRB1, ADRB2
	peptides	**COMT, PSMA4**, SULT1A1, **VIPR2**
HPA	Glucocorticoid	NR3C1
	Mineralocorticoid	**NR3C2**
	Oxytocin	**OXT**
	Oxytocin Receptor	**OXTR**
Immune response and Inflammation	Cytokines	IL-10, IL-6, **TNFα, TNFβ**
	Antigen receptors, chemokine, TF	TLR4, **CXCR4, GZMA**
Ion channels	**Purinergic**	**P2X1, P2X4, P2X7, P2Y1, P2Y2**
	Acid sensing	**ASIC1, ASIC3**
	Transient Vanilloid	**TRPV1, TRPV4**
	Potassium	**HCN2**
Mitochondria/ metabolism	**Electron Transport Chain + others**	**ATP5E, COX5B, NDUFS5, SOD2, HSPA2**
Transcription Factors	Gene expression factors	**CREB1, SIRT1, STAT5A, PPARA**, NFκB1
Neuronal health and signaling	Growth factors	**NRG1**, NPY, VEGFA
	Matrix associated	**APP, SPARC**
	Modulator	DBI

Genes highlighted in **bold** are those added in Chapter 3 study only

that examined the effects of anesthesia on depressive symptoms and cognitive function compared to ECT (included in the Appendix) (Weeks et al., 2013). We hypothesize that some "imbalances" between controls and depressed individuals found at Pretreatment may normalize following treatment and thus represent potential diagnostic markers that will allow one to monitor treatment efficacy. Chapter 3 will examine an expanded gene panel on an independent sample set of 42 patients and 38 controls. Depression severity and currently taken medications will also be examined for possible correlations with gene expression. Results from our study may reveal unique patterns of gene expression, thus fueling further research into novel mechanisms of depression as well as potential candidate drug targets.

CHAPTER 2

STRESS AND IMMUNE GENE EXPRESSION DYSREGULATION IN PATIENTS WITH MEDICATION-REFRACTORY DEPRESSION

Abstract

An extensive literature implicates immune dysregulation and Hypothalamic Pituitary Adrenal (HPA) axis dysfunction in depressive disorders (DD). Furthermore, response to medication may be related in part to its ability to normalize immune dysregulation. However, compromised immunity in medication-resistant patients is still poorly understood and to date there are no reliable immune biomarkers for DD. Because depression is a dynamic and state-dependent disease likely involving both peripheral and central mechanisms, mRNA gene expression, which reflects a combination of genetic and environmental factors, may be a means to uncover immune-related pathophysiology. White blood cells (WBCs), the circulating immune cells, can be sampled repeatedly as clinical conditions of depression patients' change. WBCs also express nearly all of the genes of interest for DD immune dysregulation. We used real-time quantitative polymerase chain reaction (qPCR) to evaluate mRNA gene expression in DD patients during a depressive episode and following treatment-induced symptom improvement, for a panel of genes related to stress and immune function previously implicated in DD. Our results suggest that DD patients during a depressive episode display significant increases for the transcription factor NFκB1, immune cytokines interleukin IL-10 and IL-6, and the $GABA_A$ modulator Diazepam Binding Inhibitor (DBI), as well as decreases in the α2A- and β1-adrenergic receptors, and the serotonin receptor 5HTR1D. Interestingly, most of these genes remained dysregulated following treatment and resultant symptom improvement. The glucocorticoid receptor NR3C1 and dopamine receptor DRD4, which were normal at Pretreatment, displayed acute decreases

immediately following treatment (Post) but returned to pretreatment levels 4 weeks later (Follow-up). Only one gene, SULT1A1, normal at pretreatment, displayed persistent decreases at Follow-up. Medications did not alter these results. These results indicate trait-dependent immune dysregulation for at least some genes in medication-refractory patients with DD, which may underlie their poor response to medication. Future work should investigate potential novel treatment targets involving the genes reported here as well as potential biological mechanisms underlying disease in this subpopulation.

Introduction

"The disease of depression remains a great mystery. It has yielded its secrets to science far more reluctantly than many of the other major ills besetting us."
-William Styron (Darkness Visible: A memoir of madness. Vintage Books, New York, 1992*)*

Depressive disorders (DD) are a rapidly growing burden on society, estimated to become the second leading cause of disability by 2020 (World Health Organization, 2004). Depression is characterized by symptoms of anhedonia, fatigue, poor concentration, memory impairment, disruptions in appetite and sleep, and increased risk for suicide. Despite the prevalence of DD and intense medical research, current first-line and second-line treatments (serotonin re-uptake and norepinephrine re-uptake inhibitors, aka SSRIs and NRIs) are still ineffective for 50% of patients, which clearly indicates disease heterogeneity (Trivedi et al., 2006).

In addition to the monoamine hypothesis, stress and immune function have been implicated in the development and recurrence of depression (Schildkraut, 1965;

Miller et al., 2009; Blume et al., 2011). Immune cell numbers and pro-inflammatory cytokines are increased and anti-inflammatory cytokines decreased in a substantial proportion of DD patients, suggesting inflammatory immune activation. On the other hand, in DD patients, proliferative responses of immune cells are blunted and chronic inflammation (presumed to increase risk of DD) (Bartolomucci and Leopardi, 2009) reduces immune responses to virus exposure, indicating immune suppression (Pike and Irwin, 2006; Blume et al., 2011; Messay et al., 2012). This paradoxical immune response is accompanied and possibly mediated by alterations in the Hypothalamic-Pituitary-Adrenal (HPA) axis. In response to acute stress, the HPA axis normally serves to modulate the immune system via corticotropin releasing factor and the production and release of cortisol. Cortisol activates the glucocorticoid receptors (GRs), causing increases in blood glucose levels while suppressing inflammation (and inflammatory cytokine release), appetite, pleasure seeking, and reproductive drive. Normally, activation of GRs also reduces the production and further release of cortisol by negative feedback, resetting these processes to normal levels after the acute stress is over. However, 50% of DD patients display hyperactive HPA, which may result in prolonged manifestation of the above symptoms. Thus, DD may be the result of impaired negative feedback of the HPA axis, with cortisol unable to turn off GR signaling, thereby causing dysregulation of immune function and long-term symptoms (Anacker et al., 2011b).

Examining changes in HPA axis receptors and immune-related pathways using gene expression in immune cells is one method to study possible dysregulation in these systems in the etiology of depression. For instance, white blood cells (WBCs), which

circulate through all tissues including the brain and mediate the immune response, express mRNA for virtually all of the receptors and cytokines of interest. Conveniently, repeated blood samples can be obtained noninvasively as the patient's clinical condition changes (Gladkevich et al., 2004; Liew et al., 2006; Sullivan et al., 2006; Le-Niculescu et al., 2008; Segman et al., 2010). Genomic DNA remains relatively stable throughout life though sections can be silenced by methylation or acetylation of specific histones. In contrast, transcription of DNA, which results in mRNA expression, is a highly dynamic and controlled process that combines genetic and environmental influences to respond to internal and external physiological changes. As such, levels of RNA for key pathway genes could provide an advantageous means to monitor changes that lead to the symptoms, if not the causes, of DD, and can be used to determine the molecular mechanisms that lead to improvement of DD following treatment (Iga et al., 2008). Previous results from both microarray and real-time quantitative polymerase chain reaction (qPCR) have demonstrated that peripheral blood cells display gene expression alterations in HPA axis and immune function genes (including pro- and anti-inflammatory cytokines, the transcription factor NFκB1, and the glucocorticoid receptor NR3C1) following acute stress as well as in depressive disorders (Bierhaus et al., 2003; Brydon et al., 2005; Matsubara et al., 2006; Iga et al., 2008; Miller et al., 2008; Di Nicola et al., 2012; Menke et al., 2012). Furthermore, exploiting the ability of taking repeated blood samples, some studies have found that changes in gene expression were related to disease states (Lai et al., 2003; Iga et al., 2005; Matuzany-Ruban et al., 2005; Iga et al., 2007a; Abe et al., 2011b; Mamdani et al., 2011).

Patients with medication-refractory depression are of particular interest because they typically have more severe depressive symptoms, and immune dysregulation has been shown to be greater in those with more severe DD (Su et al., 2009; Bufalino et al., 2012). They may also represent a more uniform subgroup of the heterogeneous population of patients with DD. In the present study, we used qPCR to compare mRNA from 14 immune-related genes on the full complement of WBCs obtained from healthy controls and from patients with medication-resistant DD, to test the hypothesis that expression of multiple immune cell genes are dysregulated in these patients during an active depressive episode (Pretreatment). A second blood sample was collected after remission of depressive symptoms due to 8-12 treatments with electroconvulsive therapy (ECT) or a novel alternative intervention involving 10 treatments of deep anesthesia with isoflurane (Posttreatment). Finally, a third sample was obtained 4 weeks after the end of treatment, when potential immediate effects of the interventions have passed, but depressive symptoms were still in remission (Follow-up). We hypothesized that 1) subjects in a depressive episode would display Pretreatment gene expression abnormalities compared to nondepressed controls, 2) some dysregulated genes would normalize with symptom improvement (state-dependent), and 3) other genes would remain abnormal despite remission of symptoms (trait-dependent) and may underlie the chronic disease state and vulnerability to relapse. Finally, since several prior studies had shown that certain medications can alter levels of cytokines or other immune markers (Sluzewska et al., 1995; Hernandez et al., 2008; Fornaro et al., 2011), we compared gene expression differences in DD patients on vs. off these medications.

Materials and Methods

All methods were approved by the University of Utah IRB and all subjects gave written consent for all methods and procedures described below.

Subjects

The Depression (DD) group included 25 individuals (12 females) in an active state of moderate to severe depression. 19 of these patients had a primary diagnosis of major depressive disorder (MDD) (9F/10M, mean age 41.7±2.4), and 6 of these patients were diagnosed with bipolar disorder (BPD) (3F/3M, mean age 41.0±7.0). Due to unsatisfactory response to three or more different antidepressant medication regimens, these patients were under consideration for an open-labeled study comparing the cognitive and antidepressant effects of an experimental treatment using deep isoflurane anesthesia (ISO) to electroconvulsive therapy (ECT) ((Weeks et al., 2013), submitted). Two patients dropped from the study leaving 15 patients who subsequently received 8-12 treatments with ECT and 8 patients who received 10 treatments with ISO, as previously described (Langer et al., 1985; Langer et al., 1995; Weeks et al., 2013). Clinical assessment was carried out and blood samples were collected before the start of treatment (“Pretreatment”, n=23), 24 hours after the last treatment (“Post,” n=23), and again 4 weeks after treatment cessation (“Follow-up”, n=21). All DD patients were clinically assessed using the Hamilton Rating Scale for Depression (HRSD-24) administered by psychiatrists or trained personnel. To ascertain gene expression changes in subjects with treatment-induced symptom improvement, we elected to

examine only those patients who displayed a greater than 30% decrease in HRSD-24 from Pretreatment to Posttreatment, deemed “treatment responders.” 13/15 subjects receiving ECT and 6/8 patients receiving ISO met these criteria [responders mean ECT 36.08 ± 1.75 Pretreatment to 9.30 ± 1.73 Posttreatment, $p < 0.001$, mean ISO 25.50 ± 1.50 to 7.17 ± 1.22, $p < 0.001$]. Pretreatment depression scores for these 19 patients broken down by diagnosis were MDD (n=15) patients averaged 32.8 ± 1.7 (range 19-48), while the BPD patients (n=4) averaged 27.8 ± 2.1 (range 23-33). Controls (CON) included 20 individuals with no current symptoms of depression (13 females, mean age 48.1 ± 2.8). All participants were nonpregnant and between 24 and 67 years old.

Table 2.1 displays a summary of clinical parameters, including medications, for the 19 treatment responders who were used for all subsequent analysis. Of the 19 DD patients, 12 were tested on an antidepressant including selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitor (NRI) and/or mixed serotonin and norepinephrine reuptake inhibitors (SNRIs). Smaller numbers of DD patients were tested on lithium (n = 1) and nonlithium antipsychotic medications (n = 7). At Pretreatment several patients were also tested on anticonvulsants (n = 7). Of the remaining 11 patients, some had stopped these medications in anticipation of treatment (n = 4), a common clinical practice, since anticonvulsants can interfere with treatment efficacy. A subset of both group, comprising 17 CON and 3 DD, were tested on no psychotropic medication. Three of the currently nondepressed CON were also antidepressant medication as a covariate in order to ensure that mRNA differences were not due to medication effects instead of the current depressive state.

Table 2.1 Demographic summary for Control and DD at baseline for treatment responders

	Control	MDD	BPD	DD (comb)
	n=20	n=15	n=4	n=19
Age(years)	48.1±2.8	40.9±2.8	33.8±8.3	39.4±2.8[a]
Gender(Male/Female)	(7/13)	(8/7)	(2/2)	(10/9)
Treatment (ECT/ISO)		(11/4)	(2/2)	(13/6)
HRSD-Pretreatment		34.1±2.0	27.8±2.1	32.8±1.7
HRSD-Post		9.4±1.3[b]	6.25±2.2[b]	8.7±1.2[b]
HRSD-Follow-up		13.9±2.0[c]	12.3±2.9[c]	13.6±1.7[c]
No medication	17	4	0	4
Antidepressants	3	10	2	12
Anticonvulsants/withdrawn	0	4/4	3/0	7/4
SSRIs	3	3	1	4
NRI	1	1	0	1
SNRI	0	7	1	8
TCA	0	0	0	0
Opioid	0	1	0	1
Antipsychotic	0	5	3	8

[a]Age was significantly lower in DD compared to control group (Student's t-test, p =0.03)

[b]HRSD was significantly decreased at Posttreatment compared to Pretreatment (p<0.001)

[c]HRSD was significantly decreased at Follow-up compared to Pretreatment (p<0.001)

SSRI (selective serotonin reuptake inhibitor), SNRI (mixed serotonin-norepinephrine reuptake inhibitor), NRI (norepinephrine reuptake inhibitor), TCA (tricyclic antidepressant)

mRNA extraction and analysis

All blood processing and analyses were performed by personnel blinded to the subjects' group. Blood was collected in EDTA tubes and kept on ice, and within 15 minutes after collection, the blood was centrifuged at 3200 rpm (1315 x g- Clay Adams Compact II Centrifuge) for 12 minutes, plasma was removed, and the white layer carefully collected in RLT+β-ME (Qiagen, Valencia, CA), then quickly frozen using a methanol-dry ice slurry, and stored at -80ºC. RNA was extracted using RNeasy mini kits (Qiagen, Valencia, CA), and treated with RNase-free DNase-I (Qiagen, Valencia, CA).

Immediately following extraction, RNA was converted to a cDNA library using the ABI High Capacity cDNA Archive Kit (Applied Biosystems/Life Technologies, Inc., Foster City, CA), followed by treatment with RNase-H (Epicentre Biotechnologies, Madison, WI). The cDNA samples were stored at -20° C until analysis. RNA integrity was assessed with a Bioanalyzer, and consistently found to have RIN values (RNA integrity numbers) greater than 9. The cycle counts for the control gene, TF2B, averaged 21.78 ±1.67 (SD) for control subjects and 22.32 ± 2.09 (SD) for patients, Student's t-test $p = 0.12$. We selected TF2B as the preferred control gene and have verified that TF2B qPCR counts do not change in freshly harvested separated human leukocytes under a variety of conditions, when RNA is processed as described here.

The cDNA libraries were analyzed using the ABI quantitative, real-time PCR system on the ABI Prism 7900 Sequence Detection System (SDS) 2.4.1 (Applied Biosystems, Inc., Foster City, CA), using ABI TaqMan Master Mix (Applied Biosystems, Inc., Foster City, CA). Master Mix/primer plus primer/probe solutions and template solutions were separately loaded onto 96 well pre-plates. Then 384 well plates were robotically loaded and mixed from the 96 well plates. Each targeted gene was examined in duplicate, with TF2B standards being run in quadruplicate. Additional control samples containing no template were also run. Primer probes for the 24 genes were obtained from TaqMan Gene Expression Assays (Applied Biosystems, Inc., Foster City, CA), were as follows: Adrenergic A2A - Hs00265081_s1; Adrenergic B-1 - Hs02330048_s1; Adrenergic B-2 - Hs00240532_s1; Diazepam Binding Inhibitor - Hs00220950_m1; Dopamine Receptor 4- Hs00609526_m1; Glucocorticoid Receptor NR3C1 –

Hs01005217_m1; IL-10 -Hs00174086_m1; IL-6 - Hs00174131_m1; Neuropeptide Y NPY – Hs00173470_m1; Nuclear Factor Kappa B 1 – Hs00765730_m1; Serotonin Receptor 1D HTR1D –Hs00704742_s1; Sulfotransferase 1A1 - Hs00738644_m1; Toll Like Receptor 4 TLR4 - Hs00152937_m1; Vascular Endothelial Growth Factor A - Hs99999070_m1; Control primer probe TF2B - Hs00155321_m1q. PCR data were processed using the SDS2 program from Applied Biosystems with count values for genes computed in the curve log-linear using a standard 0.2 threshold. Gene expression amounts were determined using the $2^{-\Delta T}$ method, where ΔT is the count difference of the candidate gene from TF2B.

Statistics

All statistical tests were made with the SPSS ver. 19 statistical software. For the purposes of this exploratory study, we combined the MDD and BPD group into one depressed (DD) group. Potential gender effects were tested using nonparametric tests and found to be not significant ($p > 0.05$) for any comparisons and so males and females were combined into a single group for all remaining comparisons. Therefore, a single DD group was used for subsequent comparisons within group as well as with the CON group. Similarly, statistics were conducted to verify that no significant differences in gene expression were present in patients in the ECT vs. ISO group both before and after treatment ($p > 0.1$) and thus we combined these two treatment groups. Between-group comparisons were done using nonparametric Mann-Whitney U tests and within-group comparisons using the Wilcoxon signed-rank test, consistent with previous similar

reports using qPCR (Iga et al., 2005; Belzeaux et al., 2010). Genes were further tested for correlations (Spearman) with other genes, as well as Pretreatment depression severity and changes following treatment response (expressed as the HRSD difference from Post or Follow-up and Pretreatment). Finally, within-and between-group comparisons examined the effects of antidepressants or anticonvulsants on Pretreatment gene expression.

Results

Differentially expressed genes in DD compared to CON

The main goal of this study was to investigate possible immune cell gene expression dysregulation in subjects with DD during a depressive episode, as well as after symptom improvement, versus CON. To this end, we selected genes representing the stress and immune pathways: the glucocorticoid receptor, cytokines, adrenergic receptors, growth factors, transcription factors, and signaling modulators. Specifically, we were interested in three separate expression patterns: (i) **"trait"** genes, which display Pretreatment mRNA dysregulation that persists even after treatment and symptom improvement, (ii) **"state"** genes, which display Pretreatment mRNA dysregulation, but whose expression moves towards CON levels following treatment, and (iii) **"treatment"** genes, which display mRNA expression that is altered acutely at Posttreatment, but reverts to Pretreatment levels at Follow-up. As described in the methods section, because no differences were seen for gender, diagnosis, or treatment groups, we formed a single DD group (see Table 2.1).

Before treatment, depressed subjects who later responded to treatment (as indicated by HRSD scores) differed significantly from CON in the expression of 7 immune cell genes (ADR2A, ADRB1, DBI, HTR1D, IL-10, IL-6, NFKB1). Between group and within group gene expression is shown in Table 2.2 and Table 2.3, respectively.

Trait genes. Four of these (ADR2A, ADRB1, DBI, and HTR1D) maintained their differences in mRNA expression both immediately Posttreatment and 4 weeks later at Follow-up, suggesting they are trait dependent genes. Three of these (ADR2A, ADRB1, HTR1D) had decreased mRNA relative to controls and DBI mRNA was increased relative to controls at all-time points (Figure 2.1).

Table 2.2 Group differences for individual mRNAs in DD vs. CON

	DD Pre vs. CON			DD Post vs. CON			DD Fol vs. CON		
GENE	**n[a]**	**FC[b]**	***p*[c]**	**n**	**FC**	***p***	**n**	**FC**	***p* value**
ADR2A	18	-2.49	***0.001***	15	-1.96	0.067	14	-1.79	**0.046**
ADRB1	13	-3.51	**0.055**	15	-4.59	***0.002***	15	-3.99	**0.023**
ADRB2	17	-1.08	0.715	12	-1.23	0.129	12	-1.02	0.785
DBI	18	1.43	***0.001***	13	1.36	***0.004***	11	1.37	***0.003***
DRD4	19	-1.03	1.000	16	-1.35	0.181	16	-1.09	0.464
HTR1D	16	-2.72	**0.046**	11	-7.10	***0.003***	11	-5.95	***0.007***
IL-10	18	2.27	**0.014**	13	1.70	0.210	11	2.48	0.201
IL-6	18	1.65	**0.019**	4	[d]ND	ND	4	ND	ND
NFKB1	18	1.34	***0.000***	9	1.15	**0.030**	6	ND	ND
NPY	17	-1.03	0.885	12	-1.21	0.301	12	-1.00	0.988
NR3C1	19	-1.15	0.431	13	-1.59	***0.006***	11	-1.28	0.160
SULT1A1	17	1.02	0.976	11	-1.17	0.141	13	-1.20	**0.043**
TLR4	16	-1.15	0.289	13	-1.04	0.792	12	-1.20	0.271
VEGFA	18	1.04	0.907	16	1.12	0.390	16	1.05	0.656

[a]n sample size number [b] Fold change (FC) was calculated by the ratio Positive fold change upregulation (DD/CON) while negative fold change downregulation (-CON/DD). [c] *p* value was calculated using Mann-Whitney U test. ***p*<0.05 in bold and *p*<0.01 in italics** [d] ND: gene was not tested for this time point since it had a sample size <8.

Table 2.3 Group differences for individual mRNAs in DD Pre vs. DD Post and DD Follow-up

	DD Post vs. Pre			DD Fol vs. Pre		
GENE	**n**[a]	**FC**[b]	**p value**[c]	**n**	**FC**	**p value**
AD2A	15	1.15	0.609	14	1.39	0.084
ADBR1	11	-1.31	0.477	13	-1.14	0.463
ADBR2	12	-1.14	0.084	12	1.06	0.875
DBI	13	-1.06	0.311	11	-1.05	0.929
DRD4	16	-1.32	**0.026**	16	-1.07	0.679
HTR1D	11	-2.61	0.075	11	-2.18	0.477
IL-10	13	-1.33	0.075	11	1.10	0.374
IL6	4	[d]ND	ND	4	ND	ND
NFKB1	9	-1.17	**0.011**	6	ND	ND
NPY	12	-1.22	0.272	12	1.03	1.000
NR3C1	13	-1.38	***0.009***	11	-1.11	0.929
SULT1A1	12	-1.200	**0.019**	10	-1.23	0.059
TLR4	12	1.12	0.638	12	-1.05	0.875
VEGFA	15	1.07	0.570	15	1.00	0.609

[a] n sample size number

[b] Fold change was calculated by the ratio of gene expression means. Positive fold change corresponds to an upregulation at Posttreatment or Follow-up while negative fold change corresponds to downregulation.

[c] *p* value was calculated using Wilcoxon paired test. ***p*<0.05 in bold and *p<0.01 in italics***

[d] ND: gene was not tested for this time point since it had a sample size <8.

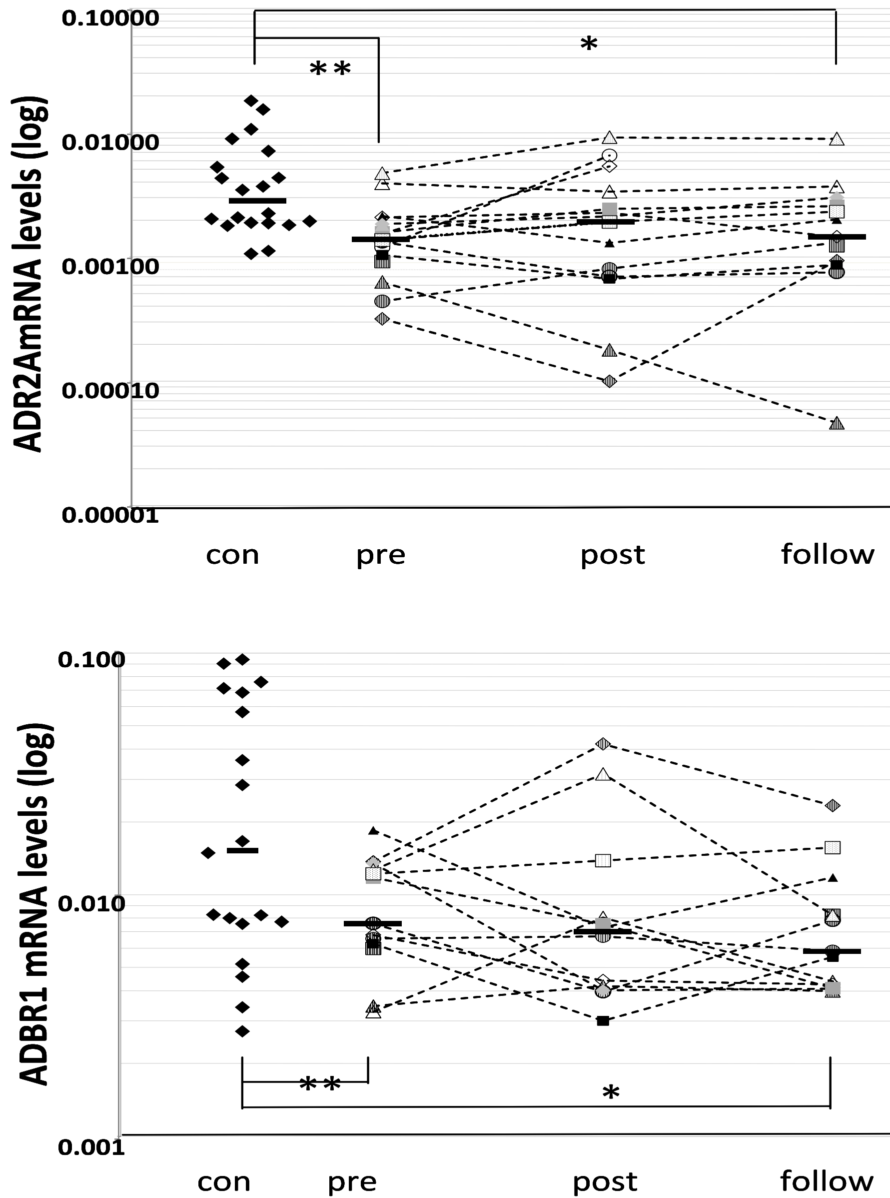
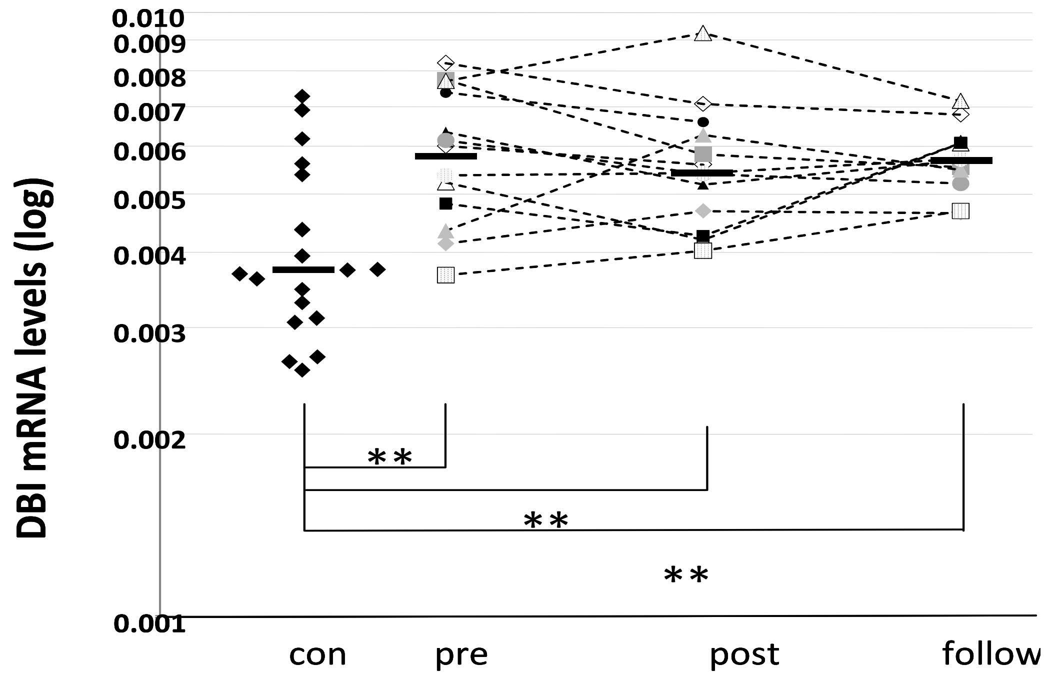


Figure 2.1 Trait-dependent gene expression dysregulation. Adrenergic receptors ADR2A and ADRB1 are decreased during a depressive episode, and remain decreased following treatment. $GABA_A$ modulator DBI remains elevated before and after treatment, while serotonin receptor HTR1D has decreased levels. Expression levels are plotted on a log scale. CON are represented as diamonds on the left and individual DD patients are connected by lines for Pretreatment, Post-treatment, and Follow-up. Medians are represented by horizontal bars. *indicates difference between groups (* $p<0.05$, **$p<0.01$) using Mann Whitney U test.



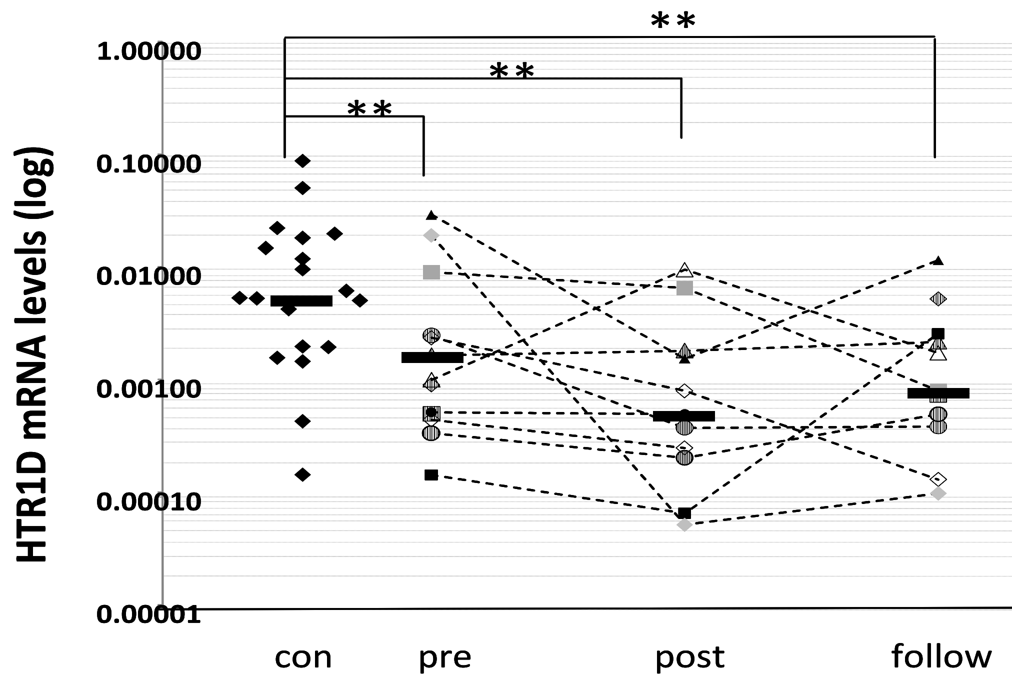


Figure 2.1 continued

State genes. NFKB1 mRNA was higher relative to controls at Pretreatment (1.34 fold higher), and was significantly decreased ($p < .011$) Posttreatment, though levels were still significantly higher than controls ($p < .03$) (Figure 2.2). Unfortunately, not enough data were collected at Follow-up to determine if the normalization towards controls was maintained at the Follow-up time-point. IL-10 mRNA was also higher than controls at Pretreatment, with some subjects displaying normalization following treatment at Posttreatment and Follow-up suggesting IL-10 may be a potential state gene. The immune cytokine IL-6 mRNA displayed significant Pretreatment increases compared to controls. We did not process enough samples to determine changes following treatment.

Treatment genes. We observed changes immediately Posttreatment for several genes that displayed no Pretreatment differences from CON. Within-group analysis indicated significant decreases immediately Posttreatment for NR3C1 ($p < 0.01$), DRD4 ($p = 0.01$), and SULT1A1 ($p = 0.02$). At follow-up, DRD4 and NR3C1 had returned to Pretreatment levels, while SULT1A1 remained decreased compared to Pretreatment levels in DD ($p = 0.04$). Expression changes for these genes are shown in Figure 2.3.

To explore whether gene expression levels were related to symptom improvements, we computed Spearman correlations between Pretreatment gene expression and depression severity as well as with depression score evolution. None of the candidate genes were related to depression severity as assessed by HRSD, nor were they predictive of depression improvement. There were, however, several genes whose Pretreatment expressions were correlated with each other, including positive

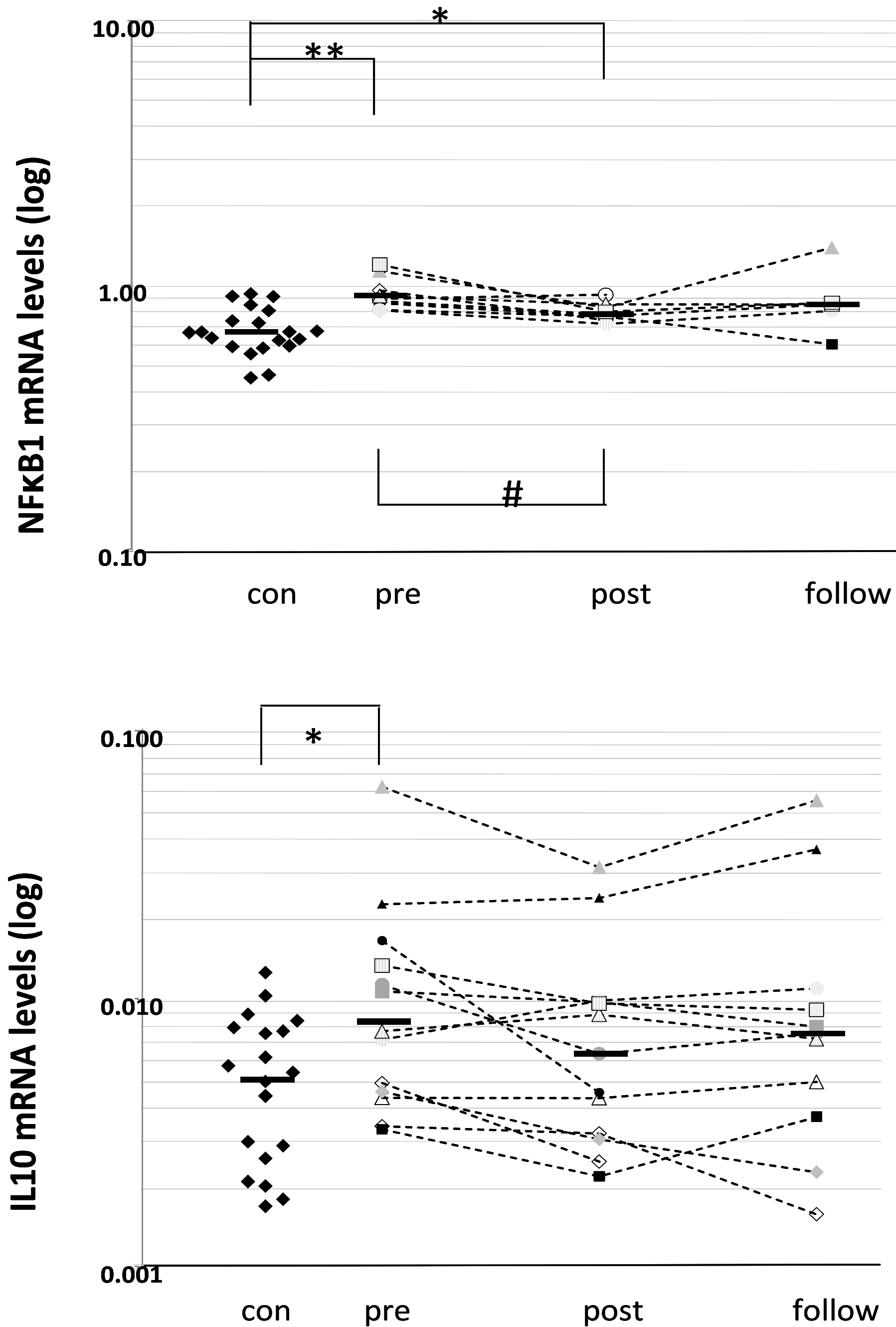


Figure 2.2 State-dependent gene expression dysregulation. NFκB1 and IL-10 are both elevated Pretreatment compared to CON but decrease Posttreatment towards CON levels. Expression levels are plotted on a log scale. CON are represented as diamonds on the left and individual DD patients are connected by lines for Pretreatment, Posttreatment, and Follow-up. Medians are represented by horizontal bars *indicates difference between groups (* $p<0.05$, **$p<0.01$) using Mann Whitney U test. # indicates difference within group (# $p<0.05$, ##$p<0.01$) using Wilcoxon rank sum test

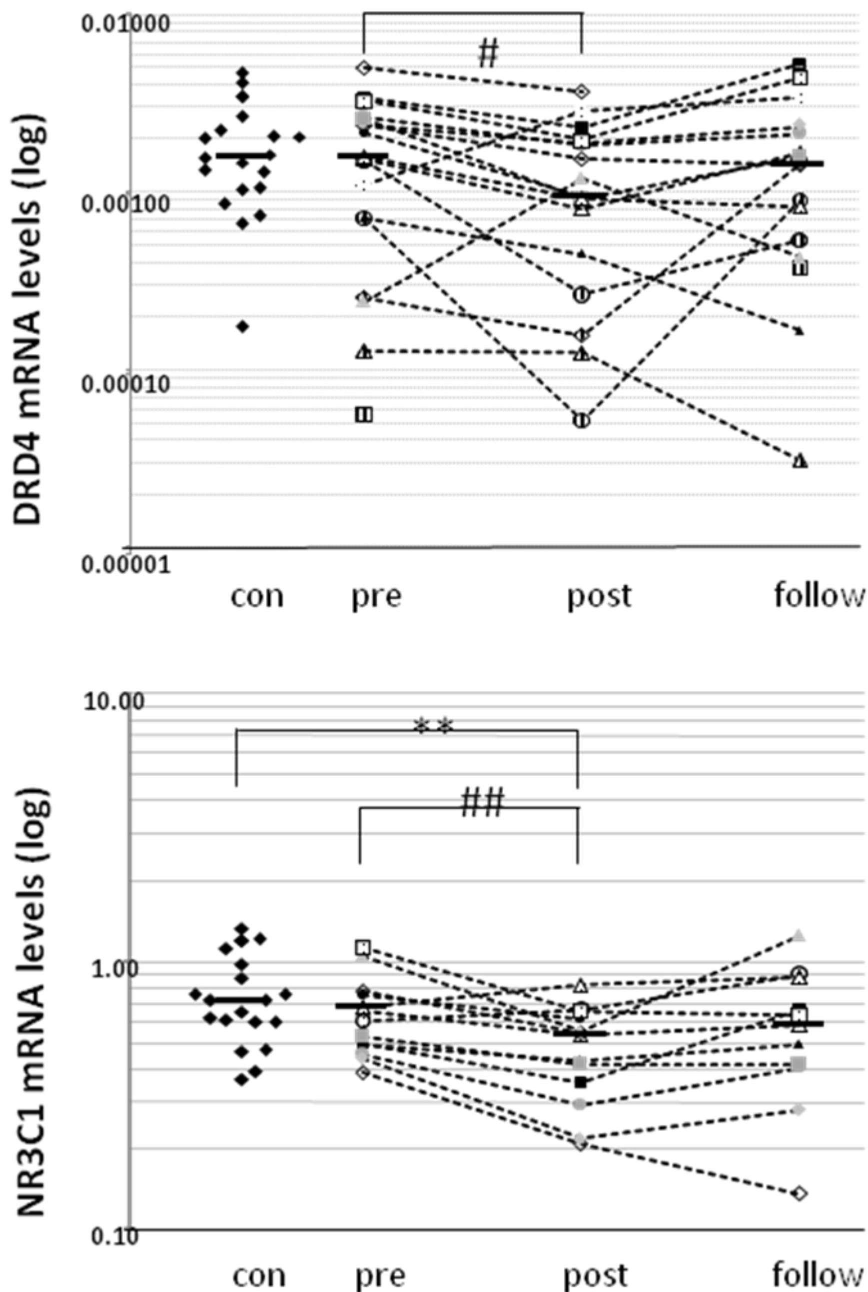


Figure 2.3 State-Treatment dependent genes that display comparable expression at baseline but dysregulation at Posttreatment or Follow-up. Expression levels are plotted on a log scale. NR3C1 and DRD4 show group decreases Posttreatment but return to Pretreatment levels at Follow-up. SULT1A shows group decreases at Posttreatment and Follow-up. CON are represented as diamonds on the left and individual DD patients are connected by lines for Pretreatment, Post, and Follow-up. Medians are represented by horizontal bars. * indicates difference between groups (* $p<0.05$, ** $p<0.01$) using Mann Whitney U test. # indicates difference within group (# $p<0.05$, ## $p<0.01$) using Wilcoxon rank sum test.

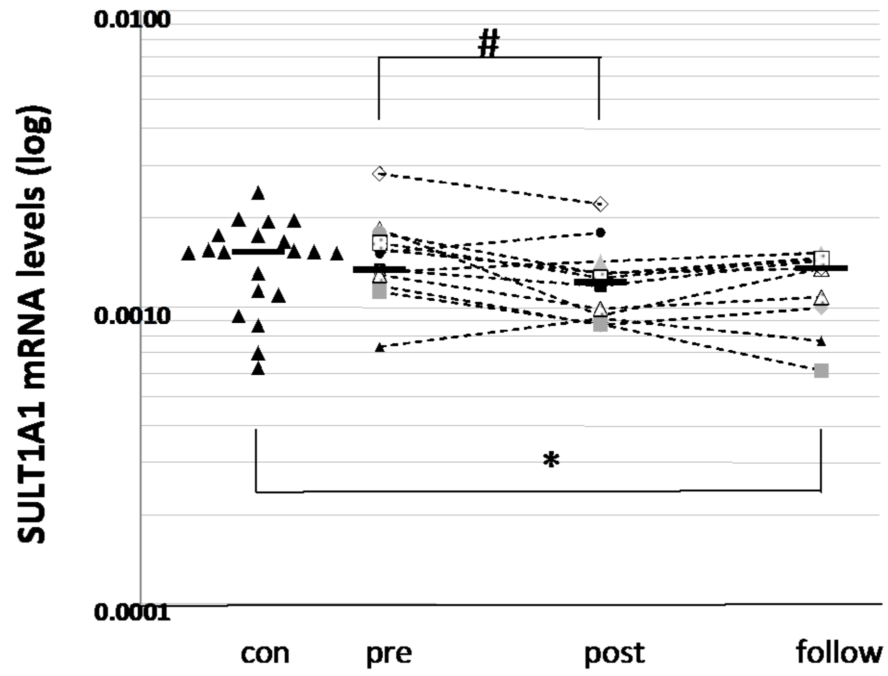


Figure 2.3 continued.

correlations for ADR2A/HTR1D (+0.529, $p = 0.035$), ADRB1 with ADRB2 (+0.706, $p = 0.01$) and with HTR1D (+0.601, $p = 0.039$), ADBR2 with DBI (+0.562, $p = 0.024$) and with IL-10 (+0.512, $p = 0.045$), and finally NFκB1 with NR3C1 (+0.649, $p = 0.004$).

Effects of medication on gene expression

We examined the effect of several classes of medications on gene expression: serotonin/norepinephrine re-uptake inhibitor antidepressants (SSRIs, SNRIs, and NRIs), anticonvulsants, and antipsychotics. For antidepressants, removal of patients and controls on SSRIs (DD/CON n=4/n=3), NRI (1/1) or SNRIs (8/0) did not alter between-group differences seen with their inclusion suggesting that reuptake inhibitors did not contribute to the mRNA differences seen in this study.

For anticonvulsants, at the Pretreatment blood draw, 4 patients had their anticonvulsants withheld in anticipation of ECT treatment, 7 were still taking them, and 8 were never on anticonvulsants. No patients were on anticonvulsants at Posttreatment. Analysis revealed that DD patients not on anticonvulsants maintained all mRNA differences observed in the entire population of patients. Thus, anticonvulsant use did not substantially contribute to the mRNA differences obtained between DD and CON groups. In fact, within-group comparisons for those patients on anticonvulsants versus those without at Pretreatment displayed trends for lower gene expression in IL-6 ($p = 0.076$), NFKB1 ($p= 0.076$), and NR3C1 ($p = 0.083$), suggesting possible immunosuppresive effects of these medications that normalize gene expression towards CON levels.

Patients not on antipsychotics maintained mRNA differences for all genes that differed between groups. Within-group analysis for those on antipsychotics compared to those without, however, demonstrated decreased mRNA gene expression for subjects on antipsychotics for IL-10 (p = 0.013), IL-6 (p = 0.013), NFκB1 (p = 0.051), and NR3C1 (p = 0.008). Similar to anticonvulsants, because these medication effects are in the opposite direction of our DD group differences, it is possible that increases in the expression of these mRNAs among the DD patients compared to controls would have been even greater if antipsychotics had been stopped in all patients. This seems to indicate that partial normalization of immune function may be one of the benefits of these drug classes in DD patients.

Discussion

There were three principal findings in the present study:

1) During the Pretreatment depressive state, patients with medication-refractory DD exhibited dysregulated gene expression in immune, HPA axis, and monoamine signaling genes. These alterations included decreased expression of adrenergic receptors ADR2A and ADRB1 and the serotonin receptor HTR1D, elevated pro- and anti-inflammatory cytokines (IL-6 and IL-10 respectively), and elevated immune transcriptional regulator NFκB1 and neurotransmission modulator DBI. These genes have known roles in stress regulation and their dysfunction may contribute to DD symptoms.

2) Dysregulated genes displayed trait, state, and treatment-dependent expression patterns following treatment-induced symptom improvement. Trait related genes, DBI (higher than controls at all time-points), and adrenergic-ADR2A, adrenergic-ADRB1, and HTR1D (which

were lower than controls at all sampled time points) were not significantly altered by treatment and/or symptom improvement, as would be expected in genes related to traits. State-related genes, NFκB1 and IL-10, had gene expression higher than CON Pretreatment and expression levels shifted toward control levels immediately Posttreatment. Thus, these genes may be related to the state that is DD, which is improved by treatment. Treatment-related genes, DRD4, NR3C1, and SULT1A1, had mRNA levels that were not different from controls at Pretreatment. However, the expression of these three genes decreased following treatment. DRD4 and NR3C1 returned to the Pretreatment levels at the Follow-up time-point, but SULT1A1 remained decreased at Follow-up. These results suggest that despite symptom improvement, these patients continue to display immune dysregulation, which may underlie their poor response to medication and their increased likelihood of disease relapse.

3) Concurrent use of antipsychotic and anticonvulsant medications does not promote, and in fact may partially mask, leukocyte gene expression differences. Within-group analysis found that subjects on these medications displayed decreases in mRNA for IL-10, IL-6, NFκB1, and NR3C1 bolstering the notion that medications may confound the search for RNA biomarkers. We did not observe any masking effects of antidepressants.

These major findings are further discussed below.

Cytokines and NFkB1 are elevated pretreatment during depressive episodes

Gene expression for the cytokines IL-10 and IL-6 were elevated at Pretreatment. Cytokines are important signaling molecules produced by brain and immune cells to induce inflammation in response to stress and foreign pathogens. IL-10, which primarily plays an anti-inflammatory role, has been found to have elevated mRNA levels in patients during a depressive episode (Belzeaux et al., 2010), though a meta-analysis suggests that protein concentrations of

IL-10 do not differ between patients with depression and controls (Dowlati et al., 2010). IL-6, which has diverse roles, including roles in depression, cancer, and asthma (Scheller and Rose-John, 2006), has been shown to have both elevated protein and mRNA levels in depression (Tsao et al., 2006; Dowlati et al., 2010) and acute increases following stressful mental tasks (Brydon et al., 2005). The combined dysregulation of both a pro- and an anti-inflammatory marker (IL-6 and IL-10 respectively) suggests immune imbalance consistent with observations of both immune activation and suppression in depressive disorders, though the functional net effect is still poorly understood (Blume et al., 2011). Interestingly, in depression patients we also observed elevated gene expression for the transcription factor NFkB1 that regulates cytokines and has an important role in mediating HPA axis stress immune response. Though only few studies have examined NFkB1 gene expression, NFkB1 binding activity has been found to be elevated following acute psychosocial stress in humans and animal model (Bierhaus et al., 2003) and upregulation of target genes was observed in family caregivers of brain cancer patients (Miller et al., 2008). Therefore, higher levels of NFkB1 could lead to imbalance in cytokine expression and chronic inflammatory response.

Adrenergic receptor gene expression for ADR2A and ADRB1, but not ADRB2, are downregulated

Catecholamines and their receptors have been shown to have key roles in the stress and immune response in both animal models and humans (Ressler and Nemeroff, 2000; Morilak et al., 2005; Levy and Tasker, 2012). We found that mRNA gene expression for ADR2A and ADRB1 (but not ADRB2) adrenergic receptors were *decreased* in depressed patients versus controls. This is in contrast to results of human and animal studies that have examined protein levels and receptor binding sites. In humans, major depression has been associated with elevated levels of

the ADR2A receptor protein and binding sites (Callado et al., 1998; Ordway et al., 2003; Garcia-Sevilla et al., 2004; Valdizan et al., 2010), though some studies suggest no change or even a decrease in binding (Maes et al., 1999; Marazziti et al., 2001). Differences may be a result of depression severity and individual disease history. In rodents, for example, acute stress was associated with an increase in NE release and activity, and with a decrease in adrenergic receptor proteins including ADR2A, ADRB1, and ADRB2 (Goddard et al., 2010). Conversely, chronic psychosocial stress results in hypo-NE activity with decreased NE release, increased ADR2A receptors binding sites, increased density of ADRB-adrenergic receptors, and changes in NE turnover and availability (Ressler and Nemeroff, 2000; Goddard et al., 2010). In humans, the ratio of ADR2A and ADRB1 binding sites has been suggested to relate to suicidality (Sastre et al., 2001), and we found a significant correlation between ADR2A and ADRB1 receptors at Pretreatment, which suggests that diagnostic markers may benefit from examination of multiple components of the adrenergic system. We did not see any difference in ADRB2 consistent with previous study by Jeanningros et al. examining ADRB2 receptor density, though they found significant decreases when examining only unipolar depression and receptor density correlated with depression severity (Jeanningros et al., 1991). Together, these results suggest that adrenergic receptors display dysfunction in MDD that may be related to depressive state and type.

Serotonin 1D receptor (HTR1D) gene expression is downregulated in refractory depression

Serotonin plays a number of important roles in the immune system, among them macrophage phagocytosis, T cell proliferation and function, and cell attraction to sites of inflammation (Ahern, 2011). Furthermore, serotonin mediates cell-type specific release of both

pro- and anti-inflammatory cytokines. Decreased expression of serotonin receptors may result in impaired ability to mediate important immune functions. However, it must be noted that decreases in mRNA gene expression does not necessarily mean decreases in receptor protein. In fact, changes in gene expression may be in response to the presence of increased membrane receptors or, conversely, low levels of serotonin could lead to understimulation of serotonin receptors and downregulation. We chose the HTR1D receptor subtype since we had previously shown that it is expressed on WBCs. We found that HTR1D gene expression was significantly decreased during a depressive episode, which with lower levels of serotonin availability may contribute to depressive symptoms. Though HTR1D was investigated in relation to the treatment of migraines, few studies have examined HTR1D in depression, and some reached contradictory conclusions, including both decreased and increased HTR1D binding depending on brain region and medication use (Arranz et al., 1994; Lowther et al., 1997). Conversely, the serotonin transporter (5HTT), which is responsible for reuptake of synaptic serotonin, has been found to be elevated in patients during a depressive episode, which in conjunction with decreased HTR1D receptor expression would greatly decrease serotonin signaling (Iga et al., 2005).

Diazepam Binding Inhibitor (DBI) is upregulated during a depressive episode

In our gene panel we included the Diazepam Binding Inhibitor (DBI), a gene which encodes a peptide that is a negative allosteric modulator of $GABA_A$ receptors with implicated roles in depression and anxiety (Corda et al., 1984; Barbaccia et al., 1986; Tsao et al., 2006). We found DBI mRNA levels to be significantly increased in the DD group at Pretreatment. This is in line with previous studies that found increased levels of DBI protein in CSF of drug-free depressed patients (Barbaccia et al., 1986; Barbaccia, 2011). Interestingly, a positive correlation

was found for DBI levels and those of CRH in depressed patients, gamblers, and controls (Roy et al., 1989). Since $GABA_A$ receptors inhibit CRH producing cells, it is possible that elevated levels of DBI lead to increased levels of CRH and ultimately elevated cortisol, a common signature of HPA dysfunction found in many patients with depression (Ferrarese et al., 1993; Levy and Tasker, 2012). Finally, DBI fragment has also been found to decrease the effect of benzodiazepines on pro-inflammatory cytokines following immune challenge, suggesting that increased levels of DBI may interfere with the efficacy of benzodiazepines for anxiolytic action and in HPA regulation (Taupin et al., 1991) and may therefore be a potential marker for treatment-refractory depression or anxiety.

ADR2A, ADRB1, HTR1D, and DBI display trait-dependent expression

We found that ADR2A and ADRB1 which were significantly decreased at Pretreatment and remained low relative to CON both immediately following treatment (Post) and 4 weeks later (Follow-up), suggesting trait dysregulation. Furthermore, these two receptors displayed a significant positive correlation at both Pretreatment and Posttreatment. However, a lack of significant change in gene expression does not mean a lack of change in protein or function. One published report on humans and several on rodents suggest that multiple sessions of electroconvulsive therapy leads to a reduction in adrenergic receptor density (Hosoda and Duman, 1993; Werstiuk et al., 1996; Seo et al., 1999). Therefore, our results in conjunction with published reports seem to imply that ECT helps with depressive symptoms in part by modulating adrenergic function, but trait imbalances persist and perhaps contribute to later depression relapse. Since norepinephrine and adrenergic function is part of the HPA axis, patients with chronic adrenergic dysfunction will continue to have dysregulated stress response. Based on our

results, adrenergic receptor gene expression may be a trait marker for depression, but is not predictive of treatment response.

We found that despite symptom improvement, HTR1D mRNA levels remained low compared to CON at both Posttreatment and Follow-up suggesting trait-dependent gene expression, and thus that patients with severe refractory depression have persistent underlying physiological imbalances in this receptor subtype. Unlike HTR1D, other serotonin receptor subtypes and the serotonin transporter do display changes following treatment. For example, Belzeaux et al. observed significant within-group increases in HTR1B and HTR2A mRNA gene expression in WBCs at 8 weeks following treatments and symptom improvement, while several groups have observed decreased neuronal receptor binding following ECT or antidepressants as assessed by neural imaging (Yatham et al., 1999; Meyer et al., 2001a; Mischoulon et al., 2002; Belzeaux et al., 2010; Yatham et al., 2012). Gene expression alterations have been observed for the serotonin transporter 5HTT, namely, increased expression relative to controls at pretreatment during a depressive episode that decreased after 8 or 12 weeks of chronic treatment with specific SSRIs (Iga et al., 2005; Tsao et al., 2006). These results suggest dysregulation in serotonin signaling that is nevertheless receptor-type and function specific.

Gene expression for the $GABA_A$ modulator DBI remained elevated at Posttreatment and Follow-up time points, suggesting that DBI levels were not confined to depressive episodes and could be a potential trait-dependent biomarker. This is in agreement with previous studies that found a lack of correlation between DBI peptide levels in the CSF and behavioral assessments, such as anxiety and depression severity, in depressed subjects (Barbaccia et al., 1986; Barbaccia, 2011). Because DBI inhibition of $GABA_A$ receptors can lead to increased CRH, chronic elevation in DBI despite symptom improvement may potentially lead to persistent HPA dysregulation and elevated cortisol, and thus continued trait immune dysregulation.

IL-10, IL-6, and NFκB1 show state-dependent expression patterns

We found that although significantly elevated at Pretreatment, IL-10 was no longer significant compared to controls following treatment and symptom improvement, which seems to indicate that IL-10 is a state-dependent marker. This would be consistent with previous findings of decreased IL-10 at 8 weeks following treatment, though ECT has been observed to result in an increase in stimulated IL-10 cytokine release (Belzeaux et al., 2010; Fluitman et al., 2011). However, in our dataset, IL-10 gene expression displayed increased variability following treatment, with patients displaying both trait-like and state-like expression patterns. Larger sample sizes are needed to address this tentative finding. Future research should also address differences that may exist between patient responders and nonresponders, as well as patient-specific changes in IL-10 in relation to other immune markers.

We were unable to follow IL-6 over time because of insufficient sample size. However, previous work has suggested that IL-6 protein serum levels normalize following the use of antidepressants, though there were differences between responders and nonresponders (Sluzewska et al., 1995; Lanquillon et al., 2000; Kenis and Maes, 2002; Fornaro et al., 2011). Conversely, ECT has been found to result in increased IL-6 production following LPS stimulation (Fluitman et al., 2011). Future studies with a larger sample size will be able to identify IL-6 gene expression changes following treatment in medication-refractory depression.

Though NFκB1 was significantly elevated in DD patients at both Pretreatment and Posttreatment compared to CON, it displayed within-group decreases following treatment, which is perhaps indicative of normalization and therefore of NFκB1 being a potential state-dependent biomarker. Since acute levels of stress lead to an increase in levels and signaling of NFκB1, it is possible that symptom improvement would elicit a decrease in expression (Bierhaus

et al., 2003; Pace et al., 2006). Examination of treatment nonresponders with persistent depressive symptoms could be informative.

NR3C1, DRD4, and SULT1A1 show treatment-dependent expression patterns

The glucocorticoid receptor (GR), which is encoded by the NR3C1 gene, has been found to be dysregulated in depression patients (Miller et al., 2009; Anacker et al., 2011a). Though comparable to controls at Pretreatment, we found that NR3C1 gene expression displayed significant Posttreatment decreases in both between- and within-group analysis, but returned to Pretreatment levels at Follow-up. This seems to indicate that ECT and ISO treatments resulted in acute gene expression changes that were not related to symptom improvement at Follow-up. Similarly, Matsubara et al. found that gene expression for the GRα isoform was not related to prolonged symptom remission in patients with depression, but contrary to our results, they found that DD subjects had significantly decreased Pretreatment expression (Matsubara et al., 2006). It is possible that concurrent medications contributed to the Pretreatment differences between our results and their study, as discussed in a section below. Finally, work by Hageman et al. in animals found that chronic stress reduced hippocampal GR mRNA, which then was restored with electroconvulsive shock (ECS), the animal equivalent of ECT (Hageman et al., 2009). This is an example of an acute effect of treatment, though GR expression following cessation of chronic stress was not examined. It must be mentioned that dysregulation of NFκB1 and GR has widespread implications, given the many physiological pathways they regulate. In microarray studies, stress in caregivers and fatigue in breast cancer survivors were associated with an over-representation of genes regulated by NFκB1 and decreased levels of NR3C1 target genes (Miller et al., 2008; Bower et al., 2011). In our studies, levels of NFkB1 and NR3C1 were positively correlated at Pretreatment (+0.649, p = 0.023) but not at Posttreatment (0.333, p =

0.419) though both genes decreased following treatment, which seems to suggest that ECT may affect these two genes differently. Because acute ECT has been shown to increase cytokines, ACTH, and cortisol in humans (Weizman et al., 1987; Kronfol et al., 1991; Fluitman et al., 2011), it is possible that the acute decreases in NFkB1 and NR3C1 are an effort to reestablish proper immune balance. Future studies should compare difference in these genes between treatment-responders and nonresponders in order to determine if Pretreatment dysregulation could be predictive of treatment response, especially prior to an invasive treatment such as ECT.

In our study, SULT1A1 displayed Pretreatment levels similar to those in healthy controls but had significant decreases Posttreatment that were maintained at Follow-up. SULT1A1 is responsible for the inactivation and ultimate metabolism of a number of compounds, including hormones, catecholamines, and drugs (Coughtrie, 2002). Thus, decreases in SULT1A1 mRNA and the enzyme product may lead to increased availability of these important signaling molecules and medications. Though research has shown that genetic variants of SULT1A1 can increase risk of breast or colorectal cancer, few studies have found differences in depressive disorders (Coughtrie, 2002; Pasqualini, 2009). One study by Marazziti et al. found variability in platelet protein levels of phenosulfotransferase (PST) (coded by SULT1A1) dependent upon mood disorder subtypes, namely, increases in obsessive compulsive disorder and patients with mania, decreases in patients with MDD or migraine, and no differences with BPD relative to controls (Marazziti et al., 1996). Future studies should examine medication-responsive patients to determine whether SULT1A1 may be an important contributor to symptoms in DD.

The dopamine receptor DRD4 showed expression levels comparable to controls at all sampled time points tested both before and following symptom improvement, but displayed a significant within-group decrease at Posttreatment. This acute change may suggest an effect on receptor expression as a result of the stress of the treatment rather than adaptive changes in

the body related to symptom improvement. This is potentially important, given that dopamine mediates some bidirectional communication between the central and immune function (Basu and Dasgupta, 2000). This is in contrast to a study that found low Pretreatment DRD4 mRNA levels that normalized to controls following treatment with paroxetine (Rocc et al., 2002), or the elevated DRD4 observed in postmortem tissue from MDD and schizophrenia patients (Stefanis et al., 1998; Xiang et al., 2008). Variations in peripheral and postmortem levels of DRD3 and dopamine transporter have also been observed, suggesting dysregulation in depressive disorders (Meyer et al., 2001b; Vogel et al., 2004)

Medications display immunosuppressive effects on gene expression

There is an extensive literature on the effects of medications on immune function, including those of multiple classes of antidepressants, mood stabilizers, and antipsychotics (Kenis and Maes, 2002; Drzyzga et al., 2006; Sullivan et al., 2006; Choi et al., 2009; Miller et al., 2009; Fornaro et al., 2011; Antonioli et al., 2012). This may help explain why in a panel 40 candidate genes previously implicated in depression, only IL-10 was found to be significantly different from controls in a depressed population currently on benzodiazepines and antipsychotic medication (Belzeaux et al., 2010). In our study, we found that patients taking anticonvulsants and antipsychotics had decreased gene expression for several immune genes, specifically IL-10, IL-6, NFκB1, which were elevated in the DD group. This is consistent with an immunosuppressive role for these medications that has been suggested to underlie their beneficial effects (Piazza et al., 1996; Pollmacher et al., 2000; Drzyzga et al., 2006; Himmerich et al., 2011). We also found that patients taking these medications had lower NR3C1 glucocorticoid receptor gene expression compared to patients not on medications, though there were no group differences between patients and controls. Differences in patient demographics and

medications may explain why we saw no GR differences at Pretreatment, whereas Matsubara et al. observed trait-like decreased GRα during depressed state as well as during remission (Matsubara et al., 2006). The effects of SSRI and SNRI antidepressants on cytokines and immune function must also be considered. Hernandez and colleagues observed that chronic use of SSRI decreased levels of anti-inflammatory cytokines and increased pro-inflammatory cytokines over the period of one year, while increasing the number of natural killer cells and B-cells in the WBCs (Hernandez et al., 2008; Hernandez et al., 2010). Results on the effects of medications on IL-6 have been mixed, with reports of increased, decreased, or no change in IL-6 serum levels in treatment responders (Sluzewska et al., 1995; Yoshimura et al., 2009; Fornaro et al., 2011). Tsao et al. found that SSRI treatment normalized elevated IFN-γ and 5HTT (Tsao et al., 2006). It must be noted, however, that Simon et al. observed increases in multiple pro- and anti-inflammatory cytokines in the serum (including IL-6, IL-8 and IL-10) in medication-naïve as well as medicated patients with MDD (Simon et al., 2008). Because our studies were done with medication-refractory patients who nevertheless responded to ECT, it is possible that gene expression differences may be masked by immunosuppressive effects of medication. In the development of biomarkers, consideration of possible medication confounders will be crucial, since they may mask the dysregulated gene expression in depressive disorders.

Study limitations

There are several limitations to this study that need to be considered in future research. First, this study examined patients with severe, treatment-resistant depression who have failed conventional treatments, while many of the studies referenced here studied patients during depressive states and following successful treatment with conventional drugs, including SSRIs and SNRIs. Though this was done to

examine a unique and more severe subtype, our results cannot be generalized to all DD. There are likely to be many subtypes of depression with different gene expression patterns depending on current depressive states and treatments. Compilation of multiple gene expression studies may reveal patient subgroups that would otherwise be lost in smaller individual samples (Kenis and Maes, 2002).

Another consideration is the influence of medications on gene expression. Antidepressants, anticonvulsants, and antipsychotics are all known to lead to changes in protein and mRNA levels (Kenis and Maes, 2002; Drzyzga et al., 2006; Choi et al., 2009; Girgenti et al., 2010; Antonioli et al., 2012). Since only three controls were on antidepressants, while 14/19 of patients were taking such medications, we cannot rule out the possibility that the observed differences in gene expression are a result of drugs alone. However, the observation that gene expression differences between DD and CON groups persisted after excluding patients on various types of medications, and that use of antipsychotics and anticonvulsants appeared to minimize rather than contribute to these differences, strongly suggest that this is not the case. Lastly, this was a hypothesis-driven pilot study that had a small sample size and a modest set of 14 candidate genes. We are currently evaluating a much larger sample with an expanded gene panel that elaborates upon immune genes and also includes other growth and transcription factors, and genes involved in metabolism and cell cycle maintenance.

Summary

We report here on findings of significant differences in mRNA gene expression in the HPA axis and immune function in subjects with medication-refractory depression. Specifically, we found changes in the glucocorticoid receptor, cytokines IL-10 and IL-6, adrenergic receptors ADR2A and ADRB1, serotonin and dopamine receptors, the transcription factor NFκB1, and the NT/ hormone modulators DBI and SULT1A1. In more detail, we found state-dependent dysregulation in NFκB1 and IL-10, which decreased following treatment and symptom improvement, showing trends towards control levels. We also found trait-like imbalances in noradrenergic receptors, serotonin receptor, as well as DBI, which suggest underlying chronic HPA dysregulation despite symptom improvement. We also identified several genes displaying acute expression changes Posttreatment that returned to Pretreatment levels at Follow-up, including the dopamine and glucocorticoid receptors. These may be related to the mechanisms of action of ECT and ISO treatment and merit further investigation. Overall, these results provide evidence of biological dysregulation detectable in blood from depressed patients who benefited from treatment. Future studies will expand upon genes involved in these pathways, and with a larger sample size will permit analyses comparing responders and nonresponders, a first step towards the development of patient-specific treatments based on objective blood biomarkers.

CHAPTER 3

A GENE EXPRESSION PROFILE COMBINING MULTIPLE PATHWAYS FOR THE STUDY OF REFRACTORY DEPRESSION

Abstract

Depressive Disorders (DD) are a great financial and social burden and yet more than 30% of patients do not respond to conventional medications. These medication-refractory patients present a unique group to discover possible mechanisms of depression. Here we identify several differences in gene expression between DD patients and age-matched controls in physiological pathways that have previously been implicated in the symptoms of depression. We used real-time quantitative polymerase chain reaction (qPCR) to evaluate mRNA gene expression from peripheral blood leukocytes for a panel of candidate genes, including a number of genes previously implicated in depression, as well as ones that may be related to co-morbid symptoms such as pain and fatigue. In our study, we included 42 currently depressed patients, specifically, 25 patients with major depressive disorder (MDD) and 17 patients with bipolar disorder (BPD), and a control group of 38 healthy, nondepressed controls. Out of a panel of 46 genes, we identified gender-specific mRNA gene expression dysregulation in APP, IL-10, IL-6, NRG1, OXTR, P2X7, P2Y1, and TRPV1, in females and ADRB2, ASIC3, and DBI in males. Concurrent medication use may have effected gene expression for NRG1, ASIC3, and TRPV1. These results help to confirm known immune dysregulation in depression as well as gene expression evidence for novel genes, including amyloid precursor protein and various ion channels involved in pain and fatigue. These gene expression differences could potentially be used to explore biological mechanisms of depression as well as for the development of drug targets.

Introduction

Depressive disorders (DD) are a devastating spectrum of diseases. including major depressive disorder (MDD) and Bipolar Disorder (BPD), which have a lifetime prevalence of 16.6% and 3.9%, respectively (Kessler et al., 2005). Women have a 70% higher risk for mood disorders compared to men (Kessler et al., 2005). Up to 30% of MDD patients have treatment-resistant or refractory depression that does not remit with currently available medications (Trivedi et al., 2006). Given that response to subsequent antidepressant medications drops precipitously with each medication failure, finding blood-based biomarkers to identify specific physiological pathways that are dysregulated in refractory depression would be of great potential help in identifying new drug targets and patient-specific treatments (Perlis, 2011).

One promising source for biomarkers is leukocyte gene expression (mRNA), since mRNA measures reflect integration of genetic, epigenetic, and environmental effects (Iga et al., 2008). Furthermore, given the interconnections between the circulating immune system and brain function, evaluation of blood leukocyte mRNA allows for a simple, noninvasive approach to measure some features of an individual's current neurophysiological state (Gladkevich et al., 2004; Sullivan et al., 2006). Previous research on associations between depression and leukocyte mRNA using real-time quantitative polymerase chain reaction (qPCR), including our own, has primarily evaluated one to two genes or an array of genes sharing the same functional class. These have included genes involved in monoamine signaling and the Hypothalamic-Pituitary-Adrenal (HPA) axis (Iga et al., 2005; Matsubara et al., 2006; Tsao et al., 2006;

Padmos et al., 2008; Iacob et al., 2013), or genetic transcription and cellular regulation, including histone deacetylase (HDAC) (Iga et al., 2007a; Hobara et al., 2010), sirtuin deacetylases (SIRT, another class in the HDAC family) (Abe et al., 2011b), cAMP response binding protein 1 (CREB-1) (Iga et al., 2007a) and vascular endothelial growth factor (VEGFA) (Iga et al., 2007b). In contrast to this approach of examining genes individually or by class, Belzeaux et al. compared mRNAs of 40 promising candidate genes linked to multiple biological pathways in 11 MDD patients and 11 matched controls. Of these 40 genes, however, only interleukin 10 (IL-10), an anti-inflammatory cytokine involved in the immune response, was significantly higher in the MDD vs. the control group (Belzeaux et al., 2010). Because DD is a heterogeneous disorder, it is logical to hypothesize that the dysregulated pathways, and thus gene expression for those pathways, may differ in different patient subgroups including related to gender, diagnosis, and current medications.

In the present exploratory study, we examined gene expression dysregulation in patients with medication-refractory DD during a moderate to severe depressive episode; they represent the subgroup of DD patients who are most difficult to treat, and for whom other pathways besides the serotonergic and noradrenergic systems, the target of most currently available medications, may underlie the disorder (Berlim et al., 2008). In view of the large gender difference in DD prevalence, it also was deemed logical to examine mRNA alterations in male and female DD patients separately. Our gene expression panel was designed to evaluate 46 mRNAs from multiple diverse biological pathways. Of these, half have some previous associations to depression while

the remaining have been implicated in other disorders that have overlapping symptoms, including schizophrenia, Alzheimer's disease, Chronic Fatigue and Fibromyalgia syndromes. These genes and their functional class are listed in Table 3.1.

They include genes related to monoaminergic signaling, in particular receptors for serotonin, dopamine, and norepinephrine (Moret and Briley, 2000; Vogel et al., 2004; Goddard et al., 2010; Albert et al., 2011; Iacob et al., 2013); the glucocorticoid receptors NR3C1 and NR3C2, both of which are involved in many of the functions of the HPA-axis (Anacker et al., 2011a); and a variety of immune cytokines and receptors to represent the inflammatory and immune response, which have also been implicated in depression (Miller et al., 2009).

Our research group and others have observed changes in adrenergic gene expression and function; for this reason we also evaluated gene expression of several subtypes, including ADR2A, ADR2C, ADRB1, and ADRB2 (Jeanningros et al., 1991; Marazziti et al., 2001; Ordway et al., 2003; Valdizan et al., 2010; Iacob et al., 2013). We also included growth factors, transcription factors, and modulators with previous association with depression, including CREB1 (Iga et al., 2007a), DBI (Barbaccia et al., 1986), SIRT1 and VEGFA (Iga et al., 2007c; Kishi et al., 2010; Abe et al., 2011a), and the VEGF receptor (VEGFR), the nuclear factor of kappa gene enhancer in B cells 1 (NFKB1) (Bierhaus et al., 2003), superoxide dismutase 2 (SOD2) (Selek et al., 2008) and sulfotransferase family cytosolic 1a (SULT1A1) that is involved in catecholamine processing (Marazziti et al., 1996). Also potentially important are genes coding for proteins involved in protein folding and proteolysis components of stress activated

Table 3.1 Implicated and novel genes for mRNA gene expression in DD*

Pathways	Classes	Genes
Monoamine and peptides	Serotonin, dopamine	HTR1d, DRD4
	Adrenergic receptors	ADR2A,ADR2C,ADRB1, ADRB2
	peptides	COMT, PSMA4, SULT1A1, VIPR2
HPA	Glucocorticoid	NR3C1
	Mineralocorticoid	NR3C2
	Oxytocin	OXT
	Oxytocin Receptor	OXTR
Immune response and Inflammation	Cytokines	IL-10, IL-6, TNFα, TNFβ
	Antigen receptors, chemokine, TF	TLR4, CXCR4, GZMA
Ion channels	Purinergic	P2X1, P2X4, P2X7, P2Y1, P2Y2
	Acid sensing	ASIC1, ASIC3
	Transient Vanilloid	TRPV1, TRPV4
	Potassium	HCN2
Mitochondria/ metabolism	Electron Transport Chain + others	ATP5E, COX5B, NDUFS5, SOD2, HSPA2
Transcription factors	Gene expression factors	CREB1, SIRT1, STAT5A, PPARA, NFκB1
Neuronal health and signaling	Growth factors	NRG1, VEGFA
	Matrix associated	APP, SPARC
	Modulator	DBI

systems including HSPA2 and PSMA4 (Vawter et al., 2006; Bousman et al., 2010). Finally, oxytocin (OXT) and the oxytocin receptor (OXTR) where included given their proposed role in appetite, anxiety, pleasure seeking, and stress (Slattery and Neumann, 2010).

A novel feature of our study is that we also examined classes of genes that were not previously studied by the qPCR method in depression, though some have been potentially linked to symptoms of depression such as low energy and enhanced fatigue.

This category consisted of genes involved in representing mitochondrial and metabolic functions such as the electron transport chain (ATP5E, COX5B, and NDUFS5) (Beech et al., 2010; Manji et al., 2012), and the growth factors Amyloid Precursor Protein (APP) which is implicated in Alzheimer's disease as well as depression (Hock et al., 1998; Jakobsson et al., 2012; Pomara et al., 2012), and also neuregulin-1 (NRG-1) which is related to susceptibility to schizophrenia and potentially DD (Green et al., 2005; Petryshen et al., 2005). Further, our gene expression panel incorporated several ligand-gated channels important in detecting metabolites potentially underlying symptoms of pain and fatigue: genes of the purinergic families (P2X ionotropic and P2Y metabotropic) that sense ATP metabolites, the transient vanilloid receptor (TRPV1, TRPV4) involved in the detection of heat, acid, pressure, and osmolarity, and the acid-sensing ion channels (ASIC1, ASIC3) important for sensing changes in protons. Changes in gene expression have been found to be associated with pain and fatigue in patients with Fibromyalgia (FM) and Chronic Fatigue Syndrome (CFS) (Light et al., 2009; Light et al., 2012a), who often have co-morbid depression. Several of the genes above have also been implicated in animal models of depression and anxiety. For example, animal studies have found that ASIC1a KO mice and WT mice treated with ASIC1a inhibitors display nondepressive and nonanxiolytic behavior (Coryell et al., 2009; Dwyer et al., 2009). Similarly, agonists and KOs of ASIC3, P2Y1, TRPV1, and P2X7 have been shown to modulate behavior in rodent tasks that simulate depressive symptoms (Kittner et al., 2003; Basso et al., 2009; Micale et al., 2009; Wu et al., 2010; Hayase, 2011; Manna and Umathe, 2012). Of these, only P2X7 has been related to functions in humans, with findings that polymorphisms of

P2X7 may predispose individuals to depressive disorders, though gene expression studies have not been conducted (Lucae et al., 2006).

Thus, the primary aim of our study was to determine if mRNA gene expression is dysregulated in both female and male medication-refractory patients for pathways previously implicated in depression, as well as novel pathways involved in depression symptoms. Results may identify new candidate mechanisms for depression-related research and potentially future targets for antidepressant drug development.

Materials and Methods

Subject

All subjects gave written consent, and protocols were reviewed and approved by the institutional review board. The Depression (DD) group included 42 individuals (23 females) in an active state of moderate to severe depression that had unsatisfactory response to 3 or more different antidepressant medication regimens. They included 25 patients with a primary diagnosis of major depressive disorder (MDD) (13F/12M) aged 21-69 years, and 17 patients with bipolar disorder (BPD) (10F/7M) aged 20-72 years (see Table 3.2).

Clinical assessment was carried out including the Hamilton Rating Scale for Depression (HRSD-24), administered by trained personnel and experienced psychiatrists, and the Quick Inventory of Depressive Symptomatology-Self Report Scale (QIDS-SR). The control group, which included 49 individuals, also completed the QIDS-SR to assess their current depressive symptoms, and provided demographic and medical history

Table 3.2 Demographic summary for CON and DD

	Control	MDD	BPD	DD(combined)
	n=38	n=25	n=17	n=42
Age(years)	43.7±2.2	43.9±2.6	41.4±3.7	42.9±2.2
Gender(Male/Female)	(19/19)	(12/13)	(7/10)	(19/23)
HRSD		36.4±2.2	32.1±2.3	34.8±5.4
No medication	36	1	0	1
Antidepressants	0	22	10	32
Anticonvulsants (N/S/C)[b]	(36/0/3)	(7/3/15)	(4/1/12)	(11/4/27)
Antipsychotic	0	14	10	24

[a]Antidep use has higher in MDD vs. BPD $\chi 2$=4.749 (p =0.03) and vs. CON (p <0.01)

[b]Anticonvulsant (N = not on, S = stopped in anticipation of ECT, C = currently on)

information including history of depression and other mental disorders. The control group was further divided into two groups: 1) CON (n = 38) had no history of depression or current symptoms of depression (19 females, mean age 43.9 ± 2.1) and 2) CON-DD (n = 11) had a history of clinical depression and/or were currently taking an antidepressant (7 females, mean age 39.4 ± 3.7). Concurrent with behavioral assessment, blood samples were obtained from each participant. All participants were nonpregnant and between 20 and 76 years old. Patients and controls were matched for age and sex, with no significant differences between groups. Antidepressant use was significantly higher in patients with MDD vs. BPD (chi square= 4.74, p = 0.03) as well as DD vs. CON (χ^2 = 49.98,

p < 0.001) (since no CON subjects were on antidepressants). There were no differences in anticonvulsant use between MDD and BPD.

mRNA extraction and analysis

All blood processing and analyses were performed by personnel blinded to the subjects' group. Blood was collected in EDTA tubes and kept on ice, and within 15 minutes after collection, the blood was centrifuged at 3200 rpm (1315 x g- Clay Adams Compact II Centrifuge) for 12 minutes, plasma was removed, and the white layer was carefully collected in RLT+β-ME (Qiagen, Valencia, CA), then quickly frozen using a methanol-dry ice slurry, and stored at -80ºC. RNA was extracted using RNeasy mini kits (Qiagen, Valencia, CA), and treated with RNase-free DNase-I (Qiagen, Valencia, CA). Immediately following extraction, RNA was converted to a cDNA library using the ABI High Capacity cDNA Archive Kit (Applied Biosystems/Life Technologies, Inc., Foster City, CA), followed by treatment with RNase-H (Epicentre Biotechnologies, Madison, WI). The cDNA samples were stored at -20° C until further use. RNA integrity was assessed with a Bioanalyzer, and consistently found to have RIN values (RNA integrity numbers) greater than 9. The cycle counts for the control gene, TF2B, averaged 23.59 ± 1.04 (SD) for CON subjects and 22.82 ± 0.69 (SD) for DD patients. We selected TF2B as the preferred control gene and have verified that TF2B qPCR counts do not change in freshly harvested separated human leukocytes under a variety of conditions, when RNA is processed as described here.

The cDNA libraries were analyzed using the ABI quantitative, real-time PCR system on the ABI Prism 7900 Sequence Detection System (SDS) 2.4.1 (Applied Biosystems, Inc., Foster City, CA), using ABI TaqMan Master Mix (Applied Biosystems, Inc., Foster City, CA). Master Mix/primer plus primer/probe solutions and template solutions were separately loaded onto 96 well pre-plates. Then 384 well plates were robotically loaded and mixed from the 96 well plates. Each targeted gene was examined in duplicate, with TF2B standards being run in quadruplicate. Additional control samples containing no template were also run. Primer probes for the 46 candidate genes were obtained from TaqMan Gene Expression Assays (Applied Biosystems, Inc., Foster City, CA) and are listed in Table 3.3. qPCR data were processed using the SDS program with count values for genes computed in the curve log-linear using a standard 0.2 threshold. Gene expression amounts were determined using the $2^{-\Delta T}$ method, where ΔT is the count difference of the candidate gene from TF2B.

Statistics

All statistical tests were made using STATA ver. 12 statistical software. Unpaired Student's t-tests for all genes were first performed to identify any differences in gene expression between MDD and BPD. It turned out that only a single gene, TNFα was significant ($p = 0.02$) in a two tailed test while all others were not significant ($p > 0.05$; range $p = 0.08$-0.97). These MDD and BPD subgroups were combined into a single DD

Table 3.3. List of ABI primers (Taqman Assays) use for qPCR

Gene Symbol	ABI numbers
TF2B	Hs00155321_m1
ADR2A	Hs00265081_s1
ADR2C	Hs03044628_s1
ADRB1	Hs02330048_s1
ADRB2	Hs00240532_s1
APP	Hs01552283_m1
ASIC1	Hs00241630_m1
ASIC3	Hs00245097_m1
ATP5E	Hs00855401_g1
COMT	Hs00241349_m1
COX5B	Hs00976765_g1
CREB1	Hs00231713_m1
CXCR4	Hs00607978_s1
DBI	Hs00220950_m1
DRD4	Hs00609526_m1
GZMA	Hs00989184_m1
HCN2	Hs00606903_m1
HSPA2	Hs00745797_s1
HTR1D	Hs00704742_s1
IL-10	Hs00174086_m1
IL-6	Hs00174131_m1
NDUFS5	Hs02578754_g1
NFKB1	Hs00765730_m1
NR3C1	Hs01005217_m1

Gene Symbol	ABI numbers
NR3C2	Hs01031809_m1
NRG1	Hs00247620_m1
OXT	Hs00792417_g1
OXTR	Hs00168573_m1
P2X1	Hs00175686_m1
P2X4	Hs00175706_m1
P2X7	Hs00175721_m1
P2Y1	Hs00704965_s1
P2Y2	Hs04176264_s1
PPARA	Hs00947537_m1
PSMA4	Hs01002583_m1
SIRT1	Hs01009006_m1
SOD2	Hs00167309_m1
SPARC	Hs00234160_m1
STAT5A	Hs00234181_m1
SULT1A1	Hs00738644_m1
TLR4	Hs00152937_m1
TNFα	Hs00174128_m1
TNFβ	Hs00236874_m1
TRPV1	Hs00218912_m1
TRPV4	Hs01099348_m1
VEGFA	Hs99999070_m1
VIPR2	Hs00173643_m1

group for subsequent analyses vs. controls. When gender subgroups were next examined, we found a significant difference in TF2B reference gene in males vs. females in the CON group (23.21 ± .19 vs. 24.05 ± .24 for females vs. males, $p < 0.001$) but not in the DD group (22.81 ± .16 vs. 22.85 ± .14 for females vs. males, $p = 0.86$). This further supported our plan to examine males and females separately.

Our primary statistical approach compared DD vs. CON males and DD vs. CON females for each mRNA under study using General Linear Models (GLM) analysis of covariance, with age and reference gene TF2B level retained as covariates in the final model if they were significant at $p < 0.05$ or if they altered the coefficient of the main variable by more than 10 %. This procedure controlled for the difference in TF2B counts we observed in females vs. males subjects. For post hoc analyses examining effects of medications on mRNA level, males and females DD patients were examined together because of insufficient sample sizes for separate analysis, and gender was included as a covariate. When our analyses yielded a significant main effect of group, subsequent mean comparisons were made using Student's t-tests with unequal variance. Genes were further tested for Pearson correlations with other genes and depression severity. Level of significance was set at $p < 0.05$ for two-tailed comparisons.

Results

Differentially expressed genes in DD compared to CON

Our 46 candidate gene panel included genes from diverse functional classes. Group differences differed by gender. For females, we observed that seven genes were

significantly increased in DD relative to controls [APP (*p* = 0.004), IL-10 (*p* = 0.035), IL-6 (*p* = 0.044), OXTR (*p* = 0.023), P2X7 (*p* = 0.048), P2Y1 (*p* = 0.002), TRPV1 (*p* = 0.023)] and NRG1 showed a trend towards increase (*p* =0.069) (see Table 3.4). Of these, only APP was effected by specific diagnosis, showing elevated levels in female BPD (7.63×10^{-1}) but not female MDD (5.98×10^{-1}) patients vs. female CON (5.65×10^{-1}, *p* = 0.003 and *p* = 0.537 respectively).

In males, none of the above genes were statistically different. Instead we observed that three other genes were significantly different between DD and controls. We found increased expression in the DD males for ASIC3 (*p* =0.04), and decreased expression for ADRB2 (*p* =0.049) and DBI (*p* =0.003). Box and whisker plots displaying significant genes in males and females are shown in Figures 3.1-3.3.

Medication effects on gene expression

Our previous report suggested that medications can affect gene expression, specifically it can mask possible differences between depressed and nondepressed populations (Iacob et al., 2013). We therefore examined the within-group effects of different classes of medication combining males and females. Because we observed gender differences for gene expression, we included gender, age, and TF2B as covariates in the regression models where appropriate.

Patients on antipsychotic medications, (24 of the 42 patients) displayed higher CREB1 (7.19×10^{-1} for patients taking antipsychotics vs. 6.36×10^{-1}, *p* = 0.03 for patients not taking antipsychotics). No other genes were affected.

Table 3.4 Group Differences for Individual mRNAs separated by gender

	FEMALES					**MALES**				
GENE	**CON**	**(SE)**	**DD**	**(SE)**	***P***	**CON**	**(SE)**	**DD**	**(SE)**	***P***
Monoamines and peptides										
HTR1D	1.16E-02	(2.54E-03)	6.84E-03	(2.51E-03)	0.138[a]	3.98E-02	(1.35E-02)	5.08E-03	(2.13E-03)	0.693[b]
DRD4	2.44E-03	(3.28E-04)	1.87E-03	(2.25E-04)	0.163	2.01E-03	(3.47E-04)	1.97E-03	(3.46E-04)	0.923
COMT	1.82E-01	(1.07E-02)	1.88E-01	(8.97E-03)	0.685	2.25E-01	(1.55E-02)	2.09E-01	(1.17E-02)	0.416
SULT1A1	6.57E-04	(5.88E-05)	5.85E-04	(3.92E-05)	0.578[b]	7.12E-04	(7.23E-05)	8.27E-04	(8.46E-05)	0.309
PSMA4	3.75E-01	(2.33E-02)	4.29E-01	(3.02E-02)	0.157	3.56E-01	(2.22E-02)	4.09E-01	(2.27E-02)	0.103
Ion channels										
ASIC1	8.97E-04	(1.34E-04)	8.32E-04	(1.12E-04)	0.604[a]	9.27E-04	(1.31E-04)	1.27E-03	(2.25E-04)	0.207[a]
ASIC3	5.63E-03	(2.84E-04)	6.45E-03	(4.19E-04)	0.112	**5.55E-03**	**(4.52E-04)**	**7.56E-03**	**(8.14E-04)**	**0.040**
HCN2	2.70E-03	(3.13E-04)	3.34E-03	(3.73E-04)	0.196	3.34E-03	(3.78E-040	3.44E-03	(3.00E-04)	0.845
P2X1	4.12E-01	(2.19E-02)	3.55E-01	(2.43E-02)	0.090	3.66E-01	(3.25E-02)	3.81E-01	(2.30E-02)	0.714
P2X4	1.48E-01	(7.76E-03)	1.67E-01	(8.99E-03)	0.119	1.69E-01	(1.17E-02)	1.63E-01	(9.50E-03)	0.717
P2X7	**4.64E-02**	**(3.22E-03)**	**5.84E-02**	**(4.89E-03)**	**0.048**	6.30E-02	(5.66E-03)	6.09E-02	(4.30E-03)	0.772
P2Y1	**7.48E-02**	**(3.89E-03)**	**9.45E-02**	**(5.82E-03)**	***0.002[b]***	9.70E-02	(9.17E-03)	8.15E-02	(5.03E-03)	0.855[b]
P2Y2	1.11E-01	(6.73E-03)	9.69E-02	(6.50E-03)	0.141	1.44E-01	(1.20E-02)	1.06E-01	(6.65E-03)	0.259[b]
TRPV1	**1.15E-02**	**(6.36E-04)**	**1.35E-02**	**(5.07E-04)**	**0.023**	1.21E-02	(7.42E-04)	1.38E-02	(6.80E-04)	0.096
TRPV4	6.98E-03	(9.65E-04)	5.84E-03	(6.90E-04)	0.345	7.53E-03	(7.78E-04)	6.90E-03	(5.10E-04)	0.505[c]

Bolded entries indicates significant difference from control subjects with ***p*** **<0.05**, and ***p<0.01 in italics.***
Relative amounts were calculated with the $2^{-\Delta t}$ method, and normalized to TF2B.
Comparisons are Student's t-tests unless they contain superscripts to indicate covariates used in GLM:
[a]Age, [b]TF2B, [c]Diagnosis (within group BPD vs. MDD).

Table 3.4 Continued

	FEMALES					MALES				
GENE	**CON**	**(SE)**	**DD**	**(SE)**	***p***	**CON**	**(SE)**	**DD**	**(SE)**	***P***
Transcription Factors, DNA Modulators, and Growth Factors										
CREB1	6.85E-01	(1.83E-02)	6.83E-01	(2.57E-02)	0.643[bc]	7.51E-01	(3.17E-02)	6.84E-01	(3.03E-02)	0.133
NFKB1	5.11E-01	(2.62E-02)	5.52E-01	(1.60E-02)	0.190	5.82E-01	(3.17E-02)	5.85E-01	(1.89E-02)	0.923
PPARA	4.17E-02	(2.28E-03)	4.60E-02	(1.63E-03)	0.136	4.99E-02	(3.49E-03)	5.19E-02	(2.77E-03)	0.658
STAT5A	5.65E-01	(4.01E-02)	6.11E-01	(2.14E-02)	0.314	6.14E-01	(4.02E-02)	6.24E-01	(1.94E-02)	0.824
SIRT1	1.95E-01	(8.22E-03)	1.90E-01	(4.63E-03)	0.656	2.06E-01	(1.09E-02)	1.99E-01	(7.57E-03)	0.608
NRG1	**8.42E-04**	**(9.61E-05)**	**1.19E-03**	**(1.60E-04)**	**0.069[c]**	7.24E-04	(9.08E-05)	9.92E-04	(1.30E-04)	0.117[ac]
VEGFA	7.35E-02	(4.49E-03)	6.95E-02	(3.91E-03)	0.496	7.11E-02	(5.94E-03)	7.08E-02	(5.04E-03)	0.964[c]
APP	**5.65E-01**	**(3.12E-02)**	**6.70E-01**	**(3.59E-02)**	***0.004[bc]***	5.99E-01	(3.61E-02)	6.68E-01	(3.70E-02)	0.189
SPARC	2.52E+00	(2.17E-01)	2.52E+00	(1.80E-01)	0.508[ac]	2.68E+00	(3.62E-01)	2.98E+00	(3.03E-01)	0.534
DBI	3.97E-03	(2.28E-04)	4.28E-03	(2.91E-04)	0.411	**5.01E-03**	**(3.17E-04)**	**3.72E-03**	**(2.41E-04)**	***0.003***
Mitochondria and Metabolism										
ATP5E	1.13E+00	(1.76E-01)	1.02E+00	(1.07E-01)	0.599	1.86E+00	(3.12E-01)	7.88E-01	(8.55E-02)	0.426[b]
COX5B	2.00E+00	(9.81E-02)	1.81E+00	(7.70E-02)	0.345[b]	1.88E+00	(1.60E-01)	1.72E+00	(1.18E-01)	0.418
NDUFS5	1.17E-01	(2.12E-02)	1.12E-01	(1.11E-02)	0.691[b]	2.31E-01	(4.68E-02)	9.31E-02	(7.05E-03)	0.814[b]
SOD2	1.67E+01	(9.32E-01)	1.46E+01	(8.72E-01)	0.111	1.31E+01	(9.69E-01)	1.40E+01	(1.36E+00)	0.564
HSPA2	4.59E-03	(8.94E-04)	3.38E-03	(8.65E-04)	0.603[ab]	1.11E-02	(3.06E-03)	2.70E-03	(7.80E-04)	0.556[b]

Bolded entries indicates significant difference from control subjects with ***p* <0.05**, and ***p<0.01 in italics.***
Relative amounts were calculated with the $2^{-\Delta t}$ method, and normalized to TF2B.
Comparisons are Student's t-tests unless they contain superscripts to indicate covariates used in GLM:
[a]Age, [b]TF2B, [c]Diagnosis (within group BPD vs. MDD).

Table 3.4 Continued

	FEMALES					MALES				
GENE	**CON**	**(SE)**	**DD**	**(SE)**	***p***	**CON**	**(SE)**	**DD**	**(SE)**	***P***
Transcription Factors, DNA Modulators, and Growth Factors										
CREB1	6.85E-01	(1.83E-02)	6.83E-01	(2.57E-02)	0.643^{bc}	7.51E-01	(3.17E-02)	6.84E-01	(3.03E-02)	0.133
NFKB1	5.11E-01	(2.62E-02)	5.52E-01	(1.60E-02)	0.190	5.82E-01	(3.17E-02)	5.85E-01	(1.89E-02)	0.923
PPARA	4.17E-02	(2.28E-03)	4.60E-02	(1.63E-03)	0.136	4.99E-02	(3.49E-03)	5.19E-02	(2.77E-03)	0.658
STAT5A	5.65E-01	(4.01E-02)	6.11E-01	(2.14E-02)	0.314	6.14E-01	(4.02E-02)	6.24E-01	(1.94E-02)	0.824
SIRT1	1.95E-01	(8.22E-03)	1.90E-01	(4.63E-03)	0.656	2.06E-01	(1.09E-02)	1.99E-01	(7.57E-03)	0.608
NRG1	**8.42E-04**	**(9.61E-05)**	**1.19E-03**	**(1.60E-04)**	$\mathbf{0.069^{c}}$	7.24E-04	(9.08E-05)	9.92E-04	(1.30E-04)	0.117^{ac}
VEGFA	7.35E-02	(4.49E-03)	6.95E-02	(3.91E-03)	0.496	7.11E-02	(5.94E-03)	7.08E-02	(5.04E-03)	0.964^{c}
APP	**5.65E-01**	**(3.12E-02)**	**6.70E-01**	**(3.59E-02)**	$\boldsymbol{0.004^{bc}}$	5.99E-01	(3.61E-02)	6.68E-01	(3.70E-02)	0.189
SPARC	2.52E+00	(2.17E-01)	2.52E+00	(1.80E-01)	0.508^{ac}	2.68E+00	(3.62E-01)	2.98E+00	(3.03E-01)	0.534
DBI	3.97E-03	(2.28E-04)	4.28E-03	(2.91E-04)	0.411	**5.01E-03**	**(3.17E-04)**	**3.72E-03**	**(2.41E-04)**	***0.003***
Mitochondria and Metabolism										
ATP5E	1.13E+00	(1.76E-01)	1.02E+00	(1.07E-01)	0.599	1.86E+00	(3.12E-01)	7.88E-01	(8.55E-02)	0.426^{b}
COX5B	2.00E+00	(9.81E-02)	1.81E+00	(7.70E-02)	0.345^{b}	1.88E+00	(1.60E-01)	1.72E+00	(1.18E-01)	0.418
NDUFS5	1.17E-01	(2.12E-02)	1.12E-01	(1.11E-02)	0.691^{b}	2.31E-01	(4.68E-02)	9.31E-02	(7.05E-03)	0.814^{b}
SOD2	1.67E+01	(9.32E-01)	1.46E+01	(8.72E-01)	0.111	1.31E+01	(9.69E-01)	1.40E+01	(1.36E+00)	0.564
HSPA2	4.59E-03	(8.94E-04)	3.38E-03	(8.65E-04)	0.603^{ab}	1.11E-02	(3.06E-03)	2.70E-03	(7.80E-04)	0.556^{b}

Bolded entries indicates significant difference from control subjects with ***p* <0.05**, and ***p<0.01 in italics.***
Relative amounts were calculated with the $2^{-\Delta t}$ method, and normalized to TF2B.
Comparisons are Student's t-tests unless they contain superscripts to indicate covariates used in GLM:
^{a}Age, ^{b}TF2B, ^{c}Diagnosis (within group BPD vs. MDD).

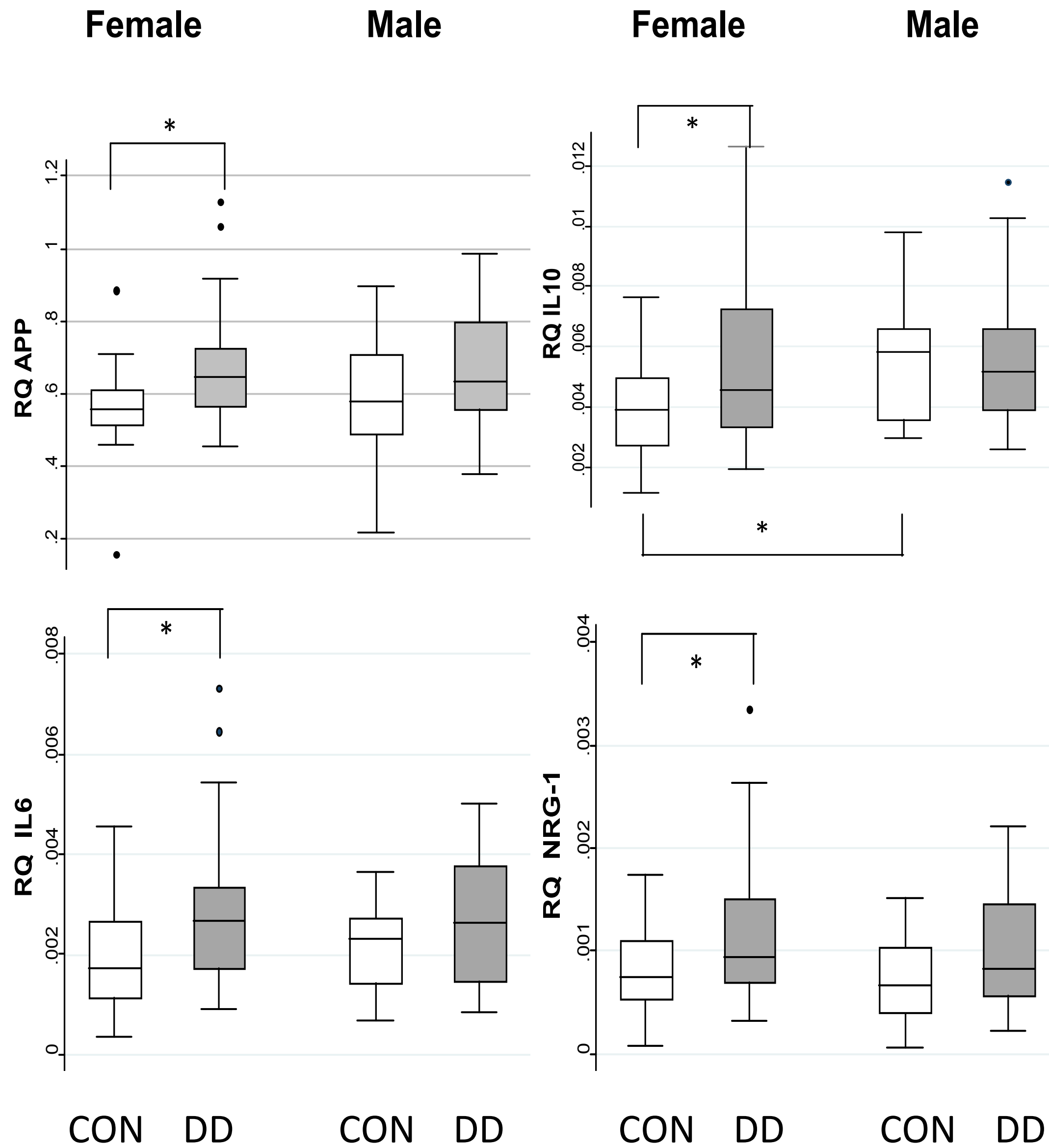


Figure 3.1 Box and whisker plots displaying mRNA gene expression Relative Quantity to TF2B (RQ). White boxes represent CON, and grey boxes represent DD. Depressed females display increased levels of APP, IL-10, IL-6 and NRG-1. *p <0.05 in Student's t-test except for APP *p <0.05 in general linear model (GLM) with TF2B as a covariate

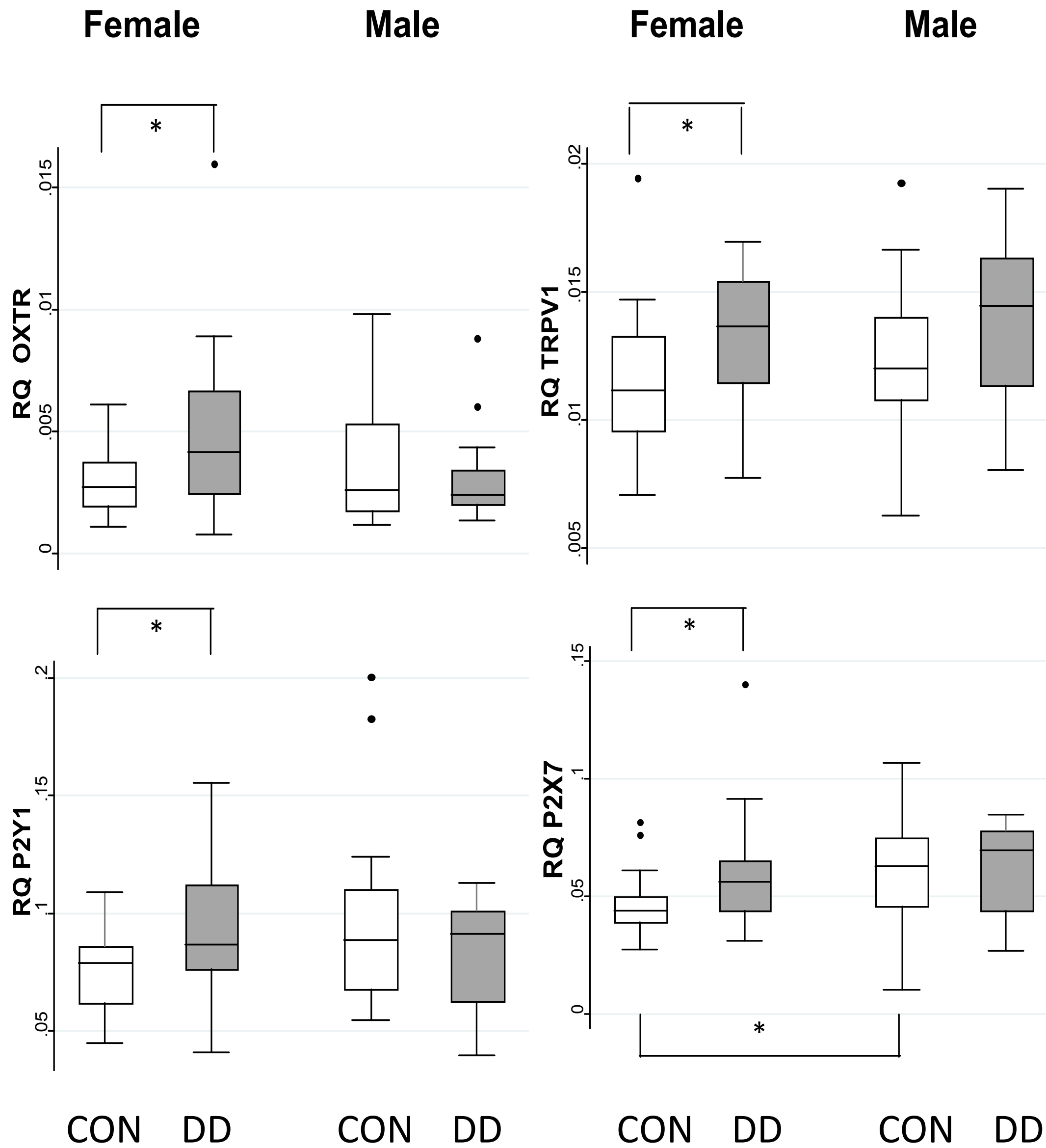


Figure 3.2 Box and whisker plots displaying mRNA gene expression Relative Quantity to TF2B (RQ). White boxes represent CON, and grey boxes represent DD. Depressed females display increased levels of OXTR, P2X7, P2Y1, and TRPV1.

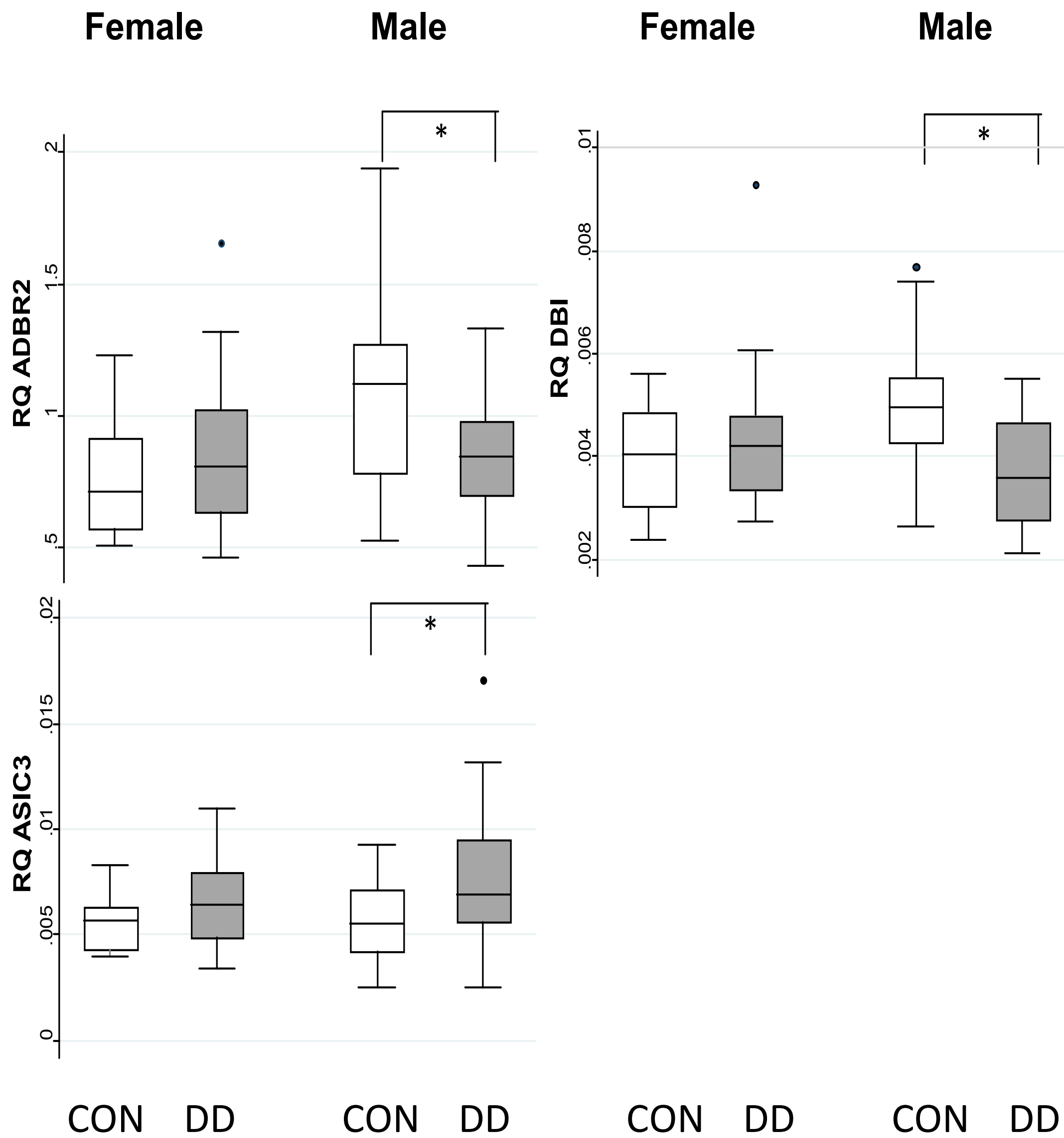


Figure 3.3 Box and whisker plots displaying mRNA gene expression Relative Quantity to TF2B (RQ). White boxes represent CON, and grey boxes represent DD. Depressed males display decreased levels of ADBR2 and DBI, and increased levels of ASIC3. *p <0.05 in Student's t-test

Patients taking any class of antidepressants (32 of 42) displayed *decreased* levels of NRG1 (9.24×10^{-4} vs. 1.67×10^{-3}, $p < 0.01$). Since DD females had marginally higher levels of NRG1 compared to CON, this suggests that medications could have a masking or normalizing effect on this gene. In fact, our Generalized Linear Model (GLM) using antidepressant use as a covariate revealed a highly significant difference between CON and DD for NRG1 for females ($p = 0.001$) and males ($p = 0.004$) (Figure 3.4).

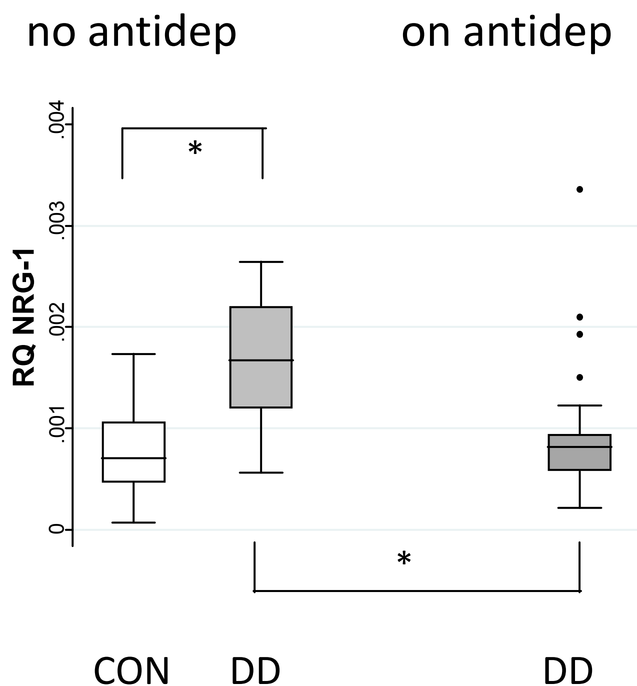


Figure 3.4 Box and whisker plots displaying mRNA gene expression Relative Quantity to TF2B (RQ). White boxes represent CON, and grey boxes represent DD. DD not taking antidepressants display significantly higher NRG-1 levels than both medication-naïve CON and medicated DD. There was no CON subject who was taking antidepressants. *p <0.05 in Student's t-test.

However, since only 4 females and 6 males in the DD group were not currently on antidepressants, subsequent research with larger samples will need to re-examine this post hoc observed possibility. Conversely, patients on antidepressants showed significantly lower P2Y2 expression compared to those not on antidepressants ($9.48x10^{-2}$ vs. $1.21x10^{-1}$, *p* = 0.03). Even with inclusion of antidepressant medication as a covariate, however, P2Y2 differences remained nonsignificant when comparing DD females or males vs. CON (*p* =0.870, *p* = 0.737 respectively).

Finally, use of anticonvulsants was examined. There were 11 patients not on anticonvulsants, 4 who stopped in anticipation of ECT treatment, and the remaining 27 were on some type of anticonvulsant. Within group ANOVA analysis for these three groups and subsequent post hoc unpaired t-tests showed that patients taking anticonvulsants displayed higher ASIC3 ($7.69x10^{-3}$ vs. $4.9x10^{-3}$, *p* <0.001), higher NR3C2 ($5.92\ x10^{-2}$ vs. $4.44x10^{-2}$, *p* < 0.005), higher SULT1A1 ($7.61x10^{-4}$ vs. $5.55x10^{-4}$, *p* = 0.02), and higher TRPV1 ($1.43\ x\ 10^{-2}$ vs. $1.21x10^{-2}$, *p* = 0.04) compared to subjects that were not on any anticonvulsants. Figure 3.5 shows the effects of anticonvulsants on ASIC3 and TRPV1.Importantly, addition of anticonvulsant use as a covariate resulted in loss of a group effect for TRPV1 (*p* = 0.286) between DD females vs. CON, and loss of group effect for ASIC3 (*p* = 0.717) between DD males vs.CON, suggesting that medication contributed to the differences seen in the primary analysis.

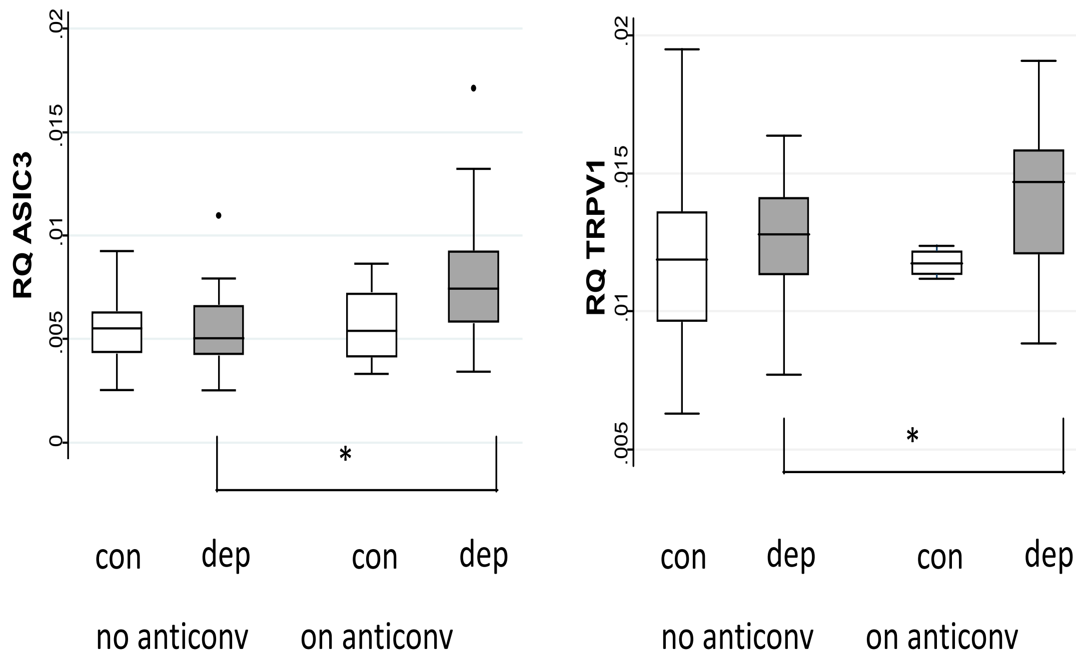


Figure 3.5 Box and whisker plots displaying mRNA gene expression Relative Quantity to TF2B (RQ). White boxes represent CON, and grey boxes represent DD. DD patients on anticonvulsants displayed increased ASIC3 and TRPV1. *p <0.05 in Student's t-test

Gene expression relationship to depression severity

To ascertain if gene expression was related to depression severity, we examined correlations between mRNA levels and HRSD score in the DD patients. Females displayed significant negative correlations for ADRB2 (-0.42, p = 0.047), NR3C1 (-0.43, p = 0.043), and SULT1A1 (-0.41, p = 0.05). For males, there were positive correlations with severity for CXCR4 (0.55, p = 0.018) and HCN2 (-0.51, p = 0.03). None of these genes showed significant differences between groups in the primary analysis.

Discussion

The principal finding of this study is that women with medication-refractory depression display circulating immune gene mRNA expression differences compared to healthy, nondepressed controls. These differences include increased expression of APP, IL-10, IL-6, NRG1, OXTR, P2Y1, and TRPV1. These genes were not differentially expressed in depressed males, who instead displayed increases in ASIC3 and decreases in ADBR2 and DBI gene expression. Furthermore, within-group analysis suggests that most of these differences were not due to concurrent anticonvulsant, antidepressant, or antipsychotic use, with the exception of TRPV1 in females and ASIC3 in males, where anticonvulsant medications appear to contribute to the observed effects. Antidepressant use masked differences in NRG1. The potential biological significance for the genes displaying differential expression is discussed below.

Immune cytokine genes IL-10 and IL-6 are upregulated in depression

Depression has long been associated with alterations in immune function and the stress response. Interestingly, although many studies report an increased immune activation with increased inflammatory markers and T-cell activation, there are also reports of immune suppression, such as decreased lymphocyte proliferation in response to mitogens and decreased natural killer cell cytotoxicity, suggestive of overall dysregulation of both processes (Miller et al., 2009; Blume et al., 2011; Messay et al., 2012). Since white blood cells mediate the immune response, helping with cross-talk between the brain and peripheral immune systems, we elected to examine several

immune genes and indeed found upregulation in IL-6 and IL-10 in females with medication-refractory depression.

Elevated blood IL-6 protein is a consistent finding in depression and some studies suggest that IL-6, and concomitant pro-inflammatory mechanisms, are specifically associated with treatment-resistant depression (Maes et al., 1997; Lanquillon et al., 2000; Simon et al., 2008; Howren et al., 2009; Dowlati et al., 2010). Few studies have examined IL-6 mRNA levels, with most of the ones that did showing increased IL-6 expression in depressed subjects (Padmos et al., 2008; Cattaneo et al., 2013; Iacob et al., 2013) though some studies report no difference (Belzeaux et al., 2010). Importantly, some studies suggest that the expression of IL-6 and several other genes is differentially reduced in patients who respond to treatment compared with those that do not (Yoshimura et al., 2009; Cattaneo et al., 2013).

IL-10 plays a primarily anti-inflammatory role by inhibiting production of pro-inflammatory cytokines and NFκB1. Previous studies have found increased serum levels of IL-10 in patients with depression, in both treated and medication naïve subjects, although other studies found no differences (Hernandez et al., 2008; Simon et al., 2008; Dowlati et al., 2010; Cattaneo et al., 2013). IL-10 mRNA has also been found to be elevated in patients with depression (Belzeaux et al., 2010; Iacob et al., 2013). Importantly, both protein and mRNA levels decreased following successful treatments (Sullivan et al., 2006; Hernandez et al., 2008; Iacob et al., 2013).

Our results together with those in the literature suggest that IL-6 and IL-10 are dysregulated in both medication refractory and medication responsive patients.

Increases in mRNA for IL-6 together with IL-10 correspond to a common underlying mechanism in depression and we believe that IL-10 and IL-6 will continue to be promising biomarkers for depression. Further examination of changes in gene expression in these and other cytokines following symptom improvement may help to determine if mRNA levels of cytokines could serve as a predictor of treatment efficacy.

Amyloid Precursor Protein is upregulated in BPD females

We observed that female patients with depression displayed elevated levels of Amyloid Precursor Protein (APP) mRNA compared to healthy nondepressed controls. Subsequent analysis with diagnosis as a covariate revealed that females with BPD, but not MDD, had higher levels of APP compared with female CON. This is of interest given that BPD and MDD patients were not distinguished by any other gene in our profile when studied during an episode of depression vs. CON.

In addition to their well-known potentially important roles in Alzheimer's disease (Rapp et al., 2006; Butters et al., 2008; Hardy, 2009; Ono and Saji, 2012), amyloid peptides have also been examined in depression. Given that depression is a common co-morbidity as well as a risk factor for Alzheimer's disease (Green et al., 2003), APP may have functional significance in MDD and other depressive disorders. First, Hock et al. observed that there were no differences in APP or amyloid Aβ peptides in the cerebral spinal fluid (CSF) of elderly Alzheimer's patients or age-matched depressed controls (Hock et al., 1998). Furthermore, studies in the elderly have found CSF Aβ40 and Aβ42 were lower in the depressed group, with levels correlating to depression severity

(Pomara et al., 2012), though both increased and decreased levels of Aβ42 have been found in human plasma (Sun et al., 2007; Moon et al., 2011). Perhaps most important is that Baba et al. observed that decreased serum Aβ42 is not restricted to the elderly depressed population: similar decreases were observed in young and middle-aged depression groups (Baba et al., 2012). Alterations in CSF APP peptides have also been observed in young patients with BPD, including decreased levels of sAPP-α and sAPP-β in Bipolar type I and changes in Aβ peptides in Bipolar type II (Jacobsen and Mork, 2004). Our results of increased APP gene expression may be due to compensation for low levels of peptides, or to nonfunctional peptides. Given the complex post transcriptional processing of APP, future studies could potentially combine imaging and blood analysis to determine the relationship between central amyloid levels and peripheral gene expression in depression patients.

Increased expression of NRG1 in refractory depression

We found a trend towards increased mRNA expression of NRG1 in DD females vs. CON, a difference that became robustly significant after inclusion of antidepressant use as a covariate. NRG1 functions through EGF-like domains to stimulate ErbB tyrosine kinases, leading to downstream events important for neural development, including growth and differentiation of neural precursor cells, axon guidance, and synaptic transmission (Falls, 2003). It is postulated that developmental dysruptions in migration and wiring potentially related to NRG1 and glutamatergic activity can lead to symptoms found in patients with schizophrenia, some of which overlap with those of affective

disorders (Mei and Xiong, 2008). NRG1 is a good example of the complex gap between mRNA and protein, since NRG1 can give rise to over 30 isoforms and it is unclear how changes in expression impact function. In rodents, subcutaneous, functional NRG1 has been shown to induce adult neurogenesis and antidepressant-like effects (Mahar et al., 2011). Genetic studies suggest that the NRG1 gene is related to susceptibility to schizophrenia (Mei and Xiong, 2008) and more recently to BPD (Green et al., 2005; Thomson et al., 2007), though Schosser et al. were not able to find a relationship to MDD (Schosser et al., 2010). Changes in mRNA for NRG1 have been observed in neural tissue, with both increases and decreases in NRG1 depending on the isoform, in schizophrenia and unipolar depression (Hashimoto et al., 2004; Bertram et al., 2007). In blood, NRG1 gene expression has been shown to be elevated in leukocytes of patients with schizophrenia (Petryshen et al., 2005). Although gene expression of NRG1 has previously been shown to be elevated in a study of 11 patients with depression (Belzeaux et al., 2010), we are the first to show upregulation of NRG1 in medication-refractory patients. Future analysis examining whether symptoms are related to NRG1 functional polymorphisms and expression could help determine if NRG1 could serve as a biomarker for at least some forms of depression.

Increased oxytocin receptor in medication-refractory females

Oxytocin, a neuropeptide synthesized and released by the hypothalamus, has been implicated in many biological functions, including social bonding, anxiety, pleasure-seeking, appetite, and stress response, all of which may be disrupted in

depression (Slattery and Neumann, 2010). The oxytocin receptor is a G-protein coupled receptor (GPCR) that signals through phospholipase C to mobilize internal calcium. Though oxytocin has been shown to be anxiolytic, antidepressant, and anti-nociceptive, plasma and CSF levels in depressed humans are inconclusive, with reports of both increased and decreased levels compared to controls (Slattery and Neumann, 2010). However, Cyranowski et al. suggest that absolute levels of oxytocin may not be as important as is its proper regulation. For example, they found that depressed females displayed greater oxytocin variability compared to controls over the course of one hour in two separate behavioral tasks, with higher levels associated with clinical and self-report depressive symptoms (Cyranowski et al., 2008). This increased variability in MDD patients is also supported by a study combining both males and females (van Londen et al., 1997). Our findings of increased oxytocin receptor mRNA may reflect receptor upregulation in response to low or poorly functioning oxytocic activity, whereas overstimulation would lead to receptor desensitization, internalization, and decreased mRNA (Gimpl and Fahrenholz, 2001). Interestingly, we did not find any other studies that have examined oxytocin receptor blood gene expression in patients with depressive disorders.

Several studies examining postmortem tissue comparing depressed patients following suicide vs. nondepressed controls found increased oxytocin immunoreactive cells in the paraventricular nucleus of the hypothalamus (Purba et al., 1996), trend towards increased hypothalamic oxytocin mRNA (Meynen et al., 2007), and trend towards increased oxytocin receptor mRNA expression (Wang et al., 2008). In their

review, Slattery et al. suggest that oxytocin activity is increased in the brain in order to offset decreased release in the periphery (Slattery and Neumann, 2010). However, since acute events such as suicide may change gene expression, it will be important for future studies to examine changes in oxytocin mRNAs before and following treatment.

Increased expression of receptors of the ASIC, TRPV, and P2 types and potential implications in pain and fatigue

Our gene expression panel also consisted of several ligand-gated channel families important in detecting ATP and other metabolites associated with aerobic and anaerobic metabolism. Of the nine receptors that we examined, four displayed differences during a depressive episode: P2X7, P2Y1, and TRPV1 were upregulated in females and ASIC3 was upregulated in males. However, our subsequent analyses showed that anticonvulsant use was associated with a significant increase in both TRPV1 and ASIC3, and when reanalyzed after controlling for this medication, only P2X7 and P2Y1 in females remained statistically significant (see medication effects below).

Our research group previously found that following a moderate exercise task, when their fatigue and pain symptoms worsen, patients with CFS (but not controls) show increased expression of ASIC3, P2X4, P2X5 and TRPV1 receptors (Light et al., 2009; Light et al., 2012a). These studies suggest a possible mechanism by which higher receptor expression leads to increased sensitivity to ATP, lactate and other metabolites, and thus the symptoms of pain and fatigue, which are also common in depression. Future research should address the possibility that ASIC3 and TRPV1 increases (which

were specifically associated with comorbid FMS pain in our CFS patients) are not directly altered by anticonvulsant use, but are instead biomarkers of those patients who will most benefit from anticonvulsant treatment; it is notable that anticonvulsants are FDA-approved treatments for FMS and neuropathic pain (Sullivan and Robinson, 2006).

Our clinical findings in regard to P2Y1, P2X7 and TRPV1 mRNAs are the first such observations in depressed humans, but are consistent with a number of preclinical studies. P2Y1 receptor agonists have been shown to display anxiolytic properties in rodents in the EPM, while P2X7 KO mice displayed normal behavior in the elevated plus maze (EPM), but nondepressed-like behavior in other tests (Kittner et al., 2003; Basso et al., 2009). In humans, it has been suggested that a polymorphisms in P2X7 may predispose individuals to MDD depressive disorders, though these findings have been disputed by some (Lucae et al., 2006; Green et al., 2009; Skaper et al., 2010). TRPV1 channels have also been implicated in animal models of depression and anxiety, with intraperitoneal injections of TRPV1 antagonists having anxiolytic effects while TRPV1 agonists were anxiogenic (Micale et al., 2009). In the brain, intracranial injections of TRPV1 agonists or antagonists were found to have antidepressant effects, perhaps by decreasing NMDA-mediated activity (Manna and Umathe, 2012).

Adrenergic receptor β2 and GABA modulator DBI

Two additional genes [adrenergic receptor β 2 (ADRB2) and diazepam binding inhibitor (DBI)] differed between DD and CON groups, but for males only. There was a decrease in the ADRB2 gene, a G-protein coupled adrenergic receptor involved in

catecholamine signaling that is important for smooth muscle relaxation in the vasculature and lungs, and for increasing heart rate when more cardiac output is necessary. The ADRβ2 agonist salbutamol has been found to have beneficial effects in a clinical trial with depressed patients (Belmaker, 1981). Furthermore, Mausbach et al. found that peripheral cell β2-receptors desensitized in individuals taking care of spouses with Alzheimer's Disease (Mausbach et al., 2008). Finally, numerous polymorphisms have been identified that may result in altered expression of β2-adrenergic receptors including in asthma, various heart conditions, and obesity (for review see (Small et al., 2003)). In fact, previous research has found that adrenergic function including α2A-, β1-, and β2-, receptors are perturbed in animal models of stress and depression with differential changes under acute and chronic stress (Goddard et al., 2010). Given the heterogeneity of depression, it is not unreasonable to assume that multiple subtypes of adrenergic receptors can be dysregulated. In the Chapter 2 study examining stress and immune-related genes, we found gene expression trait-dependent differences in ADR2A and ADRB1 but not ADRB2, though our sample size consisted primarily of unipolar depression patients, with males and females grouped together (Iacob et al., 2013). Because of their antagonistic functions, the ratio of β and α receptor expression may be important, as suggested by a postmortem study comparing suicide patients vs. controls, and thus future studies should continue to include adrenergic receptors as promising biomarkers for depression (Sastre et al., 2001).

The second gene decreased in males was the $GABA_A$ negative modulator DBI, a hormone regulated peptide that has previously been associated with anxiety and

depression, including observation of elevated CSF protein levels in depressed subjects (Barbaccia et al., 1986). We had previously observed *increased* DBI levels in refractory depression that remain elevated despite symptom improvement following ECT (Iacob et al., 2013). Because $GABA_A$ receptors act to inhibit neurons that produce corticotropin releasing hormone (which normally leads to increased cortisol), DBI dysregulation may contribute to HPA hyperactivity, observed in 50% of depressed patients. Furthermore, since DBI can interfere with the efficacy of commonly prescribed benzodiazepines, DBI dysregulation could contribute to nonresponse to medication in depressed patients with comorbid anxiety (Taupin et al., 1991). Future studies should evaluate the relationship between peripheral expression of DBI, anxiety, and treatment response.

Effects of medications and depression severity

In line with previous studies, we found that use of specific classes of medications was associated with significantly altered gene expression, masking or potentiating differences between patients and controls (Choi et al., 2009; Belzeaux et al., 2010). In the case of NRG1, use of SSRI, SNRI and NRI antidepressants may have masked differences between DD and CON for both males and females by decreasing gene expression. Dysfunctional NRG1 has been proposed to contribute to neurological disorders, including schizophrenia, by potentiating GABA and thus impairing glutamatergic signaling (Falls, 2003; Mei and Xiong, 2008). It is possible that modulation of monoamine and glutamate signaling with antidepressants leads to homeostatic induced decreases in NRG1 protein and concomitant decreases in its transcription.

Conversely, as discussed previously, anticonvulsant use may have potentiated differences in expression of some genes, specifically ASIC3 in males and TRPV1 in females. Though studies have examined the effects of anticonvulsants on gene expression, few have examined anticonvulsants in regard to depression (Tang et al., 2004; Hassel et al., 2010). Given that it is not practical or ethical to stop medications if they demonstrate at least partial therapeutic benefits, these results suggest that medications, including anticonvulsants, must be considered as potential confounders when interpreting gene expression results. Additional research examining expression of these genes before and after such antidepressant and anticonvulsant therapy is initiated would be valuable.

In our study, we did not find that depression severity was related to any of the genes that were differentially expressed in the primary analysis. However, female DD displayed negative correlations for depression severity and ADBR2, NR3C1, and SULT1A1 mRNA quantities. For DD males, CXCR4 had a positive correlation to depression severity while HCN2 had a negative correlation. Studies examining changes in gene expression following symptom improvement may be able to elucidate if these relationships are of any consequence.

Summary

The onus is on physicians to acquire better biological readouts for as yet poorly understood diseases, including depressive disorders. In this study, we examined patients with medication-refractory depression since they likely have unique mechanisms

underlying their treatment resistance. Using this method, we made observations supporting dysregulation in genes previously implicated in depression, such as immune cytokines IL-10 and IL-6, but also reported on differences for genes involved in other disorders including APP (Alzheimer's disease), NRG1 (schizophrenia) and P2X7 and P2Y1 (pain and fatigue). This is not surprising given the prevalence of depression as a common co-morbidity, and highlights the importance of research into shared biological mechanisms underlying disease. It is important to note, however, that depression remains enigmatic and difficult to treat, partially because of the diversity of disease onset, variability of symptom presentation, and demographic heterogeneity. Each different sample group may contribute a unique set of gene dysregulation and therefore, it may likely take numerous studies to uncover a gene expression signature for DD. By amassing a panel of differentially expressed genes, researchers could continue to identify novel biological mechanisms contributing to depression and potentially pave the way to new drug targets and personalized medicine. Future studies should combine examination of treatment-resistant and treatment-responsive patients in prospective studies to examine differences before and after medication as well as following moderate exercise tasks found to differentiate patients with CFS and FM from controls (Light et al., 2009; Light et al., 2012a).

Study limitation

One noteworthy limitation in this study is the observation of higher amounts of the reference gene TF2B in DD males compared to CON. If TF2B is an accurate measure

of total mRNA, it would indicate that processed blood from DD subjects had higher proportion of white blood cells. It has been suggested that patients with depression exhibit both immune suppression and activation including more reactive platelets (Musselman et al., 1996), higher monocyte counts and activation (Rothermundt et al., 2001; Beumer et al., 2012), but decreased natural killer cell cytotoxicity (Pike and Irwin, 2006; Blume et al., 2011). Changes in cell counts may also be related to immune differences observed. For example, Lanquillon et al. suggest that there may be a relationship between monocytes and IL-6 levels in treatment nonresponders (Lanquillon et al., 2000). It is important for future studies to specifically regard cell-type specific populations in changes of gene expression. Current experiments are under way to better understand the relationship between total TF2B, RNA quantity, cell types, and sample quality.

CHAPTER 4

DISCUSSION

Biological Implications of Gene Expression and Future Directions

Overview

The aim of this dissertation was to explore candidate gene expression dysregulation in depressive disorders (DD) using real-time quantitative PCR (qPCR). This was based on the hypothesis that DD display both central and peripheral dysfunction and that examination of circulating peripheral blood leukocytes can reflect brain neurophysiology (Gladkevich et al., 2004; Sullivan et al., 2006; Iga et al., 2008). Furthermore, because DD are a heterogeneous group of disorders with diverse symptomatology, we chose to examine medication-refractory patients since they would likely display the most severe symptoms and novel biological mechanisms. In Chapter 2, as part of an add-on study examining a novel treatment using anesthesia, we found that gene expression representing immune function was dysregulated prior to treatment in 19 patients with medication-refractory depression who nonetheless responded to ECT or anesthesia. Furthermore, gene expression displayed primarily two patterns following treatment: 1) trait-dependent expression with few changes suggesting continued dysregulation 2) acute changes 24 hours after ECT that recovered to original levels at one month Follow-up. However, diminishing Follow-up samples only permits conservative conclusions. In Chapter 3 we describe an exploratory study with a larger sample size of 42 patients and 38 controls and with an expanded gene expression panel of 46 genes. Because of preliminary evidence of potential gender effects, we examined gene expression separately by gender. Encouragingly, we found dysregulation in IL-10 and IL-6, consistent with the first study. Furthermore, we identified several novel genes

previously implicated in pain and fatigue, Alzheimer's disease, and neuronal development. It is likely that each sample set will have a unique gene expression profile. It will be important in future studies to continue exploring the possible implications of our findings in both clinical and animal models. qPCR results showing possible association of gene expression dysregulation with depression may lead towards a better understanding of biological pathways underlying disease.

Patients with refractory depression display state and trait-dependent gene dysregulation

In Chapter 2 we describe gene expression for immune and stress-related genes in patients before and after treatment with ECT or the anesthetic isoflurane (behavioral consequences are described in the Appendix). This study was unique in that we examined a subgroup of medication-refractory patients who all received similar care and only one of two treatments. We found that depressed subjects displayed dysregulation in adrenergic receptors ADR2A and ADRB1, the $GABA_A$ modulator DBI, and the serotonin receptor HTR1D, all of which remained dysregulated even following treatment and symptom improvement. All of these genes have been minimally evaluated in previous studies using qPCR in blood leukocytes of depressed patients though adrenergic receptors (Goddard et al., 2010), HTR1D (Arranz et al., 1994; Lowther et al., 1997) and DBI (Barbaccia et al., 1986) have all been implicated in depression and anxiety. We also detected increases in immune cytokines IL-6 and IL-10, and the immune transcription factor NFκB1. However, because of Posttreatment sample sizes,

we are unable to draw conclusions about changes in these genes following symptom improvement although IL-10 and NFκB1 show normalizing trends to control levels. Both protein and mRNA for IL-6 and IL-10 cytokines have previously been shown to be elevated in depression (Tsao et al., 2006; Belzeaux et al., 2010; Dowlati et al., 2010) and thus our studies support their use as biomarkers for depression. NFκB1 is an important transcription factor that mediates many target genes involved in pro-inflammatory and immune response (Miller et al., 2009); for instance. A study by Miller et al. describes increased number of transcripts having NFκB1 response elements in subjects under chronic stress thus further supporting the hypothesis of chronic immune dysregulation (Miller et al., 2008). Our study is the first to report on changes in mRNA levels of NFkB1 in peripheral cells. Additionally, the dopamine receptor DRD4 and glucocorticoid receptor NR3C1, which were normal at Pretreatment, displayed acute decreases immediately Posttreatment, but returned to Pretreatment levels at Follow-up. This is consistent with numerous studies that suggest ECT has acute, but not long-lasting effects (Fluitman et al., 2011). Finally, we only found one gene that displayed lasting changes following treatment, namely SULT1A1 which codes for an enzyme responsible for aiding in hormone, NT, and drug metabolism (Coughtrie, 2002). SULT1A1 as not dysregulated at Pretreatment but decreased significantly at Posttreatment and Follow-up. This is an attractive target for future studies, since it may play a role in increased medication-response seen following ECT. Therefore, in this small pilot study we identified dysregulation in mRNA gene expression in receptors and peptides previously implicated in depression, with genes primarily showing no change or only acute change

following treatment.

Novel dysregulation in patients with medication-refractory depression

In the study reported in Chapter 3 we recruited a much larger set of patients and age/gender-matched controls and examined a diverse panel of genes including expansion of immune markers, metabolite receptors implicated in pain and fatigue, genes involved in energy metabolism, and genes involved in neuronal developmental and plasticity previously implicated in such disorders as Alzheimer's disease and schizophrenia. The aim of this study was to explore possible novel biological contributors to depression and relate them to separate concurrent work examining chronic fatigue syndrome, fibromyalgia, and prostate cancer. Given the higher frequency of depression in females (Kessler et al., 2005), we opted to examine gene expression separately by gender. Out of a panel of 46 genes, encouragingly, we confirmed elevated levels for immune cytokines IL-6 and IL-10 (in depressed females). These results continue to suggest immune dysregulation in depression and the need for further research into potential adjunctive pharmacotherapy, including the proposed use of anti-inflammatory medications (Miller et al., 2009; Raison et al., 2012). Of the adrenergic receptors, we found that depressed males had decreased ADBR2 compared to controls. Previous research has found that chronic stress can lead to desensitized receptor and the β-2 receptor agonist salbutamol was shown to have therapeutic benefits in a clinical trial for depression (Belmaker, 1981; Mausbach et al., 2008). In conjunction with decreases in ADBR1 and ADA2A found in Chapter 2, these results

provide further evidence of dysregulation in norepinephrine and adrenergic function and merit further investigation as a means to monitor therapeutic response to conventional norepinephrine reuptake inhibitors (NRIs) or use of selective adrenergic agonists. Finally, males displayed decreased levels of the Diazepam Binding Inhibitor (DBI), a peptide that may have a role in indirectly activating HPA-axis and cortisol release by interfering with $GABA_A$ mediated inhibitor of CRF-releasing cells (Taupin et al., 1991). Our pilot study found that DBI expression was *increased* in patients with depression. Because of DBI's potential role in anxiety, co-morbid anxiety in different patient populations may constitute distinct gene expression signatures. Future clinical and animal studies should examine the effect of DBI activity, benzodiazepines, and treatment response.

Important to this study was inclusion of numerous genes that were not examined in the Chapter 2 study. Thus, we included here several receptors important for the detection of ATP and other metabolites associated with aerobic and anaerobic metabolism. Depressed females displayed increased expression for purinergic receptor P2X7, P2Y1, and the transient vanilloid receptor TRPV-1, all of which have roles in animal models of depression and anxiety (Kittner et al., 2003; Basso et al., 2009; Micale et al., 2009; Manna and Umathe, 2012). Furthermore, these receptors show marked increases in patients with CFS, but not in controls, following a moderate exercise, which correlated to pain and fatigue scores (Light et al., 2009; Light et al., 2012b). This suggests that acute mild stress leads to gene expression dysregulation and may underlie enhanced pain and fatigue in these patients. Therefore, depression that is characterized

by chronic stress may have hyperactive sensory activity that would benefit from medications. Future studies will examine patients with depression on this task, with the hope that the results will allow separation of this disorder from commonly misdiagnosed co-morbidities.

The amyloid precursor protein (APP), which codes for a matrix cell surface receptor and soluble peptides, has numerous roles in neuronal and synaptic development (Zheng and Koo, 2006). Unfortunately, dysfunction in peptide transport and clearance can lead to synaptic plaques, which are one of the hallmarks of Alzheimer's disease. Interestingly, while central levels are high, peripheral amounts of β-amyloid peptides are low in both Alzheimer's disease and depression (Fagan et al., 2006; Rapp et al., 2006; Baba et al., 2012). We are the first to show upregulated APP mRNA in patients with depression (in our sample it was restricted to the patients with BPD) which might be due to an effort to compensate for decreased or nonfunctional peptides. Future studies should evaluate the relationship, if any, between peripheral APP mRNA expression and central amyloid using neuroimaging in DD. Another protein involved in neuronal development is the growth factor neuregulin (NRG)-1, genetic dysfunction of which has been suggested to cause neuronal migration and disrupted glutamatergic activity that may underlie symptoms of schizophrenia and depression (Green et al., 2005; Thomson et al., 2007; Mei and Xiong, 2008). We found upregulated NRG-1 in patients with depression specifically for those who were not currently taking antidepressants. If these results can be replicated, NRG-1 expression could be used to identify a subpopulation of patients with depression who will not benefit from

antidepressants. Animal studies should further investigate the role of NRG-1 in depressive symptoms and anxiety.

Technical limitations

Though qPCR allows for quantification of small amounts of mRNA transcripts, it does have several technical limitations. First, sample preparation and separation methods can all impact gene expression, including evaluation of whole blood vs. individual cell types or poor protection from endogenous RNAses (Feezor et al., 2004; Asare et al., 2008). Additionally, the choice of internal reference genes for total mRNA normalization are critical (Bustin, 2005). Our research group has historically used TF2B, since we found that it remains stable before and following moderate exercise across multiple population groups. TF2B is a ubiquitous transcription factor necessary for transcription initiation by RNA polymerase II and has expression levels in the appropriate range compared to many candidate genes. Conversely, other commonly used reference genes like actin, GAPDH, and RNA 18S are either orders of magnitude more abundant, or have been shown to have variable expression. That being said, we found that control males in both Study 1 and Study 2 displayed lower TF2B mRNA compared to depressed males whereas these differences were absent for females. If TF2B is a proper surrogate for total mRNA, this suggests that males with depression may have higher mRNA in the buffy coat layer, which is composed of white blood cells (WBCs) and some platelets. Though WBCs contribute the majority of mRNA, platelets and red blood cells do contain mRNA transcripts (though orders of magnitude less than

WBCs) and can synthesize proteins despite lacking a nucleus (Kabanova et al., 2009; Flaumenhaft, 2011). Furthermore, platelets are known to express P2 receptors including P2X1 and P2Y1 (Clifford et al., 1998). Therefore, differences in proportion of platelets and WBCs or sample separation could lead to variation in gene expression. There is a precedence in the literature for differences in peripheral cell populations in patients with depression including increased white blood cells (Rothermundt et al., 2001), and activated platelets (Musselman et al., 1996). Therefore, changes in cell types could contribute to differences in mRNA gene expression between depressed patients and controls and must be taken into account in future studies. If immune cells become activated following stress, such as moderate exercise, we could expect increases in mRNA in processed samples. Though we did not see changes in TF2B following moderate exercise in CFS and FMS subjects, similar studies with depressed patients are underway to determine changes in gene expression.

Future directions

qPCR of peripheral blood cells permits simple, noninvasive, method to quantify gene expression in patients before and following treatment. However, in the study of a multi-faceted disease such as depression, three further lines of research are necessary. The first is to continue examining other depressed populations for candidate gene expression dysregulation. Specifically, it would be beneficial to further examine nondepressed controls currently on antidepressants or other psychotropic drugs, and more moderately depressed patients before and after successful treatment with

medications. In addition, testing depressed subjects on a moderate exercise task is of interest since the task is showing promise in differentiating patients with chronic fatigue and fibromyalgia syndromes from controls, disorders that have high rates of co-morbid depression. Current studies are underway in examining the effects of specific anticonvulsants on gene expression and symptoms in patients with CFS. The second line is biological research into the mechanisms of some of the candidate genes in animal models of depression. For example, employing animal models to examine the role of DBI in medication-resistance and anxiety symptomology; animal models of APP and NRG1 dysregulation and potential neuronal dysfunction; continued research into the role of metabotropic receptors such as the purinergic, ASIC, and TRPV receptors, in animal models of depression. A better understanding of gene expression differences between groups as well as their biological function could help lead to novel drug targets and patient-specific medical care. Finally, gene expression candidates and animal studies could lead to the development of novel therapies, which in conjunction with gene expression, could ultimately lead to patient-targeted treatments.

APPENDIX

ANTIDEPRESSANT AND NEUROCOGNITIVE EFFECTS OF ISOFLURANE ANESTHESIA VERSUS ELECTROCONVULSIVE THERAPY IN REFRACTORY DEPRESSION

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Abstract

Inconsistent prior research suggests that isoflurane anesthesia may be an effective alternative to electroconvulsive therapy (ECT) in depression. The purpose of this study was to determine if isoflurane has antidepressant effects comparable to ECT, with less adverse effects on cognition. Patients with medication-refractory depression received an average of 10 treatments of bifrontal ECT (n=20) or isoflurane (n=8) over 3 weeks. Mood and neurocognitive responses were assessed at pretreatment, posttreatment and 4-week follow-up. Both treatments produced reductions in depression scores at posttreatment ($P<0.005$) and follow-up ($P<0.05$); however, ECT had better antidepressant effect at follow-up in severity-matched patients ($P<0.05$, n=8). Immediately posttreatment, ECT (but not isoflurane) patients showed declines in memory, fluency, and processing speed ($P<0.05$); at follow-up, only autobiographical memory remained below pretreatment for ECT patients, but isoflurane patients had greater test-retest neurocognitive score improvement ($P<0.05$). Our data suggest that isoflurane has an antidepressant effect approaching ECT with less adverse neurocognitive effects.

Introduction

Although antidepressant medications are effective for many patients with depression, the rate of response to the first agent administered can be as low as 50%. For nonresponders to the first agent, the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial showed that the remission rate decreased from 37% to 13%

as successive medication alternatives were tried (Rush et al., 2006) . Electroconvulsive therapy (ECT) is generally acknowledged to be the most effective treatment for severe and medication-refractory depression(UK ECT review group, 2003). Its remission rates are between 55-90%, even in patients whose depression fails to remit with multiple trials of medications (Prudic et al., 1996; Prudic et al., 2004; Khalid et al., 2008). Significant reduction in symptoms also occurs more rapidly with ECT than medications, typically in 2-4 weeks consisting of 8-14 treatment sessions. Though effective, ECT is associated with significant adverse cognitive effects such as retrograde amnesia, problems with concentration and attention, and other cognitive sequelae. With the notable exception of autobiographical memory problems, most adverse cognitive effects of ECT are reported to resolve within two weeks (Semkovska and McLoughlin, 2010). Bifrontal ECT is equally effective to the more traditional bitemporal ECT and has less memory disruption (Dunne and McLoughlin, 2012). There is also a widespread public misunderstanding that the seizure induced by ECT may be painful and traumatic, and carries high risk of brain damage and personality change, making patients and family members reluctant to approve this treatment option (Payne and Prudic, 2009). For these reasons, this effective therapy has become a treatment of last resort (relegated to 5th, 6th or 7th step after the failure of other therapies per American Psychiatric Association guidelines) and ECT is administered to only about 100,000 patients a year (Payne and Prudic, 2009). Thus, millions of patients suffering from major depression do not receive effective therapy, and others receive relief only after many months of medication trials.

It would be highly desirable to establish an alternative therapy that has similar benefits to ECT while minimizing any adverse neurocognitive effects and having wider social acceptability. One alternative meeting these criteria that has some prior data supporting its potential effectiveness in depression is deep inhalation anesthesia with isoflurane (ISO). Like ECT, deep anesthesia with ISO induces a brief state of electrocortical quiescence (burst suppression on electroencephalogram (EEG)), but does so without inducing convulsions or other seizure symptoms. Based on two preliminary studies directed by Langer, et al in Europe (Langer et al., 1985; Langer et al., 1995), a series of six treatments with ISO had similar efficacy to six ECT sessions in reducing depressive symptoms without causing memory loss. The first study was a pilot study showing reduction of depressive symptoms in 11 patients exposed to variable numbers of ISO treatments, and the second was a small double-blind study comparing 10 depressed women treated with ISO vs. 10 treated with ECT for 6 sessions. Assessments indicated cognitive improvement in the ISO group vs. declines in ECT group after treatment. Subsequent work by Engelhardt (Engelhardt et al., 1993) demonstrated similar antidepressant effect with a combined series of ISO treatments followed by ECT. A small open-label report by Carl, et al. (Carl et al., 1988) also suggested similar antidepressant efficacy of deep ISO anesthesia compared to ECT. In contrast, however, Greenberg, et al. (Greenberg et al., 1987) showed relatively little improvement with ISO treatments in a pilot study involving 6 elderly depressed patients (including 5 aged 74-82 years). In another pilot study, Garcia-Toro, et al. (Garcia-Toro et al., 2001) treated 10 patients with four treatments of low dose sevoflurane (approximately 2.5%). While a

24% reduction in depressive symptoms was noted on average, the clinical effect was not deemed sufficient after four treatments and patients were switched to ECT. In these negative studies, methodological concerns were that the number of treatments were fewer than are typically effective for ECT, treatment effects that initially seemed promising nevertheless were stopped, and in the latter study, a low concentration of sevoflurane was used instead of a high concentration of isoflurane. There was essentially a cessation of interest in this potential treatment until recently, when studies in animal models also indicated antidepressant effects of ISO anesthesia. In a pilot study in our lab, mice treated with a single dose of either high dose ISO or desipramine and assessed 24 hours later using the forced swim test showed reduced immobility times as compared to the control group, suggesting antidepressant efficacy (Tadler SC, 2009). To assess more enduring antidepressant effects, Wang et al (Wang L, 2012) administered isoflurane (2%) or halothane (1.5%) to adult male Sprague Dawley rats continuously for 2 hours. Two weeks later, in a learned helplessness paradigm, isoflurane-treated rats had fewer failure trials and faster mean escape latency than naïve controls in the shuttle box avoidance task. Halothane-treated rats showed no antidepressant effects, suggesting that the reduced expression of learned helplessness is specific to isoflurane rather than a general effect associated with exposure to volatile anesthetics.

Given the large number of patients with medication-refractory depression (including many who are under age 70, relatively healthy, and would be deemed good candidates for ISO anesthesia), and improvements in medical monitoring and management of patients during deep anesthesia, a renewed effort to evaluate ISO

anesthesia as an effective alternative therapy to ECT is warranted. As a small open-label comparability study, the present study's primary objective was to examine whether deep ISO anesthesia shows similar efficacy to ECT in alleviating moderate to severe depression in medication-refractory patients. The secondary objective was to evaluate whether a series of 10 treatments with deep ISO anesthesia over a 3 week period results better neurocognitive function at 24-48 hours after the last treatment and 4 weeks later than treatments with bifrontal ECT, a form of ECT previously shown to have lesser effects on memory on cognition than bitemporal ECT (Dunne and McLoughlin, 2012).

Materials and Methods

Participants

This study was approved by the University of Utah Institutional Review Board (IRB Protocol No. 00025750) and all participants provided written informed consent. Participants included 28 patients aged 18-65 years with moderate to severe depression (Hamilton Rating Scale for Depression HRSD-24 score of 20 or higher). They were recruited from among patients referred for ECT to the University of Utah Neuropsychiatric Institute (UNI) based on unsatisfactory response and/or intolerance to (BPD). Exclusions included: 1) primary psychotic disorder, dysthymia, or personality disorder; 2) significant premorbid cognitive impairment (defined as Mini-mental Exam score below 24); 3) unstable cardiovascular or cerebrovascular disease, 4) any other contraindication to ISO anesthesia; 5) pregnancy or 6) inability to consent. Enrolled patients continued their usual pharmacotherapy and psychotherapy, except for

anticonvulsants which were discontinued (see Table A.1) while undergoing the study. In this open-label study, 20 patients were treated using the medical standard intervention, ECT, and 8 patients with the nonstandard intervention, ISO.

Because initial tests revealed that the ECT patients had significantly greater depression severity than the ISO patients at Pretreatment, the responses of the ISO group were also compared to a severity-matched ECT subgroup (n=8); this ECT-Matched subgroup also had the same ratio of patients with BPD vs. MDD diagnoses as the ISO group (3 BPD/5MDD). All patients completed both Pretreatment and Posttreatment testing. One patient in each group failed to return for 4-week Follow-up neurocognitive testing.

Study design

The design was an open-label, two-arm treatment trial, comparing 8-12 sessions of ECT vs. 10 sessions of ISO anesthesia treatments over a 2.5-3.5 week period. Primary outcome measures included: 1) <u>depressive symptoms</u> assessed by clinical assessment using the HRSD-24 by blinded investigators, and patient self-ratings on the 16-item Quick Inventory of Depressive Symptomatology Self Report (QIDS-SR16)
<u>2) neurocognitive function</u> assessed by tests of memory, executive function, and processing speed. HRSD-24 and cognitive assessments were completed 3 times: 1) Pretreatment, 2) 24-48 hours after the last treatment session (Posttreatment), and 3) 4 weeks after the last treatment session (Follow-up). In 12 ECT and all 8 ISO patients, QIDS-SR16 assessments of depression were obtained just prior to each treatment

Table A.1 Group Demographics, Depression Severity, Intellectual Level and Medications

	ECT-All	ECT-Matched	ISO
Gender (Male/Total)	12/20	6/8	3/8
Diagnosis (MDD/BPD)	16/4	5/3	5/3
Mean Age (SEM)	41.60 (2.80)[a]	41.38 (5.39)	35.38 (3.50)
Severity (Pretreatment HRSD-24)	36.55*	28.00	26.63
Mean WTAR (SEM)	107.90 (2.19)[a]	107.88 (4.67)	117.00 (5.01)
Pretreatment Medications (# of patients):			
SSRI	9	5	2
SNRI	8	1	3
NRI	3	1	0
Lithium/valproate	5	1	2
Antipsychotic	8	2	5
Anticonvulsant[b]	14	6	2
No psychotropic meds	3	1	1

* ECT-All > ISO, $p<0.05$, [a] Group differences nonsignificant, $p>0.12$

[b] Anticonvulsant medications were stopped during treatment sessions in all groups.

session, to provide a more continuous picture of the rate of symptom change.

Treatment procedures

Patients received standard monitoring as recommended by the American Society of Anesthesiologists (ASA).

Procedures for the ECT treatment group. Induction was performed by an anesthesiologist (KS) with methohexital (1-3mg/kg IV). A tourniquet was inflated around

a hand followed by neuromuscular blockade using succinylcholine (adjusted by height, 1-2 mg/inch). Then, 60-120 seconds later, the bifrontal ECT stimulus was administered (SpECTrum 5000Q Electroconvulsive Therapy Unit, Mecta Corportation, Tualatin, OR). Visual evidence of tonic-clonic seizure was confirmed by observation in the hand that remained isolated from the systemic circulation. ECT procedure time averaged 20 minutes, with 30-45 minutes required for postprocedure recovery. ECT treatments were repeated approximately every other day for a total of 8-12 treatments (mean 9.6, standard error (SEM) 0.34), based on clinically determined need.

Procedures for the ISO treatment group. As with ECT, anesthetic induction was achieved with methohexital (1-3 mg/kg IV) and neuromuscular blockade was accomplished with succinylcholine (adjusted by height, 1-2 mg/inch). Patients were infused with 500 ml of lactated Ringers solution to help reduce risk of hypotension. The patient's airway was secured via endotrachial intubation. The EEG-derived bispectral index (BIS) was monitored continuously using an Aspect A1000 monitor (BIS v. 3.3, Aspect Medical Systems, Newton MA), along with end-tidal concentration of isoflurane, carbon dioxide and oxygen. Isoflurane concentration was initially at 4% with high flow oxygen until >80% EEG burst suppression; it was then decreased to two times the age-adjusted Minimum Alveolar Concentration (MAC) for the patient and oxygen flows reduced. This level of burst suppression was maintained for 15 minutes (as per Langer, et al. (Langer et al., 1985; Langer et al., 1995)) and then isoflurane was discontinued and the patient allowed to awaken. ISO procedure time averaged 40-45 minutes. Emergence from anesthesia was facilitated by the ANEclear™ device (Anecare®, Salt Lake City, UT)

that rapidly removes residual inhaled anesthetics, reducing post procedure recovery time to 15-20 minutes. Significant hemodynamic alteration (change in heart rate or blood pressure more than 20% from baseline) was treated in standard fashion with appropriate agents (phenylephrine, intravenous fluids, etc.) as per the anesthesiologist's (KS's) discretion. ISO treatments were repeated approximately every other day for a total of 10 treatments.

Neurocognitive testing

At Pretreatment, all patients completed the Mini-mental Exam and the Wechsler Test of Adult Reading (WTAR) to confirm that intellectual levels were within normal limits and generally comparable across groups. Mini-mental scores were all > 26 and did not differ for the ECT-All, ECT-Matched and ISO groups (means = 28.89, 28.94 and 29.22, respectively, $P>0.38$). WTAR scores and age also did not differ significantly ($P>0.12$; Table A.1), but there was a weak trend toward higher WTAR and lower age in the ISO group. To ensure that our findings were not influenced by these subtle differences, both WTAR and age were retained as covariates in our group comparisons.

At Pretreatment, Posttreatment and Follow-up, the following battery of tests were completed. To assess retrograde amnesia, subjects completed the Autobiographical Memory Interview-Short Form (AMI) (Fraser et al., 2008; Semkovska et al., 2012). To assess anterograde amnesia, they completed the Hopkins Verbal Learning test, including Immediate Recall, Delayed Recall, and Discrimination measures (Norman et al., 2011), and the Logical Memory subtests I and II from the Wechsler Memory Scale,

3rd edition (Theisen et al., 1998; Wroolie et al., 2006). To assess speed of information processing, they completed the Symbol Search and the Digit-Symbol Coding subtests that make up the Processing Speed Index (PSI) of the Wechsler Adult Intelligence Scale (Longman et al., 2007; Wisdom et al., 2012). To screen executive function, they also completed the Delis Kaplan Verbal Fluency Test where they were asked to generate as many words as possible in 60 seconds beginning with a specific letter (Tombaugh et al., 1999; Delis, 2001).

Statistical analysis

Two sets of analyses were performed. First, ECT-All patients (n=20) were compared with the ISO patients (n=8) for HRSD-24 scores and for each of the neurocognitive performance scores at Pretreatment, Posttreatment and Follow-up using a mixed model repeated measures ANCOVA with Group (2) as a between-subjects factor and Time (3) as a within-subjects factor. For the neurocognitive measures, both age and intellectual level as indexed by WTAR score were included as covariates. For the QIDS-SR scores, which were obtained before and throughout the ECT and ISO treatment series, a Group (2) x Time (4) analysis was used, where sampling times were before the first, fourth, seventh and tenth/last treatment. When ANCOVAs indicated significant effects, between-group and within-group comparisons among means were performed; for the neurocognitive measures, these means were adjusted for age and WTAR. For measures were ANCOVAs yielded significant effects, second set of mean comparisons were performed for the ECT-Matched subgroup (n=8) vs. the ISO group. To examine relative

changes in performance after treatment between groups, Posttreatment and Follow-up means were compared between-groups after adjustment for the Pretreatment score on that test as well as WTAR. Alpha level was conservatively set at $P < 0.05$ two-tailed, even where our predicted outcomes were directional (e.g., ISO > ECT for neurocognitive measures following treatment), in an effort to limit the increase in false positive findings associated with multiple dependent variables. Cohen's d estimates of effect sizes were also generated (using pooled standard deviations) for each significant group difference.

Results

Antidepressant effects of ECT and ISO

For the HRSD-24 scores based on clinical interviews, a main effect of Times (F = 51.9, d.f. = 2,52, $p < 0.0001$) and a significant Group X Time interaction was obtained (F = 4.58, d.f.=1,52, $p < 0.05$). Subsequent comparisons showed that although the ECT-All group initially had higher depression severity than the ISO group based on Pretreatment HRSD-24 scores ($p < .008$), the scores of both groups decreased from Pretreatment levels at Posttreatment ($p < 0.0001$ for ECT, $p < 0.005$ for ISO), and these significant decreases persisted at 4-week Follow-up ($p < 0.0001$ for ECT, $p < 0.033$ for ISO). When ECT-All was compared with the ISO group, they did not differ in HRSD-24 at Posttreatment or Follow-up, with both groups reducing symptom severity to the mild range (see Figure A.1). The ECT-Matched and ISO patients (n=8 per group) had comparable HRSD-24 scores at Pretreatment and Posttreatment ($p = 0.78$), but the ECT-Matched subgroup maintained these low scores better than the ISO group at 4-week

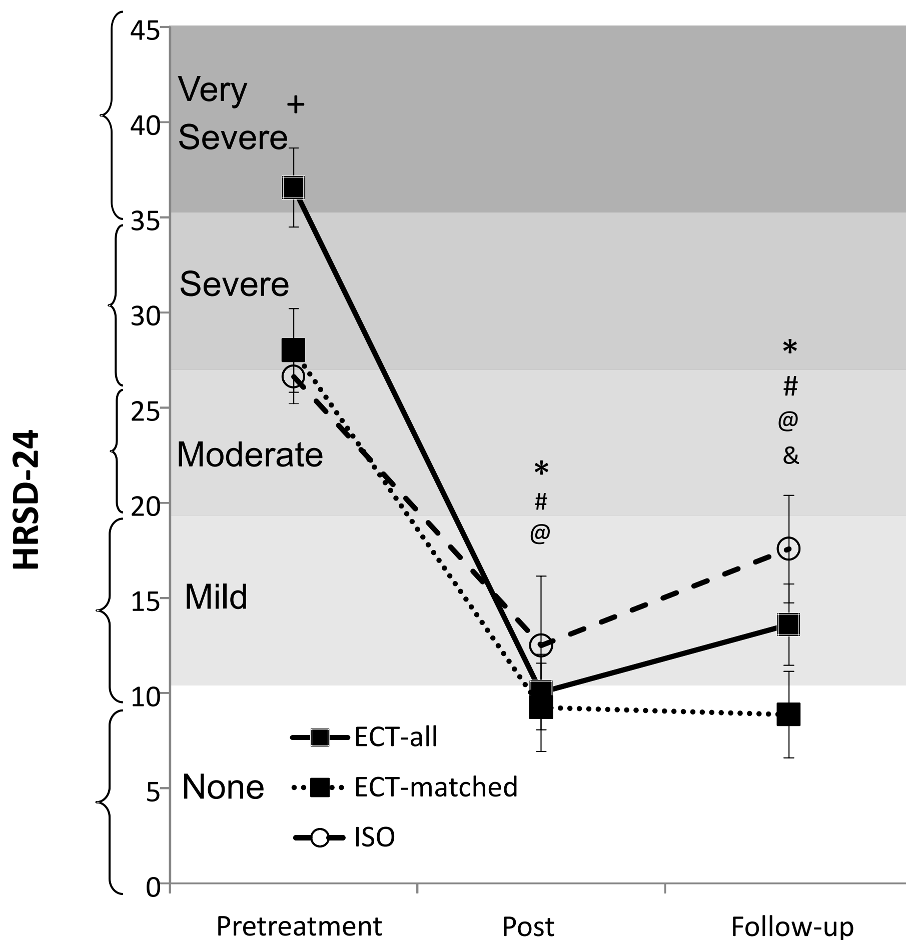

Figure A.1. Scores on Hamilton Rating Scale for Depression (HRSD-24) prior to treatment (Pretreatment), 24-48 hours after the last treatment (Post), and at a 4 week return visit (Follow-up). Treatment with ECT and ISO results in significant decrease in HRSD-24 depressive symptoms at both Post and Follow-up, compared to Pretreatment. The ECT-Matched and ISO groups had comparable decreases at Post-treatment, but ECT-Matched maintained these low scores better at Follow-up.

+ ECT-All vs. ISO $p < .05$, **&** ECT-Matched vs. ISO $p < .05$, *****Within group ECT-All Change from Pretreatment $p < .05$, **#** Within group ECT-Matched Change from Pretreatment $p < .05$, **@** Within group ISO Change from Pretreatment $p < .05$

follow-up (8.9 vs. 17.6, $p < 0.036$, d = 1.39; Figure A.1). Self-reported depressive symptoms (QIDS-SR16) obtained prior to the ECT or ISO treatment sessions did not differ between the groups at any time point (Group x Time F = 0.84, d.f.=3,70, $p = 0.55$), and there was similar steady improvement in all three groups across the treatment sessions (Main effect of Time, F = 18.65, d.f.= 3,70, $p < 0.0001$; see Figure A.2).

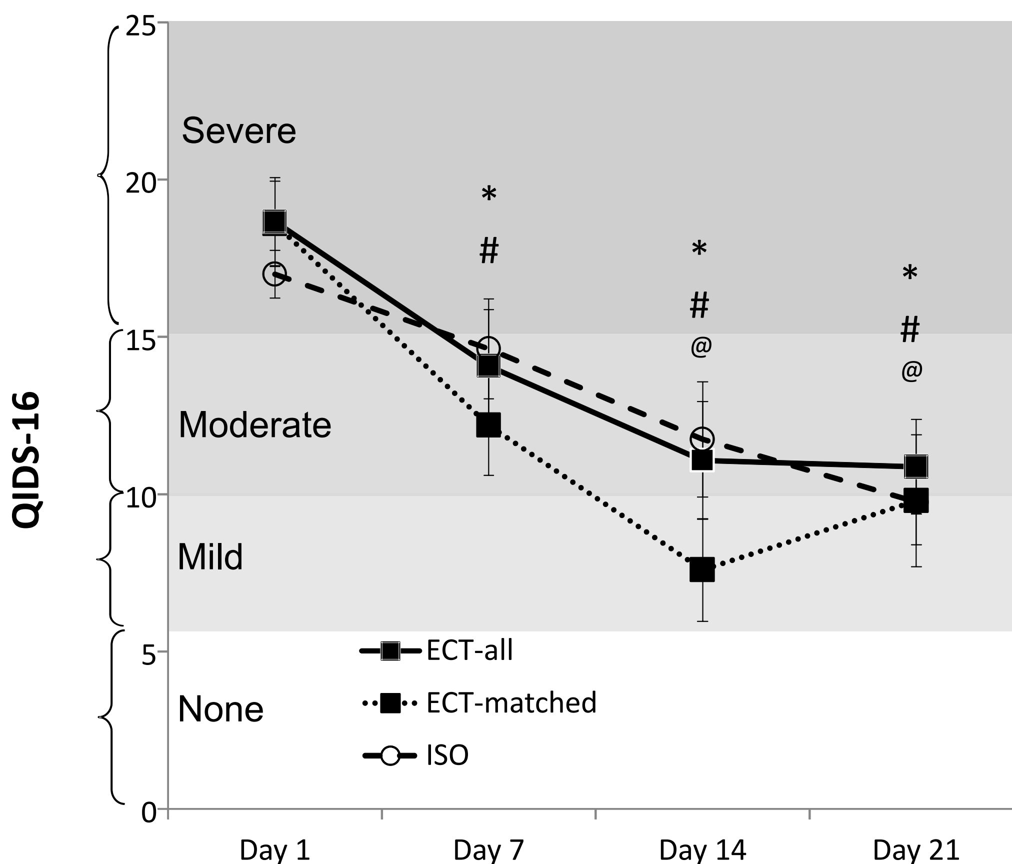

Figure A.2. Scores on the Quick Inventory of Depression Scale (QIDS-SR16) self-report form over 21 days of treatment (treatment sessions 1-10). For brevity, results are depicted showing change over each week, from Day 1 (prior to 1st treatment), Day 7 (prior to 4th treatment), Day 14 (prior to 7th treatment), Day 21 (prior to 10th treatment).

+ ECT-All vs. ISO $p < .05$, **&** ECT-Matched vs. ISO $p < .05$, *****Within-group ECT-All Change from Day 1 $p < .05$, **#** Within-group ECT-Matched Change from Day 1 $p < .05$, **@** Within-group ISO Change from Day 1 $p < .05$

Neurocognitive measures: Overall analyses

After adjustment for differences in age and WTAR, a significant Group x Time interaction was obtained for six of the eight neurocognitive measures: Hopkins Immediate Recall and Discrimination, Logical Memory I and II, Fluency and AMI (F's = 6.49, 5.23, 5.56, 7.99, 10.03 and 6.83, d.f. = 2,44, $p < 0.01$ – $p < 0.001$). For the PSI, this interaction approached significance ($p = 0.067$), but the main effect of Group was more robust (F = 8.12, d.f. = 1, 22, $p < 0.01$). For Hopkins Delayed Recall, no reliable effects were seen ($p > 0.15$). Subsequent analyses focused on comparisons of adjusted means in the seven neurocognitive measures where overall analyses were significant (see Tables A.2 and A.3).

Retrograde amnesia

For age- and WTAR-adjusted AMI scores, no group differences were obtained at Pretreatment, but the ISO group had significantly better recall for dates and details of real-life autobiographical events at Posttreatment and Follow-up than both the ECT-All and the ECT-Matched groups (Table A.2). Within-group comparisons indicated significant performance decrements between Pre- and Posttreatment in all groups ($p < 0.05$ for ISO, $p < 0.001$ for ECT-All and ECT-Matched). These decrements persisted at Follow-up. When responses after treatment were further adjusted for Pretreatment performance, the ECT-All group showed greater decline in AMI performance at both Posttreatment and Follow-up and the ECT-Matched showed greater decline at Follow and marginally greater decline at Posttreatment compared to the ISO group (TableA.3).

Table A.2. Neurocognitive Means (SEM) at Pretreatment, Posttreatment and Follow-up,Adjusted for Intellectual Level and Age

	Pretreatment		Post-Treatment		Follow-up	
	Adj. Mean (SEM)	*p*	Adj. Mean (SEM)	*p*	Adj. Mean (SEM)	*p*
Hopkins Immed Recall						
ECT-All	45.04 (2.49)	0.41	42.02 (3.10)	**0.01**	50.83 (2.57)	0.10
ECT-Matched	47.63 (3.33)	0.39	51.45 (4.77)	0.18	58.23 (3.90)	0.64
ISO	49.40 (4.20)		61.08 (5.23)		60.34 (4.58)	
Hopkins Delay Recall						
ECT-All	44.03 (2.69)	0.18	40.65 (3.22)	0.17	50.05 (2.30)	0 .31
ECT-Matched	48.25 (3.55)	0.28	52.68 (3.61)	0.41	55.17 (2.47)	0.94
ISO	51.80 (4.54)		56.76 (5.42)		55.25 (4.10)	
Hopkins Discrimination						
ECT-All	47.55 (2.38)	0.24	35.56 (2.94)	**0.001**	46.66 (1.59)	**0.01**
ECT-Matched	50.77 (3.26)	0.48	41.42 (4.87)	0.11	49.33 (2.43)	0.09
ISO	53.44 (4.01)		56.05 (4.95)		55.93 (2.83)	
Logical Memory I						
ECT-All	9.42 (0.55)	0.23	8.69 (0.73)	**0.004**	11.45 (0.69)	**0.03**
ECT-Matched	9.00 (0.79)	0.07	10.31 (0.90)	**0.03**	12.91 (1.01)	0.13
ISO	10.83 (0.94)		13.49 (1.19)		15.32	

p-values are for adjusted mean comparisons versus ISO.

Table A.2. cont

	Pretreatment		Post-Treatment		Follow-up	
	Adj. Mean (SEM)	*p*	Adj. Mean (SEM)	*p*	Adj. Mean (SEM)	*p*
Logical Memory II						
ECT-All	10.53 (0.54)	0.85	8.18 (0.83)	**0.001**	11.59 (0.58)	**0.009**
ECT-Matched	10.54 (0.81)	0.67	10.70 (1.18)	**0.02**	13.08 (0.61)	**0.03**
ISO	10.31 (0.91)		15.40 (1.35)		15.54 (1.18)	
Delis Kaplan Fluency						
ECT-All	10.32 (0.63)	0.11	6.00 (0.79)	**0.01**	9.47 (0.78)	0.75
ECT-Matched	9.94 (0.98)	0.33	6.90 (1.12)	**0.05**	10.00 (0.68)	0.89
ISO	8.20 (1.06)		11.14 (1.34)		10.02 (1.39)	
Autobiographical Mem						
ECT-All	53.39 (1.10)	0 .62	37.18 (1.88)	**0.002**	37.14 (1.93)	**0.01**
ECT-Matched	52.71 (1.94)	0.55	40.28 (2.56)	**0.04**	39.36 (2.41)	**0.04**
ISO	54.53 (1.85)		50.54 (3.17)		48.79 (3.45)	
Processing Speed Index						
ECT-All	93.22 (3.90)	**0.03**	87.40 (3.36)	**0.004**	97.89 (3.56)	**0.01**
ECT-Matched	91.16 (8.04)	0.09	91.44 (6.73)	0.09	99.71 (6.99)	0.08
ISO	112.20 (6.58)		109.75 (5.67)		119.96 (2.26)	

p-values are for adjusted mean comparisons versus ISO.

Table A.3. Neurocognitive Means (SEM) at Posttreatment and Follow-up, Adjusted for Pretreament Performance and Intellectuel Level

	Post-Treatment		Follow-up	
	Adj. Mean (SEM)	*p*	Adj. Mean (SEM)	*p*
Hopkins Immediate Recall				
ECT-All	43.48 (2.26)	**0.01**	51.48 (1.88)	**0.05**
ECT-Matched	54.49 (3.09)	0.40	60.07 (2.71)	0.84
ISO	57.41 (3.76)		59.41 (3.15)	
Hopkins Delay Recall				
ECT-All	42.70 (2.43)	0.09	51.22 (1.75)	0.65
ECT-Matched	55.49 (2.21)	0.78	56.96 (0.99)	0.28
ISO	51.61 (4.10)		52.87 (2.92)	
Hopkins Discrimination				
ECT-All	36.28 (2.97)	**0.01**	46.24 (1.63)	**0.01**
ECT-Matched	43.03 (4.49)	0.19	50.52 (1.68)	**0.046**
ISO	55.09 (5.18)		55.93 (2.83)	
Logical Memory I				
ECT-All	8.94 (0.67)	**0.005**	11.51 (0.68)	**0.04**
ECT-Matched	11.22 (0.92)	0.21	13.98 (0.77)	0.80
ISO	13.05 (1.07)		15.08 (1.37)	

p-values are for adjusted mean comparisons versus ISO.

Table A.3. cont

	Post-Treatment		Follow-up	
	Adj. Mean (SEM)	*P*	Adj. Mean (SEM)	*P*
Logical Memory II				
ECT-All	8.25 (0.72)	**0.001**	11.54 (0.54)	**0.002**
ECT-Matched	11.12 (0.88)	**0.006**	13.29 (0.44)	**0.006**
ISO	15.48 (1.12)		15.65 (1.00)	
Delis Kaplan Fluency				
ECT-All	5.87 (0.71)	**0.001**	9.18 (0.67)	0.30
ECT-Matched	6.58 (0.69)	**0.001**	9.69 (0.43)	0.37
ISO	11.44 (1.17)		10.64 (1.22)	
Autobiographical Memory				
ECT-All	37.70 (1.81)	**0.003**	37.31 (1.60)	**0.002**
ECT-Matched	41.36 (2.38)	0.07	39.60 (2.06)	**0.02**
ISO	49.24 (2.95)		48.14 (2.64)	
Processing Speed Index				
ECT-All	89.68 (2.76)	**0.02**	101.02 (2.41)	**0.02**
ECT-Matched	95.72 (5.45)	0.23	107.85 (3.99)	0.13
ISO	104.05 (4.69)		113.36 (3.93)	

p-values are for adjusted mean comparisons versus ISO.

Executive function

For the Verbal Fluency measure, no group differences were significant at Pretreatment or Follow-up, but the ISO group had significantly better performance immediately Posttreatment (Table A.2). These Posttreatment group differences were maintained after adjustment for Pretreatment scores (Table A.3). Within-group comparisons indicated that the ECT-All and ECT-Matched groups showed decrements in Fluency at Posttreatment ($p < 0.001$) while the ISO group showed a slightly enhanced performance ($p < 0.05$). Both ECT groups showed significant increases in Fluency between Posttreatment and Follow-up ($p < 0.01$) while the ISO group's Fluency score did not differ reliably between those times ($p = 0.19$).

Anterograde amnesia

Among the Hopkins Verbal Learning measures, which involve memory for spoken word lists, there were no group differences at Pretreatment after adjustment for age and WTAR. At Posttreatment, the ISO group had significantly better performance than the ECT-All group for both Immediate Recall and Discrimination scores, but did not differ significantly from the ECT-Matched group (Table A.2). After further adjustment for Pretreatment performance, the ISO group's Immediate Recall and Discrimination scores were superior to the ECT-All group at Follow-up as well as Posttreatment, and their Discrimination scores at Follow-up were better than the ECT-Matched group (Table A.3). Within-group comparisons showed that both the ISO and ECT-matched groups had higher Posttreatment vs. Pretreatment scores for Immediate Recall ($p < 0.01$ and $p <$

0.05 respectively) while the ECT-All group did not change. For Discrimination, the ECT-All and ECT-Matched groups both declined from Pretreatment to Posttreatment ($p < 0.01$ and $p < 0.05$ respectively) while the ISO group showed no real change. As noted previously, Hopkins Delayed Recall showed no significant effects.

For the Logical Memory tests I and II, which involve immediate and delayed recall of spoken passages, no significant group differences were obtained at Pretreatment after adjustment for age and WTAR. At Posttreatment, the ISO group had better performance on both tasks than either of the ECT groups (Table A.2). At Follow-up, they continued to have better delayed recall (test II) than either of the ECT groups and their immediate recall for passages (test I) was still reliably better than the ECT-All group. After adjustment for Pretreatment performance, they had better test I performance than the ECT-All group but no longer differed from the ECT-Matched group at either Posttreatment or Follow-up; however, the ISO group still showed better test II performance than either ECT-Matched or ECT-All groups at both times (Table A.3). Within-group comparisons indicated that the ISO group had significant increases and the ECT-All group had significant decreases in test II scores at Posttreatment vs. Pretreatment ($p < 0.05$). Both ISO and ECT-Matched had increases in test I scores at Posttreatment ($p < 0.05$). All groups showed better Logical Memory I and II scores at Follow-up than at Pretreatment ($p < 0.05$).

Speed of processing

For the Processing Speed Index scores, the ISO group had significantly better performance than ECT-All at Pretreatment, Posttreatment and Follow-up even with adjustment for WTAR and age ($p < 0.03$; Table A.2). Even though their responses were only marginally different from ISO ($p < 0.09$), the ECT-Matched group performed slightly worse on average than the ECT-All group, suggesting that the lack of reliable difference was due to the smaller sample size rather than depression severity. Only the ECT-All group showed a significant drop in PSI score at Posttreatment ($p < 0.05$), and this led to a significant group difference between the ISO and ECT-All group at Posttreatment after further adjustment for Pretreatment scores (Table A.3). All groups showed higher PSI scores at Follow-up vs. Posttreatment ($p < 0.05$).

Discussion

Antidepressant efficacy

The results of this open label study supported our primary hypothesis that a series of 10 sessions of deep anesthesia with ISO is effective as an antidepressant intervention for patients with medication-refractory depression. The improvement in HRSD-24 scores and QIDS-SR16 scores immediately after completion of treatment was similar in the nonstandard ISO treatment to the standard ECT treatment. Although there was somewhat better maintenance of the decrease in HRSD-24 depressive symptom scores among the 8 severity-matched ECT patients one month later, the ISO group still showed significant improvement even at that later time point.

The antidepressant effect of ISO indicated by these observations is very similar to the original reports by Langer et al. (Langer et al., 1985; Langer et al., 1995). The explanation for why our results were more positive than other later studies may be due to closer adherence to the original protocols, particularly in patient age, duration of each ISO session, and use of more ISO treatment sessions. As suggested by the QIDS-SR16 self-report data in Figure A.2, much of the beneficial effect of ISO occurred in sessions 5-10, while Garcia-Toro et al terminated their treatments after only four sessions, and they used sevoflurane which might be less effective than ISO, especially if sustained EEG burst suppression is needed for a clinical benefit (Garcia-Toro et al., 2001). We also restricted our sample to persons aged 65 and younger, in part because of the increased likelihood of adverse blood pressure and other cardiovascular concerns in elderly patients, like those studied by Greenberg et al. (Greenberg et al., 1987). We also were able to reduce side effects (nausea, vomiting, and disorientation) and shorten the time to full recovery after each session by use of the ANEclear™.

Cognitive effects

The neurocognitive assessments made as part of this study indicate that even with 10 sessions of ISO anesthesia over a three week period, there was no significant performance decrement on any of the traditional neurocognitive measures. In fact, the ISO patients showed significant improvements in word fluency, nonverbal processing speed, logical memory and immediate and delayed recall of newly learned verbal material after the intervention. These improvements are probably a result of the

combined effects of decreased depressive symptoms (which has been linked to response slowing and other performance deficits) and practice (Wroolie et al., 2006; Attix et al., 2009; Semkovska and McLoughlin, 2010). The one measure where there was any suggestion of a performance decline with ISO treatment was the Autobiographical Memory Interview. This scale has intrinsic flaws because the responses given at the initial interview are designated as correct without verification. Consequently, when retesting occurs, the individual's performance can worsen but cannot show improvement, and any answers that differ from the original ones (even if the patient has remembered more accurately when retested) are scored as errors. Recently, Semkovska et al. found that when retested after 2 months, healthy controls as well as depressed patients never treated with ECT showed similar mild declines in AMI performance, which they labeled as consistency errors rather than true memory deficits (Semkovska et al., 2012). For the ISO patients, their small Posttreatment decrements in AMI scores were similar in magnitude to expected test-retest consistency errors, and their performance was superior to the ECT patients both immediately after the last treatment and one month later. Thus, multiple sessions of ECT led to persistent AMI deficits that were not seen after multiple sessions of ISO.

The effectiveness of bifrontal ECT on depressive symptoms was never in doubt (Prudic et al., 1996; 2003; Prudic et al., 2004; Khalid et al., 2008), but our results on the time course and relative magnitude of other neurocognitive decrements are important to note. When tested within 24-48 hours after their last ECT session, the ECT-All group also showed declines in word fluency, immediate and delayed recall of verbal material,

ability to discriminate between words previously heard versus other words, and processing speed. The ECT-Matched group had fewer performance deficits, but still showed significant reductions in word fluency and discrimination at Posttreatment, and they failed to match the practice-related improvement seen in the ISO group for delayed recall of passages. After one month, however, performance of the ECT patients on all tests except for the AMI returned to or exceeded Pretreatment levels. This is consistent with the meta-analysis by Semskovska and McLoughlin (Semkovska and McLoughlin, 2010) , which noted that neurocognitive deficits linked to bifrontal ECT were mainly limited to the first 3 days Posttreatment, and at 15 days Posttreatment, there was evidence of improvement in both memory and executive function (Semkovska and McLoughlin, 2010). Comparisons between means adjusted for pretreatment task performance and intellectual level indicated, however, that the improvements 4 weeks after treatment in the ISO group were greater than the full ECT sample for several cognitive measures, including immediate recall of newly learned words and passages, word discrimination, delayed recall of passages, and processing speed. Possibly the ISO patients were able to benefit more from practice at the testing done 24-48 hours after the last treatment when many ECT patients were experiencing significant cognitive impairment. Also, the ECT-Matched group had improvements as great as the ISO group for all measures except word discrimination, delayed recall of passages and autobiographical memory. This suggests that initial depression severity contributes to lesser posttreatment improvement in immediate recall of words and passages and processing speed. Nevertheless, we cannot rule out a residual effect of ECT

influencing performance on some cognitive measures in addition to AMI at 4-week follow-up.

Study limitations

There are several study limitations to acknowledge. The study was open-label rather than randomized, and the group sizes were modest. Replication in a larger randomized trial is needed to confirm these findings. More of the severely depressed patients chose ECT than ISO, which was managed by creating a smaller ECT-Matched subgroup to examine along with the full ECT and ISO groups. The effectiveness of ISO for the most severely depressed patients is still uncertain. Also, some of the ECT patients had 8 or 12 rather than 10 treatments, titrated to clinical effectiveness, but our IRB approval restricted the ISO treatment series to 10. This factor may have contributed to the slightly better antidepressant outcome for the ECT-Matched patients at 4-week follow-up. Finally, all of the patients in the study had medication-refractory moderate to severe depression, so that future research is needed to examine whether ISO anesthesia may be an effective treatment in milder and medication-responsive depression. This small open-label study indicates the ISO may be another option for patients with moderate to severe depression (both with MDD and BPD diagnoses) who find medications ineffective or are unable to tolerate their side effects. Like transcranial magnetic and direct current stimulation, magnetic seizure therapy and other novel treatment options, it is important to have explore the comparable efficacy as well as side effect profiles of novel alternatives to ECT for seriously depressed patients (Kirov et

al., 2008; Allan et al., 2011; Loo et al., 2012). It would also add value if future studies examine whether these alternative therapies, including ISO anesthesia, convey their antidepressant effects through the same or different pathways as ECT, such as by decreasing frontal cortical connectivity (Perrin et al., 2012) or altering the expression of the same neuronal and immune genes (Iacob et al., 2013).

Implications

This small study shows that a series of 10 treatment sessions with deep inhalation isoflurane anesthesia has an antidepressant effect in moderate to severe medication-refractory depression approaching that of bifrontal ECT. Neurocognitive performance of ISO-treated patients was superior to ECT-treated patients immediately after the last treatment (when only ECT was associated with worsened performance relative to Pretreatment). ISO patients continued to perform better 4 weeks later, although performance in ECT patients showed considerable improvement between those tests. Given the small size and lack of randomization in this study, a much larger randomized and blinded study is required, preferably one designed to examine whether ECT and ISO have their effects through shared physiological pathways.

REFERENCES

(2003) Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. Lancet 361:799-808.

Abe N, Uchida S, Otsuki K, Hobara T, Yamagata H, Higuchi F, Shibata T, Watanabe Y (2011a) Altered sirtuin deacetylase gene expression in patients with a mood disorder. J Psychiatr Res 45:1106-1112.

Abe N, Uchida S, Otsuki K, Hobara T, Yamagata H, Higuchi F, Shibata T, Watanabe Y (2011b) Altered sirtuin deacetylase gene expression in patients with a mood disorder. Journal of psychiatric research 45:1106-1112.

Ahern GP (2011) 5-HT and the immune system. Current opinion in pharmacology 11:29-33.

Albert PR, Le Francois B, Millar AM (2011) Transcriptional dysregulation of 5-HT1A autoreceptors in mental illness. Mol Brain 4:21.

Allan CL, Herrmann LL, Ebmeier KP (2011) Transcranial magnetic stimulation in the management of mood disorders. Neuropsychobiology 64:163-169.

Anacker C, Zunszain PA, Carvalho LA, Pariante CM (2011a) The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology 36:415-425.

Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, Price J, Pariante CM (2011b) Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Molecular psychiatry 16:738-750.

Antonioli M, Rybka J, Carvalho LA (2012) Neuroimmune endocrine effects of antidepressants. Neuropsychiatric disease and treatment 8:65-83.

Arranz B, Eriksson A, Mellerup E, Plenge P, Marcusson J (1994) Brain 5-HT1A, 5-HT1D, and 5-HT2 receptors in suicide victims. Biological psychiatry 35:457-463.

Asare AL, Kolchinsky SA, Gao Z, Wang R, Raddassi K, Bourcier K, Seyfert-Margolis V (2008) Differential gene expression profiles are dependent upon method of peripheral blood collection and RNA isolation. BMC Genomics 9:474.

Attix DK, Story TJ, Chelune GJ, Ball JD, Stutts ML, Hart RP, Barth JT (2009) The prediction of change: normative neuropsychological trajectories. The Clinical neuropsychologist 23:21-38.

Baba H, Nakano Y, Maeshima H, Satomura E, Kita Y, Suzuki T, Arai H (2012) Metabolism of amyloid-beta protein may be affected in depression. The Journal of clinical psychiatry 73:115-120.

Barbaccia ML (2011) Much excitement about antidepressants, DBI and c-FOS. Pharmacol Res 64:333-335.

Barbaccia ML, Costa E, Ferrero P, Guidotti A, Roy A, Sunderland T, Pickar D, Paul SM, Goodwin FK (1986) Diazepam-binding inhibitor. A brain neuropeptide present in human spinal fluid: studies in depression, schizophrenia, and Alzheimer's disease. Archives of general psychiatry 43:1143-1147.

Bartolomucci A, Leopardi R (2009) Stress and depression: preclinical research and clinical implications. PloS one 4:e4265.

Basso AM, Bratcher NA, Harris RR, Jarvis MF, Decker MW, Rueter LE (2009) Behavioral profile of P2X7 receptor knockout mice in animal models of depression and anxiety: relevance for neuropsychiatric disorders. Behav Brain Res 198:83-90.

Basu S, Dasgupta PS (2000) Dopamine, a neurotransmitter, influences the immune system. Journal of neuroimmunology 102:113-124.

Beech RD, Lowthert L, Leffert JJ, Mason PN, Taylor MM, Umlauf S, Lin A, Lee JY, Maloney K, Muralidharan A, Lorberg B, Zhao H, Newton SS, Mane S, Epperson CN, Sinha R, Blumberg H, Bhagwagar Z (2010) Increased peripheral blood expression of electron transport chain genes in bipolar depression. Bipolar Disord 12:813-824.

Belmaker RH (1981) Receptors, adenylate cyclase, depression, and lithium. Biological psychiatry 16:333-350.

Belzeaux R, Formisano-Treziny C, Loundou A, Boyer L, Gabert J, Samuelian JC, Feron F, Naudin J, Ibrahim EC (2010) Clinical variations modulate patterns of gene expression and define blood biomarkers in major depression. Journal of psychiatric research 44:1205-1213.

Berlim MT, Fleck MP, Turecki G (2008) Current trends in the assessment and somatic treatment of resistant/refractory major depression: an overview. Annals of medicine 40:149-159.

Bertram I, Bernstein HG, Lendeckel U, Bukowska A, Dobrowolny H, Keilhoff G, Kanakis D, Mawrin C, Bielau H, Falkai P, Bogerts B (2007) Immunohistochemical evidence for impaired neuregulin-1 signaling in the prefrontal cortex in schizophrenia and in unipolar depression. Annals of the New York Academy of Sciences 1096:147-156.

Beumer W, Gibney SM, Drexhage RC, Pont-Lezica L, Doorduin J, Klein HC, Steiner J, Connor TJ, Harkin A, Versnel MA, Drexhage HA (2012) The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. Journal of leukocyte biology 92:959-975.

Bierhaus A, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, Ferstl R, von Eynatten M, Wendt T, Rudofsky G, Joswig M, Morcos M, Schwaninger M, McEwen B, Kirschbaum C, Nawroth PP (2003) A mechanism converting psychosocial stress into mononuclear cell activation. Proceedings of the National Academy of Sciences of the United States of America 100:1920-1925.

Blume J, Douglas SD, Evans DL (2011) Immune suppression and immune activation in depression. Brain, behavior, and immunity 25:221-229.

Bondi CO, Jett JD, Morilak DA (2010) Beneficial effects of desipramine on cognitive function of chronically stressed rats are mediated by alpha1-adrenergic receptors in medial prefrontal cortex. Progress in neuro-psychopharmacology & biological psychiatry 34:913-923.

Bousman CA, Chana G, Glatt SJ, Chandler SD, Lucero GR, Tatro E, May T, Lohr JB, Kremen WS, Tsuang MT, Everall IP (2010) Preliminary evidence of ubiquitin proteasome system dysregulation in schizophrenia and bipolar disorder: convergent pathway analysis findings from two independent samples. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 153B:494-502.

Bower JE, Ganz PA, Irwin MR, Arevalo JM, Cole SW (2011) Fatigue and gene expression in human leukocytes: increased NF-kappaB and decreased glucocorticoid signaling in breast cancer survivors with persistent fatigue. Brain, behavior, and immunity 25:147-150.

Brydon L, Edwards S, Jia H, Mohamed-Ali V, Zachary I, Martin JF, Steptoe A (2005) Psychological stress activates interleukin-1beta gene expression in human mononuclear cells. Brain, behavior, and immunity 19:540-546.

Bufalino C, Hepgul N, Aguglia E, Pariante CM (2012) The role of immune genes in the association between depression and inflammation: A review of recent clinical studies. Brain, behavior, and immunity.

Bustin SA (2005) Real-time, fluorescence-based quantitative PCR: a snapshot of current procedures and preferences. Expert Rev Mol Diagn 5:493-498.

Bustin SA, Mueller R (2005) Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. Clin Sci (Lond) 109:365-379.

Butters MA, Klunk WE, Mathis CA, Price JC, Ziolko SK, Hoge JA, Tsopelas ND, Lopresti BJ, Reynolds CF, 3rd, DeKosky ST, Meltzer CC (2008) Imaging Alzheimer pathology in late-life depression with PET and Pittsburgh Compound-B. Alzheimer disease and associated disorders 22:261-268.

Callado LF, Meana JJ, Grijalba B, Pazos A, Sastre M, Garcia-Sevilla JA (1998) Selective increase of alpha2A-adrenoceptor agonist binding sites in brains of depressed suicide victims. Journal of neurochemistry 70:1114-1123.

Capuron L, Fornwalt FB, Knight BT, Harvey PD, Ninan PT, Miller AH (2009) Does cytokine-induced depression differ from idiopathic major depression in medically healthy individuals? Journal of affective disorders 119:181-185.

Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, Miller AH (2003) Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. Biological psychiatry 54:906-914.

Carl C, Engelhardt W, Teichmann G, Fuchs G (1988) Open comparative study with treatment-refractory depressed patients: electroconvulsive therapy--anesthetic therapy with isoflurane (preliminary report). Pharmacopsychiatry 21:432-433.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301:386-389.

Cattaneo A, Gennarelli M, Uher R, Breen G, Farmer A, Aitchison KJ, Craig IW, Anacker C, Zunsztain PA, McGuffin P, Pariante CM (2013) Candidate Genes Expression Profile Associated with Antidepressants Response in the GENDEP Study: Differentiating between Baseline 'Predictors' and Longitudinal 'Targets'. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 38:376.

Choi KH, Higgs BW, Weis S, Song J, Llenos IC, Dulay JR, Yolken RH, Webster MJ (2009) Effects of typical and atypical antipsychotic drugs on gene expression profiles in the liver of schizophrenia subjects. BMC psychiatry 9:57.

Clifford EE, Parker K, Humphreys BD, Kertesy SB, Dubyak GR (1998) The P2X1 receptor, an adenosine triphosphate-gated cation channel, is expressed in human platelets but not in human blood leukocytes. Blood 91:3172-3181.

Corda MG, Ferrari M, Guidotti A, Konkel D, Costa E (1984) Isolation, purification and partial sequence of a neuropeptide (diazepam-binding inhibitor) precursor of an anxiogenic putative ligand for benzodiazepine recognition site. Neuroscience letters 47:319-324.

Coryell MW, Wunsch AM, Haenfler JM, Allen JE, Schnizler M, Ziemann AE, Cook MN, Dunning JP, Price MP, Rainier JD, Liu Z, Light AR, Langbehn DR, Wemmie JA (2009) Acid-sensing ion channel-1a in the amygdala, a novel therapeutic target in depression-related behavior. J Neurosci 29:5381-5388.

Coughtrie MW (2002) Sulfation through the looking glass--recent advances in sulfotransferase research for the curious. Pharmacogenomics J 2:297-308.

Covington HE, 3rd, Maze I, LaPlant QC, Vialou VF, Ohnishi YN, Berton O, Fass DM, Renthal W, Rush AJ, 3rd, Wu EY, Ghose S, Krishnan V, Russo SJ, Tamminga C, Haggarty SJ, Nestler EJ (2009) Antidepressant actions of histone deacetylase inhibitors. J Neurosci 29:11451-11460.

Cyranowski JM, Hofkens TL, Frank E, Seltman H, Cai HM, Amico JA (2008) Evidence of dysregulated peripheral oxytocin release among depressed women. Psychosomatic medicine 70:967-975.

Delis DC, Kaplan E., and Kramer J.H. (2001) Delis Kaplan D-KEFS Executive Function System: Examiners Manual. The Psychological Corporation, San Antonio, TX.

Di Nicola M, Cattaneo A, Hepgul N, Di Forti M, Aitchison KJ, Janiri L, Murray RM, Dazzan P, Pariante CM, Mondelli V (2012) Serum and gene expression profile of cytokines in first-episode psychosis. Brain, behavior, and immunity.

Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctot KL (2010) A meta-analysis of cytokines in major depression. Biological psychiatry 67:446-457.

Dror N, Klein E, Karry R, Sheinkman A, Kirsh Z, Mazor M, Tzukerman M, Ben-Shachar D (2002) State-dependent alterations in mitochondrial complex I activity in platelets: a potential peripheral marker for schizophrenia. Molecular psychiatry 7:995-1001.

Drzyzga L, Obuchowicz E, Marcinowska A, Herman ZS (2006) Cytokines in schizophrenia and the effects of antipsychotic drugs. Brain, behavior, and immunity 20:532-545.

Dunne RA, McLoughlin DM (2012) Systematic review and meta-analysis of bifrontal electroconvulsive therapy versus bilateral and unilateral electroconvulsive therapy in depression. World J Biol Psychiatry 13:248-258.

Dwyer JM, Rizzo SJ, Neal SJ, Lin Q, Jow F, Arias RL, Rosenzweig-Lipson S, Dunlop J, Beyer CE (2009) Acid sensing ion channel (ASIC) inhibitors exhibit anxiolytic-like activity in preclinical pharmacological models. Psychopharmacology 203:41-52.

Engelhardt W, Carl G, Hartung E (1993) Intra-individual open comparison of burst-suppression-isoflurane-anaesthesia versus electroconvulsive therapy in the treatment of severe depression. Eur J Anaesthesiol 10:113-118.

Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, LaRossa GN, Spinner ML, Klunk WE, Mathis CA, DeKosky ST, Morris JC, Holtzman DM (2006) Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Annals of neurology 59:512-519.

Falls DL (2003) Neuregulins: functions, forms, and signaling strategies. Experimental cell research 284:14-30.

Feezor RJ, Baker HV, Mindrinos M, Hayden D, Tannahill CL, Brownstein BH, Fay A, MacMillan S, Laramie J, Xiao W, Moldawer LL, Cobb JP, Laudanski K, Miller-Graziano CL, Maier RV, Schoenfeld D, Davis RW, Tompkins RG (2004) Whole blood and leukocyte RNA isolation for gene expression analyses. Physiol Genomics 19:247-254.

Ferrarese C, Appollonio I, Bianchi G, Frigo M, Marzorati C, Pecora N, Perego M, Pierpaoli C, Frattola L (1993) Benzodiazepine receptors and diazepam binding inhibitor: a possible link between stress, anxiety and the immune system. Psychoneuroendocrinology 18:3-22.

Flaumenhaft R (2011) Platelets get the message. Blood 118:1712-1713.

Fluitman SB, Heijnen CJ, Denys DA, Nolen WA, Balk FJ, Westenberg HG (2011) Electroconvulsive therapy has acute immunological and neuroendocrine effects in patients with major depressive disorder. Journal of affective disorders 131:388-392.

Fornaro M, Martino M, Battaglia F, Colicchio S, Perugi G (2011) Increase in IL-6 levels among major depressive disorder patients after a 6-week treatment with duloxetine 60 mg/day: a preliminary observation. Neuropsychiatric disease and treatment 7:51-56.

Forster T, Roy D, Ghazal P (2003) Experiments using microarray technology: limitations and standard operating procedures. J Endocrinol 178:195-204.

Fraser LM, O'Carroll RE, Ebmeier KP (2008) The effect of electroconvulsive therapy on autobiographical memory: a systematic review. J ECT 24:10-17.

Garcia-Sevilla JA, Ventayol P, Perez V, Rubovszky G, Puigdemont D, Ferrer-Alcon M, Andreoli A, Guimon J, Alvarez E (2004) Regulation of platelet alpha 2A-adrenoceptors, Gi proteins and receptor kinases in major depression: effects of mirtazapine treatment. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 29:580-588.

Garcia-Toro M, Segura C, Gonzalez A, Perello J, Valdivia J, Salazar R, Tarancon G, Campoamor F, Salva J, De La Fuente L, Romera M (2001) Inefficacy of burst-suppression anesthesia in medication-resistant major depression: a controlled trial. J ECT 17:284-288.

Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiological reviews 81:629-683.

Girgenti MJ, Nisenbaum LK, Bymaster F, Terwilliger R, Duman RS, Newton SS (2010) Antipsychotic-induced gene regulation in multiple brain regions. Journal of neurochemistry 113:175-187.

Gladkevich A, Kauffman HF, Korf J (2004) Lymphocytes as a neural probe: potential for studying psychiatric disorders. Progress in neuro-psychopharmacology & biological psychiatry 28:559-576.

Goddard AW, Ball SG, Martinez J, Robinson MJ, Yang CR, Russell JM, Shekhar A (2010) Current perspectives of the roles of the central norepinephrine system in anxiety and depression. Depress Anxiety 27:339-350.

Green EK, Raybould R, Macgregor S, Gordon-Smith K, Heron J, Hyde S, Grozeva D, Hamshere M, Williams N, Owen MJ, O'Donovan MC, Jones L, Jones I, Kirov G, Craddock N (2005) Operation of the schizophrenia susceptibility gene, neuregulin 1, across traditional diagnostic boundaries to increase risk for bipolar disorder. Archives of general psychiatry 62:642-648.

Green EK, Grozeva D, Raybould R, Elvidge G, Macgregor S, Craig I, Farmer A, McGuffin P, Forty L, Jones L, Jones I, O'Donovan MC, Owen MJ, Kirov G, Craddock N (2009) P2RX7: A bipolar and unipolar disorder candidate susceptibility gene? American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 150B:1063-1069.

Green RC, Cupples LA, Kurz A, Auerbach S, Go R, Sadovnick D, Duara R, Kukull WA, Chui H, Edeki T, Griffith PA, Friedland RP, Bachman D, Farrer L (2003) Depression as a risk factor for Alzheimer disease: the MIRAGE Study. Archives of neurology 60:753-759.

Greenberg LB, Gage J, Vitkun S, Fink M (1987) Isoflurane Anesthesia Therapy: A Replacement for ECT in Depressive Disorders? Convuls Ther 3:269-277.

Gundersen BB, Blendy JA (2009) Effects of the histone deacetylase inhibitor sodium butyrate in models of depression and anxiety. Neuropharmacology 57:67-74.

Hageman I, Nielsen M, Wortwein G, Diemer NH, Jorgensen MB (2009) Electroconvulsive stimulations normalizes stress-induced changes in the glucocorticoid receptor and behaviour. Behav Brain Res 196:71-77.

Hardy J (2009) The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. Journal of neurochemistry 110:1129-1134.

Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR (2004) Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Molecular psychiatry 9:299-307.

Hassel B, Tauboll E, Shaw R, Gjerstad L, Dingledine R (2010) Region-specific changes in gene expression in rat brain after chronic treatment with levetiracetam or phenytoin. Epilepsia 51:1714-1720.

Hayase T (2011) Differential effects of TRPV1 receptor ligands against nicotine-induced depression-like behaviors. BMC pharmacology 11:6.

Heilig M (2004) The NPY system in stress, anxiety and depression. Neuropeptides 38:213-224.

Heinz A, Jones DW, Mazzanti C, Goldman D, Ragan P, Hommer D, Linnoila M, Weinberger DR (2000) A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. Biological psychiatry 47:643-649.

Hernandez ME, Martinez-Fong D, Perez-Tapia M, Estrada-Garcia I, Estrada-Parra S, Pavon L (2010) Evaluation of the effect of selective serotonin-reuptake inhibitors on lymphocyte subsets in patients with a major depressive disorder. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 20:88-95.

Hernandez ME, Mendieta D, Martinez-Fong D, Loria F, Moreno J, Estrada I, Bojalil R, Pavon L (2008) Variations in circulating cytokine levels during 52 week course of treatment with SSRI for major depressive disorder. European

neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 18:917-924.

Himmerich H, Schonherr J, Fulda S, Sheldrick AJ, Bauer K, Sack U (2011) Impact of antipsychotics on cytokine production in-vitro. Journal of psychiatric research 45:1358-1365.

Hobara T, Uchida S, Otsuki K, Matsubara T, Funato H, Matsuo K, Suetsugi M, Watanabe Y (2010) Altered gene expression of histone deacetylases in mood disorder patients. Journal of psychiatric research 44:263-270.

Hock C, Golombowski S, Muller-Spahn F, Naser W, Beyreuther K, Monning U, Schenk D, Vigo-Pelfrey C, Bush AM, Moir R, Tanzi RE, Growdon JH, Nitsch RM (1998) Cerebrospinal fluid levels of amyloid precursor protein and amyloid beta-peptide in Alzheimer's disease and major depression - inverse correlation with dementia severity. European neurology 39:111-118.

Hosoda K, Duman RS (1993) Regulation of beta 1-adrenergic receptor mRNA and ligand binding by antidepressant treatments and norepinephrine depletion in rat frontal cortex. Journal of neurochemistry 60:1335-1343.

Howren MB, Lamkin DM, Suls J (2009) Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosomatic medicine 71:171-186.

Iacob E, Light KC, Tadler SC, Weeks HR, Hughen RW, Smith KW, White AT, Bushnell LA, Light AR (2013) Stress and Immune Gene Expression Dysregulation in Patients with Refractory Depression. Brain, behavior, and immunity submitted.

Iga J, Ueno S, Ohmori T (2008) Molecular assessment of depression from mRNAs in the peripheral leukocytes. Annals of medicine 40:336-342.

Iga J, Ueno S, Yamauchi K, Numata S, Kinouchi S, Tayoshi-Shibuya S, Song H, Ohmori T (2007a) Altered HDAC5 and CREB mRNA expressions in the peripheral leukocytes of major depression. Progress in neuro-psychopharmacology & biological psychiatry 31:628-632.

Iga J, Ueno S, Yamauchi K, Motoki I, Tayoshi S, Ohta K, Song H, Morita K, Rokutan K, Ohmori T (2005) Serotonin transporter mRNA expression in peripheral leukocytes of patients with major depression before and after treatment with paroxetine. Neuroscience letters 389:12-16.

Iga J, Ueno S, Yamauchi K, Numata S, Tayoshi-Shibuya S, Kinouchi S, Nakataki M, Song H, Hokoishi K, Tanabe H, Sano A, Ohmori T (2007b) Gene expression and association analysis of vascular endothelial growth factor in major depressive disorder. Progress in neuro-psychopharmacology & biological psychiatry 31:658-663.

Iga J, Ueno S, Yamauchi K, Numata S, Tayoshi-Shibuya S, Kinouchi S, Nakataki M, Song H, Hokoishi K, Tanabe H, Sano A, Ohmori T (2007c) Gene expression and association analysis of vascular endothelial growth factor in major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 31:658-663.

Jacobsen JP, Mork A (2004) The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. Brain research 1024:183-192.

Jakobsson J, Zetterberg H, Blennow K, Johan Ekman C, Johansson AG, Landen M (2012) Altered Concentrations of Amyloid Precursor Protein Metabolites in the Cerebrospinal Fluid of Patients with Bipolar Disorder. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.

Jeanningros R, Mazzola P, Azorin JM, Samuelian-Massa C, Tissot R (1991) Beta-adrenoceptor density of intact mononuclear leukocytes in subgroups of depressive disorders. Biological psychiatry 29:789-798.

Kabanova S, Kleinbongard P, Volkmer J, Andree B, Kelm M, Jax TW (2009) Gene expression analysis of human red blood cells. Int J Med Sci 6:156-159.

Kenis G, Maes M (2002) Effects of antidepressants on the production of cytokines. Int J Neuropsychopharmacol 5:401-412.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Archives of general psychiatry 62:593-602.

Khalid N, Atkins M, Tredget J, Giles M, Champney-Smith K, Kirov G (2008) The effectiveness of electroconvulsive therapy in treatment-resistant depression: a naturalistic study. J ECT 24:141-145.

Kirov G, Ebmeier KP, Scott AI, Atkins M, Khalid N, Carrick L, Stanfield A, O'Carroll RE, Husain MM, Lisanby SH (2008) Quick recovery of orientation after magnetic seizure therapy for major depressive disorder. The British journal of psychiatry : the journal of mental science 193:152-155.

Kishi T, Yoshimura R, Kitajima T, Okochi T, Okumura T, Tsunoka T, Yamanouchi Y, Kinoshita Y, Kawashima K, Fukuo Y, Naitoh H, Umene-Nakano W, Inada T, Nakamura J, Ozaki N, Iwata N (2010) SIRT1 gene is associated with major depressive disorder in the Japanese population. J Affect Disord 126:167-173.

Kittner H, Franke H, Fischer W, Schultheis N, Krugel U, Illes P (2003) Stimulation of P2Y1 receptors causes anxiolytic-like effects in the rat elevated plus-maze:

implications for the involvement of P2Y1 receptor-mediated nitric oxide production. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28:435-444.

Klempan TA, Sequeira A, Canetti L, Lalovic A, Ernst C, ffrench-Mullen J, Turecki G (2009) Altered expression of genes involved in ATP biosynthesis and GABAergic neurotransmission in the ventral prefrontal cortex of suicides with and without major depression. Molecular psychiatry 14:175-189.

Klimek V, Schenck JE, Han H, Stockmeier CA, Ordway GA (2002) Dopaminergic abnormalities in amygdaloid nuclei in major depression: a postmortem study. Biological psychiatry 52:740-748.

Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. Nature 455:894-902.

Krishnan V, Nestler EJ (2010) Linking molecules to mood: new insight into the biology of depression. The American journal of psychiatry 167:1305-1320.

Kronfol Z, Hamdan-Allen G, Goel K, Hill EM (1991) Effects of single and repeated electroconvulsive therapy sessions on plasma ACTH, prolactin, growth hormone and cortisol concentrations. Psychoneuroendocrinology 16:345-352.

Kuromitsu J, Yokoi A, Kawai T, Nagasu T, Aizawa T, Haga S, Ikeda K (2001) Reduced neuropeptide Y mRNA levels in the frontal cortex of people with schizophrenia and bipolar disorder. Brain research Gene expression patterns 1:17-21.

Lai IC, Hong CJ, Tsai SJ (2003) Expression of cAMP response element-binding protein in major depression before and after antidepressant treatment. Neuropsychobiology 48:182-185.

Langer G, Neumark J, Koinig G, Graf M, Schonbeck G (1985) Rapid psychotherapeutic effects of anesthesia with isoflurane (ES narcotherapy) in treatment-refractory depressed patients. Neuropsychobiology 14:118-120.

Langer G, Karazman R, Neumark J, Saletu B, Schonbeck G, Grunberger J, Dittrich R, Petricek W, Hoffmann P, Linzmayer L, et al. (1995) Isoflurane narcotherapy in depressive patients refractory to conventional antidepressant drug treatment. A double-blind comparison with electroconvulsive treatment. Neuropsychobiology 31:182-194.

Lanquillon S, Krieg JC, Bening-Abu-Shach U, Vedder H (2000) Cytokine production and treatment response in major depressive disorder. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 22:370-379.

Le-Niculescu H, McFarland MJ, Ogden CA, Balaraman Y, Patel S, Tan J, Rodd ZA, Paulus M, Geyer MA, Edenberg HJ, Glatt SJ, Faraone SV, Nurnberger JI, Kuczenski R, Tsuang MT, Niculescu AB (2008) Phenomic, convergent functional genomic, and biomarker studies in a stress-reactive genetic animal model of bipolar disorder and co-morbid alcoholism. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 147B:134-166.

Lee JS, Jang DJ, Lee N, Ko HG, Kim H, Kim YS, Kim B, Son J, Kim SH, Chung H, Lee MY, Kim WR, Sun W, Zhuo M, Abel T, Kaang BK, Son H (2009) Induction of neuronal vascular endothelial growth factor expression by cAMP in the dentate gyrus of the hippocampus is required for antidepressant-like behaviors. J Neurosci 29:8493-8505.

Levy BH, Tasker JG (2012) Synaptic regulation of the hypothalamic-pituitary-adrenal axis and its modulation by glucocorticoids and stress. Frontiers in cellular neuroscience 6:24.

Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA (2006) The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. J Lab Clin Med 147:126-132.

Light AR, White AT, Hughen RW, Light KC (2009) Moderate exercise increases expression for sensory, adrenergic, and immune genes in chronic fatigue syndrome patients but not in normal subjects. J Pain 10:1099-1112.

Light AR, Bateman L, Jo D, Hughen RW, Vanhaitsma TA, White AT, Light KC (2012a) Gene expression alterations at baseline and following moderate exercise in patients with Chronic Fatigue Syndrome and Fibromyalgia Syndrome. J Intern Med 271:64-81.

Light AR, Bateman L, Jo D, Hughen RW, Vanhaitsma TA, White AT, Light KC (2012b) Gene expression alterations at baseline and following moderate exercise in patients with Chronic Fatigue Syndrome and Fibromyalgia Syndrome. J Intern Med 271:64-81.

Longman RS, Saklofske DH, Fung TS (2007) WAIS-III percentile scores by education and sex for U.S. and Canadian populations. Assessment 14:426-432.

Loo CK, Alonzo A, Martin D, Mitchell PB, Galvez V, Sachdev P (2012) Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial. The British journal of psychiatry : the journal of mental science 200:52-59.

Lopez-Munoz F, Alamo C (2009) Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. Curr Pharm Des 15:1563-1586.

Lowther S, Katona CL, Crompton MR, Horton RW (1997) 5-HT1D and 5-HT1E/1F binding sites in depressed suicides: increased 5-HT1D binding in globus pallidus but not cortex. Molecular psychiatry 2:314-321.

Lucae S, Salyakina D, Barden N, Harvey M, Gagne B, Labbe M, Binder EB, Uhr M, Paez-Pereda M, Sillaber I, Ising M, Bruckl T, Lieb R, Holsboer F, Muller-Myhsok B (2006) P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major depressive disorder. Hum Mol Genet 15:2438-2445.

Ma XM, Mains RE, Eipper BA (2002) Plasticity in hippocampal peptidergic systems induced by repeated electroconvulsive shock. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 27:55-71.

Maes M, Van Gastel A, Delmeire L, Meltzer HY (1999) Decreased platelet alpha-2 adrenoceptor density in major depression: effects of tricyclic antidepressants and fluoxetine. Biological psychiatry 45:278-284.

Maes M, Bosmans E, De Jongh R, Kenis G, Vandoolaeghe E, Neels H (1997) Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine 9:853-858.

Mahar I, Tan S, Davoli MA, Dominguez-Lopez S, Qiang C, Rachalski A, Turecki G, Mechawar N (2011) Subchronic peripheral neuregulin-1 increases ventral hippocampal neurogenesis and induces antidepressant-like effects. PloS one 6:e26610.

Maletic V (2007) What clinicians need to know about neuroprotective issues in bipolar disorder. The Journal of clinical psychiatry 68:e29.

Maletic V, Robinson M, Oakes T, Iyengar S, Ball SG, Russell J (2007) Neurobiology of depression: an integrated view of key findings. Int J Clin Pract 61:2030-2040.

Mamdani F, Berlim MT, Beaulieu MM, Labbe A, Merette C, Turecki G (2011) Gene expression biomarkers of response to citalopram treatment in major depressive disorder. Translational psychiatry 1:e13.

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nature reviews Neuroscience.

Manna SS, Umathe SN (2012) A possible participation of transient receptor potential vanilloid type 1 channels in the antidepressant effect of fluoxetine. European journal of pharmacology 685:81-90.

Marazziti D, Palego L, Dell'Osso L, Batistini A, Cassano GB, Akiskal HS (1996) Platelet sulfotransferase in different psychiatric disorders. Psychiatry research 65:73-78.

Marazziti D, Baroni S, Masala I, Di Nasso E, Giannaccini G, Betti L, Lucacchini A, Cassano GB (2001) Correlation between platelet alpha(2)-adrenoreceptors and symptom severity in major depression. Neuropsychobiology 44:122-125.

Matsubara T, Funato H, Kobayashi A, Nobumoto M, Watanabe Y (2006) Reduced Glucocorticoid Receptor alpha Expression in Mood Disorder Patients and First-Degree Relatives. Biological psychiatry 59:689-695.

Matuzany-Ruban A, Avissar S, Schreiber G (2005) Dynamics of beta-arrestin1 protein and mRNA levels elevation by antidepressants in mononuclear leukocytes of patients with depression. Journal of affective disorders 88:307-312.

Mausbach BT, Aschbacher K, Mills PJ, Roepke SK, von Kanel R, Patterson TL, Dimsdale JE, Ziegler MG, Ancoli-Israel S, Grant I (2008) A 5-year longitudinal study of the relationships between stress, coping, and immune cell beta(2)-adrenergic receptor sensitivity. Psychiatry research 160:247-255.

Mehta D, Menke A, Binder EB (2010) Gene expression studies in major depression. Curr Psychiatry Rep 12:135-144.

Mei L, Xiong WC (2008) Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nature reviews Neuroscience 9:437-452.

Menke A, Arloth J, Putz B, Weber P, Klengel T, Mehta D, Gonik M, Rex-Haffner M, Rubel J, Uhr M, Lucae S, Deussing JM, Muller-Myhsok B, Holsboer F, Binder EB (2012) Dexamethasone stimulated gene expression in peripheral blood is a sensitive marker for glucocorticoid receptor resistance in depressed patients. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 37:1455-1464.

Messay B, Lim A, Marsland AL (2012) Current understanding of the bi-directional relationship of major depression with inflammation. Biology of mood & anxiety disorders 2:4.

Meyer JH, Kapur S, Eisfeld B, Brown GM, Houle S, DaSilva J, Wilson AA, Rafi-Tari S, Mayberg HS, Kennedy SH (2001a) The effect of paroxetine on 5-HT(2A) receptors in depression: an [(18)F]setoperone PET imaging study. The American journal of psychiatry 158:78-85.

Meyer JH, Kruger S, Wilson AA, Christensen BK, Goulding VS, Schaffer A, Minifie C, Houle S, Hussey D, Kennedy SH (2001b) Lower dopamine transporter binding potential in striatum during depression. Neuroreport 12:4121-4125.

Meynen G, Unmehopa UA, Hofman MA, Swaab DF, Hoogendijk WJ (2007) Hypothalamic oxytocin mRNA expression and melancholic depression. Molecular psychiatry 12:118-119.

Micale V, Cristino L, Tamburella A, Petrosino S, Leggio GM, Drago F, Di Marzo V (2009) Anxiolytic effects in mice of a dual blocker of fatty acid amide hydrolase and transient receptor potential vanilloid type-1 channels. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:593-606.

Miller AH, Maletic V, Raison CL (2009) Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biological psychiatry 65:732-741.

Miller GE, Chen E, Sze J, Marin T, Arevalo JM, Doll R, Ma R, Cole SW (2008) A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. Biological psychiatry 64:266-272.

Minelli A, Zanardini R, Abate M, Bortolomasi M, Gennarelli M, Bocchio-Chiavetto L (2011) Vascular Endothelial Growth Factor (VEGF) serum concentration during electroconvulsive therapy (ECT) in treatment resistant depressed patients. Progress in neuro-psychopharmacology & biological psychiatry 35:1322-1325.

Mischoulon D, Dougherty DD, Bottonari KA, Gresham RL, Sonawalla SB, Fischman AJ, Fava M (2002) An open pilot study of nefazodone in depression with anger attacks: relationship between clinical response and receptor binding. Psychiatry research 116:151-161.

Moon YS, Kang SH, No HJ, Won MH, Ki SB, Lee SK, Kim do H (2011) The correlation of plasma Abeta42 levels, depressive symptoms, and cognitive function in the Korean elderly. Progress in neuro-psychopharmacology & biological psychiatry 35:1603-1606.

Morales-Medina JC, Dumont Y, Quirion R (2010) A possible role of neuropeptide Y in depression and stress. Brain research 1314:194-205.

Moret C, Briley M (2000) The possible role of 5-HT(1B/D) receptors in psychiatric disorders and their potential as a target for therapy. European journal of pharmacology 404:1-12.

Morilak DA, Barrera G, Echevarria DJ, Garcia AS, Hernandez A, Ma S, Petre CO (2005) Role of brain norepinephrine in the behavioral response to stress. Progress in neuro-psychopharmacology & biological psychiatry 29:1214-1224.

Musselman DL, Tomer A, Manatunga AK, Knight BT, Porter MR, Kasey S, Marzec U, Harker LA, Nemeroff CB (1996) Exaggerated platelet reactivity in major depression. The American journal of psychiatry 153:1313-1317.

Naydenov AV, MacDonald ML, Ongur D, Konradi C (2007) Differences in lymphocyte electron transport gene expression levels between subjects with bipolar disorder and normal controls in response to glucose deprivation stress. Archives of general psychiatry 64:555-564.

Nemeroff CB, Vale WW (2005) The neurobiology of depression: inroads to treatment and new drug discovery. The Journal of clinical psychiatry 66 Suppl 7:5-13.

Nemeroff CB, Owens MJ (2009) The role of serotonin in the pathophysiology of depression: as important as ever. Clin Chem 55:1578-1579.

Norman MA, Moore DJ, Taylor M, Franklin D, Jr., Cysique L, Ake C, Lazarretto D, Vaida F, Heaton RK (2011) Demographically corrected norms for African Americans and Caucasians on the Hopkins Verbal Learning Test-Revised, Brief Visuospatial Memory Test-Revised, Stroop Color and Word Test, and Wisconsin Card Sorting Test 64-Card Version. J Clin Exp Neuropsychol 33:793-804.

Ono M, Saji H (2012) Molecular approaches to the treatment, prophylaxis, and diagnosis of Alzheimer's disease: novel PET/SPECT imaging probes for diagnosis of Alzheimer's disease. Journal of pharmacological sciences 118:338-344.

Ordway GA, Schenk J, Stockmeier CA, May W, Klimek V (2003) Elevated agonist binding to alpha2-adrenoceptors in the locus coeruleus in major depression. Biological psychiatry 53:315-323.

Otsuki K, Uchida S, Watanuki T, Wakabayashi Y, Fujimoto M, Matsubara T, Funato H, Watanabe Y (2008) Altered expression of neurotrophic factors in patients with major depression. Journal of psychiatric research 42:1145-1153.

Pace TW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, Miller AH, Heim CM (2006) Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. The American journal of psychiatry 163:1630-1633.

Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ, de Ridder D, Kupka RW, Nolen WA, Drexhage HA (2008) A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. Archives of general psychiatry 65:395-407.

Pajer K, Andrus BM, Gardner W, Lourie A, Strange B, Campo J, Bridge J, Blizinsky K, Dennis K, Vedell P, Churchill GA, Redei EE (2012) Discovery of blood transcriptomic markers for depression in animal models and pilot validation in subjects with early-onset major depression. Translational psychiatry 2:e101.

Pandey GN, Janicak PG, Davis JM (1987) Decreased beta-adrenergic receptors in the leukocytes of depressed patients. Psychiatry research 22:265-273.

Papakostas GI, Shelton RC, Kinrys G, Henry ME, Bakow BR, Lipkin SH, Pi B, Thurmond L, Bilello JA (2011) Assessment of a multi-assay, serum-based biological diagnostic test for major depressive disorder: a Pilot and Replication Study. Molecular psychiatry.

Pasqualini JR (2009) Estrogen sulfotransferases in breast and endometrial cancers. Annals of the New York Academy of Sciences 1155:88-98.

Payne NA, Prudic J (2009) Electroconvulsive therapy: Part I. A perspective on the evolution and current practice of ECT. J Psychiatr Pract 15:346-368.

Pena S, Baccichet E, Urbina M, Carreira I, Lima L (2005) Effect of mirtazapine treatment on serotonin transporter in blood peripheral lymphocytes of major depression patients. Int Immunopharmacol 5:1069-1076.

Perlis RH (2011) Translating biomarkers to clinical practice. Molecular psychiatry 16:1076-1087.

Perona MT, Waters S, Hall FS, Sora I, Lesch KP, Murphy DL, Caron M, Uhl GR (2008) Animal models of depression in dopamine, serotonin, and norepinephrine transporter knockout mice: prominent effects of dopamine transporter deletions. Behav Pharmacol 19:566-574.

Perrin JS, Merz S, Bennett DM, Currie J, Steele DJ, Reid IC, Schwarzbauer C (2012) Electroconvulsive therapy reduces frontal cortical connectivity in severe depressive disorder. Proceedings of the National Academy of Sciences of the United States of America 109:5464-5468.

Petryshen TL et al. (2005) Support for involvement of neuregulin 1 in schizophrenia pathophysiology. Molecular psychiatry 10:366-374, 328.

Piazza PV, Barrot M, Rouge-Pont F, Marinelli M, Maccari S, Abrous DN, Simon H, Le Moal M (1996) Suppression of glucocorticoid secretion and antipsychotic drugs have similar effects on the mesolimbic dopaminergic transmission. Proceedings of the National Academy of Sciences of the United States of America 93:15445-15450.

Pike JL, Irwin MR (2006) Dissociation of inflammatory markers and natural killer cell activity in major depressive disorder. Brain, behavior, and immunity 20:169-174.

Pollmacher T, Haack M, Schuld A, Kraus T, Hinze-Selch D (2000) Effects of antipsychotic drugs on cytokine networks. Journal of psychiatric research 34:369-382.

Pomara N, Bruno D, Sarreal AS, Hernando RT, Nierenberg J, Petkova E, Sidtis JJ, Wisniewski TM, Mehta PD, Pratico D, Zetterberg H, Blennow K (2012) Lower CSF amyloid beta peptides and higher F2-isoprostanes in cognitively intact elderly individuals with major depressive disorder. The American journal of psychiatry 169:523-530.

Prudic J, Olfson M, Marcus SC, Fuller RB, Sackeim HA (2004) Effectiveness of electroconvulsive therapy in community settings. Biol Psychiatry 55:301-312.

Prudic J, Haskett RF, Mulsant B, Malone KM, Pettinati HM, Stephens S, Greenberg R, Rifas SL, Sackeim HA (1996) Resistance to antidepressant medications and short-term clinical response to ECT. Am J Psychiatry 153:985-992.

Purba JS, Hoogendijk WJ, Hofman MA, Swaab DF (1996) Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. Archives of general psychiatry 53:137-143.

Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, Haroon E, Miller AH (2012) A Randomized Controlled Trial of the Tumor Necrosis Factor Antagonist Infliximab for Treatment-Resistant Depression: The Role of Baseline Inflammatory Biomarkers. Archives of general psychiatry:1-11.

Rapp MA, Schnaider-Beeri M, Grossman HT, Sano M, Perl DP, Purohit DP, Gorman JM, Haroutunian V (2006) Increased hippocampal plaques and tangles in patients with Alzheimer disease with a lifetime history of major depression. Archives of general psychiatry 63:161-167.

Ressler KJ, Nemeroff CB (2000) Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress Anxiety 12 Suppl 1:2-19.

Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, Zink M, Hortnagl H, Flor H, Henn FA, Schutz G, Gass P (2005) Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. J Neurosci 25:6243-6250.

Rocc P, De Leo C, Eva C, Marchiaro L, Milani AM, Musso R, Ravizza L, Zanalda E, Bogetto F (2002) Decrease of the D4 dopamine receptor messenger RNA

expression in lymphocytes from patients with major depression. Progress in neuro-psychopharmacology & biological psychiatry 26:1155-1160.

Rothermundt M, Arolt V, Fenker J, Gutbrodt H, Peters M, Kirchner H (2001) Different immune patterns in melancholic and non-melancholic major depression. European archives of psychiatry and clinical neuroscience 251:90-97.

Roy A, Pickar D, Gold P, Barbaccia M, Guidotti A, Costa E, Linnoila M (1989) Diazepam-binding inhibitor and corticotropin-releasing hormone in cerebrospinal fluid. Acta Psychiatr Scand 80:287-291.

Rush AJ, Trivedi MH, Wisniewski SR, Stewart JW, Nierenberg AA, Thase ME, Ritz L, Biggs MM, Warden D, Luther JF, Shores-Wilson K, Niederehe G, Fava M (2006) Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. N Engl J Med 354:1231-1242.

Sarchiapone M, Carli V, Camardese G, Cuomo C, Di Giuda D, Calcagni ML, Focacci C, De Risio S (2006) Dopamine transporter binding in depressed patients with anhedonia. Psychiatry research 147:243-248.

Sastre M, Guimon J, Garcia-Sevilla JA (2001) Relationships between beta- and alpha2-adrenoceptors and G coupling proteins in the human brain: effects of age and suicide. Brain research 898:242-255.

Scaglia F (2010) The role of mitochondrial dysfunction in psychiatric disease. Dev Disabil Res Rev 16:136-143.

Scheller J, Rose-John S (2006) Interleukin-6 and its receptor: from bench to bedside. Medical microbiology and immunology 195:173-183.

Schildkraut JJ (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. The American journal of psychiatry 122:509-522.

Schildkraut JJ, Gordon EK, Durell J (1965) Catecholamine metabolism in affective disorders. I. Normetanephrine and VMA excretion in depressed patients treated with imipramine. Journal of psychiatric research 3:213-228.

Schosser A, Cohen-Woods S, Gaysina D, Chow PC, Martucci L, Farmer A, Korszun A, Gunashinghe C, Gray J, Jones L, Craddock N, Owen MJ, Craig IW, McGuffin P (2010) NRG1 gene in recurrent major depression: no association in a large-scale case-control association study. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 153B:141-147.

Segman RH, Goltser-Dubner T, Weiner I, Canetti L, Galili-Weisstub E, Milwidsky A, Pablov V, Friedman N, Hochner-Celnikier D (2010) Blood mononuclear cell gene

expression signature of postpartum depression. Molecular psychiatry 15:93-100, 102.

Selek S, Savas HA, Gergerlioglu HS, Bulbul F, Uz E, Yumru M (2008) The course of nitric oxide and superoxide dismutase during treatment of bipolar depressive episode. Journal of affective disorders 107:89-94.

Semkovska M, McLoughlin DM (2010) Objective cognitive performance associated with electroconvulsive therapy for depression: a systematic review and meta-analysis. Biol Psychiatry 68:568-577.

Semkovska M, Noone M, Carton M, McLoughlin DM (2012) Measuring consistency of autobiographical memory recall in depression. Psychiatry Res 197:41-48.

Seo DO, Shin CY, Seung CH, Han SY, Ko KH (1999) Effects of chronic electroconvulsive shock on the expression of beta-adrenergic receptors in rat brain: immunological study. Biochemistry and molecular biology international 47:195-203.

Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA, Mirnics K (2011) Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. Molecular psychiatry 16:751-762.

Simon NM, McNamara K, Chow CW, Maser RS, Papakostas GI, Pollack MH, Nierenberg AA, Fava M, Wong KK (2008) A detailed examination of cytokine abnormalities in Major Depressive Disorder. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 18:230-233.

Skaper SD, Debetto P, Giusti P (2010) The P2X7 purinergic receptor: from physiology to neurological disorders. FASEB J 24:337-345.

Slattery DA, Neumann ID (2010) Oxytocin and Major Depressive Disorder: Experimental and

Clinical Evidence for Links to Aetiology and Possible Treatment. Pharmaceuticals (Basel) 3:702-724.

Sluzewska A, Rybakowski JK, Laciak M, Mackiewicz A, Sobieska M, Wiktorowicz K (1995) Interleukin-6 serum levels in depressed patients before and after treatment with fluoxetine. Annals of the New York Academy of Sciences 762:474-476.

Small KM, McGraw DW, Liggett SB (2003) Pharmacology and physiology of human adrenergic receptor polymorphisms. Annual review of pharmacology and toxicology 43:381-411.

Stefanis NC, Bresnick JN, Kerwin RW, Schofield WN, McAllister G (1998) Elevation of D4 dopamine receptor mRNA in postmortem schizophrenic brain. Brain research Molecular brain research 53:112-119.

Su S, Zhao J, Bremner JD, Miller AH, Tang W, Bouzyk M, Snieder H, Novik O, Afzal N, Goldberg J, Vaccarino V (2009) Serotonin transporter gene, depressive symptoms, and interleukin-6. Circ Cardiovasc Genet 2:614-620.

Sullivan MD, Robinson JP (2006) Antidepressant and anticonvulsant medication for chronic pain. Physical medicine and rehabilitation clinics of North America 17:381-400, vi-vii.

Sullivan PF, Fan C, Perou CM (2006) Evaluating the comparability of gene expression in blood and brain. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 141B:261-268.

Sun X, Wang JF, Tseng M, Young LT (2006) Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. J Psychiatry Neurosci 31:189-196.

Sun X, Mwamburi DM, Bungay K, Prasad J, Yee J, Lin YM, Liu TC, Summergrad P, Folstein M, Qiu WQ (2007) Depression, antidepressants, and plasma amyloid beta (Beta) peptides in those elderly who do not have cardiovascular disease. Biological psychiatry 62:1413-1417.

Tadler SC LAaHR (2009) Isoflurane Demonstrates Antidepressant-like Activity in a Mouse Model of Depression Anesthesia and Analgesia 108:212.

Tang Y, Glauser TA, Gilbert DL, Hershey AD, Privitera MD, Ficker DM, Szaflarski JP, Sharp FR (2004) Valproic acid blood genomic expression patterns in children with epilepsy - a pilot study. Acta neurologica Scandinavica 109:159-168.

Taupin V, Herbelin A, Descamps-Latscha B, Zavala F (1991) Endogenous anxiogenic peptide, ODN-diazepam-binding inhibitor, and benzodiazepines enhance the production of interleukin-1 and tumor necrosis factor by human monocytes. Lymphokine and cytokine research 10:7-13.

Theisen ME, Rapport LJ, Axelrod BN, Brines DB (1998) Effects of practice in repeated administrations of the Wechsler Memory Scale Revised in normal adults. Assessment 5:85-92.

Thomson PA, Christoforou A, Morris SW, Adie E, Pickard BS, Porteous DJ, Muir WJ, Blackwood DH, Evans KL (2007) Association of Neuregulin 1 with schizophrenia and bipolar disorder in a second cohort from the Scottish population. Molecular psychiatry 12:94-104.

Tombaugh TN, Kozak J, Rees L (1999) Normative data stratified by age and education for two measures of verbal fluency: FAS and animal naming. Arch Clin Neuropsychol 14:167-177.

Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, Fava M (2006) Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. The American journal of psychiatry 163:28-40.

Tsao CW, Lin YS, Chen CC, Bai CH, Wu SR (2006) Cytokines and serotonin transporter in patients with major depression. Progress in neuro-psychopharmacology & biological psychiatry 30:899-905.

Turnbull AV, Rivier CL (1999) Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiological reviews 79:1-71.

UK ECT review group (2003) Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. Lancet 361:799-808.

Valdizan EM, Diez-Alarcia R, Gonzalez-Maeso J, Pilar-Cuellar F, Garcia-Sevilla JA, Meana JJ, Pazos A (2010) alpha(2)-Adrenoceptor functionality in postmortem frontal cortex of depressed suicide victims. Biological psychiatry 68:869-872.

van Londen L, Goekoop JG, van Kempen GM, Frankhuijzen-Sierevogel AC, Wiegant VM, van der Velde EA, De Wied D (1997) Plasma levels of arginine vasopressin elevated in patients with major depression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 17:284-292.

Vawter MP, Tomita H, Meng F, Bolstad B, Li J, Evans S, Choudary P, Atz M, Shao L, Neal C, Walsh DM, Burmeister M, Speed T, Myers R, Jones EG, Watson SJ, Akil H, Bunney WE (2006) Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. Molecular psychiatry 11:615, 663-679.

Vogel M, Pfeifer S, Schaub RT, Grabe HJ, Barnow S, Freyberger HJ, Cascorbi I (2004) Decreased levels of dopamine D3 receptor mRNA in schizophrenic and bipolar patients. Neuropsychobiology 50:305-310.

Walker MS, Hughes TA (2008) Messenger RNA expression profiling using DNA microarray technology: diagnostic tool, scientific analysis or un-interpretable data? Int J Mol Med 21:13-17.

Wang L BP, Elmer GI, Mayo CL, Gould TD, Shepard PD (2012) Isoflurane impedes the development of a depression-like phenotype in rats. Society for Neuroscience Annual Meeting Planner 665.06/X17.

Wang SS, Kamphuis W, Huitinga I, Zhou JN, Swaab DF (2008) Gene expression analysis in the human hypothalamus in depression by laser microdissection and real-time PCR: the presence of multiple receptor imbalances. Molecular psychiatry 13:786-799, 741.

Weeks H, Tadler S, Smith K, Iacob E, Saccoman M, White A, Landvatter J, Chelune G, Suchy Y, Clark E, Cahalan M, Bushnell L, Sakata D, Light A, Light K (2013) Antidepressant and Neurocognitive Effects of Isoflurane Anesthesia versus Electroconvulsive Therapy in Refractory Depression

Weizman A, Gil-Ad I, Grupper D, Tyano S, Laron Z (1987) The effect of acute and repeated electroconvulsive treatment on plasma beta-endorphin, growth hormone, prolactin and cortisol secretion in depressed patients. Psychopharmacology 93:122-126.

Werstiuk ES, Coote M, Griffith L, Shannon H, Steiner M (1996) Effects of electroconvulsive therapy on peripheral adrenoceptors, plasma, noradrenaline, MHPG and cortisol in depressed patients. The British journal of psychiatry : the journal of mental science 169:758-765.

Wisdom NM, Mignogna J, Collins RL (2012) Variability in Wechsler Adult Intelligence Scale-IV subtest performance across age. Arch Clin Neuropsychol 27:389-397.

World Health Organization (2004) The Global Burden of Disease.

Wroolie TE, Williams KE, Keller J, Zappert LN, Shelton SD, Kenna HA, Reynolds MF, Rasgon NL (2006) Mood and neuropsychological changes in women with midlife depression treated with escitalopram. J Clin Psychopharmacol 26:361-366.

Wu WL, Lin YW, Min MY, Chen CC (2010) Mice lacking Asic3 show reduced anxiety-like behavior on the elevated plus maze and reduced aggression. Genes, brain, and behavior 9:603-614.

Xiang L, Szebeni K, Szebeni A, Klimek V, Stockmeier CA, Karolewicz B, Kalbfleisch J, Ordway GA (2008) Dopamine receptor gene expression in human amygdaloid nuclei: elevated D4 receptor mRNA in major depression. Brain research 1207:214-224.

Yalcin I, Aksu F, Belzung C (2005) Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. European journal of pharmacology 514:165-174.

Yatham LN, Liddle PF, Sossi V, Erez J, Vafai N, Lam RW, Blinder S (2012) Positron emission tomography study of the effects of tryptophan depletion on brain serotonin(2) receptors in subjects recently remitted from major depression. Archives of general psychiatry 69:601-609.

Yatham LN, Liddle PF, Dennie J, Shiah IS, Adam MJ, Lane CJ, Lam RW, Ruth TJ (1999) Decrease in brain serotonin 2 receptor binding in patients with major depression following desipramine treatment: a positron emission tomography study with fluorine-18-labeled setoperone. Archives of general psychiatry 56:705-711.

Yoshimura R, Hori H, Ikenouchi-Sugita A, Umene-Nakano W, Ueda N, Nakamura J (2009) Higher plasma interleukin-6 (IL-6) level is associated with SSRI- or SNRI-refractory depression. Progress in neuro-psychopharmacology & biological psychiatry 33:722-726.

Zhang HT, Whisler LR, Huang Y, Xiang Y, O'Donnell JM (2009) Postsynaptic alpha-2 adrenergic receptors are critical for the antidepressant-like effects of desipramine on behavior. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:1067-1077.

Zheng H, Koo EH (2006) The amyloid precursor protein: beyond amyloid. Molecular neurodegeneration 1:5.

Zunszain PA, Hepgul N, Pariante CM (2012) Inflammation and Depression. Curr Top Behav Neurosci.

Zunszain PA, Anacker C, Cattaneo A, Carvalho LA, Pariante CM (2011) Glucocorticoids, cytokines and brain abnormalities in depression. Progress in neuro-psychopharmacology & biological psychiatry 35:722-729.