Immune Alterations in Psychosis: Focus on the JAK-STAT1 Pathway

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THESIS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BMI	body mass index
C4	complement component 4
ChIP	chromatin immunoprecipitation
CNS	central nervous system
COX	cyclooxygenase
CRP	c-reactive protein
CSF	cerebrospinal fluid
DGE	differential gene expression
DSM	diagnostic and statistical manual of mental disorders
ELISA	enzyme linked immunosorbent assay
FDR	false discovery rate
GABA	gamma aminobutyric acid
GSEA	gene set enrichment analysis
IFN	interferon
IL	interleukin
IRF	interferon regulatory factor

LIST OF ABBREVIATIONS (continued)

JAK	janus kinase
LAI	long acting injectable
LPS	lipopolysaccharide
mRNA	messenger RNA
NF-κB	nuclear factor kappa B
NMDA	n-methyl d-aspartate
PANSS	positive and negative syndrome scale
PBMC	peripheral blood mononuclear cell
pSTAT1	phosphorylated STAT1
qRT-PCR	quantitative real time polymerase chain reaction
RDoC	research domain criteria
SCID	structured clinical interview for DSM
STAT	signal transducer and activator of transcription
TFEA	transcription factor enrichment analysis
Th	T helper
TLR	toll like receptor
TNF	tumor necrosis factor

SUMMARY

Despite decades of literature indicating alterations to immune parameters in psychosis, understanding the connections between these findings and pathophysiology is not simple. Recently, there has been a surge in research and interest in the interactions of the immune and central nervous systems across the course of the lifetime, from development to adult homeostasis, and in injury and illness. Circulating and peripherally-derived immune cells are understood to communicate with neurons and glia either directly or via interaction with cells at the parenchymal borders, contributing to neuronal signaling and behavior. While there is some indication of activation of proinflammatory signaling in circulating immune cells in psychosis, data are lacking. One piece of the puzzle in determining whether and how these alterations are involved in the pathophysiology of psychotic disorders is an understanding of alterations to specific immune parameters across the cross the course of the illness in relation to symptom acuity and treatment.

The molecular focus of this thesis was the JAK-STAT1 signaling pathway. Interferon-gamma mediated JAK-STAT1 signaling controls the expression of a wide variety of genes involved in immune activation and induces a proinflammatory cellular phenotype in monocytes and macrophages. In the first research chapter, measures of activation of the JAK-STAT1 signaling pathway were quantified in peripheral blood cells from participants with psychosis and examined in relation to illness duration, acuity and measures of symptomology. The results demonstrated alterations to JAK-STAT1 signaling in psychosis. Specifically, a JAK-STAT1 transcriptional signature (IFNG, CXCL10, IRF1, STAT1, TLR4) was decreased in participants with psychosis who were earlier in illness and those who had a higher illness acuity compared to non-psychiatric controls. This signature was positively correlated with illness duration such that as illness duration increased so did expression of the JAK-STAT1 transcriptional signature. This increase in expression appeared to be specific to participants who were chronically ill, but not acutely symptomatic.

Additionally, there was a negative correlation of expression of specific JAK-STAT1 signature genes, but not others, with measures of symptomology.

Antipsychotic treatment has immunomodulatory properties that are not well characterized, but likely influence measures of immune alterations in adults with psychosis. In the second research chapter the JAK-STAT1 signature was compared between participants with psychosis who were treated with antipsychotic medications and untreated participants. Additionally, the effects of risperidone, the most commonly prescribed antipsychotic in the clinical sample, on JAK-STAT1 signaling, were investigated using the THP-1 human monocyte cell line. Comparisons within the clinical sample demonstrated that the JAK-STAT1 transcriptional signature was increased in risperidone treated participants who had a longer illness duration compared to participants with a shorter illness duration and compared to untreated participants regardless of illness duration. Results of the in-vitro experiments showed that risperidone increases expression of JAK-STAT1 signature genes in monocytes and monocyte-derived macrophages that are exposed to stimuli that induce both a proinflammatory or a 'tolerized' anti-inflammatory and tissue remodeling cellular phenotype. Collectively these data indicate that risperidone may contribute to the increased expression of JAK-STAT1 signature genes in participants with a longer illness duration. Additionally, if antipsychotics like risperidone also skew myeloid cells within the brain parenchyma and at its borders towards a proinflammatory phenotype, this may have implications for findings of central nervous system (CNS) immune activation in psychosis.

In addition to the 'tolerized' myeloid phenotype modeled in the THP-1 cells, there are multiple other physiological states and stimuli that are associated with anti-inflammatory and tissue remodeling functions and accompanied by decreased IFN-γ mediated JAK-STAT1 activity. These include Th2 mediated immunity and acute psychological stress. In the third and final research chapter, genome wide

transcriptional profiling was carried out using isolated monocytes from chronically symptomatic participants with psychosis. Transcriptional signatures of each of the described stimuli were compiled, along with IFN-γ (Type II) and IFN-α (Type I) interferon mediated JAK-STAT1 and chronic stress signatures, which were tested for enrichment in relation to psychosis diagnosis and illness duration. The results indicated a monocyte phenotype in psychosis predominantly consistent with an anti-inflammatory tissue remodeling skew earlier in illness, that reverses later in illness. However, the results were not entirely clear-cut with some indication of a mixed monocyte phenotype that requires further research. Transcription factor enrichment analysis highlighted an important role of the IRF8-IRF1 regulome, downstream of IFN-γ mediated JAK-STAT1 signaling, in driving the monocyte phenotypic differences between shorter and longer illness duration participants with psychosis.

Going forward, these alterations to the JAK-STAT1 signature in circulating immune cells in psychosis should be further investigated in relation to cellular function, illness stage and acuity. In particular it would be useful to determine whether the JAK-STAT1 signature is or is not suppressed prior to illness onset, as well as how this measure of monocyte activity is associated with serum and CSF markers of inflammation and blood brain barrier dysfunction as well as psychopathology.

INTRODUCTION

1.1 Psychosis

1.1.1 Terminology

The term 'psychosis' or 'psychotic disorder' is used throughout this thesis to refer to major psychiatric illness in which one of the primary defining features is the presence of hallucinations and/or delusions. Under the current Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-V) this encompasses diagnoses of schizophrenia, schizoaffective disorder and bipolar disorder with psychosis. The method of categorizing psychotic disorders is frequently debated, with many researchers and clinicians using a spectrum or continuum approach to conceptualizing psychotic illness (Craddock, O'Donovan, and Owen 2009; Guloksuz and Os 2018). This discussion is born of the fact that there is high heterogeneity within these diagnostic categories as well as transdiagnostic overlap with regards to symptom presentation, genetics, molecular signatures, environmental risk factors and phenomenology (Owen, Sawa, and Mortensen 2016; Gandal et al. 2018). At the individual level, many individuals with psychosis experience a constellation of symptoms that do not easily fit within the schizophrenia and bipolar disorder diagnostic dichotomy. The National Institute of Mental Health introduced the Research Domain Criteria (RDoC) as a conceptual framework to aid research of neuropsychiatric disorders (Cuthbert and Insel 2010). The RDoC encourages a dimensional approach that moves away from a focus on traditional diagnostic boundaries and instead places emphasis on a mechanistic understanding of particular biological alterations present in individuals with psychiatric disorders and their relationship to symptoms and behavior.

The majority of the published literature relating to psychotic disorders to date has used these traditional diagnostic categories, usually limited solely to schizophrenia (with or without the inclusion of

schizoaffective disorder) (Guloksuz and Os 2018), and for research relating to bipolar disorder, data are rarely presented in relation to the presence or absence of psychosis (Burton et al. 2018). The remaining background will therefore focus primarily on the schizophrenia literature, with discussion related to bipolar disorder with psychosis and psychotic disorders overall where available.

1.1.2 Prevalence and symptomology

Schizophrenia is usually reported to have an estimated prevalence of approximately 1%, though recent reports demonstrate variability across study populations that cannot be attributed to methodological differences (Owen, Sawa, and Mortensen 2016). The first episode of psychosis in schizophrenia usually occurs in late adolescence or early adulthood, and the illness is characterized by positive, negative and cognitive symptoms. Positive symptoms include hallucinations, which are sensory perceptions that lack corresponding stimuli, delusions, which are fixed false beliefs categorized by types such as persecutory and grandiose delusions and delusions of control, and though disorder, which is inferred from disorganized speech (Arciniegas 2015). Negative symptoms include social withdrawal and loss of motivation, and recently, the prevalence of mood instability and depressive symptoms in schizophrenia have been highlighted (Owen, Sawa, and Mortensen 2016; Upthegrove, Marwaha, and Birchwood 2017). Cognitive symptoms are also now considered a core feature of schizophrenia, with deficits in executive function, working memory and attention (Aas et al. 2014; Cabrera et al. 2016). Bipolar I disorder has an estimated prevalence of 1% and the presence of psychosis in bipolar disorder is approximately 53% (Burton et al. 2018; Grande et al. 2016). Bipolar disorder with psychosis has an overlapping symptom profile but is currently clinically differentiated by the predominance of symptomology that is characterized by episodes of elevated mood and episodes of depressed mood in addition to the presence of hallucinations and/or delusions.

1.1.3 Neurobiology

While the neurobiology of psychotic disorders is still poorly understood, some progress has been made largely due to advances in imagining techniques (O. Howes, Mccutcheon, and Stone 2015). Neurostructural changes in psychosis include grey matter density reductions, that range from broadly distributed to small localized changes, particularly in frontotemporal regions (Ivleva et al. 2017). These alterations appear to cluster with psychosis biotypes, which are characterized by cognitive and neuropsychological profiles rather than classical symptom profiles. Dendritic spine loss in the dorsolateral prefrontal cortex has also been demonstrated in both schizophrenia and bipolar disorder (Konopaske et al. 2017).

Historically, elevated dopamine was thought to be the primary altered neurotransmitter system underlying the development of schizophrenia. This was based on the finding that dopamine agonists increased symptom severity in individuals with psychosis and sometimes led people without a diagnosis to experience symptoms of psychosis, as well as the understanding that antipsychotic drugs worked primarily through inhibiting dopamine D2 receptors (O. D. Howes et al. 2017). Post-mortem evidence indicates elevated levels of the rate-limiting enzyme involved in dopamine synthesis, tyrosine hydroxylase, in the substantia nigra in schizophrenia (O. Howes, Mccutcheon, and Stone 2015). Additionally, multiple studies using in-vivo imaging now demonstrate increased dopamine synthesis capacity and elevated striatal dopamine release in schizophrenia, and more recently the same results were shown in bipolar disorder with psychosis (Jauhar et al. 2017). These changes related to subcortical dopamine are associated with positive symptoms (Kesby et al. 2018). The predominant hypothesis linking these neurotransmitter changes to the phenomenology of positive symptoms in psychosis is the salience hypothesis, which focuses on the role of dopamine in inappropriately ascribing salience to internally and externally generated perceptual stimuli (O. Howes, Mccutcheon, and Stone 2015; Corlett et al. 2011).

Some evidence suggests that elevated striatal dopamine may also play a role in negative and cognitive symptoms in psychosis, though alterations to prefrontal activity have also been implicated (O. Howes, Mccutcheon, and Stone 2015). Importantly, it is now thought that changes in glutamatergic and (gamma aminobutyric acid) (GABA)-ergic signaling are also involved in psychosis. In particular N-methyl D-aspartate (NMDA) receptor hypofunction appears to play a crucial role, with some authors proposing that dopaminergic changes may be secondary to glutamatergic alterations (Cannon 2015). Acute and chronic NMDA receptor antagonists lead to a variety of psychotic-like symptoms, and effects are thought to be mediated by decreased GABAergic interneuron activity resulting in increased pyramidal neuron firing (O. Howes, Mccutcheon, and Stone 2015). NDMA receptor antagonists result in alterations to dopamine signaling similar to those seen in psychosis, and imagining studies indicate increased glutamate in the prefrontal cortex in psychosis (O. Howes, Mccutcheon, and Stone 2015; Cannon 2015). A model based on integration of current data pertaining to dopaminergic, glutamatergic and GABAergic alterations, posits two mechanisms by which dopamine release in the striatum could be dysregulated (O. D. Howes et al. 2017). First, decreased cortical dopamine release may result in increased glutamatergic activity projecting to the midbrain and subsequent elevated striatal dopamine release. Second, NMDA receptor hypofunction on GABAergic interneurons may disrupt frontal inhibitory control of midbrain dopamine neurons, also leading to increased striatal dopamine release.

Linking these findings back to the observed neurostructural data, post-mortem studies demonstrate that dendritic spine loss is most pronounced in glutamatergic pyramidal neurons, and imaging studies show cortical thinning is greatest in the superior and medial prefrontal cortex (Cannon 2015). The mechanisms by which genetic and environmental factors contribute to these neurostructural and transmitter alterations are not well understood, and are likely temporally complex and different for each individual. Neuroimmune processes are implicated, and excessive immune activation is proposed to

be a contributing factor to illness development and exacerbation in psychosis (Cannon 2015; B. J. Miller and Goldsmith 2016).

1.2 The immune system in psychosis

One of the most prevalent themes in psychosis research are findings and theories that involve the immune system. These findings include the presence of peripheral and central immune alterations preceding illness onset and spanning the course of the illness, as well as genetic and environmental risk factors that impact immune function. In this section these findings will be outlined and discussed in relation to current theories of illness development and pathophysiology.

1.2.1 Immune related risk factors

The heritability of schizophrenia and bipolar disorder are estimated to be approximately 80% and 85%, respectively (Owen, Sawa, and Mortensen 2016). Large-scale genome-wide association studies demonstrate that psychotic disorders are highly polygenic, with many common variants conferring risk of small effect and highlight overlap in genes that contribute risk for schizophrenia and bipolar disorder (Purcell et al. 2009; Frost et al. 2016). Results implicate genes within the major histocompatibility complex region on chromosome 6, which includes genes involved in antigen presentation, as well as those that code for complement proteins, cytokines and other immune effectors as well as other loci with genes involved in immune function (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014; Stefansson et al. 2009).

A range of environmental factors are associated with the development of schizophrenia and related psychoses. These include maternal infections and stress as well as postnatal infections and psychosocial stressors that extend throughout childhood and adolescence (Khandaker et al. 2015; B. J. Miller and Goldsmith 2016a). Children of mothers diagnosed with a variety of viral and bacterial infections, or who have elevated serum levels of C-reactive protein (CRP), tumor necrosis factor (TNF)-α and

interleukin (IL)-8 during pregnancy, are at an increased risk of developing schizophrenia (B. J. Miller et al. 2011; Khandaker and Dantzer 2015). Maternal infection and early life stressors have also been associated with bipolar disorder (Leboyer et al. 2016; Marangoni, Faedda, and Baldessarini 2018; Khandaker et al. 2015). IL-6 expression levels in childhood is associated with a two-fold increased risk of development of a psychotic disorder, and longitudinal studies indicate that higher CRP in adolescence is associated with development of psychosis (Khandaker et al. 2015; Metcalf et al. 2017). Additionally, population based studies found that a diagnosis of an autoimmune disease or a prior hospitalization for infection significantly increased the risk for both schizophrenia and bipolar disorder with psychosis (Benros et al. 2014). The neurodevelopmental hypothesis of schizophrenia posits that an interaction of these genetic and environmental risk factors at various time points affect critical neurodevelopmental processes (Owen, Sawa, and Mortensen 2016; Catts et al. 2013).

The effects of some of the prenatal and premorbid risk factors discussed in relation to psychosis have been tested using animal models. For example, maternal immune activation models demonstrate that offspring whose mothers are subjected to an immune insult during pregnancy, exhibit peripheral and central immune changes and neuroanatomical abnormalities as well as behavioral deficits related to neuropsychiatric illness in adulthood (Estes and McAllister 2015; Fineberg and Ellman 2013; B. J. Miller and Goldsmith 2016). Interestingly, some studies have demonstrated that these alterations can result from multiple 'hits', such that subtle prenatal immune activation and adolescent stressors on their own have minimal long-term consequences, but in combination result in persistent cytokine alterations and behavioral changes, such a sensorimotor gating deficits, thought to be relevant for psychosis (S Giovanoli et al. 2016). While murine models of the effects of prenatal and premorbid risk factors demonstrate the profound impact of these insults on the developing nervous system and endophenotypes related to psychiatric illness, there are major limitations regarding the extent to which human psychotic illness can be modeled in rodents.

1.2.2 Serum immune alterations

Published findings of altered expression of proinflammatory cytokines in serum date back to the 1970s, and by 2001 there were hundreds of publications regarding cytokine levels in psychotic disorders (Libikova et al. 1979; Hinze-Selch and Pollmächer 2001). Meta-analyses conducted in the last 5 years indicate that serum cytokine levels and other measures of inflammation such as CRP are indeed altered in a substantial proportion of individuals with schizophrenia and also in bipolar disorder, predominantly demonstrating increases in proinflammatory cytokines, though findings across meta-analyses are not fully consistent with regards to individual markers (B. J. Miller et al. 2011; Upthegrove, Manzanares-Teson, and Barnes 2014; Goldsmith, Rapaport, and Miller 2016). Some cytokines appear to fluctuate with illness stage ('state markers'), while others remain consistently altered ('trait markers') throughout illness. In the periphery, though the dissociation of specific cytokines as state or trait markers should be confirmed with longitudinal studies, these findings imply that immune dysfunction in psychosis is more nuanced than a systemic and chronic inflammatory profile.

While the proportion of studies that include measures of psychopathology are lacking (B. J. Miller and Goldsmith 2016), there are reports of associations of peripheral immune markers with measures of symptomology and psychopathology. For example, IL-6 messenger RNA (mRNA) expression correlated with positive symptom severity as measured by the positive and negative syndrome scale (PANSS) in participants with schizophrenia (Chase et al. 2016). Additionally, evidence suggests a negative correlation between peripheral markers of inflammation and measures of cognition in first-episode psychosis (Martínez-Cengotitabengoa et al. 2012) and schizophrenia (Bulzacka et al. 2016). In bipolar disorder, alterations in certain serum cytokines also demonstrate fluctuations with symptomology (Barbosa et al. 2014).

1.2.3 Immune alterations at the brain borders

Associations between peripheral inflammation and symptomology indicate communication between peripheral and central compartments, but the routes of communication are still being elucidated. Animal models demonstrate that peripheral immune activation can contribute to neuronal and behavioral changes either directly or via inducing immune activation within the central nervous system, and various immune to brain communication pathways have been described (Kirkpatrick and Miller 2013; Khandaker and Dantzer 2015; Perry and Holmes 2014). While the blood brain barrier limits the entry of large molecules to the brain, research now suggests that the parenchymal borders including the meninges and choroid plexus are areas of constant communication between peripheral immune cells and effector molecules, perivascular meningeal and choroid plexus macrophages and endothelial cells, and glial and neuronal cells within the brain parenchyma (Rejane Rua and McGavern 2018; Herz et al. 2017). The meninges are highly vascularized and though vessels within the subarachnoid space contain tight junctions, the dural vessels are fenestrated, and the dura mater is populated by a variety of immune cells (Rejane Rua and McGavern 2018). Immune cells from circulation communicate with resident cells at these borders under steady-state conditions where secreted cytokines and other immune effectors from the periphery can influence neuronal and glial function (Yirmiya and Goshen 2011; Pollak et al. 2017). However, the exact mechanisms underlying these interactions are still being elucidated. Under certain inflammatory conditions in which there is blood brain barrier dysfunction, entry of larger molecules and even cells to the leptomeninges and parenchyma can occur (Rejane Rua and McGavern 2018).

Certain markers of blood brain barrier abnormalities, such as serum albumin, and cytokines, particularly IL-6 and IL-1 β, have been demonstrated in the cerebrospinal fluid in psychotic disorders (Khandaker and Dantzer 2015; Bergink, Gibney, and Drexhage 2014; Orlovska-waast et al. 2018). Additionally, an earlier study reported an increased proportion of macrophages in the cerebrospinal fluid during acute psychosis in schizophrenia (Nikkilä et al. 1999) The most recent meta-analyses show some

concordance between blood and cerebrospinal fluid markers of inflammation, though they highlight continuing methodological limitations such as small sample sizes and confounding factors, concluding that further studies are needed (Orlovska-waast et al. 2018; Wang and Miller 2018). Direct investigations of the brain borders in psychosis are scarce (Pollak et al. 2017), though a recent study that carried out transcriptome sequencing of the choroid plexus revealed upregulation of immune and inflammatory genes in schizophrenia (Kim et al. 2016). As yet, there have been no reports directly testing whether there is immune cell infiltration to the parenchyma in psychosis, although one study found evidence of t-cells infiltration in the hippocampus (Pollak et al. 2017; Van Kesteren et al. 2017).

1.2.4 Central immune alterations

Immune signaling molecules and receptors play critical roles in the CNS during development, homeostasis and in illness (Boulanger 2009; Yirmiya and Goshen 2011). During development, cytokines and other immune proteins have been demonstrated to perform vital roles in neuronal differentiation, synapse formation and synaptic pruning (Stevens et al. 2007; Réaux-Le Goazigo et al. 2013), and in the healthy adult brain cytokines including IL-1, TNF-α and IFN-γ are involved in processing underlying memory, synaptic scaling and social behavior (Yirmiya and Goshen 2011; Filiano et al. 2016). Under pathological conditions some of these same immune molecules are implicated in neuroinflammation and CNS damage (Boulanger 2009). Because microglia, which are derived from myeloid precursors in embryonic development, are considered the primary immune cell residing in the brain parenchyma they are thought to play an important role in neuroinflammation (Herz et al. 2017). In the healthy brain, microglia patrol the CNS and maintain homeostasis through interactions with neurons and other glial cells (Knuesel et al. 2014). In response to pathogen or damage associated ligands and inflammatory cytokines microglia are activated, leading to either damaging or protective effects depending on the time point and context (Bergink, Gibney, and Drexhage 2014; Khandaker et al. 2015). Some effector functions are similar to those of monocytes and macrophages in the periphery and include phagocytosis and secretion of both

pro- and anti-inflammatory proteins. In addition, microglia provide critical trophic support to neurons and regulate synaptic plasticity. An exaggerated proinflammatory phenotype can have deleterious consequences.

Post-mortem studies have reported elevated protein and mRNA expression of cytokines and other inflammatory markers in the frontal cortex in schizophrenia and bipolar disorder, though findings have not all been consistent with regards to specific markers (Fillman et al. 2013; Jagadeesh Sridhara Rao et al. 2013; J S Rao et al. 2010). A number of recent gene expression profiling studies have now demonstrated dysregulated immune activity in the CNS in psychosis (Darby, Yolken, and Sabunciyan 2016; Bergon et al. 2015; Hess et al. 2016). As these expression studies used whole tissue samples the findings are not specific to any particular cell type. Interestingly, a post-mortem expression study that followed up with immunohistochemistry of specific markers found that some of the alterations related to immune gene expression in the brain appears to derive from monocytes within vessels and from vessel endothelial cells (Hwang et al. 2013). There have been a number of attempts to determine whether there are signs of microglial activation in psychosis, and some post mortem studies have found increased expression of markers and morphological characteristics indicative of activation of microglia in the frontal cortex of individuals with schizophrenia (Fillman et al. 2013; Jagadeesh Sridhara Rao et al. 2013). A recent meta-analysis found evidence of increased microglial density as well as increased levels of proinflammatory cytokines in schizophrenia (Van Kesteren et al. 2017), though they and others note significant heterogeneity in results regarding markers of activated microglia (Laskaris et al. 2016; Trépanier et al. 2016). These findings are complicated by methodological and theoretical issues regarding the specificity of current microglial markers for this cell type and for particular cellular activation states (Filiou et al. 2014). It is also worth noting that markers used to measure microglia also do not differentiate between microglia and peripherally derived monocytes/macrophages, and while it is thought that monocytes do not infiltrate the parenchyma in psychosis this has not been directly tested (Van Kesteren et al. 2017).

These heterogeneous post-mortem findings are mirrored by those in the microglial imaging literature. Positron emission tomography imaging of ligands which bind to activated microglia show both increased binding, no difference in binding, and even decreased binding in psychotic disorders (van Berckel et al. 2008; Doorduin et al. 2009; Haarman et al. 2014). Additionally, as with the post-mortem brain markers, the specificity of some of these markers for activated microglia has been questioned, nor do they distinguish between microglial phenotypes (Laskaris et al. 2016; De Picker et al. 2017).

More recently, attempts have been made to link immune activation across compartments and with neurostructural and neurochemical data. For example, one group recently reported a negative correlation of in-vivo imaging of a marker of activated microglia and cortical volume reductions in schizophrenia (Selvaraj et al. 2018). In another study, higher levels of serum proinflammatory cytokines in clinical high risk subjects predicted grey matter reduction rates in the prefrontal cortex in those who converted to psychosis (Cannon 2015). Additionally, many studies demonstrate that peripheral immune alterations can impact dopaminergic signaling, with corresponding effects on reward-related behaviors, as well as glutamatergic signaling and associated mood and cognitive changes that are of relevance for neuropsychiatric illness (Felger and Treadway 2016; A. H. Miller, Haroon, and Felger 2017). These effects can be mediated directly by the actions of immune effectors on neurons and glia or indirectly, for example, by altering activity of the kynurenine pathway which impacts levels of downstream metabolites. Increased levels of kynurenic acid, the only naturally occurring NMDA receptor antagonist, are seen in both schizophrenia and bipolar disorder with psychosis (Erhardt, Schwieler, and Imbeault 2017; Müller et al. 2015). Furthermore, chronically increased kynurenic acid was demonstrated to lead to increased striatal dopamine release (Erhardt, Schwieler, and Imbeault 2017).

Furthermore, one study demonstrated that increased transcription of immune and inflammatory genes in the choroid plexus in psychosis were positively correlated with protein levels of markers of

immune activation in both the serum and frontal cortex (Kim et al. 2016). Interestingly, while the majority of macrophages within the brain and at the parenchymal borders do not appear to be replenished by monocytes from circulation, macrophages in the choroid plexus, an area that is thought to be important for communication between peripheral and central compartments demonstrate replenishment from the periphery (Goldmann et al. 2016).

1.2.5 Peripheral immune cells alterations

Recent advances in understanding communication pathways between the peripheral immune and central nervous system have highlighted the presence and functions of peripherally derived immune cells at the brain borders, and their influence on neuronal activity and behavior. In animal studies, for example, t-cells in the meninges seem to be critical for normal social behavior and are thought to act via secretion of IFN-γ that binds to neurons in the frontal cortex (Filiano et al. 2016). The stress literature highlights recruitment of proinflammatory monocytes to the brain by activated microglia, and a role in anxiety-like behaviors (Wohleb and Delpech 2017). Additionally, following immune activation, monocytes were demonstrated to mediate cortical dendritic spine loss and impaired learning, implicating these cells and TNF-α in sickness induced behavioral alterations (Garré et al. 2017). This is an emerging literature and further research will better elucidate under which conditions these cells are important for CNS activity during health and illness, as well as mechanisms by which communication occurs at the parenchymal borders. Nethertheless, these findings indicate that understanding the contribution of peripheral immune alterations to pathophysiology neuropsychiatric illness will require an elucidation of specific immune cellular changes in psychosis preceding illness onset and across the course of the illness.

The overwhelming majority of studies have measured overall levels of cytokines in circulation in psychosis, and thus don't provide cell specific data. However, some reports indicate alterations to lymphocyte subsets as well as evidence of monocytosis, both of which may vary with illness stage (B. J.

Miller et al. 2013). Using isolated peripheral blood mononuclear cells (PBMCs), which includes lymphocytes (t-cells, b-cells and natural killer (NK) cells), monocytes and dendritic cells, it has been shown that there are alterations in mRNA expression of proinflammatory cytokines in schizophrenia (X.-Q. Song et al. 2009; García-Bueno et al. 2014; Chase et al. 2016). Nuclear factor kappa B (NF-κB) is often considered the prototypical proinflammatory signaling pathway as its activation induces the expression of proinflammatory cytokines and other effectors of inflammation (Lucas and Maes 2013). This pathway is activated following ligation of toll-like receptors (TLRs) by bacterial ligands as well as endogenous cellular damage associated ligands (collectively termed 'danger signals') on pathogen sensing cells such as monocytes and macrophages (Venkatasubramanian and Debnath 2013; Fleshner, Frank, and Maier 2017). NF-κB is also activated by proinflammatory cytokines such as TNF-α and IL-1β, and increased activation of this pathway is seen in a number of chronic inflammatory conditions (Venkatasubramanian and Debnath 2013). In addition to cytokines, increased levels of activated NF-κB and downstream inflammatory mediators such as cyclooxygenase (COX)-2 and inducible nitric oxide synthase have been reported in PBMCs from participants with schizophrenia (García-Bueno et al. 2014; Cabrera et al. 2016; X.-Q. Song et al. 2009).

There have also been some experiments carried out using isolated monocytes. Monocytes from participants with schizophrenia and bipolar disorder demonstrated increased expression of select inflammatory genes, indicating activation of these cells to a proinflammatory phenotype in psychosis (Padmos et al. 2008; Drexhage et al. 2010). Another study reported that levels of IL-1β and TNF-α were significantly increased in monocytes from participants with schizophrenia after stimulation with the bacterial ligand lipopolysaccharide (LPS) (Kowalski et al. 2001). However, another demonstrated increased monocyte cell surface levels of specific TLRs, but decreased levels of some cytokines (IL-1β and IL-6) and no difference in others (TNF-α, IL-10) at baseline (Krause et al. 2012; Müller et al. 2012). Here, stimulation with LPS indicated potential alterations in responsivity of immune cells in schizophrenia, but the results

were overall unclear. Thus, while some evidence points to an 'inflammatory set point' of circulating monocytes, and even potentially the presence of inflammatory monocytes at the parenchymal borders, the quantity of data is lacking and overall findings are somewhat mixed.

1.2.6 Cause and consequence of immune alterations

Risk factors related to immune function along with findings of peripheral and central immune alterations, while compelling, do not allow inferences of causation regarding the cause or consequence of immune alterations in adults with psychosis. As discussed, some animal models indicate that prenatal alone, or a combination of prenatal and premorbid psychosis risk factors can lead to long term peripheral and central immune alterations and relevant behavioral changes in adulthood (Sandra Giovanoli et al. 2013). These data support a neurodevelopmental hypothesis, highlighting the importance of immune perturbations during development. However, others show that specific gene expression changes seen in schizophrenia post-mortem brain are recapitulated by peripheral immune activation in adult but not prenatally challenged mice (Volk et al. 2015). Additionally, in adults with psychosis immune findings may also reflect the effects of comorbidities, metabolic factors and psychosocial factors (Bergink, Gibney, and Drexhage 2014; B. J. Miller and Goldsmith 2016). It is important to appreciate that the peripheral immune system is highly dynamic; it both responds to and impacts exogenous stimuli, systemic factors, and CNS functioning throughout the lifetime. Thus, immune system changes seen at any given point in adulthood may reflect genetic background and the long-term peripheral and central consequences of immune insults occurring during development, the impact of systemic biological and psychosocial factors in adulthood, or most likely a combination of these influences among others. Vulnerability-stress models propose that stressors, many of which influence immune function, continue to impact psychosis pathophysiology throughout the course of the illness (De Picker et al. 2017). Though it's clear from the animal and human literature that peripheral immune activation in adulthood results in central immune and behavioral

changes, whether and how peripheral immune alterations in psychosis contribute to illness pathophysiology requires further testing.

1.2.7 Immune targeting treatments

One way to determine if immune changes influence pathophysiological mechanisms in adulthood is to assess whether immune targeting treatments alleviate symptoms. Findings of immune alterations in psychosis have resulted in numerous trials of adjunctive treatment with currently available immune modifying drugs, and some results indicate an improvement in symptoms (Melbourne et al. 2017). For example, non-steroidal anti-inflammatory drugs such as aspirin and celecoxib have shown promise as adjunctive therapies in schizophrenia, and some meta-analyses indicate that participants with acute psychosis given aspirin or celecoxib demonstrate overall improvement in symptoms (Zheng et al. 2017; Sommer et al. 2014). Another recent meta-analysis to assess the outcome of trials with minocycline, one effect of which is inhibition of macrophages, demonstrated improvement in all PANSS symptom domains, whereas previous meta-analysis showed no efficacy (Xiang et al. 2017; Sommer et al. 2014). While some efficacy has been demonstrated, findings are heterogeneous, and improvements were only seen in some participants (B. J. Miller et al. 2011; Wang and Miller 2018). Furthermore, these drugs, while widely utilized have off-target effects that make results difficult to interpret with regard to the effects of immune activation. More recently, trials of monoclonal antibodies that target inflammatory cytokines directly are being investigated. Though sample sizes are too small for interpretation, improvements in some symptoms domains have been noted thus far (B. J. Miller and Buckley 2017).

Interpreting the effects of adjunctive treatment, and findings of immune alterations in psychosis in general, are complicated by the fact that antipsychotics also have immunomodulatory properties. There are now a substantial number of reports of cytokine alterations in first episode psychosis prior to initiation of treatment that indicate that immune dysfunction is not due solely to the effects of antipsychotic

treatment (B. J. Miller and Goldsmith 2016). However, findings relating to immune activity in participants with psychosis treated with antipsychotic mediations need to consider the influence of treatment. The exact nature of antipsychotic effects on immune measures is unclear, as some data indicate decreases in proinflammatory cytokines following the initiation of treatment whereas others do not demonstrate an effect or even indicate increases in markers of inflammation (B. J. Miller et al. 2011; Witte et al. 2014; M.-L. Chen et al. 2013). Differing results may depend on specific drug effects and length of treatment, as well which immune markers were assayed. In the periphery, as with most measures, focus has been on serum cytokines.

1.3 Summary and project overview

1.3.1 Summary

The extent to which the peripheral immune changes observed in psychosis may play a primary causative role in illness development, contribute to secondary pathophysiological mechanisms, lead to symptom exacerbation, or represent an epiphenomenon, remains an ongoing area of research with many questions outstanding: What are the nature of peripheral immune alterations in psychosis with regards to specific cell types and functions? How do these immune alterations vary across different stages of illness, with illness severity and with symptomology? How do current treatments impact peripheral immune measures and does this affect pathophysiology? Do peripheral immune changes associate with measures of CNS immune activation, and neurostructural and neurochemical findings? What are the mechanisms by which immune cells and soluble factors affect brain and behavior in psychosis? Can treatments that target the immune system improve symptoms in psychosis?

The purpose of this thesis is to contribute a small piece to this puzzle. Recent research indicates that circulating immune cells play a critical role in central nervous system functioning in the steady state and in the context of disease, but while findings of alterations to serum cytokines are prevalent in

psychosis, these are blunt measures with which to attempt to understand the activity of circulating immune cells. Some data using PBMCs and isolated cell types indicate a proinflammatory phenotype in psychosis, particularly in monocytes, but this area is understudied. Furthermore, changes to immune cell markers across the course of the illness and the relationship with measures of psychopathology and with treatment are largely unexplored. Some of these gaps will be addressed with particular focus on the Janus kinase (JAK) – signal transducer and activator of transcription 1 (STAT1) signaling pathway.

1.3.2 The JAK-STAT1 pathway

JAK-STAT1 signaling in immune cells, historically understood in relation to its antiviral functions, is now known to be critical for the orchestration of multiple aspects immunity and inflammation (Hu and Ivashkiv 2009). Type I (α/β/others) and II (γ only) interferons (IFNs) play a pivotal role in activation of the JAK-STAT1 pathway (Schindler, Levy, and Decker 2007). While type I and II IFNs have some overlapping functions, IFN-γ mediated JAK-STAT1 signaling in particular is important for inflammatory cellular activity, particularly in myeloid cells such as monocytes and macrophages (Schroder et al. 2004). IFN-γ stimulation initiates a transcriptional program that upregulates the expression of genes involved in antigen presentation, antiviral functions, complement, chemotaxis and adhesion.

Additionally, there is crosstalk of JAK-STAT1 and NF-κB signaling such that expression of genes regulated by both transcription factors is greatly amplified when these pathways are jointly activated (Hu and Ivashkiv 2009). Advances in sequencing techniques which allow for the characterization of genome-wide transcription factor binding have demonstrated that following IFN-γ stimulation STAT1 binds to many regions of chromatin in addition to the promoters of genes that it transcriptionally regulates (Satoh and Tabunoki 2013). In monocytes and macrophages these include regulatory elements upstream of genes encoding proinflammatory cytokine such as IL-6, TNF-α and IL-12, and correspond with open

chromatin modifications and increased and prolonged recruitment of NF-κB, and a significantly increased response to the TLR4 ligand LPS (Qiao et al. 2013).

Thus, in addition to initiating a transcriptional program critical for many aspects of the immune response, IFN-γ mediated JAK-STAT1 signaling is understood to induce permissive epigenetic modifications that underlie the proinflammatory monocyte/macrophage cellular phenotype as well as restrictive modifications that inhibit an anti-inflammatory cellular phenotype (Qiao et al. 2016; Villarino, Kanno, and O'Shea 2017). IFN-γ is secreted by natural killer cells, important initial effectors in an innate immune response, and T-helper (Th) 1 cells, which primarily encourage proinflammatory responses to intracellular pathogens and are implicated in certain autoimmune disorders, as well as a range of other cell types (Schroder et al. 2004; Berger 2000). In addition to the described myeloid cell functions IFN-γ also induces Th1 cellular differentiation.

1.3.3 Project overview

This overarching aim of this thesis is to characterize alterations to peripheral immune cellular phenotype in individuals with psychosis, with primary focus on the IFN-γ mediated JAK-STAT1 pathway. In the first research chapter, markers of JAK-STAT1 pathway activity will be measured in PBMCs from participants with psychosis and alterations to this pathway will be assessed in relation to illness stage, duration and measures of symptomology. In the second research chapter, the impact of antipsychotics on the JAK-STAT1 pathway will be addressed by examining the relationship with treatment in the clinical sample and tested directly using immune cell models. In the third research chapter, monocytes will be isolated from a second cohort of participants with psychosis and alterations to the JAK-STAT1 signature will be assessed alongside other phenotype defining signatures using genome-wide transcriptomics.

THE JAK-STAT1 SIGNATURE IN PSYCHOSIS

2.1 Background

Altered serum cytokine expression is a well-documented phenomenon in psychotic disorders such as schizophrenia and bipolar disorder with psychosis (Goldsmith, Rapaport, and Miller 2016). Alongside genome-wide association studies, epidemiological and post-mortem data, these findings have precipitated the formation of multiple theories regarding the potential contribution of immune activation to psychotic illness development and exacerbation (B. J. Miller and Goldsmith 2016; Khandaker and Dantzer 2015; Bergink, Gibney, and Drexhage 2014). Conceptually, elevated levels of inflammatory cytokines points to activation of peripheral immune cells, yet a pathophysiological role in psychosis remains to be established. This is partly because until recently the brain was considered to be immune privileged, with minimal interaction of these two systems. However, data are emerging that demonstrate that peripheral immune cells along with the effectors they secrete play important roles in CNS functions both in health and disease (Filiano et al. 2016; Garré et al. 2017). For example, manipulation of the peripheral immune system in animal models results in neurobiological and behavioral changes relevant to psychiatric illness (B. J. Miller and Goldsmith 2016). Conversely, in clinical subjects pharmacological treatments that target the immune system show early promise for improving symptoms in psychotic disorders (Melbourne et al. 2017).

Thus, an understanding of the current activity of immune cells in psychosis and how activity relates to clinical presentation may shed light both on pathophysiology and potential treatment targets. While there is some consensus that proinflammatory cytokines, such as IL-6, TNF-α and interferon (IFN)-γ, are elevated in the serum in psychotic disorders, there are discrepancies amongst studies (Goldsmith, Rapaport, and Miller 2016; Upthegrove, Manzanares-Teson, and Barnes 2014; Capuzzi et al. 2017). This is particularly true for IFN-γ. The inconsistencies are partly a consequence of variability in clinical cohorts,

but also likely due to the unresolved source of serum cytokines. Cytokines may derive from multiple tissue and cellular sources, and assays often lack the sensitivity to adequately quantify expression. If the findings of altered cytokines in psychosis reflect functional alterations to cellular signaling activity, there should be measurable changes at the genomic level. Measuring a molecular signature, here mRNA expression, in an isolated tissue type such as PBMCs both resolves this issue of source specificity and allows for inference of intracellular signaling activity. Results from the few studies that have so far used this approach seem to support an proinflammatory signature in psychosis (Drexhage et al. 2010; Chase et al. 2015; X.-Q. Song et al. 2009). These include findings of increased expression of genes encoding cytokines, such as IL-6 and TNF, as well as other immune effector genes that indicate activation of the NF-κB signaling pathway, an important mediator of proinflammatory cellular activity.

Another critical cell signaling system that regulates proinflammatory activity is the JAK-STAT1 pathway. IFN-γ is a key soluble activator of the JAK-STAT1 pathway, orchestrating the induction of a type 1 immune response, characterized by activation of T-helper 1, natural killer and myeloid cells such as monocytes and macrophages. Activation of the JAK-STAT1 pathway by IFN-γ induces a transcriptional program of genes involved in antigen presentation, antiviral functions, the complement cascade, chemotaxis and cell adhesion (Schoenborn and Wilson 2007) as well inducing changes to the chromatin landscape that prime and sustain the myeloid cell proinflammatory phenotype and response to pathogenic and endogenous NF-κB activating ligands (Qiao et al. 2013) (Figure 1). Altered activity of the JAK-STAT1 pathway has been associated with multiple autoinflammatory and autoimmune disorders, and is being actively investigated as a potential treatment target in these conditions (Schoenborn and Wilson 2007). While immune activity is hypothesized to contribute to neuropsychiatric illness development and exacerbation, this signaling system has not been directly investigated in individuals with psychosis.

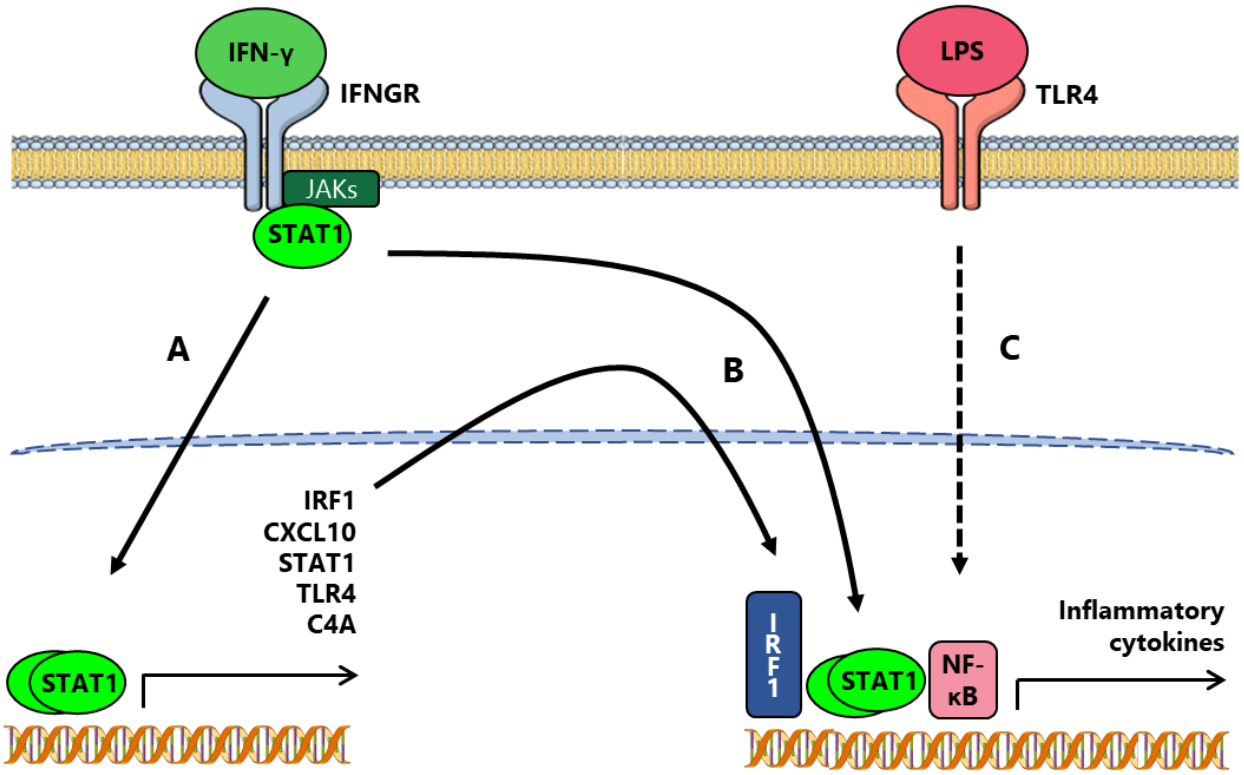


Figure 1. The JAK-STAT1 signaling pathway. A) IFN-γ activates the JAK-STAT1 signaling pathway by binding to its membrane receptor IFNGR. STAT1 is phosphorylated, forms a dimer and enters the nucleus where it initiates transcription of genes including IRF1, CXCL10, STAT1, TLR4 and C4A, as well as numerous other effector genes involved in functions such as antigen presentation, chemotaxis and adhesion. B) In addition to initiation of transcription, activation of the JAK-STAT1 pathway induces chromatin remodeling at proinflammatory gene promoters in myeloid cells such as monocytes and macrophages. C) Now, activation of the NF-κB pathway, for example by the bacterial ligand LPS binding to TLR4, results in a strong induction of transcription at these proinflammatory gene promoters.

To test the hypothesis that JAK-STAT1 signaling is elevated in peripheral blood cells from participants with psychosis, two strategies were used. Firstly, levels of phosphorylated STAT1 (pSTAT1) were measured in nuclear extracts of isolated PBMCs. Secondly, a panel of genes representative of genomic activation of this pathway were selected for measurement using quantitative real-time PCR (qRT-PCR): the gene for IFN-γ itself (IFNG), and the prototypical IFN-γ response genes CXCL10, IRF1, STAT1, and TLR4 (Satoh and Tabunoki 2013; Bosisio et al. 2002). In addition to these four canonical genes, complement component 4A (C4A) was also selected. Interestingly, apart from its inclusion in the IFN-γ-JAK-STAT1 response gene list, C4A has recently been implicated in schizophrenia (Sekar et al. 2016). The authors demonstrated that common structural variants of the C4 gene region which lead to the greatest mRNA expression of C4A also confer risk for developing schizophrenia. Additionally, C4A mRNA expression was elevated in multiple areas of post-mortem brain from individuals with schizophrenia compared to controls. As C4A is strongly induced by activation of the JAK-STAT1 pathway (Satoh and Tabunoki 2013), it raises the possibility that elevated expression in schizophrenia, in addition to the influence of gene structural variation, may be secondary to altered activation of this pathway. In the CNS, C4A is involved in synaptic pruning, which features strongly in neurodevelopmental theories of psychosis (Keshavan et al. 2014). Previous reports that measured serum C4 protein levels in psychotic disorders have had mixed results (Mayilyan, Weinberger, and Sim 2008; dos Santos Soria et al. 2012; Wadee et al. 2002), however these studies did not distinguish between C4A and C4B protein isoforms.

While particular immune markers appear to vary based on clinical status and have been associated with specific aspects of psychopathology, these relationships are understudied in psychosis (B. J. Miller and Goldsmith 2016; Goldsmith, Rapaport, and Miller 2016; Chase et al. 2016; Cabrera et al. 2016; Hope et al. 2015). An understanding of how immune alterations relate to illness stage, severity and symptomology will be critical for elucidating mechanisms underlying immune system involvement in psychosis pathophysiology. Thus, in addition to investigating overall differences between individuals with

psychosis and control participants, measures of JAK-STAT1 pathway activity were examined in relation to clinical characteristics including illness duration, acuity and measures of psychopathology.

2.2 Method

2.2.1 Participant Information and Clinical Measures

Participant demographic and clinical characteristics for the pSTAT1 protein and JAK-STAT1 transcriptional signature are outlined in Tables I and II. Subjects were recruited from the University of Illinois at Chicago (UIC) Medical Center and surrounding urban community including mental health clinics. The study was approved by the Institutional Review Board of the University of Illinois, and signed consent was obtained prior to the initiation of study procedures. Inclusion criteria for the study included persons who met SCID/DSM-IV diagnostic criteria for schizophrenia, bipolar disorder with psychosis or persons who did not meet the criteria for a psychiatric diagnosis and had no history of a psychiatric disorder. Exclusion criteria included treatment with valproic acid, carbamazepine, or clozapine in the previous 30 days, reported infections or autoimmune diseases, substance abuse/dependency within the past 2 months, seizure disorders, pregnancy and neurological conditions. Diagnosis was established by the clinical and research team using the Structured Clinical Interview for DSM Disorders (SCID-IVtr) (First et al. 2002) and available collateral information. For the pSTAT1 protein sample, all participants with psychosis met the DSM-IV diagnostic criteria for schizophrenia, and for the JAK-STAT1 transcriptional signature 64 participants with psychosis met the diagnostic criteria for schizophrenia and 25 for bipolar disorder with psychosis. The clinical samples primarily consisted of persons with current, and often chronic and persistent psychotic symptoms.

Clinical symptomology was measured using the PANSS (Kay, Fiszbein, and Opler 1987). The PANSS consists of 30 items scored along a continuum of severity between 1 (asymptomatic) to 7 (extreme symptom severity). Scores were calculated for three-factors assessing positive symptoms (delusions,

conceptual disorganization, hallucinatory behavior, excitement, grandiosity, suspiciousness/persecution, hostility), negative symptoms (blunted affect, emotional withdrawal, poor rapport, passive/apathetic social withdrawal, lack of spontaneity and flow of conversation, active social avoidance), and general psychopathology.

			Control	Psychosis (Schizophrenia)	pSTAT1 Group	
					Low	High
Total (n)			13	22	13	9
Age (M ± SD)			38.08 ± 11.95	38.32 ± 12.08	39.5 ± 12.5	38.2 ± 11.9
Sex*	Female (n)		8	5	3	2
	Male (n)		5	17	10	7
Race	Caucasian, non-Hispanic (n)		1	1	0	1
	Black, non-Hispanic (n)		8	18	10	8
	Asian or other Pacific Islander (n)		2	0	0	0
	Hispanic (n)		2	3	3	0
BMI (M ± SD)			29.85 ± 7.55	33.50 ± 9.00	35.1 ± 8.6	31.2 ± 9.6
Antipsychotic Use		Yes (n)	0	21	13	8
		No (n)	13	1	0	1
Illness Status		Inpatient (n)	NA	16	8	8
		Outpatient (n)	NA	6	5	1
Illness Duration (M ± SD)			NA	20.42 ± 13.12	21.08 ± 12.61	19.29 ± 14.91
PANSS Scores**##		Positive (M ± SD)	7.54 ± .78	29.23 ± 4.94	29.00 ± 5.32	21.7 ± 11.33
		Negative (M ± SD)	7.62 ± .96	23.95 ± 5.59	24.23 ± 5.40	23.56 ± 6.17
		General (M ± SD)	19.85 ± 3.31	51.32 ± 10.11	51.31 ± 12.30	51.33 ± 6.42

Table I. Participant demographic and clinical characteristics: pSTAT1 ELISA. M=mean, SD=standard deviation. *p<.05, **p<.001 psychosis compared with controls, ##p<.001 pSTAT1 groups and controls

		Control	Psychosis	Illness Status Groups		Illness Duration Groups		
				Inpatient	Outpatient	Short	Medium	Long
Total (n)		44	89	33	56	42	24	15
Age (M ± SD)##~~		38.77 ± 12.82	40.88 ± 13.14	30.33 ± 9.39	47.09 ± 10.90	32.33 ± 10.13	47.08 ± 7.34	57.53 ± 3.60
Sex#	Female (n)	25	37	9	28	19	8	6
	Male (n)	19	52	24	28	23	16	9
Race**## ~~	Caucasian, non-Hispanic (n)	12	9	5	9	7	0	2
	Black, non-Hispanic (n)	18	67	45	67	26	22	12
	Asian or other Pacific Islander (n)	10	2	2	2	2	0	0
	Hispanic (n)	4	11	7	4	7	2	1
BMI (M ± SD)*#		28.63 ± 7.96	31.66 ± 8.39	28.63 ± 7.96	33.53 ± 8.21	31.30 ± 9.07	32.50 ± 6.11	30.72 ± 10.36
Illness Duration (M ± SD)^		NA	19.96 ± 14.42	10.59 ± 11.31	24.65 ± 13.57	7.83 ± 5.52	27.58 ± 4.57	41.73 ± 3.99
Illness Status	Inpatient (n)	NA	33	33	0	22	4	1
	Outpatient (n)	NA	56	0	56	20	20	14
PANSS Scores**##~~	Positive (M ± SD)	7.68 ± 1.36	24.40 ± 6.66	29.27 ± 4.12	21.54 ± 6.20	25.05 ± 5.93	24.21 ± 7.09	22.47 ± 6.44
	Negative (M ± SD)	7.80 ± 1.84	19.91 ± 7.64	24.15 ± 7.90	17.41 ± 6.32	20.24 ± 8.02	20.96 ± 7.53	16.67 ± 6.42
	General (M ± SD)	20.09 ± 4.50	42.79 ± 10.48	50.09 ± 8.25	38.48 ± 9.22	43.60 ± 10.74	43.13 ± 10.60	38.87 ± 6.83

Table II. Participant demographic and clinical characteristics: JAK-STAT1 transcriptional signature. M=mean, SD=standard deviation. *p<.05 **p<.001 psychosis compared with controls, #p<.05 ##p<.001 illness status groups and controls, ^^p<.001 inpatient compared with outpatients, ~~p<.001 illness duration groups and controls

2.2.2 Sample Collection and Processing

Blood was collected from all participants in the early morning, via sterile venipuncture into a tube with 0.5M EDTA (Invitrogen). PBMCs were isolated using Ficoll-Paque (GE Healthcare) density gradient centrifugation.

2.2.3 Protein Extraction and pSTAT1 ELISA

Nuclear proteins were extracted by homogenizing cells in phosphate buffered saline/PMSF followed by incubation with hypotonic lysis buffer, resuspension in H_2SO_4, precipitation with TCA and washing with acetone. Protein levels were quantified using the Bradford method with bovine serum albumin. Levels of phosphorylated STAT1 at tyrosine 701 were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Abcam #126456). Levels of total histone 3 (H3) protein were measured (ActiveMotif #53110) for normalization.

2.2.4 RNA extraction, cDNA synthesis and qRT-PCR

RNA was extracted using TRIzol reagent (Invitrogen) and DNase (Ambion) treated to remove any contaminating genomic DNA. RNA was reverse transcribed using the Applied Biosystems High Capacity Archive Kit. Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) was used for detection of PCR product and mixtures were run on a Thermo Scientific PikoReal PCR machine. mRNA expression values were calculated relative to the mean of the housekeeping genes GAPDH and ACTB. Primers were designed using NCBI primer-BLAST and synthesized by Integrated DNA Technologies. Primer sequences are listed in Table III.

	Forward Primer	Reverse Primer
IFNG	5’-TCTTTTGGATGCTCTGGTCA-3’	5’-TTCAGCTCTGCATCGTTTTG-3’
CXCL10	5’- ATTTGCTGCCTTATCTTTCTG-3’	5’- TCTCACCCTTCTTTTTCATTGTAG-3’
IRF1	5’- ATGAGACCCTGGCTAGAG-3’	5’-AAGCATCCGGTACACTCG-3’
STAT1	5’-GCCAAAGGAAGCACCAGAGCCAAT-3’	5’-AGGAGACATGGGGAGCAGGTTGT-3’
TLR4	5’-TGGAAGTTGAACGAATGGAATGTG-3’	5’-ACCAGAACTGCTACAACAGATACT-3’
C4A	5’-GGCTCACAGCCTTTGTGTTG-3’	5’-CCCTGCATGCTCCTGTCTAA-3’
GAPDH	5’-CGAGATCCCTCCAAAATCAA-3’	5’-TTCACACCCATGACGAACAT-3’
ACTB	5’-TGAAGGTAGTTTCGTGGATGC-3’	5’-TCCCTGGAGAAGAGCTACGA-3’

Table III. Primer sequences for qRT-PCR experiments.

2.2.5 Statistical Analyses

SPSS (version 24.0 for Windows) was used for all statistical analyses. A probability level of $p<0.05$ was the criterion used to achieve statistical significance. Group differences for demographic and clinical data were analyzed by using Chi Square tests, independent samples t-tests and one-way analysis of variance (ANOVA). For ANOVA that yielded significant results, Tukey post hoc tests or planned comparisons were used to identify significant pair-wise group differences. For the pSTAT1 results, bimodality was tested assuming mixtures of normal distributions (Holzmann and Vollmer 2008; J. Chen and Li 2009). Spearman correlations were used to determine associations between mRNA relative expression levels, demographic variables and clinical metrics. Based on the overall pattern of gene expression for the selected genes a composite JAK-STAT1 signature score was computed for each participant. Composite scores are often used in the biomedical sciences to represent a biological process, such as activation of intracellular signaling pathways (Berglund, Welsh, and Eschrich 2017). The JAK-STAT1 composite score was calculated for each gene expression data point followed by averaging the z-scores of the five genes (IFNG, STAT1, IRF1, TLR4 and CXCL10) for each participant (M. Song et al. 2013). Lastly, a 3-stage hierarchical multiple linear regression model was performed to predict the JAK-STAT1 composite signature scores, examining

specifically the relative contribution of individual descriptive variables, clinical symptomology, duration of illness and illness acuity in participants with psychosis (schizophrenia and bipolar disorder with psychosis).

2.3 Results

2.3.1 Demographics – ELISA

There were no significant differences when comparing controls and participants with psychosis (schizophrenia) for demographic characteristics including age, race and body mass index (BMI), however, there were sex differences between the two groups ($\chi^2(1)=5.27$, p=.02). Control, schizophrenia low pSTAT1 and schizophrenia high pSTAT1 groups demonstrated no differences in relation to age, sex, race or BMI. Illness status and duration were not significantly different between the schizophrenia low pSTAT1 and high pSTAT1 groups. PANSS Positive (*F(2,32)*=118.91, p<.001), Negative (*F(2,32)*=52.50, p<.001, and General (*F(2,32)*=56.84, p<.001) subscales were significantly different between the three groups, with lower scores in controls (p<.001 for all comparisons) compared to participants with schizophrenia, but no differences between the low and high pSTAT1 groups. Furthermore, there was no significant association of age, sex, race, BMI, illness status, duration or PANSS scores and pSTAT1 levels.

2.3.2 Demographics – qRT-PCR

Within the JAK-STAT1 transcriptional signature participant sample, there was no significant difference between controls and participants with psychosis (schizophrenia and bipolar disorder with psychosis) for age or sex. Race ($\chi^2(3)=24.90$, p<.001), BMI ($t_{130}=-1.99$, p=.05) and PANSS Positive ($t_{131}=-16.47$, p<.001), Negative ($t_{131}=-10.35$, p<.001) and General ($t_{131}=-13.74$, p<.001) subscales were different between the controls and participants with psychosis. Between the control, inpatient and outpatient groups, there was a significant difference for age (*F(2,130)*=23.59, *p*<.001), with control participants older than inpatients (p=.004) but younger than outpatients (p=.001), and inpatients younger than outpatients

(p<.001). There were also group differences for sex (χ^2(2)=7.06, p=.03), race (χ^2(6)=29.58, p<.001) and BMI (*F(2,129)*=6.06, *p*=.003), as outpatients had a higher BMI than both controls (p=.009) and inpatients (p=.02). PANSS scores were also significantly different for Positive (*F(2,130)*=225.31, p<.001), Negative (*F(2,130)*=78.90, p<.001) and General (*F(2,130)*=151.05, p<.001) subscales for all post-hoc comparisons (p<.001). Inpatients compared to outpatients had significantly different illness durations (t_{79}=-4.6, p<.001). When comparing the control and illness duration groups there were significant differences for age (*F(3,121)*=26.45, *p*<.001) for all post-hoc comparisons (p<.001 – p=.02) and race (χ^2(9)=28.72, p=.001), but no differences for sex or BMI. PANSS scores were significantly different for Positive (*F(3,121)*=98.67, p<.001), Negative (*F(3,121)*=37.10, p<.001, and General (*F(3,121)*=66.97, p<.001) subscales with differences between controls and all illness duration groups (p<.001) but no differences between illness duration groups.

Though BMI was different between groups, there was no correlation of BMI with mRNA expression of any of the JAK-STAT1 signature genes or C4A. Additionally, mRNA expression did not differ significantly by race for IFNG, STAT1, IRF1, TLR4, or C4A though there was a significant difference for CXCL10 (*F(3,122)*=3.27, p=.02), with increased expression in Caucasian participants compared to Black (p=.05) and Hispanic participants (p=.04). There was no difference in mRNA expression for any of the genes or JAK-STAT1 signature scores between male and female participants. However, there was an association of gene expression of IFNG (r_{130}=.30, p<.001), IRF1 (r_{130}=.20, p=.02) STAT1 (r_{129}=.31, p<.001), TLR4 (r_{130}=.19, p=.03) and JAK-STAT1 composite score (r_{130}=.28, p=.001), but not CXCL10 or C4A, with age. Breakdown of this association by presence of psychosis demonstrated an association of IFNG (r_{86}=.47, p<.001), IRF1 (r_{86}=.25, p=.02), STAT1 (r_{86}=.41, p<.001), TLR4 (r_{88}=.35, p=.001) and the JAK-STAT1 composite score (r_{87}=.45, p<.001), but not CXCL10 or C4A, with age in participants with psychosis. No associations with age were present in the control participants.

2.3.3 pSTAT1 levels in psychosis

There was no significant difference in pSTAT1 levels when comparing the control and schizophrenia groups. However, as seen in figure 2, the data suggested a bimodal distribution within the group of participants with schizophrenia, which testing for bimodality confirmed ($\mu1 = 0.015$, $\sigma1 = 0.124$; $\mu2 = 0.310$, $\sigma2 = 0.206$; $p<.001$). Participants with schizophrenia with a measurement above the $\mu2 = 0.310$ value were coded as having high pSTAT1 while the remainder of the schizophrenia sample was coded as having low pSTAT1 ($\mu2 < 0.310$). As noted in table I there was no significant difference in gender, race or age between groups. In the schizophrenia high pSTAT1 subgroup, levels of pSTAT1 were significantly higher than the control participants (Figure 2). These findings support the hypothesis of increased activation of the JAK-STAT1 signaling pathway in psychosis, at least in a subset of individuals.

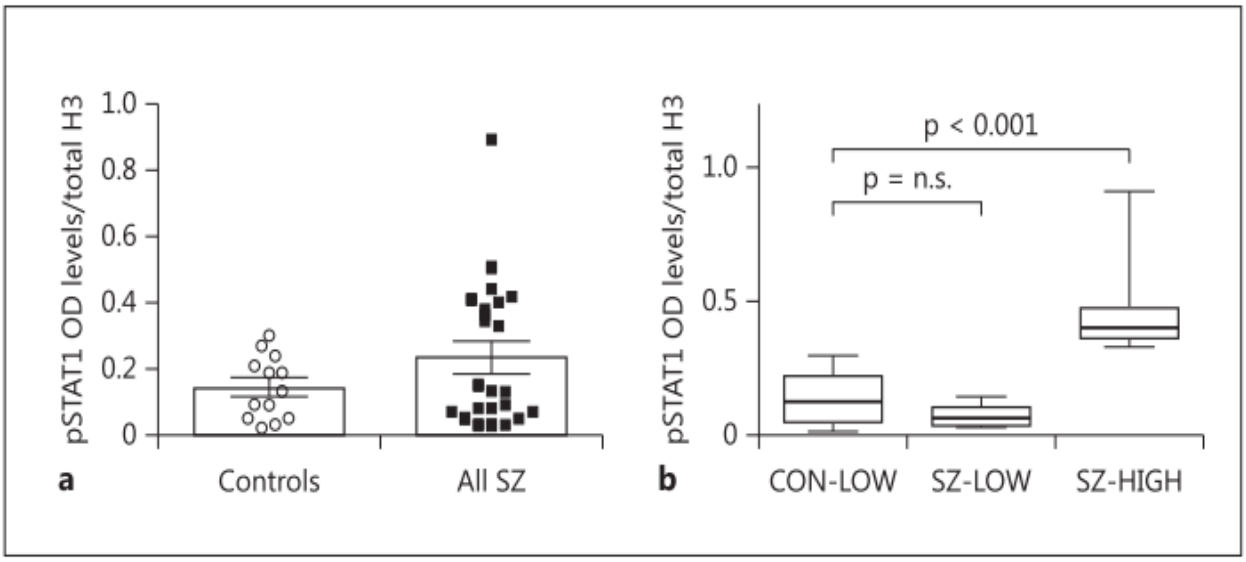


Figure 2. pSTAT1 levels in psychosis. A) pSTAT1 levels in PBMCs from participants with schizophrenia demonstrated a bimodal distribution. B) There was a subgroup of participants with schizophrenia with significantly increased pSTAT1 levels compared to control participants. Error bars represent standard error of the mean (SEM). Data published in Sharma et al, 2016.

2.3.4 Co-expression of JAK-STAT1 signature genes

mRNA expression of the JAK-STAT1 signature genes IFNG, CXCL10, IRF1, STAT1 and TLR4 were correlated with one another across the entire participant sample (Table IV). Expression of C4A was correlated with IRF1, STAT1, and TLR4, but not IFNG or CXCL10.

	IFNG	CXCL10	IRF1	STAT1	TLR4
CXCL10	.26 .003 124				
IRF1	.25 .005 128	.28 .002 125			
STAT1	.43 <.001 127	.31 .001 125	.51 <.001 128		
TLR4	.31 <.001 127	.24 .008 123	.29 .001 127	.55 <.001 126	
C4A	.08 .38 128	-.07 .44 124	.29 .001 128	.21 .02 127	.22 .01 127

Table IV. Correlation of JAK-STAT1 signature genes mRNA expression within all participants. Displayed as Spearman's rho, p-value, number of participants.

2.3.5 mRNA expression of JAK-STAT1 signature genes in psychosis

Next, JAK-STAT1 signature genes were compared between participants with psychosis and controls. Contrary to our expectation that the JAK-STAT1 transcriptional signature would be elevated in psychosis, mRNA expression of IFNG (t_{128}=2.71, p=.008), CXCL10 (t_{124}=3.69, p<.001) and IRF1 (t_{128}=3.06, p=.003) were significantly decreased in participants with psychosis (Figure 3). STAT1 (t_{127}=1.45, p=.15), TLR4 (t_{128}=0.50, p=.62) and C4A (t_{128}=-.26, p=.79) expression did not demonstrate a significant difference between participants with psychosis and controls.

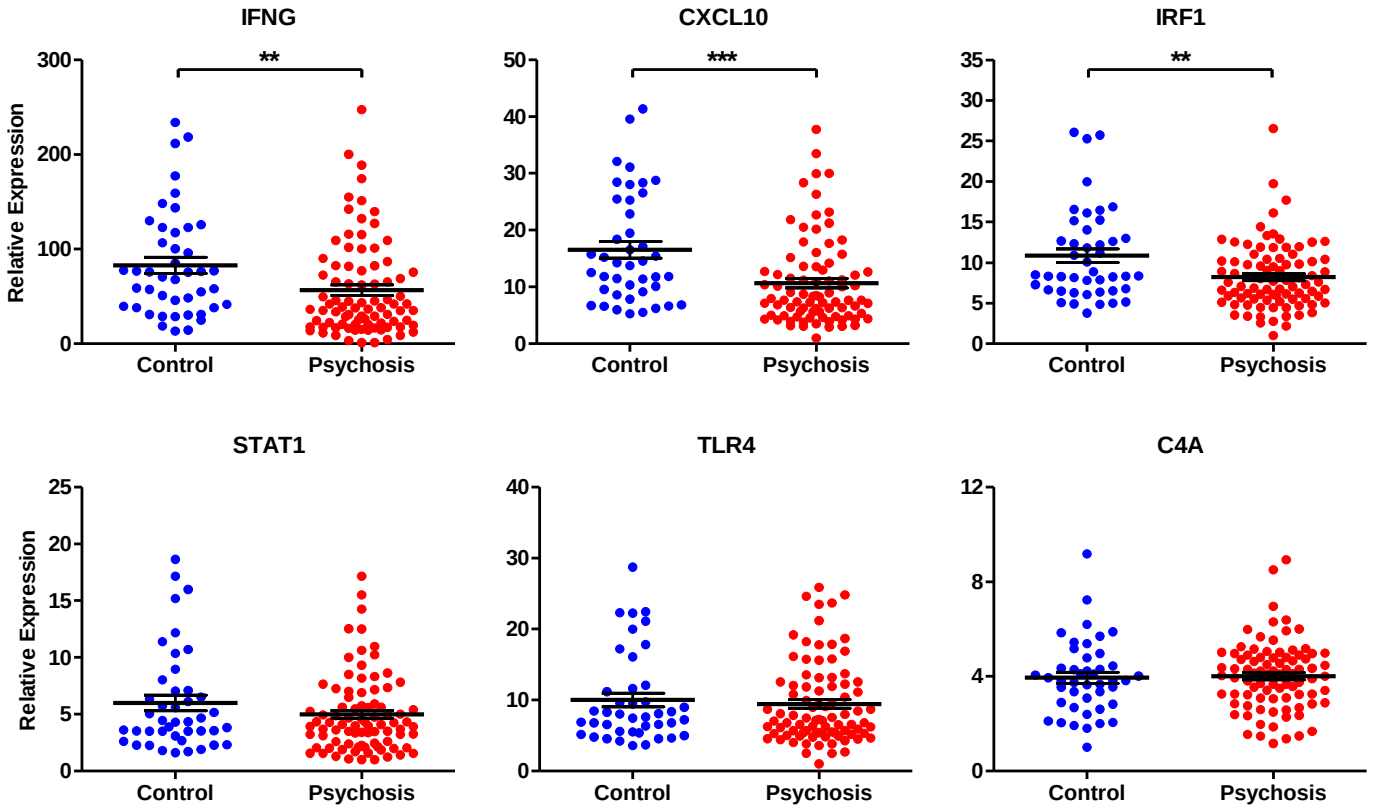


Figure 3. Relative mRNA expression of JAK-STAT1 signature genes displayed by diagnostic group. Statistically significant t-tests denoted by **p<.01, ***p<.001. Error bars represent SEM.

2.3.6 The JAK-STAT1 signature and illness duration

When the JAK-STAT1 transcriptional signature was analyzed in relation to illness duration in participants with psychosis, mRNA expression of IFNG (r_2=.48, p<.001, n=78), IRF1 (r_2=.32, p=.004, n=78), STAT1 (r_2=.34, p=.003, n=78), and TLR4 (r_2=.25, p=.03, n=80) demonstrated a positive correlation with duration of illness (onset of psychosis to time of blood draw), but this relationship was not statistically significant for CXCL10 (r_2=.21, p=.07, n=76) or C4A (r_2=.10, p=.35, n=78).

In order to assess the association of illness duration and JAK-STAT1 signature gene expression in relation to control participants, illness duration was converted to a categorical variable. Participants with psychosis were split into three groups based on years since psychosis onset: 0-17 years (n=42), 18-34 years (n=24), and 35-51 years (n=15). Three planned comparisons were made to determine differences in JAK-STAT1 signature gene expression in controls versus the short illness duration group, controls versus the long illness duration group and the short versus long illness duration groups. There was a significant difference in mRNA expression between controls and participants with an illness duration of 0-17 years for IFNG, CXCL10, IRF1 and STAT1 but this was not significantly different for TLR4 or C4A (Figure 4). mRNA expression of all genes except for C4A demonstrated a significant difference when comparing participants with a short versus long illness duration. Furthermore, there were no significant differences when comparing control participant mRNA expression with the longest illness duration group of 35-51 years for any gene examined.

Overall, these data suggest that expression of the canonical JAK-STAT1 signature genes is suppressed early in illness course and increases over the duration of illness. Unlike the other five genes, expression of C4A did not change with illness duration.

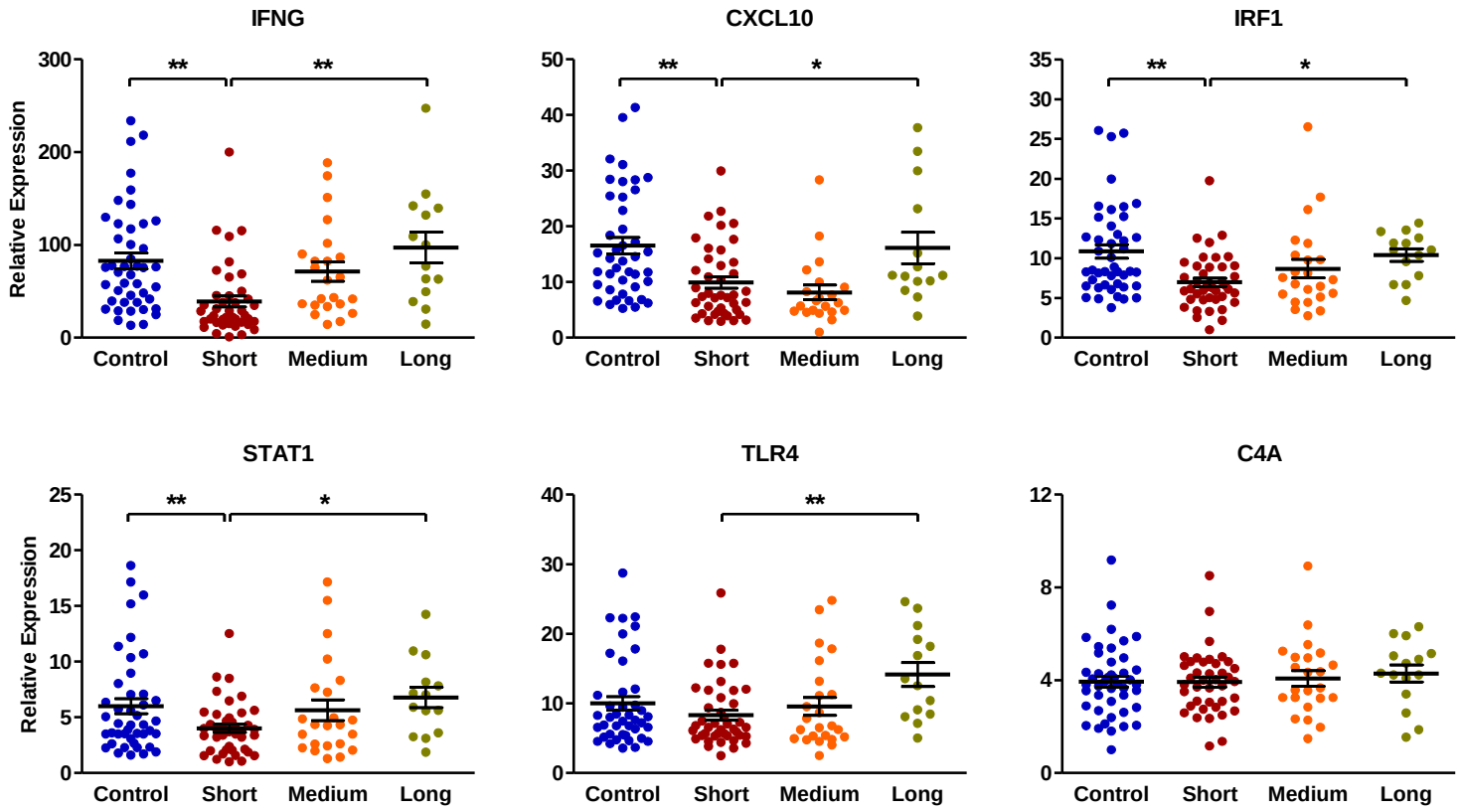


Figure 4. Relative mRNA expression of JAK-STAT1 signature genes displayed by illness duration group. L to R: Control - control participants; Short - 0-17 year illness duration; Medium: 18-34 year illness duration; Long: 35-51 year illness duration. Statistically significant planned comparisons denoted by *p<.05, **p<.01. Error bars represent SEM.

2.3.7 Relationship of the JAK-STAT1 signature and illness acuity

When we compared the JAK-STAT1 transcriptional signature genes in these high versus low illness acuity groups we found that mRNA expression of IFNG, CXCL10, IRF1 and STAT1 were decreased in inpatients compared to controls (Figure 5). IFNG, STAT1 and TLR4 expression were decreased in inpatients compared with outpatients, whereas CXCL10 and IRF1 mRNA expression was decreased in both inpatients and outpatients compared to controls. Thus, high illness acuity (inpatient status) is associated with a suppressed JAK-STAT1 transcriptional signature but results for low illness acuity (outpatient status) were more varied. Once again, C4A does not appear to vary in the same manner as the other JAK-STAT1 signature genes, demonstrating no difference in expression between the inpatient group and controls or an effect of illness acuity in participants with psychosis.

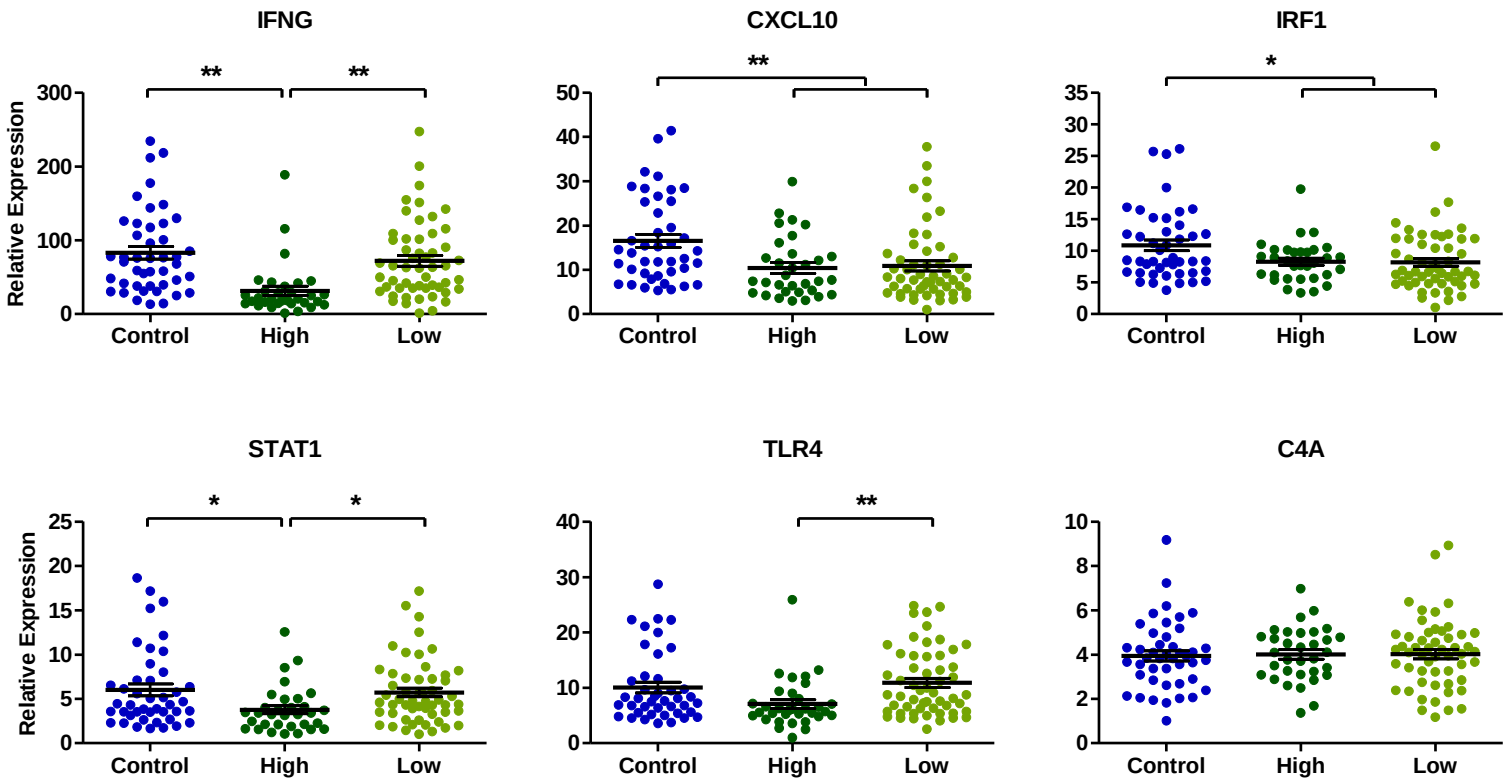


Figure 5. Relative mRNA expression of JAK-STAT1 signatures genes displayed by illness acuity group. Statistically significant post-hoc comparisons denoted by*p<.05, **p<.01. Error bars represent SEM.

2.3.8 Association of JAK-STAT1 signature genes with psychopathology

Grouping by inpatient outpatient status is one strategy with which to determine illness acuity. Additionally, the presence and severity of specific symptom domains can be quantified using the PANSS. While PANSS scores did not significantly vary with illness duration, scores were greater in the high illness acuity (inpatient) group compared to low illness acuity (outpatient) group (t_{87}=6.39, p<.001). PANSS scores were first used to examine for a relationship of JAK-STAT1 mediated gene expression with measures of psychopathology on a continuum from control participants to those with a diagnosis of psychosis. IFNG (n=130) expression demonstrated a negative correlation with PANSS Positive (r_2=-.342, p<.001), Negative (r_2=-.321, p=<.001) and General (r_2=-.347, p<.001) subscales, as did CXCL10 (n=126) with PANSS Positive (r_2=-.258, p=.004), Negative (r_2=-.248, p=.005) and General (r_2=-.256, p=.004) subscales. However, expression levels of IRF1, STAT1, TLR4 and C4A were not correlated with these measures of psychopathology.

When the same analyses was conducted in only the participants with psychosis, there was a negative correlation of IFNG (n=86) expression with PANSS Positive (r_2=-.327, p=.002), Negative (r_2=-.284, p=.008) and General (r_2=-.312, p=.003) subscales, and a positive correlation of C4A expression with the PANSS Positive (r_2=.222, p=.04) subscale but no association with the Negative or General subscales. Expression levels of CXCL10, IRF1, STAT1 and TLR4 were not associates with PANSS in participants with psychosis.

2.3.9 Interaction of illness duration and acuity in relation to the JAK-STAT1 signature

IFNG, CXCL10, IRF1, STAT1 and TLR4 demonstrated co-expression within the participant sample, and comparable group differences and associations in relation to clinical characteristics. Co-expression of these genes within the participant sample indicates shared signaling mechanisms and supports their use as a combined measure of cellular phenotype, in this case activation of the IFN-γ regulated JAK-STAT1

pathway. Thus, a composite JAK-STAT1 signature score, combining IFNG, CXCL10, IRF1, STAT1 and TLR4, was computed for each participant and used as an overall measure of transcriptional activity of the JAK-STAT1 pathway in subsequent analyses. C4A was not included in the JAK-STAT1 composite score due to a lack of consistent co-expression and relationship with clinical variables. The JAK-STAT1 composite score demonstrated a positive correlation with illness duration (r_2=.28, p=.001, n=80), and was significantly lower in the shortest illness duration group compared to controls and to the longest illness duration group (Figure 6). In relation to illness acuity, the JAK-STAT1 composite score was decreased in inpatients (high acuity) compared to controls and to the outpatient group (low acuity). Finally, across the entire sample the JAK-STAT1 composite score (n=130) demonstrated a negative correlation with PANSS Positive (r_2=-.262, p=.003), Negative (r_2=-.220, p=.012) and General (r_2=-.250, p=.004) subscales.

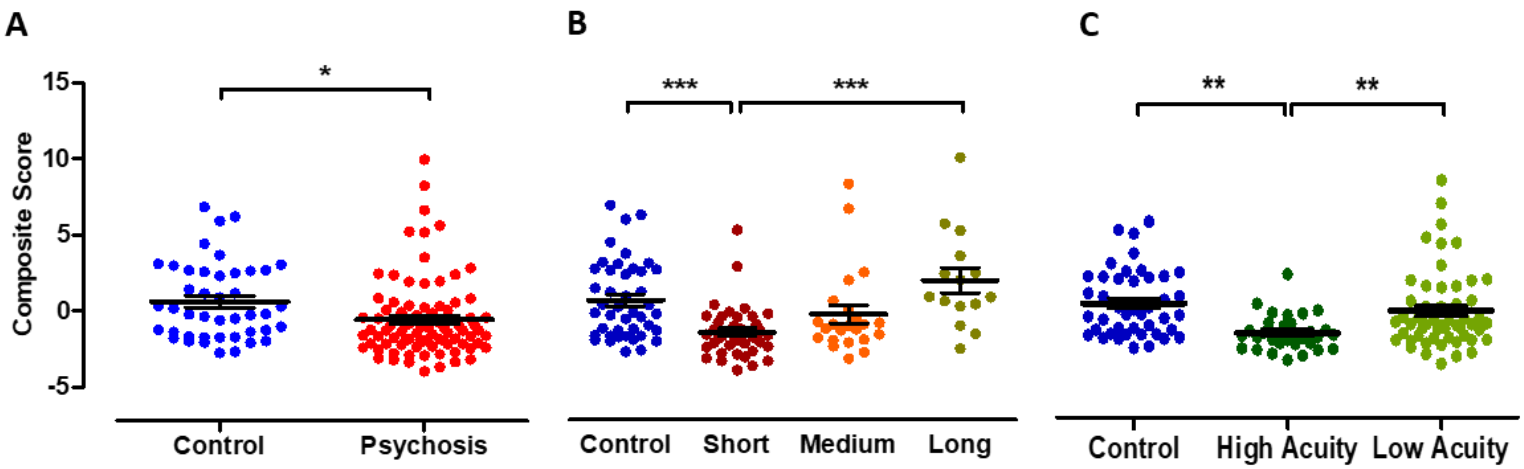


Figure 6. JAK-STAT composite score group differences. A) Comparison of participants with psychosis and controls. B) Illness duration subgroup comparisons. C) Illness acuity group comparisons. Statistically significant planned and post-hoc comparisons denoted by*p<.05, **p<.01, ***p<.001. Error bars represent SEM.

In order to determine whether participants demonstrated a suppressed JAK-STAT1 signature during periods of greater acuity, regardless of illness duration, the association of the JAK-STAT1 signature composite score with illness duration was assessed in each illness acuity group. Within the inpatient group alone (high acuity), there was no correlation of the JAK-STAT1 signature score with illness duration (Figure 7). When outpatients alone were selected, there was a strong correlation with illness duration, demonstrating that it is this group of participants driving the positive association of JAK-STAT1 signature expression with illness duration. Thus, the results demonstrate that both high illness acuity and shorter illness duration are associated with suppressed JAK-STAT1 signature gene expression. Whereas, in the presence of chronic and persistent but not acute psychopathology, there is greater expression of JAK-STAT1 signature genes.

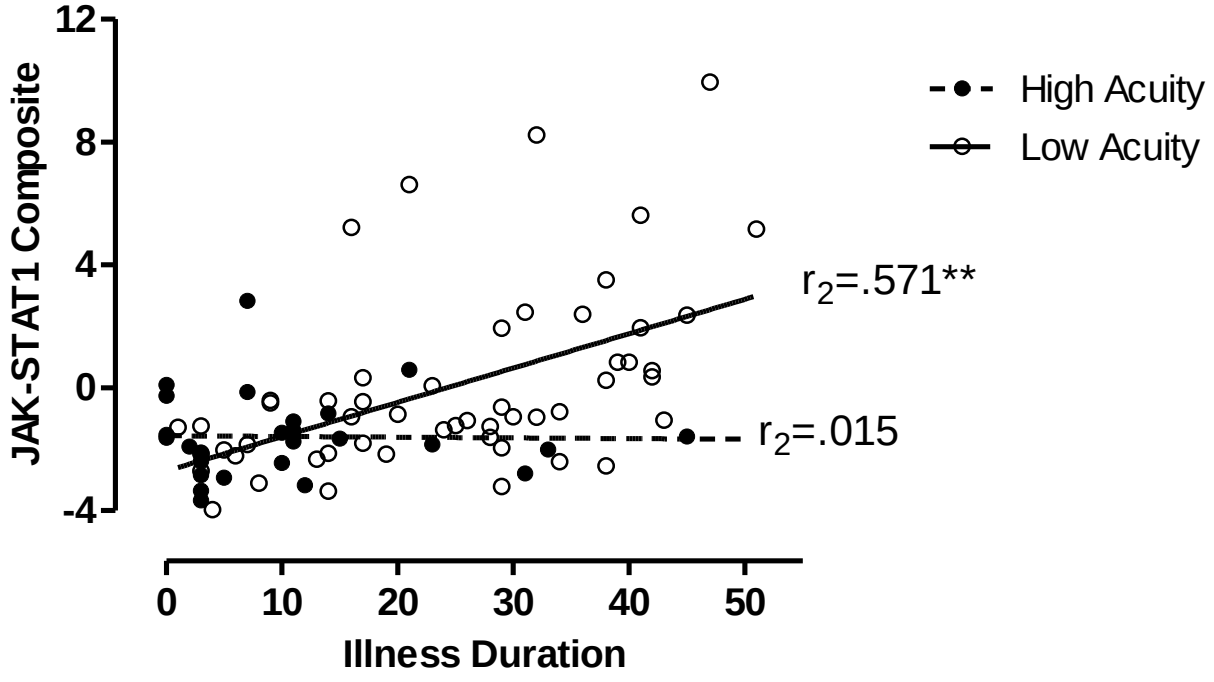


Figure 7. Correlation of the JAK-STAT1 composite score and illness duration for high and low illness acuity groups. **p<.01

2.3.10 Illness duration predicts JAK-STAT1 signature gene expression in psychosis

The findings outlined so far demonstrate that JAK-STAT1 signature gene expression varies with clinical measures including illness duration, acuity and psychopathology. To examine which contributing factors best explain variation in the JAK-STAT1 composite score, a hierarchical regression was used in the sample of participants with psychosis (n=79). Prior to conducing a hierarchical multiple regression, the relevant assumptions of this statistical analysis were tested (Tabachnick and Fidell 2007). The collinearity statistics (i.e., Tolerance and VIF) were all within accepted limits, thus the assumptions of multicollinearity were satisfied (Coakes 2005). An examination of the Mahalanobis distance scores indicated no multivariate outliers. Residual and scatter plots indicated the assumptions of normality, linearity and homoscedasticity were all satisfied (Hair et al. 1998). A three-stage hierarchical multiple regression was conducted with the JAK-STAT1 composite score as the dependent variable. Descriptive variables (age, sex, race and BMI) were entered at stage one of the regression. PANSS positive, negative and general subscales were entered at stage two, and illness duration and illness status were entered at stage three. The regression statistics are reported in Table V.

The hierarchical multiple regression revealed that at stage one, descriptive variables contributed significantly to the regression model, ($F(5,72)$=3.90, p=.004) accounting for 21% of the variance in JAK-STAT1 signature gene expression. Introducing the clinical symptomology variables at Stage 2 also significantly contributed to the regression model (F(8,69)=2.38, p=.02) accounting for less than 1% of the variance in JAK-STAT1 signature gene expression. Finally, in stage 3, the addition of illness duration and illness acuity explained an additional 7% of the variation and this change in R^2 was significant (F(10,67) = 2.71, p=.007). When considering the full model in stage 3, we observe that only illness duration (p=.01) significantly contributed to the prediction of alterations in JAK-STAT1 signature gene expression.

Thus, none of the demographic variables, including age, sex, race or BMI significantly predicted JAK-STAT1 signature gene expression in participants with psychosis when all independent variables were included. In section 3.5 high illness acuity was associated with decreased JAK-STAT1 signature expression compared to control, but when included in the full model in participants with psychosis only illness acuity did not significantly predict the JAK-STAT1 composite score. As highlighted, illness duration uniquely predicted JAK-STAT1 signature gene expression in participants with psychosis.

Stage	Variable	*B*	*SE*	β	*t*	*p*	*VIF*
1.	Age	-.007	.043	-.034	-.161	.873	4.238
	Sex	.953	.611	.176	1.559	.124	1.200
	Race	-.081	.210	-.041	-.368	.700	1.078
	BMI	-.029	.036	-.090	-.798	.427	1.206
2.	PANSS positive subscale	.003	.086	.007	.034	.973	3.884
	PANSS negative subscale	-.011	.060	-.029	-.175	.861	2.578
	PANSS general subscale	.018	.064	.065	.279	.781	5.101
3.	Illness status	.457	.908	.081	.503	.617	2.434
	Illness duration	**.091**	**.036**	**.494**	**2.539**	**.013**	**3.564**
Note. Stage 1 R^2= .213, p= .004; Stage 2 R^2= .216, p= .03; Stage 3 R^2= .288, p= .007.							

Table V. Hierarchical multiple regression stage 3: Prediction of JAK-STAT1 signature gene expression.

2.3.11 Diagnostic specificity

The approach used here to investigate the JAK-STAT1 signature and C4A mRNA expression in psychosis did not separate the sample based on traditional diagnostic categories of schizophrenia and bipolar disorder. This is due to questions regarding the utility of these categories in relation to underlying biological alterations in psychosis, and as per RDoC guidelines (Cuthbert and Insel 2010; Guloksuz and Os 2018). To confirm that these findings relate to psychosis and not a specific diagnosis, we used the JAK-STAT1 composite score and C4A mRNA expression to confirm our primary findings in participants with a diagnosis of schizophrenia and those with a diagnosis of bipolar disorder with psychosis.

The JAK-STAT1 composite score was significantly decreased in inpatients (M=-1.46, n=24) compared to controls (M=.62, p=.007, n=43) and to outpatients (M=.17, p=.05, n=39) in schizophrenia (*F(2,103)*=4.93, p=009), and in inpatients (M=-2.10, n=8) compared to controls (p=.008) in bipolar disorder with psychosis (*F(2,64)*=5.06, p=.009). With regards to illness duration in schizophrenia, there was a positive correlation of the JAK-STAT1 composite score and illness duration (r_{59}=.45, p<.001), and the JAK-STAT1 composite score was decreased in the short illness duration group (M=-1.60, n=30) compared to controls (M=.62, p=<.001, n=43), the short compared to long illness duration group (M=2.55, p<.001, n=11), and the control compared to long illness duration group (p=.02; *F(3,98)*=8.78, p<.001). In bipolar disorder with psychosis there was a positive correlation of the JAK-STAT1 composite score with illness duration (r_{24}=.60, p=.005, n=20). The ANOVA was not significant for the illness duration groups (p=.08), though there was a significant difference in the planned comparison between controls (M=.62) and the short illness duration group (M=-1.20, p=.03, n=11). The sample sizes for the medium (M=-1.20, n=5) and long (M=.15, n=4) illness duration groups were small for participants diagnosed with bipolar disorder with psychosis. Finally, the relationship of C4A with positive symptomology was present in both schizophrenia (r_{64}=.26, p=.04) and bipolar disorder with psychosis (r_{23}=.43, p=.04). Overall these findings demonstrate

that the presence of a relationship of the JAK-STAT1 transcriptional signature with illness duration and acuity, and of C4A mRNA expression with positive symptomology is not diagnosis dependent.

2.4 Discussion

The potential involvement of the immune system in the pathophysiology of psychotic disorders is receiving renewed interest in recent years. It has become apparent that both peripheral and central immune cells, which are predominantly considered for their role in host defense against pathogens, are also intricately involved in multiple aspects of CNS development and homeostasis. Much of the data to date indicates that psychosis may be associated with mild but chronic systemic inflammation. However, there is a paucity of data illustrating alterations to immune cellular phenotype and the manner in which these immune measures relate to clinical characteristics (B. J. Miller and Goldsmith 2016). Here, we chose to investigate the JAK-STAT1 signature as this signaling pathway plays a vital role in multiple aspects of immunity, including activation of myeloid cells to a proinflammatory state (Hu and Ivashkiv 2009; Rauch, Müller, and Decker 2013). In addition, we measured the expression of C4A alongside this signature as the literature highlights genetic risk and altered expression of this JAK-STAT1 regulated gene in psychosis.

2.4.1 pSTAT1 and the JAK-STAT1 transcriptional signature

We found that a subgroup of participants with psychosis demonstrated increased levels of pSTAT1 in peripheral blood cells. Furthermore, we demonstrated that expression of a panel of genes representative of IFN-γ mediated JAK-STAT1 activity indeed revealed alterations to this transcriptional signature in psychosis. Interestingly however, while we had hypothesized overall increased activity of the JAK-STAT1 pathway based on its known role in inflammation, we instead found that this signature was decreased in participants with psychosis who were early in illness duration and in those who had high illness acuity compared to controls. Our data further demonstrated that this initial suppression of JAK-STAT1 transcriptional activity reverses over the course of the illness, with gene expression increasing with

the number of years of illness duration. When we examined the relationship of JAK-STAT1 signature gene expression with illness duration in each illness acuity group we found that when acuity is high, regardless of the duration of the illness, the JAK-STAT1 signature is suppressed, whereas in the presence of chronic and persistent but not acute psychopathology, expression of JAK-STAT1 signature genes increases. Additionally, some but not all of the JAK-STAT1 signature genes were negatively correlated with measures of symptom presence and severity as measured by the PANSS. Only IFN-γ was associated with these psychopathology scores both across the entire sample and in the participants with psychosis. A hierarchical regression was used to determine the relative contribution of each of the described variables to JAK-STAT1 mediated transcriptional activity. Controlling first for demographic characteristics, we demonstrate that illness duration is the variable that predicts JAK-STAT1 signature gene expression in participants with psychosis. pSTAT1 levels did not mirror these changes seen for the JAK-STAT1 transcriptional signature in relation to clinical characteristics. This is likely due to the fact that pSTAT1 is not specific to IFN-γ mediated JAK-STAT1 signaling, phosphorylation of STAT1 occurs following binding of a number of ligands, including both type I and II IFNs, and various interleukins and growth factors (Banerjee et al. 2017; Sharma et al. 2016).

The variability seen in immune cell gene expression over the course of the illness is perhaps not surprising given the high degree of phenotypic plasticity these cells exhibit in response to microenvironmental perturbations. Indeed, some serum cytokine levels also seem to demonstrate alterations based on illness stage and have thus been designated state or trait markers (Goldsmith, Rapaport, and Miller 2016). However, the suppressed JAK-STAT1 signature we found early in illness and with greater acuity is unexpected given that these time points and states are often reported to have increased proinflammatory cytokines in serum. To our knowledge this is the first study to directly investigate the JAK-STAT1 transcriptional signature in psychosis, and thus it provides important insight into the functional state of circulating immune cells.

The decreased JAK-STAT1 signature expression observed early in illness and with increased illness acuity may be indicative of an immune state in which these circulating cells, particularly myeloid cells, are skewed more towards an anti-inflammatory or tissue remodeling phenotype. These phenotypic changes are induced by environmental signals that lead to epigenetic remodeling, including heterochromatin formation at gene promoters whose expression is characteristic of a proinflammatory cellular phenotype (Hoeksema and de Winther 2016). A decreased JAK-STAT1 signature is seen under a variety of conditions, the best characterized include the presence of the T-helper 2 cytokines IL-4 and IL-13, high levels of bacterial ligands or TNF-α, and glucocorticoids (O. M. Pena et al. 2011; Yona and Gordon 2007; Huber et al. 2017). Stress is an important consideration given that acute and chronic stressors are shown to impact immune function in animal models, and alterations to immune parameters are present in humans who are experiencing or have experienced stress (Wohleb et al. 2015). Further, stress and immune alterations are both present in and considered risk factors for psychosis (Fillman et al. 2014). While acute glucocorticoids limit overall inflammation, the effects of certain chronic stressors are more complex, and are frequently associated with glucocorticoid resistance and elevated proinflammatory activity.

Whether these peripheral immune cell changes are pathophysiologically relevant for psychosis or are an epiphenomenon of the illness remains to be established. The interaction between the peripheral immune system and CNS is an area of intense investigation, and routes of communication in both directions have been elucidated (Dantzer 2018). This allows for speculation that the immune stimulus is emanating from the center and will be registered in circulating cells that are equipped to survey these signals. Furthermore, immune stimuli that originate in the periphery, such as bacterial ligands and cytokines, are shown to affect the blood brain barrier, which demonstrates some alterations in psychosis (Pollak et al. 2017; Varatharaj and Galea 2017). Peripherally derived immune cells are found at these brains boundaries, including the meninges and choroid plexus (Korin et al. 2017) and play complex roles in CNS function. Interestingly, it was recently demonstrated that IFN-γ secreted by t-cells in the meninges

mediates normal social behavior in mice, and further that these social behavioral changes were mediated by the effects of IFN-γ on GABAergic interneuron firing in the prefrontal cortex (Filiano et al. 2016). Other studies highlight the importance of IFNs including IFN-γ for appropriate immune surveillance at parenchymal borders (Kunis et al. 2013). Thus, our findings of decreased expression of the IFN-γ-JAK-STAT1 transcriptional signature in circulating immune cells in psychosis in the earlier stages of illness and during periods of illness exacerbation are of theoretical interest. Here we focused our investigation on one gene signature; IFN-γ mediated JAK-STAT1 signaling. The results presented here highlight the temporal and functional complexity of immune alterations in psychosis. Future studies should investigate other relevant immune and physiological signatures simultaneously in order to paint a comprehensive picture of the dynamic changes to immune activity in relation to stage, severity and treatment of psychosis.

2.4.2 C4A expression and relationship with psychopathology

To our knowledge this is the first study to specifically measure C4A mRNA expression in PBMCs from participants with psychosis (Melbourne et al. 2018). This is important given recent findings relating C4A gene structural variants to schizophrenia risk and findings of elevated mRNA expression (Sekar et al. 2016), as well as the literature that indicates a critical role for C4 and other complement proteins in processes such as synaptic refinement and pruning in the developing central nervous system (Stevens et al. 2007; Sekar et al. 2016; Orsini et al. 2014). While we had hypothesized that previously reported elevations in C4A mRNA expression in schizophrenia might be a consequence of altered JAK-STAT1 activity in psychosis, C4A was not as strongly co-expressed as the selected JAK-STAT1 signature genes and did not demonstrate comparable diagnostic findings or relationship clinical variables and with measures of psychopathology. We therefore infer that our findings with C4A are not coordinated with overall JAK-STAT1 signaling. Additionally, compared with previous findings in the CNS (Sekar et al. 2016), we did not find a similar overall elevation in mRNA expression of C4A in psychosis (or schizophrenia alone), though

we find a noteworthy association with psychopathology. Across all participants with psychosis we demonstrated a positive association of C4A mRNA expression and positive symptomology using the PANSS, such that as C4A mRNA expression increased, so did positive symptom scores. Two potential explanations are that C4A expression varies with current positive symptom severity (a state marker), or that it is consistently elevated in individuals with a more severe psychosis presentation (a trait marker), potentially due to genetic variation. This requires further testing, particularly to directly investigate whether gene structural variation at the C4 locus is associated with severity of psychopathology.

RISPERIDONE EFFECTS ON THE JAK-STAT1 SIGNATURE

3.1 Background

As discussed in Chapters 1 and 2, individuals with psychosis are frequently shown to have alterations to immune parameters both peripherally and centrally. While much of the literature posits an overall systemic inflammatory state in psychosis based on elevated serum proinflammatory cytokines, the data outlined in Chapter 2 indicate a more nuanced alteration to immune cellular phenotype that is related to clinical characteristics including illness duration and acuity. The results showed that blood mononuclear cells exhibited a suppressed JAK-STAT1 transcriptional signature in participants with psychosis who were earlier in illness and in those currently experiencing a more acute psychotic manifestation. Furthermore, expression of this JAK-STAT1 signature increased over the duration of illness in participants who were chronically, but not acutely, symptomatic. This transcriptional signature was selected for investigation because elevated peripheral cytokine levels in psychosis suggest activation of circulating immune cells such as monocytes to a proinflammatory phenotype, a canonical function of IFN-γ signaling via the JAK-STAT1 pathway. Additionally, the stimuli, intracellular signaling components and genomic targets of the JAK-STAT1 pathway are well defined, particularly in myeloid cells such as monocytes and macrophages that are crucial for orchestration of the inflammatory response.

Myeloid cells display high phenotypic plasticity in response to homeostatic, modulatory and pathogen or damage-associated signals, and integration of local and systemic stimuli present in the cells environmental milieu results in a spectrum of functional states. In addition to endogenous stimuli, these cells are also sensitive to many pharmacological agents. Consequently, one critical factor to consider in relation to the alterations seen in circulating immune cells in participants with psychosis is medication use. Antipsychotic drugs are the mainstay pharmacotherapy for psychosis, and of the participants who

took part in the study outlined in Chapter 2, the majority of those with a psychiatric diagnosis reported current treatment with antipsychotic medications. Many studies have demonstrated immunomodulatory properties of antipsychotic drugs, however, whether treatment ultimately has anti-inflammatory or pro-inflammatory effects remains unresolved. In clinical populations meta-analyses have focused on serum cytokines, with some concluding that antipsychotic treatment has overall anti-inflammatory effects, whereas others find no overall change in proinflammatory cytokines and a decrease in anti-inflammatory cytokines (B. J. Miller et al. 2011; Witte et al. 2014). Effects appear to depend largely on the particular antipsychotic drug investigated, the context in which they are tested and the immune parameters measured (Melbourne et al. 2017). The impact of antipsychotics on JAK-STAT1 signaling specifically has received minimal attention.

Medication effects on JAK-STAT1 signature gene expression can be assessed in the clinical sample by examining differences between participants who report no current treatment with antipsychotic medications and those who report antipsychotic medication treatment. The obvious advantages of this approach are that these samples come from individuals living with psychosis, and changes to the JAK-STAT1 signature can be assessed in relation to both antipsychotic treatment and clinical variables of interest such as illness duration. However, observational studies do not allow an inference of cause and effect. In-vitro cell culture models are a complementary technique that allow for investigation of the direct effects of medication on cellular activity under controlled experimental conditions. An additional advantage of using a monocyte cell line is that these cells can be further differentiated to a state more closely resembling tissue macrophages, which are rarely accessible in living subjects. In the body, monocytes perform effector functions in circulation and following recruitment to tissues where they can further differentiate to macrophages (Italiani and Boraschi 2014). Some tissue macrophages are thus derived from circulating monocytes; however, others are replenished by embryologically-derived local progenitors. In the CNS, microglia are the only myeloid cell residing within the brain parenchyma, but

perivascular, meningeal and choroid plexus macrophages, as well as monocytes, are found at the parenchymal borders (Prinz and Priller 2017; Herz et al. 2017). These cells are of mixed lineage, but all play vital roles in CNS homeostatic functions as well as following injury or illness and thus are likely involved in the pathophysiology of neuropsychiatric illnesses (Herz et al. 2017). For example, microglia provide trophic support to neurons and mediate processes such as synaptic pruning, perivascular macrophages maintain the integrity of the blood brain barrier, and circulating monocytes are responsible for learning and memory deficits after viral infection (Herz et al. 2017; Garré et al. 2017).

Monocytes and macrophages, both peripheral and central, exist across a spectrum of phenotypes that are dependent on the combination of stimuli in the cells' local environment and prior exposures (Mitchell, Roediger, and Weninger 2014). Phenotypes range from pro-inflammatory to anti-inflammatory and tissue remodeling functional extremes. These extremes are often termed M1 and M2 respectively, and while an over-simplification of the reality of activation states in-vivo (Glass and Natoli 2015) they provide a useful and well-characterized cell model with which to test the effects of antipsychotic medication (Figure 8). The M1 proinflammatory phenotype is induced following JAK-STAT1 pathway activation by IFN-γ, and fully polarized with an additional NF-κB activating inflammatory stimulus such as the bacterial ligand LPS (Das et al. 2018). As such, the M1 phenotype is characterized by the expression of JAK-STAT1 signature genes such as CXCL10, IRF1 and STAT1 as well as proinflammatory cytokines. The M2 phenotype is subdivided based on the polarizing stimulus used. We focus here on the M2 'tolerized' ($M2^{tol}$) phenotype induced by high levels of the NF-κB activating stimulus LPS (O. M. Pena et al. 2011; Porta et al. 2009). In this phenotype cells become refractory to inflammatory stimuli, and expression of many M1 characteristic genes are suppressed by repressive chromatin modifications, while expression of genes with anti-inflammatory and tissue remodeling functions are elevated. Blood monocytes, including the human THP-1 monocyte cell line, express dopamine and serotonin receptors and are responsive to antipsychotics in culture (Gaskill et al. 2012; M.-L. Chen et al. 2013). Results from in-vitro studies of the effects of

antipsychotics on myeloid cells vary similarly to clinical data, with some demonstrating decreases while other find increases in proinflammatory cytokines in monocyte and macrophage cell lines (M.-L. Chen et al. 2013; Da Cruz Jung et al. 2016).

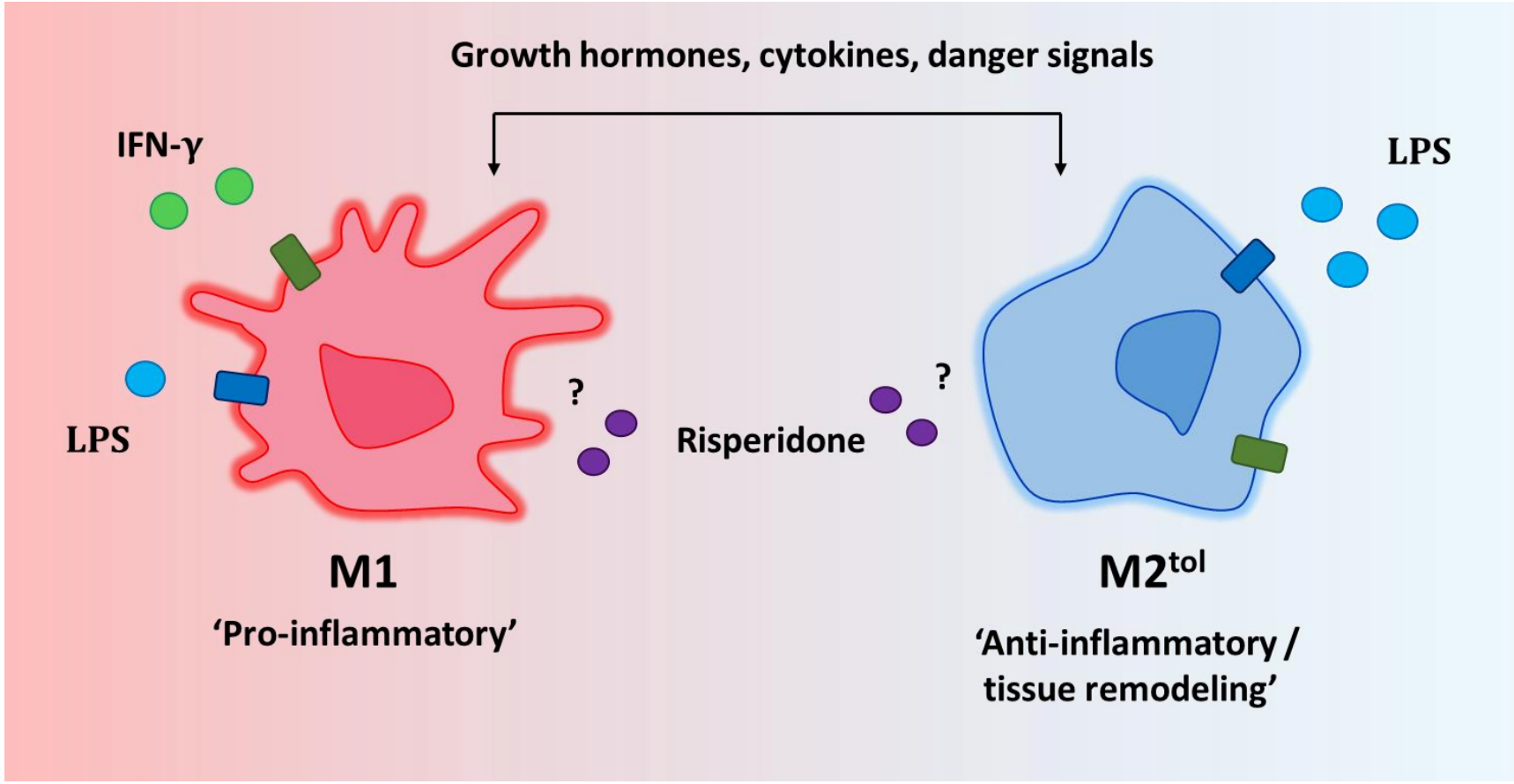


Figure 8. Myeloid cell M1 and $M2^{tol}$ phenotypes. Monocytes and macrophages display a spectrum of functional phenotypes, ranging from pro-inflammatory (M1) to anti-inflammatory and tissue remodeling (M2). M1 polarization is induced with IFN-γ and low-dose LPS. An M2 state can be induced with multiple polarizing stimuli, one of which is high-dose LPS leading to a tolerized M2 ($M2^{tol}$) phenotype. The effects of the presence of risperidone on these phenotypes will be investigated in the present study.

The objective of the present study, to investigate antipsychotic effects on the JAK-STAT1 signature in immune cells, was carried out using two approaches. Firstly, using the clinical sample we compared JAK-STAT1 signature gene expression in PBMCs from untreated versus treated participants using any antipsychotic medication. As risperidone was the most frequently prescribed medication in these antipsychotic treatment participants, a subsample of participants who reported risperidone monotherapy were selected for comparison with the untreated group. We further explored differences amongst these untreated versus risperidone treated participants in relation to illness duration. In the second approach, THP-1 cell culture models were used to determine the effects of risperidone treatment on the JAK-STAT1 transcriptional signature in monocytes and macrophages exposed to M1 and $M2^{tol}$ polarizing stimuli.

3.2 Methods

3.2.1 Clinical Measures

Participant recruitment and diagnostic information is detailed in Chapter 2. Demographic and clinical characteristics are outlined by antipsychotic status group in Table X. Information pertaining to antipsychotic medication treatment was collected from participants with psychosis at study recruitment. Participants who were included in this analysis reported the current antipsychotic treatment as follows: daily oral monotherapy with risperidone (n=15), quetiapine (n=7), ziprasidone (n=6), aripiprazole (n=6), lurasidone (n=5), olanzapine (n=5), paliperidone (n=2), haloperidol (n=2), fluphenazine (n=2), perphenazine (n=2), and iloperidone (n=1), monotherapy with aripiprazole long acting injectable (LAI; n=2), risperidone LAI (n=1), paliperidone LAI (n=1), or haloperidol LAI (n=1), treatment with more than one type of antipsychotic (n=9), or unknown (n=1). Monotherapy was defined as participants who reported current treatment with only one antipsychotic medication. A subsample of participants treated with risperidone monotherapy was selected for further analysis from the group of participants who reported treatment with any antipsychotic medication. The sample was selected by matching risperidone

treated participants optimally on mean and range of duration of illness with the untreated group (untreated M=17.82 years and range from illness onset to 42 years; risperidone monotherapy treated M=18.36 years and range from illness onset to 42 years).

		No Current Antipsychotic Treatment	Current Antipsychotic Treatment	
			Antipsychotic Treated	Risperidone Treated Subsample
Total (n)		11	68	11
Age (M ± SD)		38.82 ± 13.91	41.96 ± 12.95	43.27 ± 14.37
Female (n)		5	28	6
Male (n)		6	40	5
Race*#	Caucasian, non-Hispanic (n)	4	5	0
	Black, non-Hispanic (n)	5	54	10
	Asian or other Pacific Islander (n)	0	2	1
	Hispanic (n)	2	7	0
BMI (M ± SD)*#		26.02 ± 5.51	33.03 ± 8.45	34.05 ± 9.05
Illness Duration (M ± SD)		17.82 ± 16.32	20.40 ± 14.19	18.36 ± 15.28
Illness Status	Inpatient (n)	5	21	3
	Outpatient (n)	6	47	8
PANSS	Positive (M ± SD)	23.81 ± 4.31	24.53 ± 6.53	26.64 ± 5.33
	Negative (M ± SD)	16.09 ± 7.35	20.26 ± 7.18	20.82 ± 6.49
	General (M ± SD)	43.73 ± 7.46	42.25 ± 10.10	43.45 ± 7.03

Table VI. Participant demographic and clinical characteristics: antipsychotic treatment. *p<.05 comparison of no antipsychotic with antipsychotic treated group, #p<.05 comparison of no antipsychotic with risperidone treated subsample. M=mean, SD=standard deviation.

Illness duration was then used to split participants into the following low and high illness duration groups: untreated low duration (n=5, M=2.20 years, range from illness onset to 5 years), untreated high duration (n=6, M=30.83, range from 19 to 42 years), risperidone treated low duration (n=6, M=6.33, range from illness onset to 11 years) and risperidone treated high duration (n=5, M=32.80, range from 21 to 42 years). The JAK-STAT1 transcriptional signature composite score (the standardized combined mRNA expression of IFNG, CXCL10, IRF1, STAT1 and TLR4) was used as the measure of the JAK-STAT1 transcriptional signature for the clinical analysis, and only participants with data pertaining to illness duration were included. Here the terms 'untreated' and 'treated' are used to refer to treatment with antipsychotic medication, and do not include other types of medication or forms of treatment for psychosis.

3.2.2 THP-1 Monocyte Culture and Treatment

Human monocytic THP-1 cells (ATCC TIB-202) were maintained at 37^{o}C in a humidified incubator at 5% CO_2. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 0.1 mg/ml l-glutamine and 50 U/mL each of penicillin and streptomycin (all Invitrogen). THP-1 monocytes were plated at 10^6 cells per well using 6-well plates. Cells were stimulated with 10ng/µL recombinant IFN-γ (BD Pharmingen) for 7 hours to activate the JAK-STAT1 pathway. 10µM risperidone (Sigma-Aldrich) was added to the cell culture medium for the final hour. It has previously been shown that risperidone at this concentration is immunomodulatory but does not affect cell viability in human monocyte and murine macrophage cell lines, whereas higher concentrations demonstrated toxicity (M.-L. Chen et al. 2013; Schmidt et al. 2010). Appropriate vehicle controls were used for each condition.

3.2.3 Macrophage Differentiation, Polarization and Treatment

THP-1 cells were plated at 10^6 cells per well using 6-well plates. Resting macrophages (M0) were generated by treating THP-1 monocytes with 15nM phorbol 12-myristate-13 acetate (PMA; Sigma) for 24

hours to induce macrophage differentiation, followed by 24 hours rest in fresh media (Genin et al. 2015). The M1 proinflammatory phenotype was induced by treating M0 macrophages with 10ng/µL recombinant IFN-γ and low dose (1ng/mL) LPS (E. coli O111:B4; Sigma-Aldrich) for 24 hours. The $M2^{tol}$ phenotype was induced by treating M0 macrophages with a tolerizing dose (100ng/mL) of LPS for 24 hours (Murphy et al. 2015; O. M. Pena et al. 2011). M0 cells were treated with 10µM risperidone alongside these M1 and $M2^{tol}$ or vehicle polarizing stimuli. For each experiment cells were harvested, washed with phosphate buffered saline (Gibco), and pelleted by centrifugation prior to RNA extraction.

3.2.4 RNA Extraction and qRT-PCR

RNA extraction, cDNA synthesis and qRT-PCR for the cell culture experiments were carried out using the same methods previously outlined in Chapter 2. Three of the JAK-STAT1 signature genes used for the composite score, CXCL10, IRF1 and STAT1, were selected as a readout representative of activity of this pathway for the cell culture experiments. Primer sequences are listed in Chapter 2 Table III.

3.2.5 Statistical Analyses

Statistical analyses were carried out using SPSS for the clinical sample and GraphPad Prism for cell culture experiments. Demographic differences and medication effects between groups and in relation to the JAK-STAT1 composite score in the clinical sample were assessed using chi-squared tests, independent samples t-tests and ANOVA with post-hoc Tukey's. Bivariate correlations for the risperidone monotherapy analyses were carried out using Pearson's r after confirming that variables met the assumption of normality using the Shapiro-Wilk test. Spearman's rho was used for variables that did not meet the assumption of normality, which was the case for the JAK-STAT1 composite score in the group of participants treated with any antipsychotic medication. For the cell culture experiments, at least two biological and two technical replicates were used. Expression results for CXCL10 were calculated using 2^-Δct, and presented as relative expression, as this gene was not expressed under non-stimulated

conditions. Expression results for IRF-1 and STAT1 were calculated using 2^-ΔΔct (fold change relative to the unstimulated condition). Differences between conditions were assessed using one- and two-sample t-tests and ANOVA. Tukey post-hoc tests were used for ANOVA that yielded significant results.

3.3 Results

3.3.1 Demographics

Demographic and clinical characteristics are outlined by antipsychotic status group in Table VI. There was no significant difference in age (t_{77}=-.74, p=.46), sex (χ^2(1)=.071, p=.79), illness duration (t_{77}=-.55, p=.59), illness status (χ^2 (1)=.91, p=.34) or PANSS Positive (t_{77}=-.35, p=.73), Negative (t_{77}=-1.78 p=.08) or General psychopathology (t_{77}=.46, p=.64) subscales when comparing untreated with all antipsychotic treated participants. However, race (χ^2(3)=9.30, p=.03) and BMI (t_{76}=-2.54, p=.01) were different between the two groups. When comparing untreated participants with risperidone monotherapy treated participants there was no significant difference in age (t_{20}=-.74, p=.47), sex (χ^2(1)=.18, p=.67), illness duration (t_{20}=-.08, p=.94), illness status (χ^2(1)=.79, p=.38), or PANSS positive (t_{20}=-1.36, p=.19), negative (t_{20}=-1.60, p=.13), general (t_{20}=-.09, p=.93) subscales. Again, race (χ^2(3)=8.67, p=.03) and BMI were unmatched between the two groups (t_{19}=-2.42, p=.03). There were no significant associations of the JAK-STAT1 composite score with either race or BMI.

3.3.2 Antipsychotic medication and the JAK-STAT1 signature in psychosis

Medication use is a critical variable to consider when assessing alterations to immune function in clinical populations. It is possible that treatment with antipsychotics may contribute to the suppression of the JAK-STAT1 signature earlier in illness, or the increase in expression seen over the duration of illness. When comparing the JAK-STAT1 composite score in participants with psychosis who were untreated (M=-1.35, SD=1.73) and participants who reported treatment with any antipsychotic medication (M=-.36, SD=2.77) there was no significant difference between the groups (t_{77}=-1.14, p=.26) (Figure 9A).

Participants in this comparison group were treated with one or more of 12 different antipsychotic drugs, with risperidone being the most frequently prescribed antipsychotic. Given that immunomodulatory properties have been demonstrated to differ depending on the particular antipsychotic drug investigated, a subsample of participants treated with risperidone monotherapy were selected for further analyses. These participants were matched with the group of untreated participants based on illness duration, as this variable was demonstrated to predict JAK-STAT1 signature gene expression in the previous chapter. While the mean JAK-STAT1 composite score was greater in the risperidone treated subsample (M=-1.35, SD=1.73) compared to untreated participants (M=.70, SD=3.15), this difference was not statistically significant (t_{20}=-1.89, p=.07; Figure 9B).

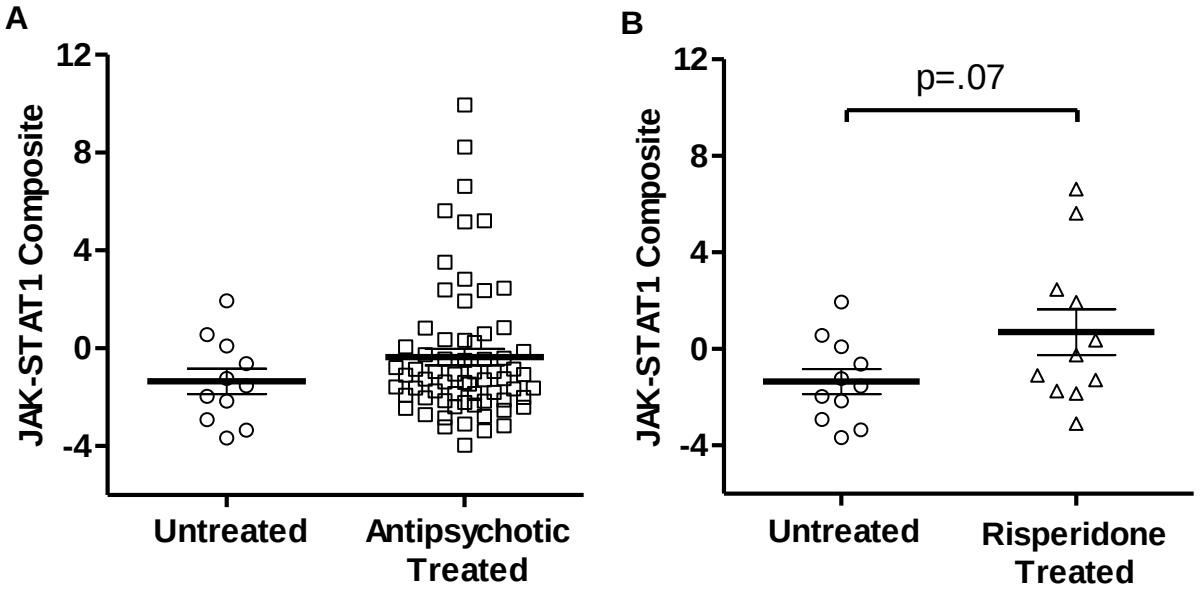


Figure 9. JAK-STAT1 signature score grouped by antipsychotic treatment. A) Untreated participants with psychosis compared to participants who reported treatment with any antipsychotic medication. B) Untreated participants with psychosis compared to the risperidone monotherapy treated subsample. Error bars represent SEM.

3.3.3 Risperidone treatment and the JAK-STAT1 signature in participants with psychosis

Untreated and risperidone monotherapy treated participants were split into low and high illness duration groups. Comparison of the JAK-STAT1 composite score between these groups revealed significant differences ($F(3,18)=10.84$, $p<.001$) (Figure 10). Post-hoc comparisons demonstrated that there was no difference in the JAK-STAT1 composite score between untreated participants with a low compared to high illness duration, or between untreated participants and treated participants both with a low illness duration. The treated participants with a high illness duration had an elevated JAK-STAT1 composite score compared to untreated participants with low illness duration ($p<.001$), untreated participants with a high illness duration ($p=.007$) and treated participants with a low illness duration ($p=.001$).

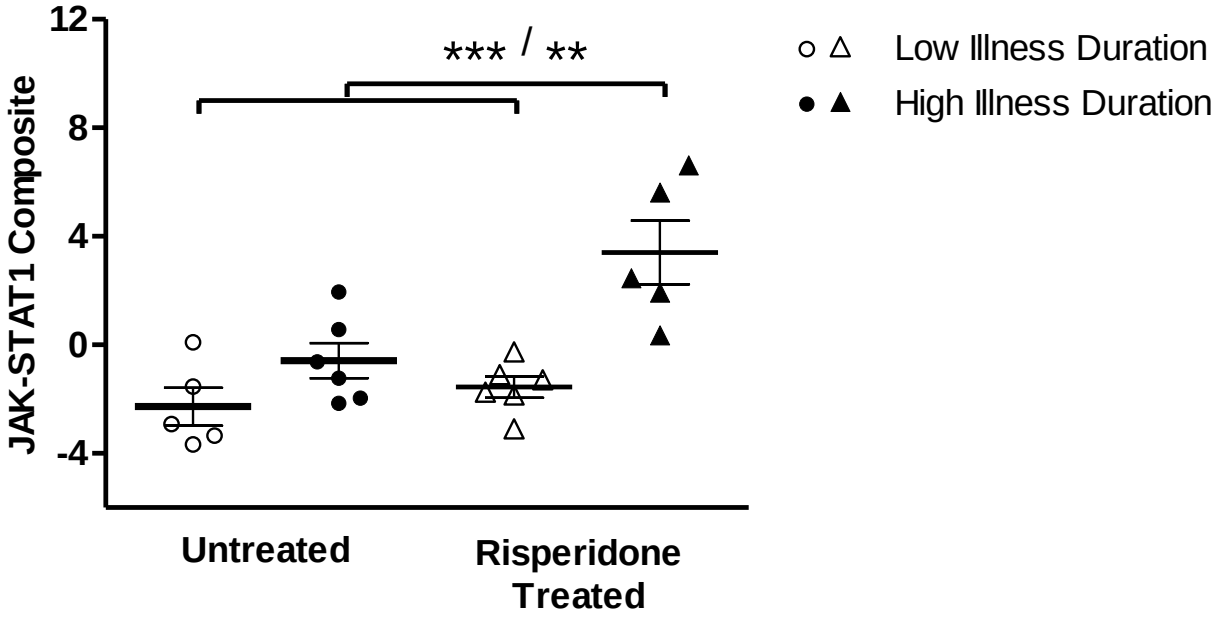


Figure 10. JAK-STAT1 signature score grouped by risperidone treatment and illness duration. Untreated participants compared to the risperidone monotherapy treated subsample grouped by low and high illness duration. ***p<.001, **p<.01. Error bars represent SEM.

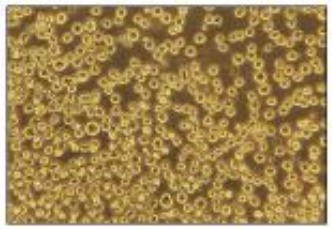
Thus, risperidone treated participants only demonstrated increased JAK-STAT1 signature gene expression if they had a longer illness duration. One possible interpretation is that the effects of risperidone treatment are only manifest after long-term antipsychotic use. Alternatively, it might be the case that risperidone has a different effect on immune cells in individuals with chronic illness. As these data are observational cause and effect cannot be established, however, these findings support the hypothesis that antipsychotics such as risperidone may contribute to some of the changes seen the in the JAK-STAT1 transcriptional signature. Interestingly, when the association of the JAK-STAT1 composite score and illness duration was tested, there was a significant positive correlation in both the untreated (r_{11}=.66, p=.03), and risperidone monotherapy treated (r_{11}=.64, p=.04) groups. Thus, the JAK-STAT1 signature gene expression was associated with illness duration regardless of antipsychotic treatment**,** but risperidone treated participants who had a longer illness duration had overall increased JAK-STAT1 signature expression compared to untreated participants.

3.3.4 THP-1 cell model: morphology and JAK-STAT1 signature gene expression

To determine the direct effects of risperidone on JAK-STAT1 signature gene expression in monocytes and macrophages an in-vitro cellular assay was used. The expression of the JAK-STAT1 signature genes CXCL10, IRF1 and STAT1 were selected as readouts of activity of this pathway. Representative images, culture conditions and JAK-STAT1 signature gene expression for each phenotype are presented in Table 11. As expected, treatment of THP-1 monocytes with PMA induced morphological changes expected of transition to macrophages (M0), including decreased proliferation, cell adherence to the culture plate and spreading (Figure 11A) (Richter et al. 2016). Furthermore, polarization with M1 stimuli resulted in the development of filopodia whereas the M2-like cells were rounded or spindle shaped, as previously reported (Ploeger et al. 2013). In undifferentiated THP-1 monocytes CXCL10 mRNA is not expressed, whereas IRF1 and STAT1 are constitutively expressed (Figure 11B). Resting macrophages showed similar expression of JAK-STAT1 signature genes. As expected, CXCL10, IRF1 and STAT1 were

strongly induced in M1 polarized macrophages, which is induced partly through activation of the JAK-STAT1 pathway by IFN-γ. To confirm an M2 endotoxin tolerized ($M2^{tol}$) phenotype, M0 cells were first challenged with 4 hours acute LPS, followed by tolerization with LPS for 24 hours, and further challenge with 4 hours acute LPS. In the M2 paradigm, acute LPS challenge resulted in increased expression of CXCL10 and IRF1 compared to M0 macrophages. Following $M2^{tol}$ polarization with LPS, CXCL10 and IRF1 mRNA expression was decreased compared to the acute LPS stimulus. Furthermore CXCL10 and IRF1 expression remained suppressed following re-stimulation with acute LPS, demonstrating endotoxin tolerance (Biswas and Lopez-Collazo 2009). On the other hand, STAT1 expression was not responsive to acute LPS and did not demonstrate tolerization.

A

Phenotype	Monocyte	Resting Macrophage	Inflammatory Macrophage	Tolerized Macrophage
Name	'THP-1'	'M0'	'M1'	'M2^{tol}'
Morphology				
Stimulus	THP-1 cell line, none	+ 24h PMA + 24h rest	+ 24h 10ng/µL rIFN-γ & 1ng/µL LPS	+ 24h 100ng/µL LPS

B

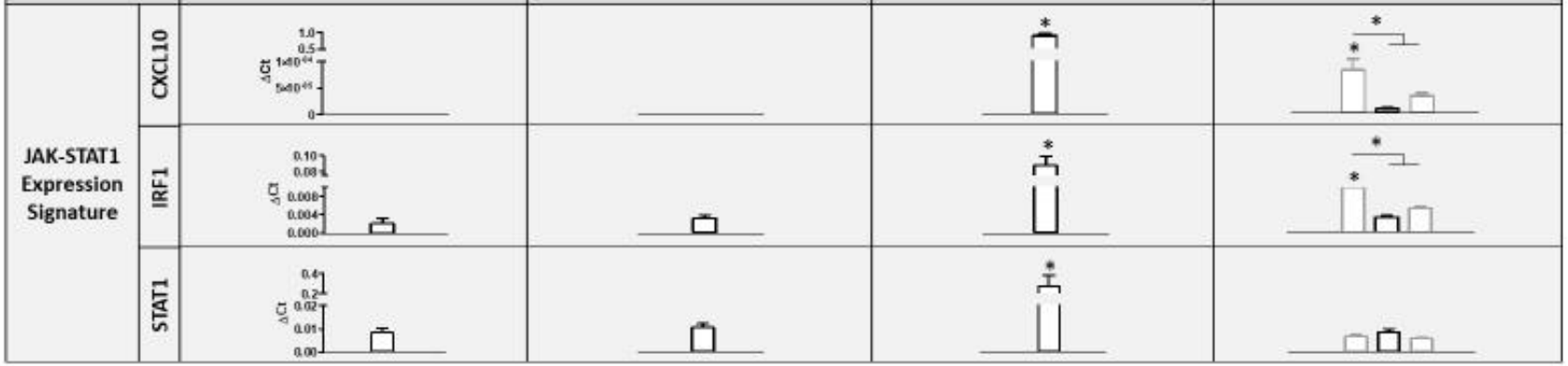


Figure 11. Myeloid cell culture and JAK-STAT1 signature gene expression. A) Phenotype modelled, representative image and stimuli for each culture condition. B) JAK-STAT1 expression signature for each phenotype. In the M2^{tol} column gray bars indicate acute LPS stimulus. L to R: M0 with acute LPS challenge; M2^{tol}; M2^{tol} with acute LPS challenge. *p<.05. Error bars represent SEM.

3.3.5 Risperidone effects on the JAK-STAT1 signature in THP-1 monocytes

Data from some cell culture experiments has shown an anti-inflammatory effect of antipsychotic treatment. However, to our knowledge the in vitro effect of risperidone on the JAK-STAT1 signature in monocytes has not been investigated. One possible explanation for the decreased JAK-STAT1 transcriptional signature in peripheral blood cells from participants early in illness and with hospitalization is a suppressive effect of antipsychotic treatment on this pathway. To test the effects of risperidone treatment on the JAK-STAT1 transcriptional signature in monocytes, THP-1 cells were stimulated with IFN-γ to activate the JAK-STAT1 pathway, followed by treatment with risperidone. As expected, IFN-γ alone increased expression of all three JAK-STAT1 signature genes (Figure 12). When cells were stimulated with IFN-γ and treated with risperidone, expression of all three genes was significantly increased compared to the IFN-γ stimulus alone. These data demonstrate that risperidone has a potentiating effect on JAK-STAT1 signature gene expression in THP-1 monocytes. Thus, these results, as in the clinical analysis do not support a suppressive effect of risperidone treatment on JAK-STAT1 signature gene expression.

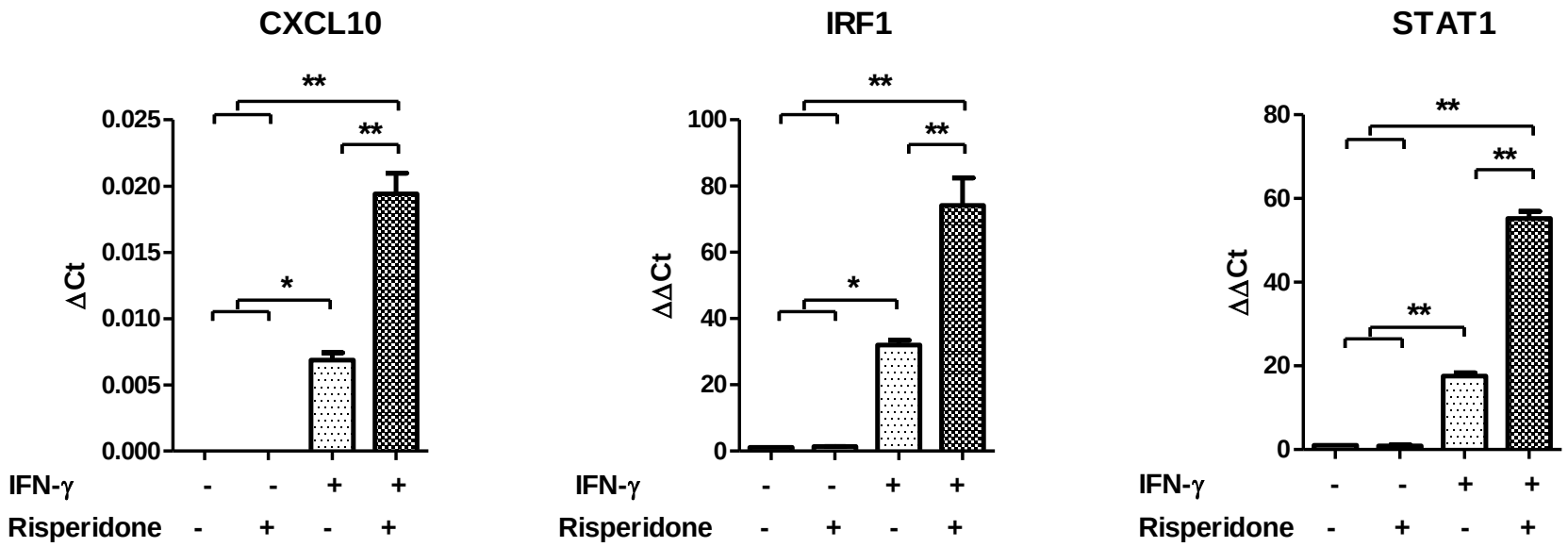


Figure 12. Effects of risperidone on THP-1 monocytes. THP-1 monocytes were stimulated with IFN-γ for 7hours and treated with risperidone for the final hour. Risperidone treatment increased the expression of JAK-STAT1 signature genes in IFN-γ stimulated monocytes. *p<.05, **p<.01. Error bars represent SEM.

3.3.6 Risperidone effects on the JAK-STAT1 signature during M1 macrophage polarization

Next the effects of risperidone on THP-1 derived macrophages exposed to M1 polarizing stimuli was tested. M0 cells were treated with IFN-γ and low-dose LPS with and without risperidone. In differentiated macrophages that were exposed to the canonical M1 polarizing stimuli IFN-γ and LPS, expression of JAK-STAT1 signature genes were elevated (Figure 13). Risperidone alone did not have a significant effect on JAK-STAT1 signature gene expression in unstimulated macrophages. We found that concurrent treatment with M1 polarizing stimuli and risperidone resulted in significantly increased mRNA expression of CXCL10, IRF-1 and STAT1. Thus, as was the case in the THP-1 monocyte condition, risperidone potentiated expression of JAK-STAT1 signature genes in THP-1 derived macrophages and strengthened this M1 macrophage signature.

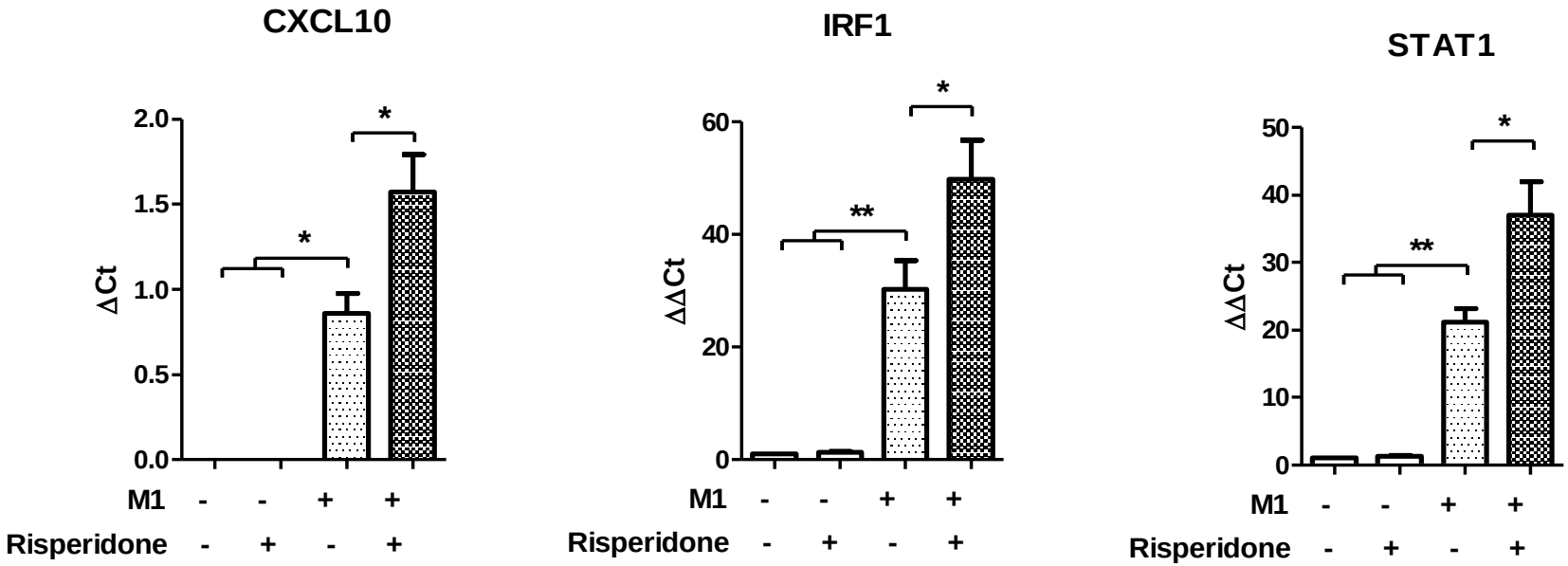


Figure 13. Effects of risperidone on M1 macrophages. M0 macrophages were polarized to M1 in the presence and absence of risperidone. Risperidone treatment increased the expression of JAK-STAT1 signature genes compared to M1 stimuli alone. *p<.05, **p<.01. Error bars represent SEM.

3.3.7 Risperidone effects on the JAK-STAT1 signature during $M2^{tol}$ macrophage polarization

In both of the previous conditions, cultured cells were exposed to JAK-STAT1 pathway activating stimuli in conjunction with risperidone. In this experiment, expression of JAK-STAT1 signature genes were decreased, similar to the pattern of expression seen in the clinical data early in illness and with increased acuity. M0 macrophages were exposed to the $M2^{tol}$ polarizing stimulus high-dose LPS for 24 hours with and without risperidone treatment. Again, cells treated with risperidone showed increased expression of CXCL10, IRF1 and STAT1 (Figure 14). For CXCL10 and IRF1 this equated to blocking or reversal of the tolerization of these genes, though as the results for STAT1 demonstrate, this increase in expression due to risperidone in cells exposed to an $M2^{tol}$ polarizing stimulus occurs regardless of whether the gene is tolerized.

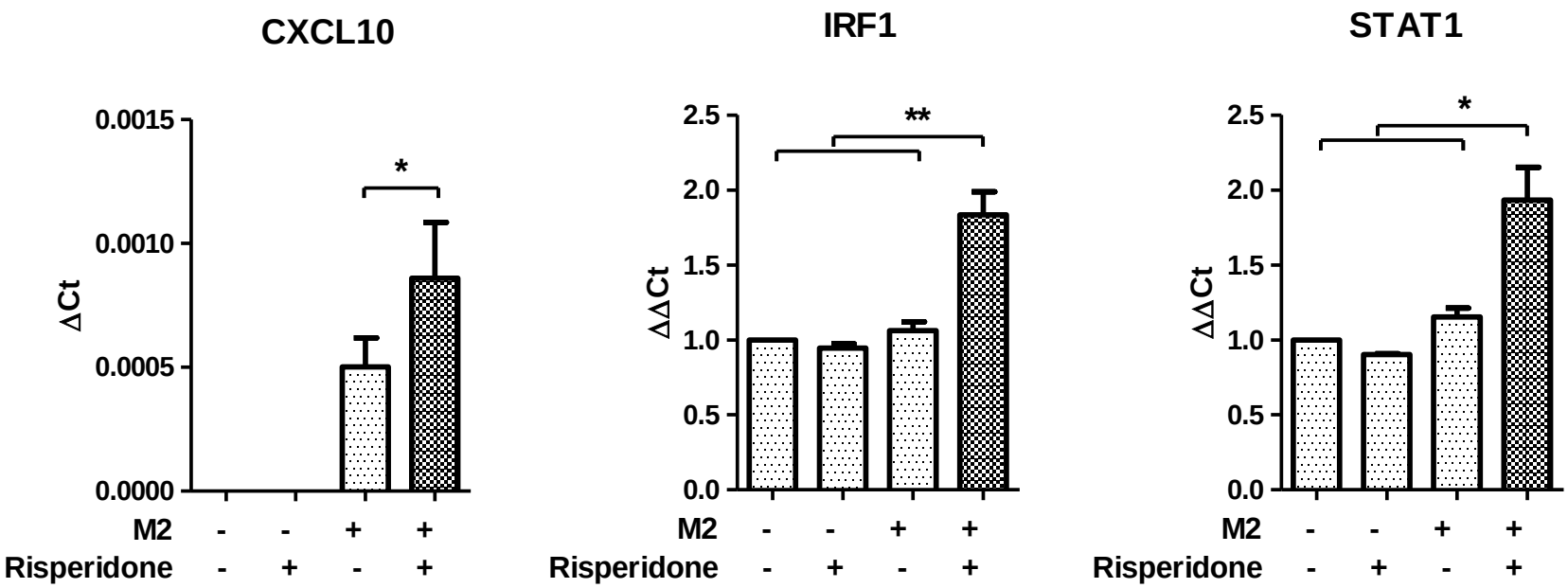


Figure 14. Effects of risperidone on M2tol macrophages. M0 macrophages were polarized to $M2^{tol}$ in the presence and absence of risperidone. In the presence of risperidone, all JAK-STAT1 genes are increased compared to M2 alone. CXCL10 and IRF1 no longer demonstrate endotoxin tolerance. *p<.05, **p<.01. Error bars represent SEM.

3.4 Discussion

In this novel study two approaches, one clinical and one in-vitro cell model, were used to investigate the effects of the commonly prescribed antipsychotic medication risperidone, on JAK-STAT1 signature gene expression in immune cells. In the clinical sample, there were no statistically significant overall differences in the previously measured JAK-STAT1 transcriptional signature when comparing PBMCs from participants with psychosis who reported no current treatment with antipsychotic medication and a) participants who reported treatment with any antipsychotic medication or b) a subsample treated with risperidone monotherapy. However, when differences in untreated and risperidone monotherapy treated participants were assessed in relation to illness duration, the risperidone treated individuals who were later in illness had significantly increased JAK-STAT1 signature gene expression compared to untreated participants with both low and high illness duration as well as risperidone treated participants who were early in illness. Thus, risperidone treatment is associated with an increase in JAK-STAT1 signature gene expression but only in individuals who are later in illness.

In the second approach, in-vitro models using the human THP-1 monocyte cell line were used to determine the effects of risperidone on the mRNA expression of the JAK-STAT1 signature genes CXCL10, IRF1 and STAT1. Overall, the data from these cell culture experiments demonstrated that risperidone increases the JAK-STAT1 transcriptional signature. This result was consistent across different cellular differentiation states, including both THP-1 monocytes and THP-1 monocyte-derived macrophages, and opposing polarizing conditions characterized by high (pro-inflammatory M1) or low (anti-inflammatory and tissue remodeling $M2^{tol}$) expression of JAK-STAT1 signature genes. These findings imply that treatment with risperidone skews myeloid cells away from an anti-inflammatory (M2) and towards a more pro-inflammatory (M1) phenotype. Interestingly, it appears that risperidone is able to prevent or reverse the suppressed expression of endotoxin tolerized genes, which in itself indicates risperidone inducing an M1 response even in an M2 tolerized state.

None of the results presented here, either in the clinical sample or the monocyte and macrophage cell models, indicate an inhibitory effect of antipsychotic treatment on JAK-STAT1 signature gene expression. Thus, it is unlikely that the suppressed JAK-STAT1 signature seen earlier in illness and with greater acuity is due to the effects of antipsychotic medication. Instead, the data with risperidone indicate that certain antipsychotics may contribute to the increase in JAK-STAT1 signature gene expression observed over the course of the illness. In the THP-1 monocyte and macrophage cell models there was a consistent potentiating effect of risperidone on JAK-STAT1 signature gene expression. In the clinical sample, however, only risperidone treated participants with a longer illness duration had an increased JAK-STAT1 transcriptional signature compared to untreated participants, whereas risperidone treated participants with a shorter illness duration demonstrated no difference in expression. It might be the case that in vivo changes to the JAK-STAT1 activity in immune cells are only manifest following long-term antipsychotic treatment. An alternative interpretation is that physiological differences that are present later in illness result in a stronger response to risperidone treatment in these individuals. Interestingly, the JAK-STAT1 transcriptional signature was positively correlated with illness duration in both the untreated and risperidone treated groups, and therefore other factors likely also contribute to this relationship.

Finally, the finding that antipsychotics such as risperidone can skew myeloid cells towards a proinflammatory phenotype may have relevance for understanding the effects of psychotropic medications on myeloid cells within the brain parenchyma and at its borders. Myeloid cells present in the CNS exhibit certain molecular and phenotypic characteristics similar to those observed in M1 and M2 in-vitro polarized macrophages (Prinz and Priller 2014). Such an interpretation is consistent with data showing that long-term in-vivo treatment with antipsychotic medications in rodents resulted in microglia with an activated phenotype, though specific phenotypic signatures were not measured (Cotel et al. 2015). Regardless of whether the findings presented here translate to embryologically-derived resident

macrophages including microglia, the implications for bone marrow derived myeloid cells remain relevant for neuropsychiatric illness. These peripherally derived cells are understood to mediate CNS homeostasis from the parenchymal borders through interaction with resident cells, modulating a vast array of neurobiological processes (Korin et al. 2017; Herz et al. 2017).

In conclusion, the results presented here demonstrate that risperidone treatment is associated with an increase in JAK-STAT1 signature gene expression. In the clinical population this increase was observed only in risperidone treated participants who were later in illness. Given the small sample size in the untreated and risperidone monotherapy groups, better powered studies are required to confirm these findings. Additionally, the influence of other antipsychotic drugs on the JAK-STAT1 signature remain unexplored. Finally, while there were no significant differences in PANSS scores we cannot exclude the possibility that there were inherent baseline differences with regards to illness presentation between study participants not currently treated with antipsychotics and those who were. Overall, the present analysis highlights a consistent potentiating effect of risperidone on the JAK-STAT1 transcriptional signature in human immune cells both at the in-vitro cellular level and in naturalistically treated individuals with chronic psychosis.

GENOME-WIDE TRANSCRIPTIONAL PROFILING OF MONOCYTES IN PSYCHOSIS

4.1 Background

Findings of increased proinflammatory cytokines and related immune alterations are prevalent in psychosis, as are immune-related risk factors (B. J. Miller and Goldsmith 2016; Goldsmith, Rapaport, and Miller 2016). Understanding the nature of alterations to immune activity in psychosis may prove critical for elucidating underlying pathophysiological processes that contribute to illness development and exacerbation, and for the development of new treatment options, which are currently lacking (Girgis, Kumar, and Brown 2014). Recent research highlights the importance of peripherally derived immune cells in mediating immune-brain communication, particularly at the parenchymal borders, where they've been shown to influence a wide range of processes such as learning and memory, social behavior and mood, under both normal physiological as well as pathological conditions (Korin et al. 2017; Prinz and Priller 2017). Myeloid cells such as monocytes and macrophages in particular are the subject of intense investigation as they can drive inflammation, perform phagocytosis and cause tissue damage (R. Rua and McGavern 2015; Papavasiliou et al. 2016). It has recently been demonstrated in rodents that following immune activation inflammatory monocytes are recruited to brain borders such as the choroid plexus, and that TNF-α secreted by circulating monocytes drives sickness induced learning deficits (Garré et al. 2017; María et al. 2017). Additionally, while the majority of resident myeloid cells in the CNS and at its borders are now thought to be embryologically derived and thus distinct from adult bone marrow derived cells, recent lineage tracing studies indicate that macrophages that reside in the choroid plexus are replaced by these circulating monocytes under steady state conditions (Greter 2016). There are limited gene expression data in the literature specific to monocytes in psychosis. One 2010 study found increased mRNA expression of inflammatory mediators such as cytokines and chemokines in monocytes from participants with schizophrenia (Drexhage et al. 2010). Interestingly, data also indicate that some of the

changes related to immune gene expression in schizophrenia post-mortem brain may be due to the presence of circulating monocytes in vessels (Hwang et al. 2013).

The immunological literature highlights the role of IFN-γ mediated activation of JAK-STAT1 signaling in orchestrating multiple aspects of immunity, and particularly the induction and stabilization of the myeloid cell proinflammatory phenotype (Villarino, Kanno, and O'Shea 2017; Schroder et al. 2004). The results presented in Chapter 2 demonstrate the presence of a decreased IFN-γ-JAK-STAT1 transcriptional signature in PBMCs from participants with psychosis who have a shorter illness duration and in those who have high illness acuity relative to non-psychiatric controls. Furthermore, expression of IFN-γ-JAK-STAT1 signature genes increased with illness duration. These results were somewhat unexpected given the described proinflammatory effects of this pathway, and thus highlight the temporal and contextual complexity of immune alterations in psychosis. As the majority of clinical participants reported current treatment with antipsychotic medications, which are known to have immunomodulatory properties, antipsychotic effects were one possible explanatory factor for alterations to immune parameters. In Chapter 3, expression of IFN-γ-JAK-STAT1 signature genes were examined in the clinical sample in relation to antipsychotic treatment with focus on the atypical antipsychotic risperidone, which was the most commonly prescribed antipsychotic, and the direct effects of risperidone on JAK-STAT1 signature genes were tested in a human monocyte and macrophage cell model. Overall the results suggested that risperidone may contribute to increases in JAK-STAT1 signature gene expression later in illness but that treatment with this antipsychotic does not explain the suppressed signature seen earlier in illness or during acute episodes of psychosis relative to non-psychiatric controls. The study outlined in Chapter 2 was carried out using total PBMCs, and while it is likely that monocytes contribute to the measured JAK-STAT1 transcriptional signature, confirmation is required that the findings are reflective of alterations to the JAK-STAT1 signature in this cell type.

Myeloid cells, including monocytes and macrophages, are highly sensitive to environmental stimuli, which in combination influence the cells overall functional phenotype (Mitchell, Roediger, and Weninger 2014). The prototypical proinflammatory skewed monocyte and macrophage cellular phenotype, frequently termed 'M1', is induced by activation of the JAK-STAT1 pathway by IFN-γ in conjunction with an NF-κB activating stimulus such as LPS. Conversely, monocytes and macrophages that are skewed towards an anti-inflammatory and tissue remodeling phenotype are often called 'M2'. While these terms imply bipolarity, a spectrum or multipolar model of possible activation states and functions is now considered to be more conceptually useful (Guilliams and van de Laar 2015). M2-skewed monocytes and macrophages can be induced by a variety of physiological states and stimuli, including the Th2-associated cytokines IL-4 and IL-13, glucocorticoids in response to psychological stress, and high levels of NF-κB activating stimuli such as LPS in the absence of IFN-γ, which leads to a cellular state called endotoxin tolerance (Yona and Gordon 2007). These cellular phenotypes are characterized, in part, by decreased expression of IFN-γ mediated JAK-STAT1 signature genes (Piccolo et al. 2017; O. M. Pena et al. 2011; Curtin et al. 2009), and thus the suppressed JAK-STAT1 signature in the earlier stages of illness and with greater acuity in psychosis could be indicative of an M2-skew in circulating monocytes. The impact of stress on myeloid cells is complex, as while acute stress and glucocorticoids generally have an anti-inflammatory effect, chronic stress has been associated with an elevated proinflammatory NF-κB mediated signature and in chronic social stress a decreased type I IFN (IFN-α/β) signature in circulating monocytes (G. E. Miller et al. 2014; Powell et al. 2013). While we have focused on the type II IFN signature, which is IFN-γ, both type I and II IFNs use components of the JAK-STAT1 pathway and the transcriptional signatures demonstrate some degree of overlap. To our knowledge the type I IFN signature has not been measured in psychosis.

Recent advances in sequencing technology have improved the accessibility and accuracy of genome-wide transcriptional profiling, which provides the enhanced molecular depth needed for

bioinformatic analyses that are sensitive to small perturbations in the overall pattern of gene expression. Transcriptomics is increasingly used to define myeloid cell phenotypes in various tissues in response to a variety of environmental signals and corresponding activation states under steady state conditions as well as in the context of injury and illness (Glass and Natoli 2015; Sevenich 2018). Here, we carried out RNA sequencing of isolated monocytes in chronically symptomatic participants with psychosis, all of whom had a diagnosis of schizophrenia, as well as non-psychiatric controls. Participants with schizophrenia were split into two illness duration groups: 'medium illness duration' and 'long illness duration'. Gene expression was compared between these illness duration groups and with control participants. Transcriptional signatures for each of the described cellular phenotypes or phenotype determining stimuli were compiled (see Table VIII in Methods) and assessed for enrichment in relation to diagnosis and illness duration. Finally, analyses were carried to determine which transcription factors might drive the changes in the IFN-γ mediated JAK-STAT1 transcriptional signature in relation to illness duration.

4.2 Method

4.2.1 Participant Characteristics

Participant demographic and clinical characteristics are outlined in Table VII. Participants with psychosis who met SCID-IVtr (First et al. 2002) diagnostic criteria for schizophrenia were recruited from a large urban university medical center that included referrals from community treatment facilities. All participants with psychosis took part as outpatients. Control participants were recruited from the surrounding urban community and had no current or past history of psychiatric illness. The study was approved by the Institutional Review Board of the University of Illinois at Chicago, and all participants gave signed written consent prior to initiation of any research procedures. Inclusion and exclusion criteria were the same as for Chapter 2. Clinical symptomology was measured using the PANSS (Kay, Fiszbein, and Opler 1987). Finally, to assess the effects of illness duration on monocyte gene expression, the sample of

participants with schizophrenia were split into two evenly sized illness duration categories (medium and long illness duration). Illness duration in the short group ranged from 25-31 years and in the long group from 32-46 years. Group differences between all participants with schizophrenia and controls were assessed using independent samples t-tests and chi-squared. Group differences between two illness duration groups and controls were assessed using one-way ANOVA followed by Tukey's post-hoc tests if significant. Differences in illness duration between the two illness duration groups were confirmed using an independent samples t-test.

		Control	Schizophrenia	Illness Duration Group	
				Medium	Long
Total (n)		14	14	7	7
Age (M ± SD)		49.00 ± 11.64	51.79 ± 6.34	51.29 ± 7.18	52.29 ± 5.91
Sex	Female (n)	7	7	4	3
	Male (n)	7	7	3	4
Race	Caucasian, non-Hispanic (n)	2	5	2	3
	Black, non-Hispanic (n)	12	8	4	4
	Hispanic (n)	0	1	1	0
BMI (M ± SD)		28.91 ± 8.07	32.91 ± 12.67	33.70 ± 5.38	32.11 ± 4.53
Illness Duration (M ± SD)		N/A	32.36 ± 6.86	27.29 ± 2.14**#**	37.43 ± 6.11**#**
Current Antipsychotic Use (n)		0	13	6	7
PANSS	Positive (M ± SD)	8.93 ± 1.69	29.71 ± 3.58******	28.71 ± 3.45******	30.71 ± 3.68******
	Negative (M ± SD)	9.07 ±1.86	22.00 ± 5.56******	21.71 ± 5.53******	22.29 ± 6.02******
	General (M ± SD)	24.07 ± 3.02	49.00 ± 7.63******	48.43 ± 6.58******	49.57 ± 9.05******

Table VII. Participant demographics and clinical characteristics: monocyte RNAseq. ******p<.001 compared to controls; **#**p<.01 comparison of medium and long illness duration groups. M=mean, SD=standard deviation.

4.2.2 Sample Collection

The blood draw and PBMC isolation for each participant were carried out using the same methodology described in Chapter 2. CD14, a cell surface marker expressed on monocytes and macrophages (Ziegler-Heitbrock and Ulevitch 1993), was used to isolate monocytes from the PBMCs. Cells were labelled with anti-CD14 coated microbeads, and positively selected using magnetic cell sorting (Miltenyi Biotech). Monocytes were washed with phosphate buffered saline, pelleted by centrifugation, and stored at -80°C prior to extraction.

4.2.3 RNA Extraction

RNA was extracted from the CD14+ primary monocytes using the Qiagen miRNeasy mini kit with Qiagen on-column DNase treatment. RNA integrity was assessed using the Agilent tapestation, and was considered satisfactory for 3' library preparation.

4.2.4 Library Preparation and Sequencing

The cDNA library was prepared by the Core Genomics Facility at the University of Illinois at Chicago using the Lexogen QuantSeq FWD kit. cDNA libraries were sequenced using an Illumina HiSeq system by DNA Services, also at the University of Illinois at Chicago. QuantSeq generates one cDNA complementary to the 3'-end sequence, which corresponds to one read per transcript. This technology therefore allows for improved quantification of gene expression and does not require the usual gene length normalization steps that alternative RNAseq methodologies require.

4.2.5 Differential Gene Expression

Read count matrices were generated using the Bluebee bioinformatics pipeline. Briefly, adapters trimming was carried out with bbduk, FastQC was used for read quality control, STAR for alignment and HTSeq for read counting. Genes with read counts with a mean expression under 10 across all samples were removed, as were genes expressed in less than 75% of the participants. Differential gene expression

(DGE) analyses were conducted in Bluebee using DESeq2 (Bioconductor) (Love, Huber, and Anders 2014) for the following group comparisons: all participants with schizophrenia versus controls, medium illness duration versus controls, long illness duration versus controls and medium illness duration versus long illness duration.

4.2.6 Gene Set Enrichment Analysis

In order to carry out gene set enrichment analysis (GSEA) a rank file for each group comparison was created. Genes were ranked starting with greatest increase in gene expression down to the greatest decrease in expression using the DESeq2 Wald statistic pair-wise comparison output (Subramanian et al. 2005). Seven gene set 'signatures' representative of the cellular phenotypes outlined in the background were compiled (Table VIII). The analysis was carried out using Broad Institute GSEA Preranked software with the recommended default settings of 1000 permutations and weighted enrichment. For each preselected gene set, the GSEA software computes an 'enrichment score' (ES). The ES is calculated by walking down the list of genes ranked by differential expression and creates a running score. When a gene from the gene set being analyzed is in the rank file the score increases, and if not present the score decreases. The resulting ES, the maximum deviation from zero, indicates whether or not a gene set is enriched amongst the genes at either end of the rank file, i.e. whether it is enriched amongst the genes whose expression is increased or decreased in the phenotype of interest. The ES is first calculated for each gene set individually, followed by normalization to allow for comparison between gene sets of different sizes. A gene set was considered significantly enriched if both the nominal p-value was under .05 and the False Discovery Rate (FDR) under .25. 'Leading edge' analyses were carried out for significantly enriched genes sets in each comparison, also using the Broad Institute GSEA Software (Subramanian et al. 2005). The leading-edge subset of genes for a differentially enriched gene set consists of the genes leading up to, and therefore contributing to, the maximum ES. Any leading-edge genes that are present in two or more of the significantly enriched gene sets are highlighted.

Gene Set	No. of Genes	Cell Type	Conditions	Data Acquisition	Source
IFN-γ Signature	500	Human primary monocyte-macrophages	Culture IFN-γ 24h	RNA-seq	(Qiao et al. 2016)
IFN-α Signature	200	Human primary monocyte-macrophages	Culture IFN-α 4h	Microarray	(Greenwell-Wild et al. 2009)
LPS Acute Signature	330	Human primary monocyte-macrophages	Culture LPS 4h	RNA-seq	(Novakovic et al. 2016)
ET Signature	76	Human PBMCs	In-vivo sepsis	Microarray	(Pena et al. 2014)
IL-4 Signature	200	Human primary monocyte-macrophages	Culture IL-4 24h	Microarray	(Szanto et al. 2010)
GC Acute Signature	87	Human primary monocyte-macrophages	Culture dexamethasone 1-24h	Microarray	(Jubb et al. 2016)
Chronic Stress Signature	315	Human primary monocytes	In-vivo chronic caregiver stress	Microarray	(Miller et al., 2014)

Table VIII. Gene sets compiled from the published literature that were used for GSEA. ET: endotoxin tolerance, GC: glucocorticoid

4.2.7 Transcription Factor Enrichment Analysis

Transcription factor enrichment analysis (TFEA) was carried out for each of the IFN-γ signature leading edge subsets. The most recent chromatin immunoprecipitation (ChIP)-X database (ChEA 2016) was used and implemented in Enrichr which carries out over representation analysis (Lachmann et al. 2010; Kuleshov et al. 2016; E. Y. Chen et al. 2013). This method determines whether genes that are regulated by a particular transcription factor are over-represented within the subset of leading edge genes. The data are presented using the combined score output, which is representative of the p-value and the z-score that assesses deviation from the expected rank. The ChIP-X database contains lists of transcription factor target genes compiled using results of published genome-wide ChIP experiments (currently 645 gene lists). Additionally, GSEA was carried out using the Broad Institute software for the medium versus high ranked gene list using the ChIP-X database as the gene sets list.

4.3 Results

4.3.1 Demographics

There was no difference in age (t_{26}=-.79, p=.44), sex ($\chi^2(1)$=.00, p=1.00), race ($\chi^2(2)$=3.09, p=.21) or BMI (t_{26}=-1.00, p=.33) when comparing participants with schizophrenia and controls. PANSS positive (t_{26}=-19.64, p<.001), negative (t_{26}=-8.25, p<.001) and general (t_{26}=-11.37, p<.001) subscale scores were greater in participants with schizophrenia than non-psychiatric controls. The control participants and participants with schizophrenia in the medium and long illness duration groups demonstrated no differences in age (F(2,25)=.32, p=.73), sex ($\chi^2(2)$=.29, p=.87), race ($\chi^2(4)$=5.37, p=.25), or BMI (F(2,25)=.52, p=.60). Again, PANSS positive (F(2,25)=200.12, p<.001), negative (F(2,25)=32.84, p<.001) and general (F(2,25)=62.54, p<.001) scores were greater in both illness duration groups relative to controls (p<.001 for all subscales), but there were no differences in PANSS scores between the two illness duration groups.

Illness duration was significantly greater in the long illness duration group than the medium illness duration group (t_{12}=-4.15, p=.004).

4.3.2 Transcriptome analysis of primary monocytes in schizophrenia

Comparison of participants with schizophrenia and controls by DESeq2 revealed 389 protein-coding genes differentially expressed in primary monocytes, 214 increased in schizophrenia and 175 decreased at p<.05 (Figure 15).

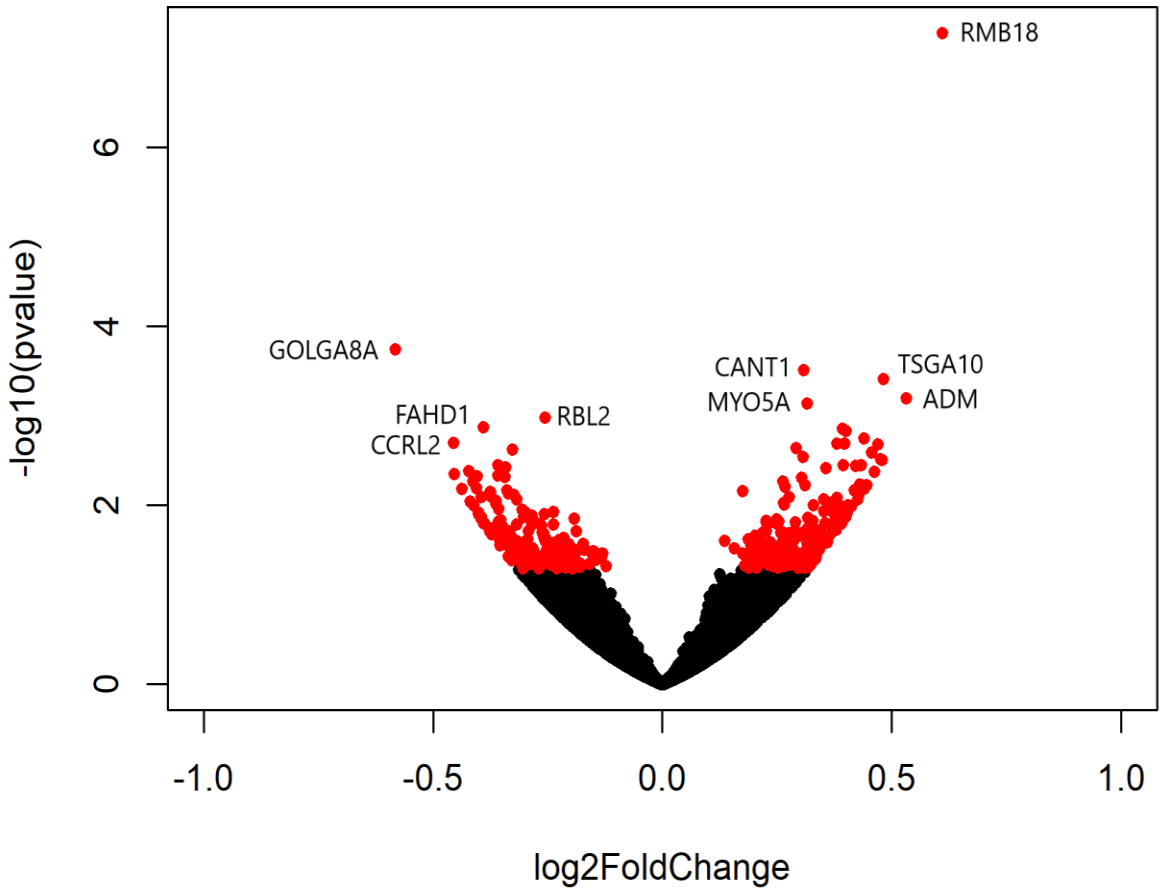


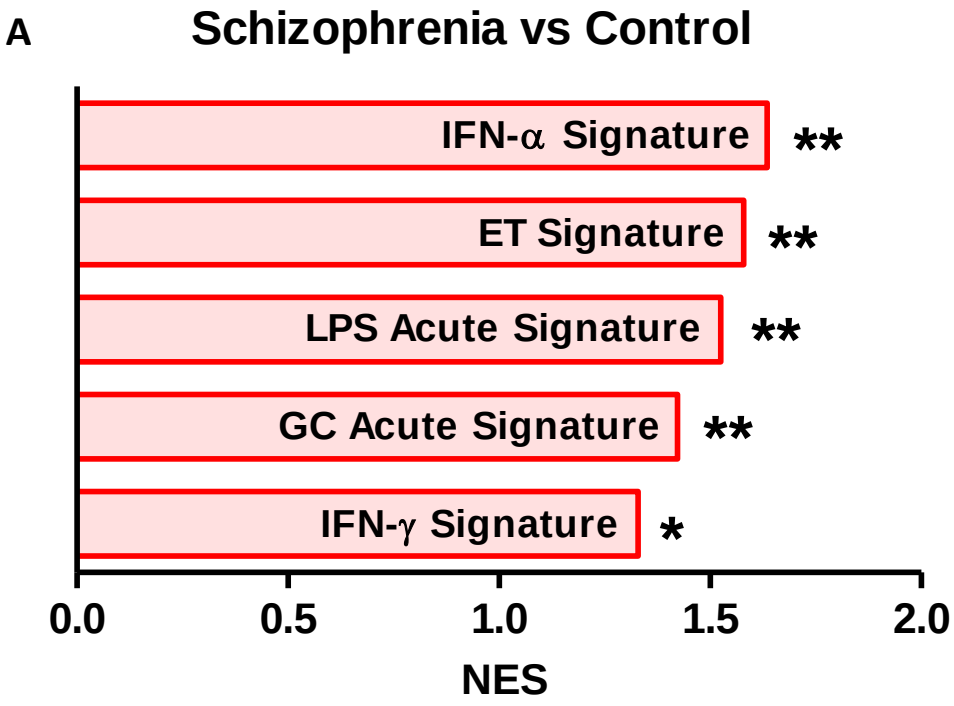
Figure 15. Volcano plot of DGE: schizophrenia versus controls. p<.05 labelled red

The DGE output was ranked from the genes with the greatest increase in expression to genes with the greatest decrease in expression in schizophrenia compared to control participants. GSEA was carried out to determine whether any of the seven selected gene sets were enriched in schizophrenia compared to control participants and vice versa. The IFN-γ signature was enriched amongst the genes increased in schizophrenia (Figure 16A, Figure 17A). In addition to the IFN-γ signature, the IFN-α, ET, LPS acute, GC acute were also enriched amongst the increased genes differentially expressed in schizophrenia (Figure 16A). These results indicate that both the type I ('IFN-α signature') and type II ('IFN-γ signature') IFN signatures, the primary drivers of JAK-STAT1 signaling, are increased in schizophrenia compared to controls. Interestingly, genes that are characteristic of an in-vivo endotoxin tolerant state ('ET signature') and genes that are expressed following in-vitro acute LPS challenge ('acute LPS signature') were both enriched in schizophrenia. Finally, there was also enrichment of the cellular response to glucocorticoids ('GC acute signature'), but no evidence of in-vivo transcriptional changes observed in individuals experiencing chronic caregiver stress ('chronic stress signature').

Leading edge analysis for the enriched gene sets demonstrated overlap of genes driving the IFN-γ (16.39%) and IFN-α (37.04%) signature enrichment in participants with schizophrenia (Figure 16B). There was also some overlap of the IFN-γ (13.93%) and IFN-α (27.78%) signatures with the LPS acute signature leading edge subset (24.29% and 21.43% respectively). A proportion of the genes contributing to the ET (25.00%) and GC acute (23.53%) enrichment were also present in the IFN-γ (3.28% for both sets) leading edge subset. Additionally, there was overlap of the genes driving the LPS acute (5.71%) and GC acute (23.53%) signature enrichment. There was minimal overlap for the remaining gene set comparisons (0-12.5%).

TFEA for the 122 IFN-γ signature leading edge subset genes demonstrated that interferon regulated factor (IRF) 8 is the most likely transcription factor candidate driving the enrichment of this

signature in schizophrenia. RELA and IRF1 regulated genes were also in the top 4 enriched gene lists (Figure 17B).



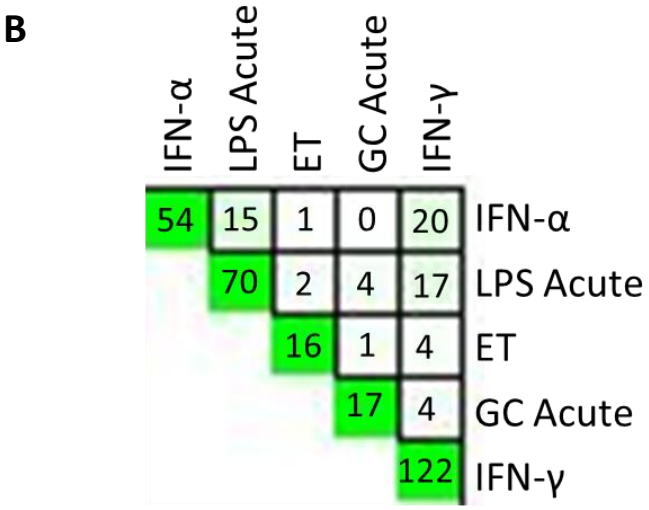


Figure 16. GSEA and leading-edge analysis: schizophrenia versus control. A) Gene sets that were enriched in the schizophrenia group compared to controls. **FDR<.05, *FDR<.25. NES=normalized enrichment score. B) Leading-edge analysis for gene sets enriched amongst genes overexpressed in schizophrenia. The number of leading edge genes that are present in both sets are presented. The green gradient illustrates the degree of overlap between the corresponding gene sets.

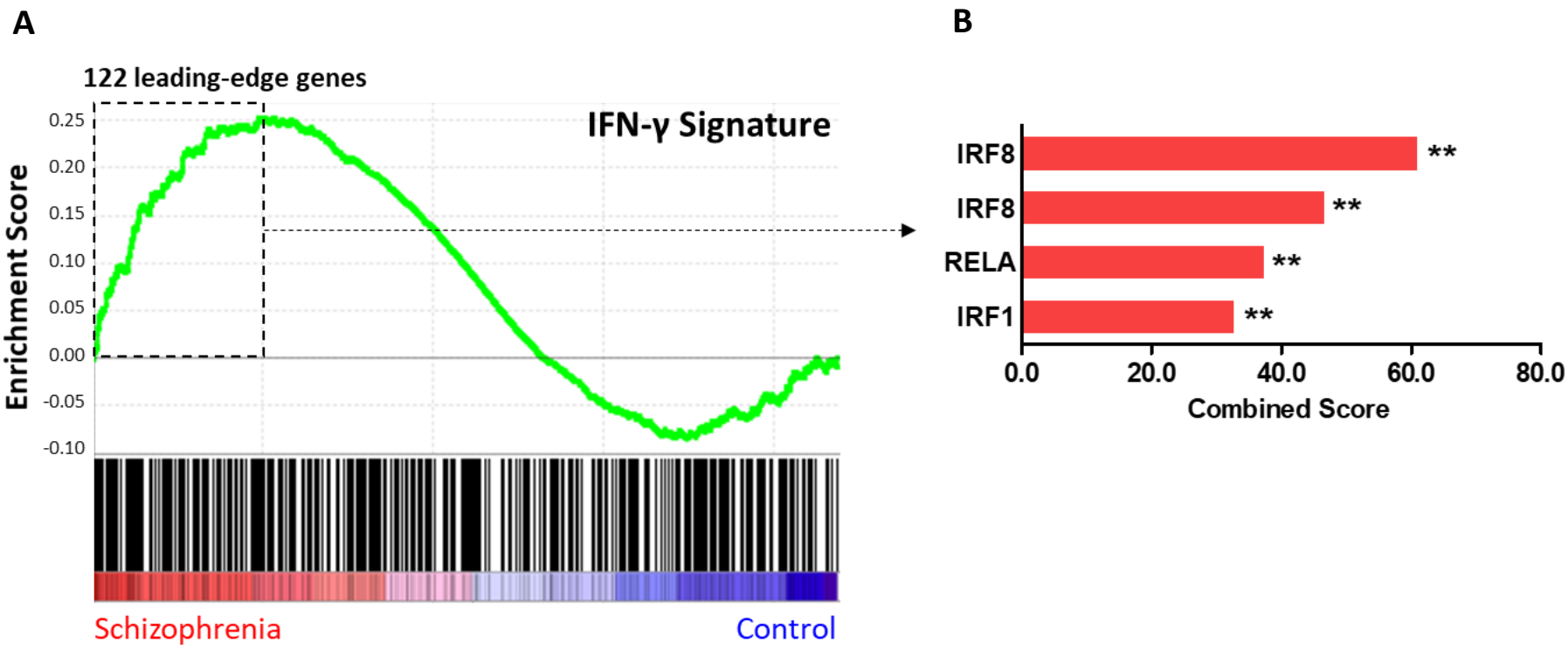


Figure 17. IFN-γ signature enrichment plot and TFEA: schizophrenia versus control. A) Enrichment plot for the IFN-γ signature in participants with schizophrenia compared to controls. Genes are ranked from greatest increase to the greatest decreased in expression in schizophrenia compared to controls. Black lines indicate the location of genes that are in both the ranked list and gene set. B) Top four results from the transcription factor enrichment analysis of the leading-edge subset of genes in the IFN-γ signature. **p<.001 and adjusted p<.001.

4.3.3 Transcriptome analysis: medium illness duration

The data presented in Chapter 2 indicate that expression of the IFN-γ mediated JAK-STAT1 transcriptional signature varies with illness duration. To confirm these findings using a genome-wide approach in isolated monocytes, participants with schizophrenia were split into two groups based on illness duration. In participants with schizophrenia who had a shorter illness duration 197 protein coding genes were increased and 329 decreased compared to controls at p<.05 (Figure 18).

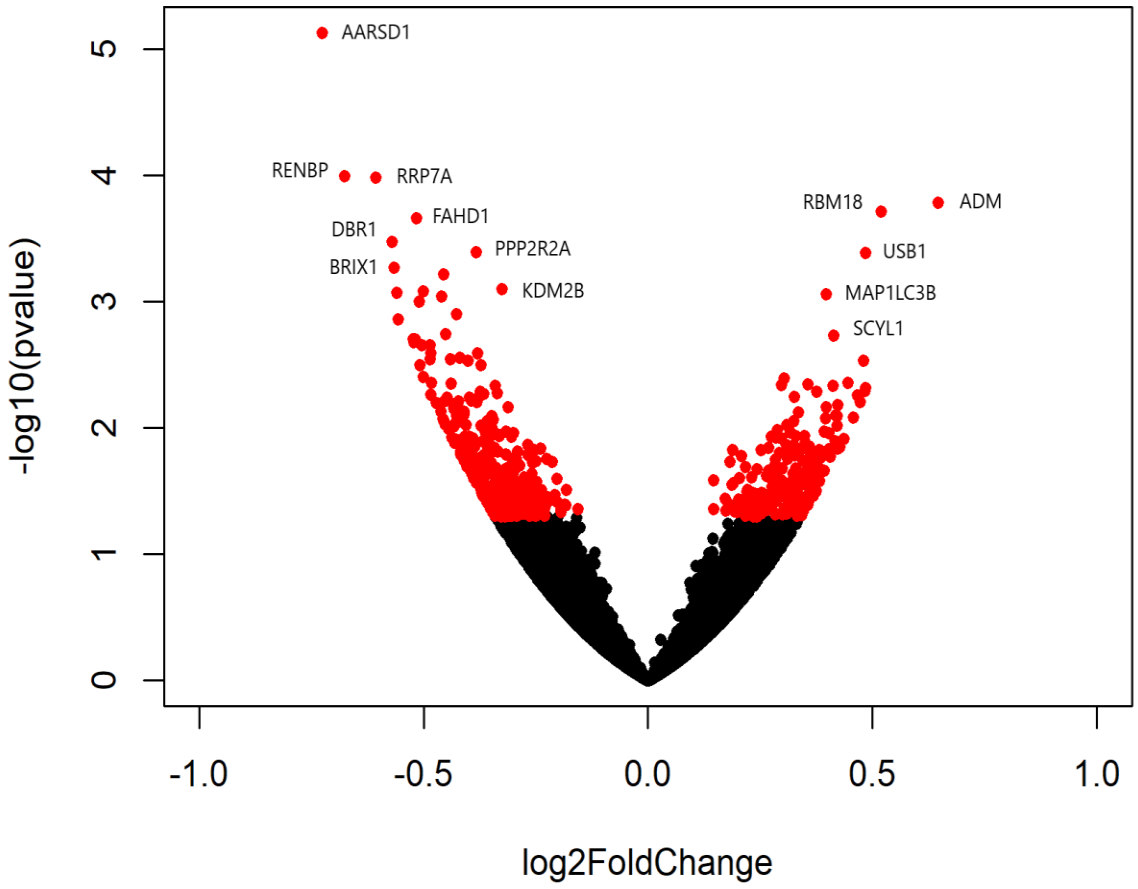
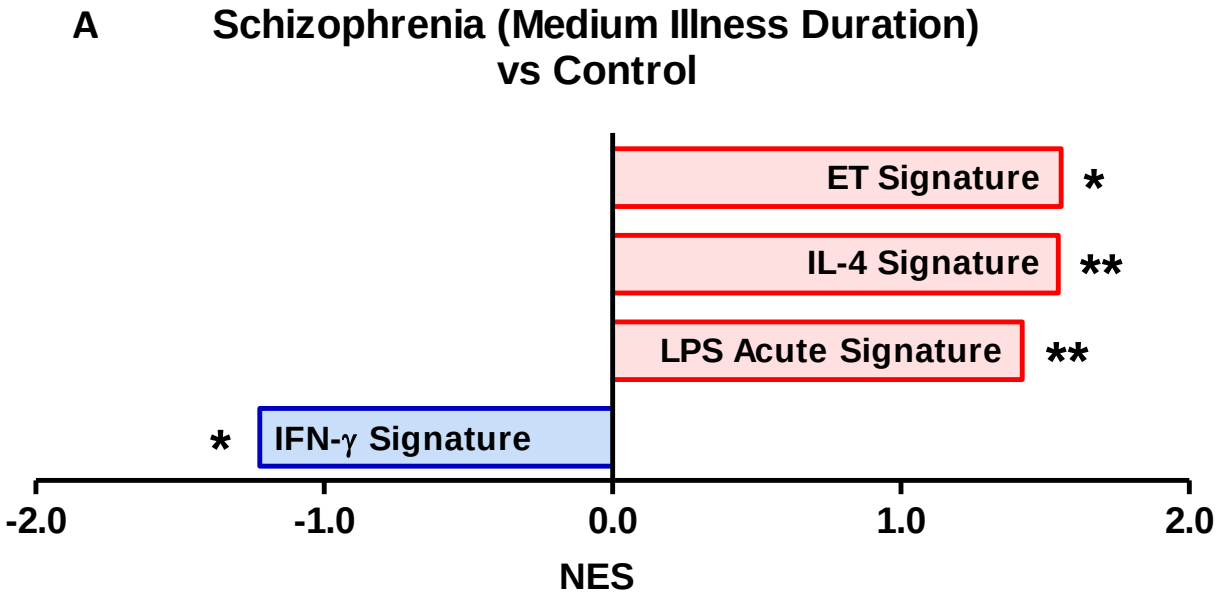


Figure 18. Volcano plot of DGE: medium illness duration versus controls. p<.05 labelled red

GSEA revealed that the IFN-γ signature was enriched amongst the genes increased in controls compared to participants with schizophrenia in the earlier illness duration category (Figure 19A, Figure 20A). In contrast, the ET, IL-4 and LPS acute signatures were all enriched amongst the genes increased in the earlier illness duration group of participants with schizophrenia. There was no enrichment of either the GC acute or chronic stress signatures. Leading edge subset analysis for the three gene sets enriched in the early illness duration group demonstrated minimal overlap of genes driving enrichment (0-10%) (Figure 19B). Finally, TFEA for the 118 leading edge subset genes driving enrichment of the IFN-γ signature in controls compared to the earlier illness duration group revealed that IRF8 regulated genes were the most strongly enriched, followed by IRF1 (Figure 20B).



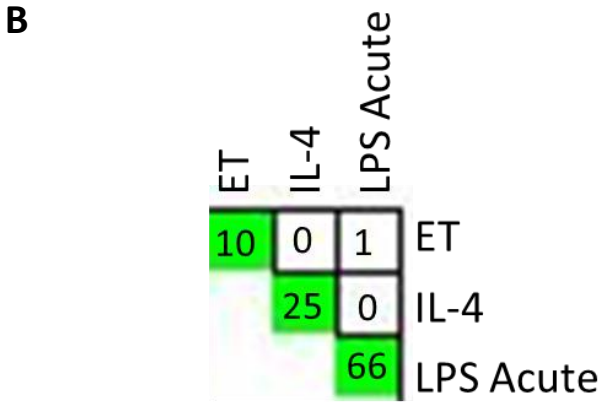


Figure 19. GSEA and leading-edge analysis: medium illness duration versus control. A) Gene sets that were enriched in the medium illness duration group compared to controls. **FDR<.05, *FDR<.25. B) Leading-edge analysis for gene sets enriched amongst genes increased in the medium illness duration group compared to controls. The number of leading edge genes that are present in both sets are presented and the green gradient illustrates the degree of overlap.

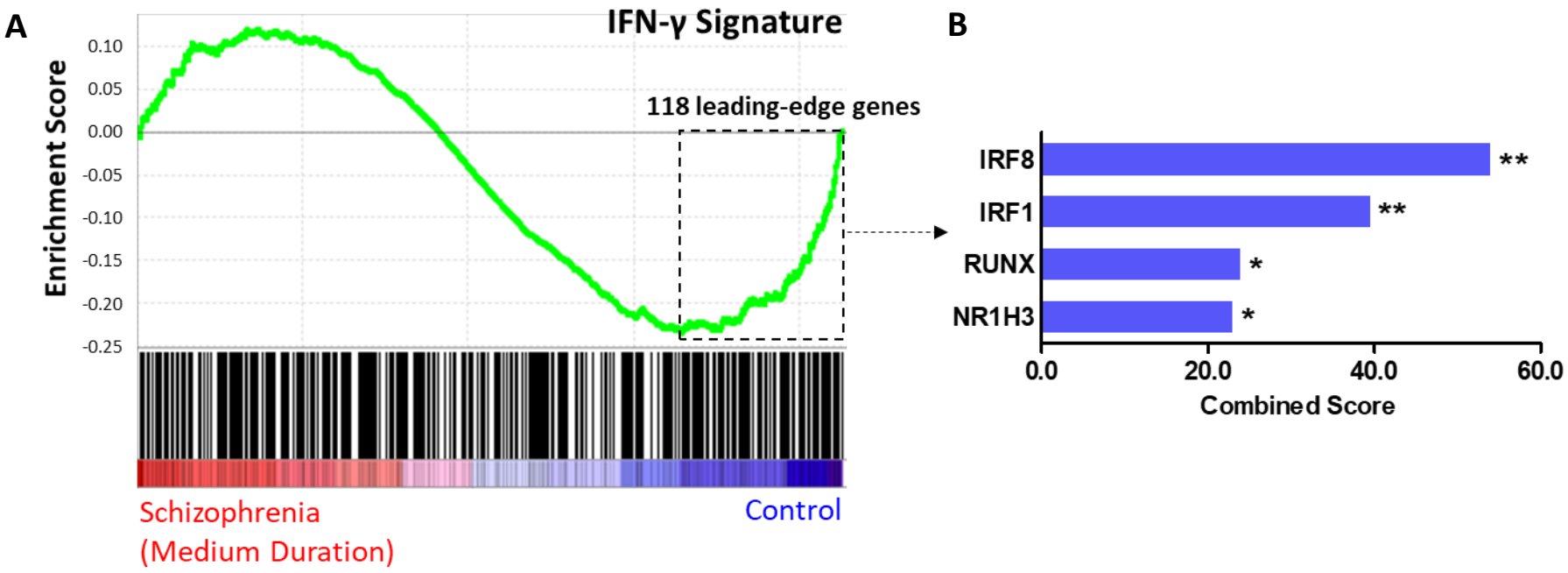


Figure 20. IFN-γ signature enrichment plot and TFEA: medium illness duration versus control. A) Enrichment plot for the IFN-γ signature in the medium illness duration group compared to controls. Genes are ranked from greatest increase to the greatest decreased in expression in the medium illness duration group compared to controls. Black lines indicate the location of genes that are in both the ranked list and gene set. B) Top four results from the transcription factor enrichment analysis of the leading-edge subset of genes in the IFN-γ signature. **p<.001 and adjusted p<.001, *adjusted p<.01

4.3.4 Transcriptome analysis: long illness duration

Next, differential gene expression analysis was carried out for the longer illness duration group compared to controls. There were 237 protein coding genes increased and 143 genes decreased compared to controls (Figure 21).

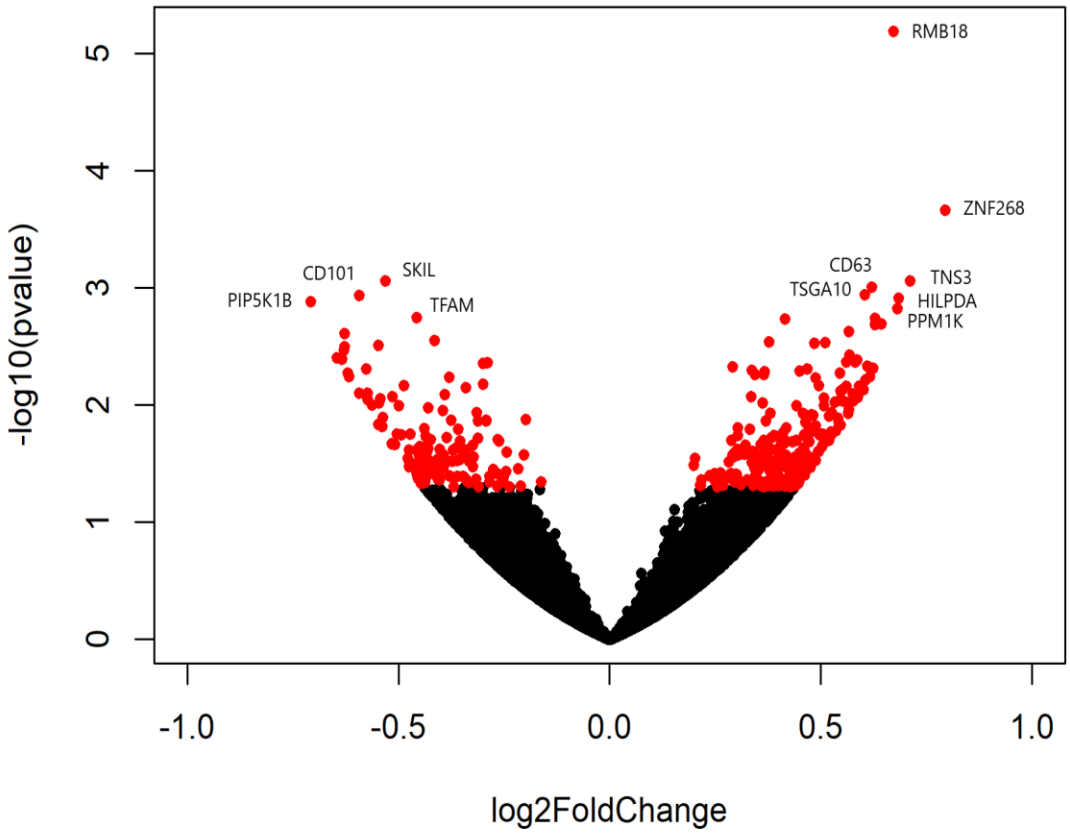
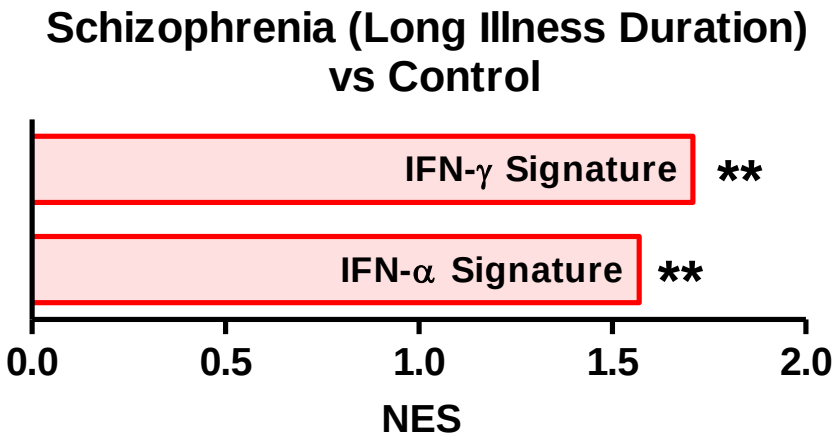


Figure 21. Volcano plot of DGE: long illness duration participants versus controls. p<.05 labelled red

GSEA demonstrated an enrichment of the IFN-γ signature amongst the genes increased in the participants with schizophrenia in the longer illness duration group compared to controls (Figure 22A, Figure 23A). The IFN-α signature was also enriched in this later illness duration category (Figure 22A). There was no significant enrichment of any of the remaining gene sets. Leading edge analysis demonstrated overlap of the IFN-γ (14.96%) and IFN-α (33.33%) signature leading edge subset genes (Figure 22B). TFEA of the 127 IFN-γ leading edge subset genes once again demonstrated enrichment of IRF8 regulated genes followed by IRF1 (Figure 23B).

A



B

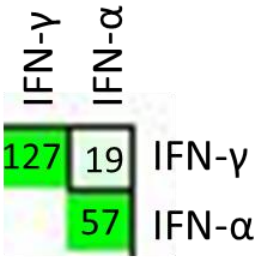


Figure 22. GSEA and leading-edge analysis: long illness duration versus control. A) Gene sets that were enriched in the long illness duration group compared to controls. **FDR<.05, *FDR<.25. B) Leading-edge analysis for gene sets enriched amongst genes increased in the long illness duration group compared to controls. The number of leading edge genes that are present in both sets are presented. The green gradient illustrates the degree of overlap.

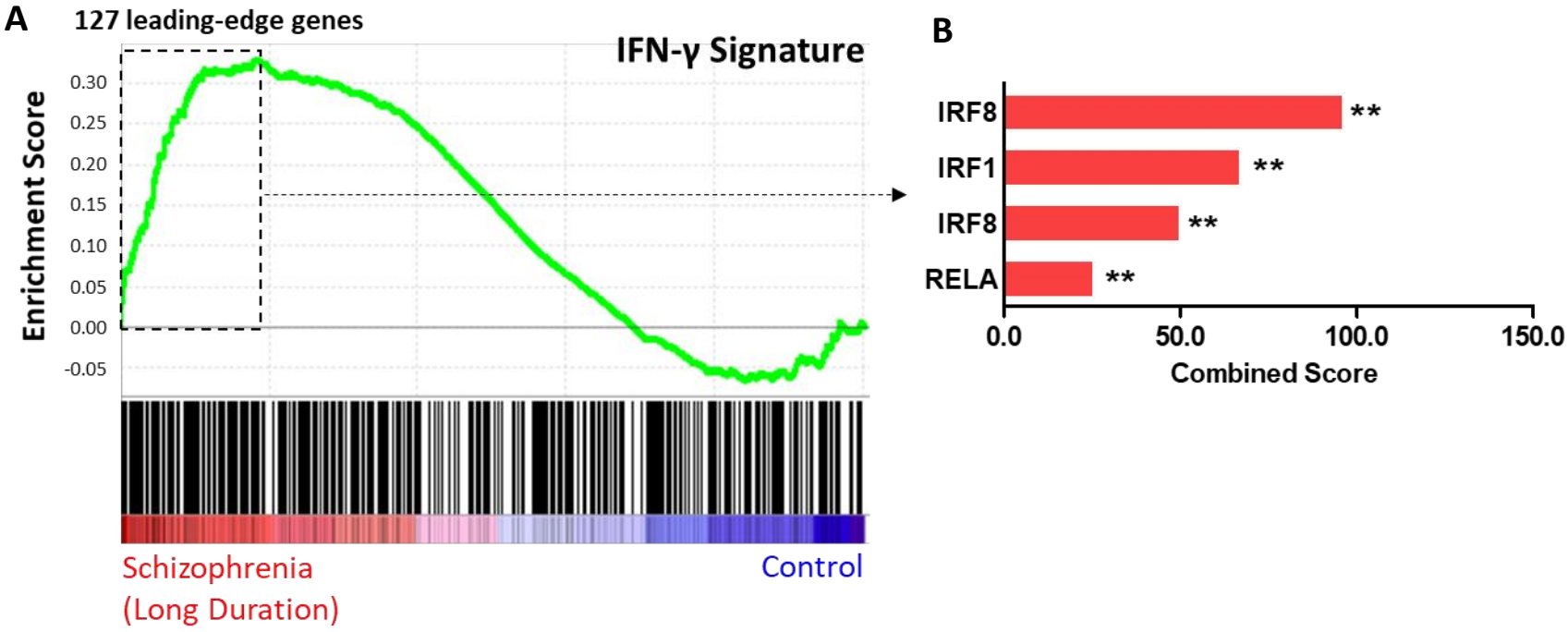


Figure 23. IFN-γ signature enrichment plot and TFEA: long illness duration versus control. A) Enrichment plot for the IFN-γ signature in the long illness duration group compared to controls. Genes are ranked from greatest increase to the greatest decreased in expression in the long illness duration group compared to controls. Black lines indicate the location of genes that are in both the ranked list and gene set. B) Top four results from the transcription factor enrichment analysis of the leading-edge subset of genes in the IFN-γ signature. **p<.001 and adjusted p<.001.

4.3.5 Transcriptome analysis: medium versus long illness duration

Differential gene expression analysis comparing the two illness duration groups resulted in 151 protein coding genes increased and 304 genes decreased in the shorter compared to the longer illness duration group (Figure 24).

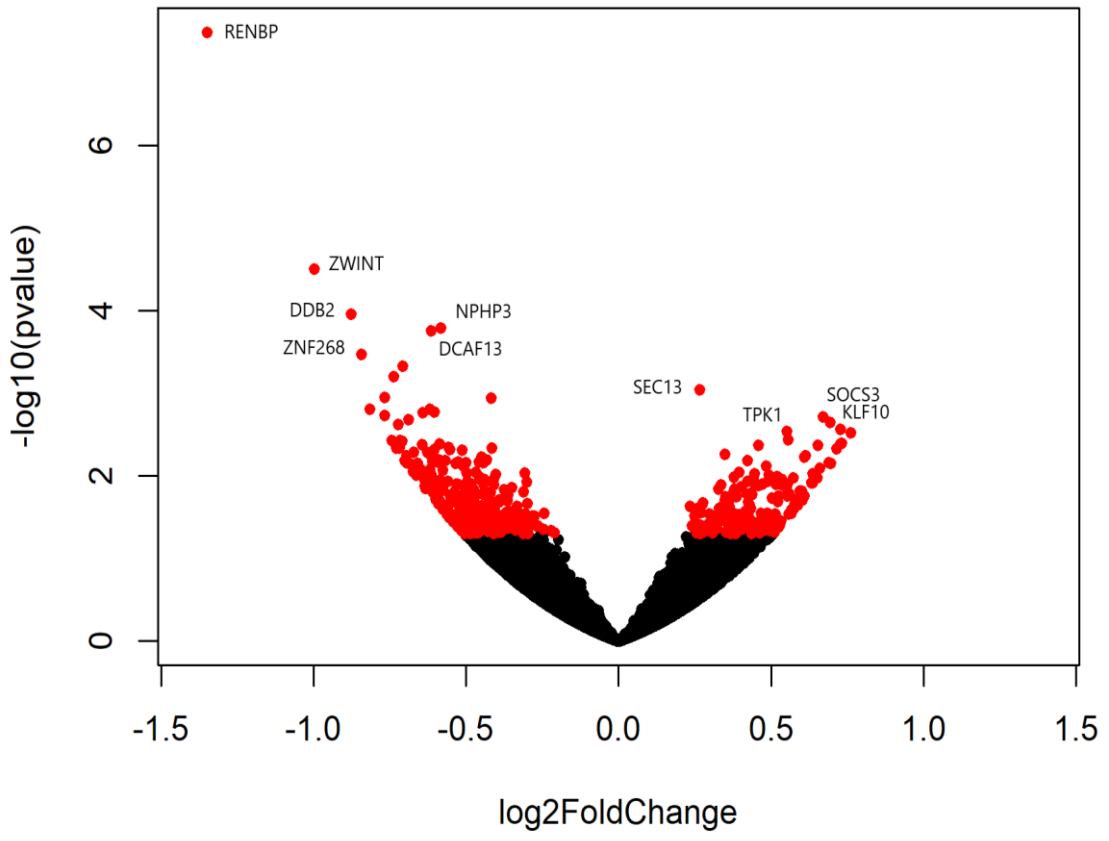


Figure 24. Volcano plot of DGE: medium versus long illness duration participants. p<.05 labelled red

GSEA revealed that the IFN-γ signature was enriched in the group of participants with a longer compared to shorter illness duration (Figure 25A, Figure 26A). In contrast, the IL-4, GC acute and LPS acute signatures were enriched amongst the genes increased in the participants with a shorter compared to longer illness duration (Figure 25A). Leading edge subset analysis for the three gene sets enriched in the shorter illness duration group demonstrated some overlap of the GC acute (18.75%) and LPS acute (5.08%) leading edge subset genes (Figure 25B). There was minimal overlap for the remaining leading-edge subset genes (1.69-6.25%). TFEA of the 148 IFN-γ leading edge subset genes again demonstrated enrichment of IRF8 regulated genes followed by IRF1 (Figure 26B).

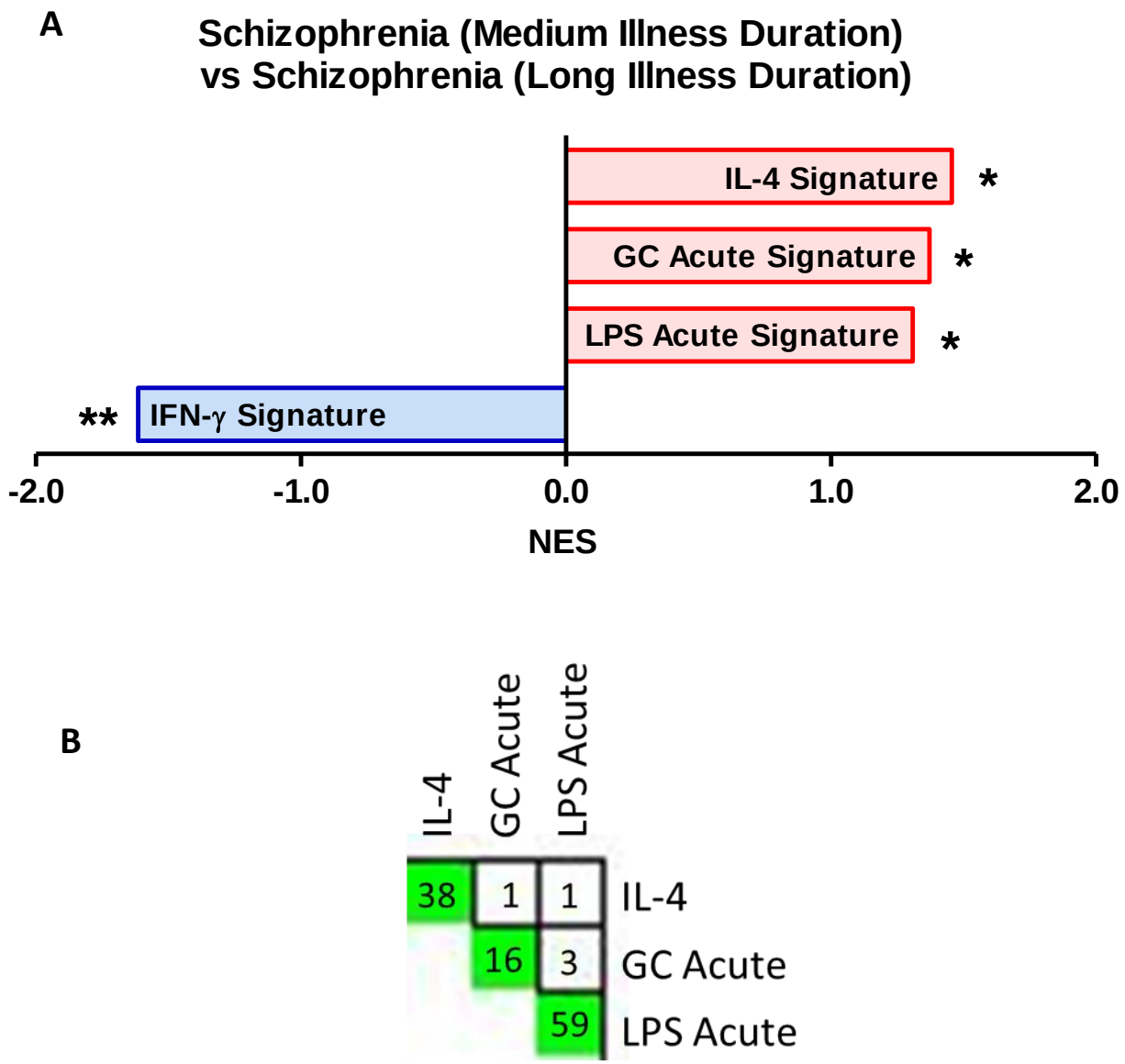


Figure 25. GSEA and leading-edge analysis: medium versus long illness duration. A) Gene sets that were enriched in the medium illness duration group compared to the long illness duration group. **FDR<.05, *FDR<.25. B) Leading-edge analysis for gene sets enriched amongst genes increased in the medium illness duration group compared to controls. The number of leading edge genes that are present in both sets are presented. The green gradient illustrates the degree of overlap.

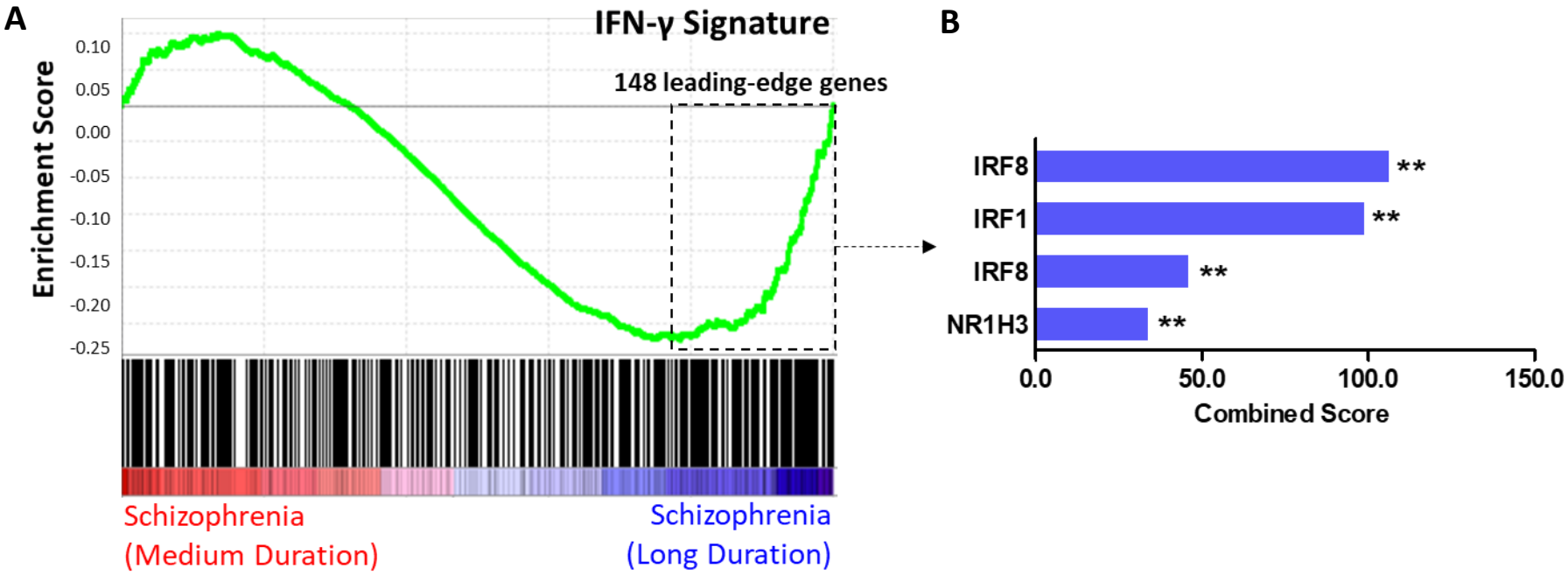


Figure 26. IFN-γ signature enrichment plot and TFEA: medium versus long illness duration. A) Enrichment plot for the IFN-γ signature in the long illness duration group compared to controls. Genes are ranked from greatest increase to the greatest decreased in expression in the long illness duration group compared to controls. Black lines indicate the location of genes that are in both the ranked list and gene set. B) Top three results from the transcription factor enrichment analysis of the leading-edge subset of genes in the IFN-γ signature. **p<.001 and adjusted p<.001

4.3.6 Transcription factor analysis: medium versus long illness duration

Finally, GSEA was carried out using the entire ranked gene list for the medium versus high illness duration group comparison with the 2016 ChIP-X database as the gene set file to determine which transcription factors may be driving the decreased expression in the lower illness duration group. The results revealed that IRF8 and IRF1 regulated genes were the top two enriched sets (Figure 27), strengthening the implication that these signaling components are involved in monocyte transcriptional alterations related to illness duration in schizophrenia. However, it should be noted that no gene set was significant at an FDR<.25.

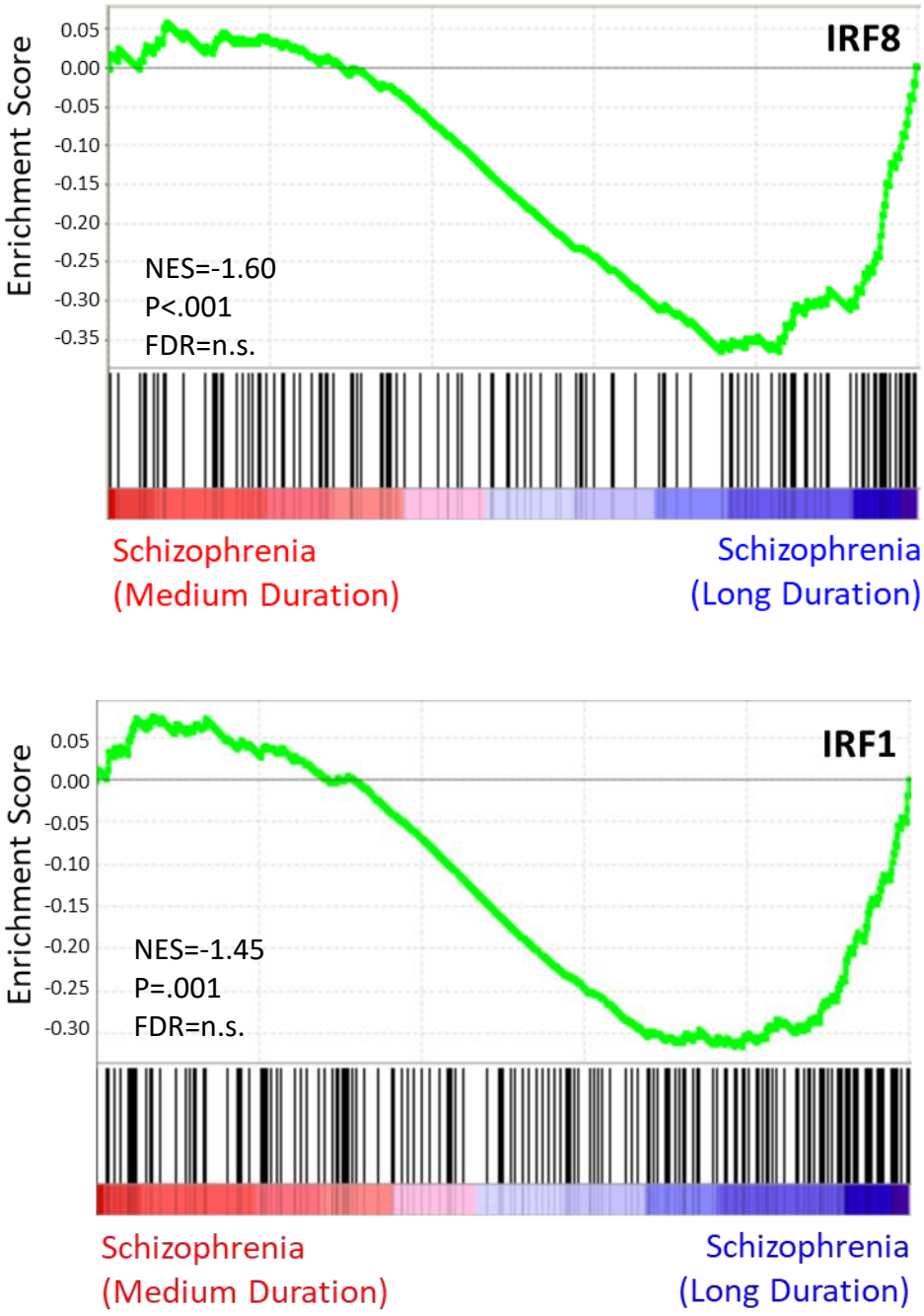


Figure 27. TF enrichment plots: medium versus long illness duration. Genes are ranked from greatest increase to the greatest decreased in expression in the long illness duration group compared to controls. Black lines indicate the location of genes that are in both the ranked list and gene set.

4.4 Discussion

To our knowledge, this is the first investigation of the monocyte transcriptome in schizophrenia using genome-wide RNA sequencing. An understanding of the changes to monocyte phenotype and function in psychosis is critical, given recent research that highlights the role of these immune cells in CNS homeostasis, neuroinflammation, and in neurological and neuropsychiatric disorders. We confirmed the presence of IFN-γ mediated JAK-STAT1 signature alterations in isolated monocytes from individuals with psychosis. Furthermore, the results presented here indicate a monocyte phenotypic switch that occurs with illness progression.

4.4.1 Type I and II IFN signatures

Using an independent cohort we replicated the findings presented in Chapter 2 that the IFN-γ mediated JAK-STAT1 signature is decreased earlier in illness compared both to control participants and to participants with a longer illness duration. In Chapter 2 this was demonstrated in total PBMCs, here we confirm this signature and relationship with illness duration in isolated monocytes. Additionally, we found that this IFN-γ signature was significantly enriched in the participant group with a longer illness duration compared to controls, and that overall there was an increased IFN-γ signature in the sample of participants with psychosis compared to controls. The increased IFN-γ signature (type II IFN) in the longer illness duration group and in all participants with schizophrenia compared to controls was also mirrored by increases in the type I IFN ('IFN-α signature') signature. However, the decreased IFN signature in the shorter illness duration group relative to controls and to the longer illness duration group was specific to the IFN-γ signature as there were no significant changes to the IFN-α signature in these comparisons.

4.4.2 LPS and ET signatures

Alterations to TLR4 mediated NF-κB signaling (the 'LPS acute signature'), an important proinflammatory pathway that controls expression of cytokines and other effectors of inflammation, have

been previously described in schizophrenia. Activation of this pathway alongside IFN-γ mediated JAK-STAT1 signaling is understood to underlie a proinflammatory myeloid cellular phenotype. The LPS acute signature was increased overall in schizophrenia and in the lower illness duration group but not the higher illness duration group compared to controls. Furthermore, this signature was enriched in the shorter compared to longer illness duration group. Thus, while both the IFN-γ and LPS acute signatures were elevated overall in schizophrenia, they diverge in relation to illness duration, with enrichment of the LPS acute signature present only earlier in illness and enrichment of the IFN-γ signature present only later in illness. One potential explanation we had considered for the decreased JAK-STAT1 transcriptional signature was a tolerized cellular state that can occur in response to high levels of NF-κB activating stimuli including LPS and TNF-α (Olga M. Pena et al. 2014; Huber et al. 2017). Examination of the ET signature revealed an enrichment overall in participants with schizophrenia and in the lower illness duration group as was seen with the LPS acute signature, with minimal overlap of genes contributing to the enrichment. While counterintuitive to see both LPS acute and ET signature enrichment, this corresponds to what the group that described the in-vivo ET signature found in participants with sepsis (Pena et al. 2014). Potential explanations will be discussed in the following sections.

4.4.3 IL-4 signature

In addition to NF-κB mediated tolerizing stimuli, Th2 cytokines such as IL-4 have been demonstrated to induce a myeloid cellular phenotype that is skewed towards anti-inflammatory and tissue remodeling functions and characterized in part by decreased JAK-STAT1 signature gene expression. The IL-4 signature was not significantly enriched overall in participants with schizophrenia or controls but was enriched in the lower illness duration group both compared with controls and with the longer illness duration group. This pattern of enrichment was the opposite of that seen for the IFN-γ signature in relation to illness duration. IL-4 mediated monocyte and macrophage functions include tissue repair and remodeling as well

as inhibition of inflammation (Mitchell, Roediger, and Weninger 2014), and could represent a protective phenotypic skew induced in individuals with a shorter illness duration.

4.4.4 Acute and chronic stress signatures

Finally, stress has been demonstrated to impact the phenotype of circulating monocytes and is a risk factor for psychosis as well as important consideration in adults with major psychiatric illness. While acute stress is predominantly anti-inflammatory, chronic stress is associated with a specific 'genomic fingerprint' in monocytes, with increased pro-inflammatory activity and glucocorticoid resistance (G. E. Miller et al. 2014). Therefore, gene signatures for the glucocorticoid acute response and chronic in-vivo caregiver stress were assessed. The glucocorticoid acute response was enriched overall in schizophrenia compared to controls, and in the lower illness duration group compared to the higher illness duration group, but not in the illness duration group comparisons with controls. The in-vivo chronic stress signature did not demonstrate enrichment in any of the comparisons. Thus, the inflammatory profile and glucocorticoid resistance that can develop as a result of chronic stress does not map onto the changes seen in immune cell signaling in psychosis.

4.4.5 Summary of signature enrichment

The primary findings following GSEA of gene expression data in schizophrenia are as follows. Firstly, the IFN-γ signature was uniquely suppressed in the shorter illness duration group both relative to controls and to the longer illness duration group. Secondly, while IFN-γ and LPS are described in the literature as proinflammatory stimuli, and together underlie the 'M1' proinflammatory myeloid cell phenotype, when assessed separately these two signatures did not show coordinated expression with one another in circulating monocytes. Thirdly, in the participants who had a shorter illness duration the suppressed IFN-γ signature corresponded to increases of the ET, IL-4 and LPS acute signatures when compared to controls, and of the IL-4, GC acute and LPS acute signatures when compared to the higher illness duration group.

Thus, in participants with a shorter illness duration the monocyte transcript expression profile is characterized predominantly by distinct anti-inflammatory and tissue remodeling (ET, IL-4, GC) 'M2' signatures, with a decrease in the 'M1' pro-inflammatory IFN-γ signature, all indicative of an M2-skew, but also demonstrates an enrichment of the proinflammatory (LPS acute) signature indicative of an M1-skew. Finally, in participants with a longer illness duration the monocyte phenotype consisted of an interferon response (both type I and II) signature, but there was no significant enrichment of any of the other gene sets.

4.4.7 IFN-γ signature transcription factor analysis

The leading-edge subset genes that drove changes in the IFN-γ signature were analyzed by TFEA. The results demonstrated that IRF8 and IRF1 were the primary transcription factors known to regulate the leading edge IFN-γ signature genes in each comparison. Furthermore, IRF8 and IRF1 were the top two transcription factors regulating genes that were decreased in the shorter compared to longer illness duration groups when using the entire ranked gene list and the same transcription factor binding database in GSEA. IRF8 and IRF1 are transcription factors that interact with STAT1 following IFN-γ stimulation and are vital for the activation of transcriptional programs underlying both myeloid cell development and the coordination of a wide range of immune and proinflammatory cellular functions (Langlais, Barreiro, and Gros 2016). They have also been associated with an M1 cellular skew (Günthner and Anders 2013). The regulation of myeloid cell transcriptional programs as well as effects on chromatin structure under stimulated and basal conditions have been termed the IRF8/IRF1 regulome. Our findings suggest that alterations to activity of the IRF8/IRF1 regulome may underlie the JAK-STAT1 signature changes seen at different time points throughout illness in individuals with psychosis, and particularly the phenotypic switch that occurs during illness progression.

4.4.6 Mixed cellular phenotypes

While some of the signature enrichment in the illness duration group comparisons fit into an M1 versus M2 framework, indicating a primarily M2-like skew in the participants with a shorter illness duration, there was also indication of mixed cellular phenotypes with the increased enrichment of both signatures that are described as proinflammatory (LPS acute) and those described as anti-inflammatory and tissue remodeling (ET, IL-4, GC), as well as divergence of specific proinflammatory signatures (IFN-γ and LPS acute). A concurrent enrichment of proinflammatory and endotoxin tolerance signatures was also seen in circulating cells from participants with sepsis (Olga M. Pena et al. 2014). The authors hypothesized that the presence of both signatures might be due to the mixed cell population from which they derived the signature. As here we describe the same phenomenon in an isolated cell type, an related explanation is that a mixed pro-and anti-inflammatory signature enrichment may be due to the fact that circulating monocytes are in various stages of development and thus enrichment may be representative of different stages of response to the same ligands. However, testing these possibilities would require a single-cell transcriptomics approach. Additionally, this co-occurrence of signatures associated with particular phenotypes also likely reflects the complexity of cellular responses. The mixed phenotype that these results indicate mirrors profiles seen in in-vivo myeloid cells such as microglia in other disorders (Morganti, Riparip, and Rosi 2016). This may partly reflect the complexity of the environmental milieu to which these cells are exposed, but also the fact that while a specific signature may induce the expression of proinflammatory genes, the reality is that the cellular response to particular ligands, such as IFN-γ and LPS, induces transcriptional programs that underlie multiple cellular functions. Thus, further analysis of functions for genes that do or do not contribute to the enrichment of a particular signature is required.

4.4.8 Implications and further considerations

How these transcriptomic changes in peripheral cells relate to cellular activity throughout the course of the illness in psychosis requires further testing, and the impact of this indicated shift in cellular

phenotype on pathophysiology in psychosis remains speculative. Genetic variants and environmental factors that influence the IRF8/IRF1 myeloid cell regulome are thought to contribute to a number of inflammatory diseases, and activation of the IRF8/IRF1 regulome is associated with neuroinflammation in certain animal models where loss-of-function mutants for these transcription factors can be protective (Langlais, Barreiro, and Gros 2016). It is increasingly clear that peripherally derived cells are present in and act at the brain borders under steady state conditions in addition to increased infiltration in the context of disease (Sevenich 2018). Additionally, while the increased infiltration of monocytes to the brain has historically been assumed detrimental, and indeed these cells have been demonstrated to contribute to neuroinflammation under many conditions, data suggest that peripherally-derived monocytes skewed towards an M2-like phenotype can also serve neuroprotective and reparative functions (Deczkowska, Baruch, and Schwartz 2016; Herz et al. 2017; Shechter and Schwartz 2013). Interestingly, recently single cell profiling studies have been used to investigate myeloid cell alterations in the CNS across the course of the illness in murine models of Alzheimer's disease, and results similarly suggest a shift in phenotype in earlier versus later illness (Sevenich 2018).

In this analysis we cannot account for antipsychotic medication effects as only one participant was untreated at the time of the blood draw. Based on the results of Chapter 3, it may be the case that cumulative effects of antipsychotic treatment contribute to the increased JAK-STAT1 signature enrichment seen later in illness, but this requires further testing. Here we have limited our sample of participants with psychosis to individuals with chronic, but not acute, illness. These analyses relating to illness duration should be further extended to participants with recent onset psychosis. It would be interesting to determine whether these temporal shifts in monocyte phenotype at different illness stages relate to measures of blood brain barrier dysfunction and immune activation in the CNS. Post-mortem data highlight immune alterations to the blood brain barrier and choroid plexus, but to our knowledge

the relationship of these measures with illness duration has not been assessed (Pollak et al. 2017; Kim et al. 2016).

DISCUSSION

5.1 Introduction

The primary aim of this thesis was to examine alterations to the JAK-STAT1 pathway in peripheral immune cells over the course of the illness in psychosis. Markers of JAK-STAT1 activity were assessed in PBMCs from participants with psychosis in relation to illness duration, acuity, symptomology and treatment. The effects of treatment, specifically the atypical antipsychotic risperidone, were then assessed using human myeloid cell models. Finally, transcriptional profiling was carried out using isolated monocytes from an independent cohort of participants with psychosis, and the JAK-STAT1 signature was assessed in relation to illness duration and other related cellular signatures. The results will be outlined and interpreted, and future directions discussed.

5.2 The JAK-STAT1 signature in psychosis

In the first research chapter (Chapter 2) the JAK-STAT1 pathway was assessed in peripheral blood cells from participants with psychosis. Firstly, levels of pSTAT1, the downstream signaling component activated following stimulation of this pathway, were measured by ELISA. The results demonstrated that a subset of participants with psychosis had increased levels of pSTAT1 in PBMCs compared to controls. These initial results suggested altered activity of the JAK-STAT1 signaling pathway in psychosis. A panel of genes representative of the IFN-γ mediated JAK-STAT1 signature (IFNG, CXCL10, IRF1, STAT1, TLR4) were measured in PBMCs and analyzed in relation to illness duration, acuity and measures of psychopathology in participants with psychosis. Contrary to our expectations, the genes selected to represent the JAK-STAT1 transcriptional signature demonstrated suppressed expression in participants with psychosis who were earlier in illness and in those who had greater illness acuity relative to controls. Furthermore, we demonstrated these JAK-STAT1 signature genes increased over the course of the illness, and that some

but not all of the selected genes were negatively associated with psychopathology scores encompassing positive, negative and general symptoms.

A composite score representative of the combined expression of all five genes was created for further analysis. Examination of the association between the composite score and illness duration in relation to illness acuity suggested that high illness acuity corresponded to a suppressed JAK-STAT1 transcriptional signature regardless of illness duration, and therefore that the positive association of gene expression with illness duration was driven by participants with chronic and persistent, but not acute, psychopathology. However, there were only a small number of participants in the high illness acuity group who were also comparatively later in illness. When the influence of demographic and clinical characteristics were included in a multiple regression model for just the participants with psychosis, illness duration was the sole predictor of the JAK-STAT1 composite score. We had expected to find increased activity of the JAK-STAT1 pathway in circulating immune cells based on its well characterized proinflammatory functions, and the elevated peripheral inflammation frequently reported in psychosis. There are multiple factors that might explain our results that instead demonstrated suppression of the IFN-γ mediated JAK-STAT1 transcriptional signature in relation to the clinical characteristics outlined, which will be discussed in the following sections.

C4A was also measured alongside these JAK-STAT1 signature genes. Recently, schizophrenia risk was shown to be associated with C4A structural variants that lead to increased mRNA expression, and mRNA expression was elevated in post-mortem brain tissue in schizophrenia. As C4A is also induced by activation of the JAK-STAT1 pathway by IFN-γ we hoped to replicate this increased C4A expression, and to demonstrate co-expression with other JAK-STAT1 regulated genes. However, C4A was only co-expressed with some of the other JAK-STAT1 regulated genes and demonstrated no variability across different stages of illness. In fact, there was an opposing relationship with psychopathology in that C4A

mRNA expression was positively correlated with measures of positive symptomology. These results indicate that C4A expression is not coordinated with IFN-γ mediated JAK-STAT1 signaling in circulating immune cells. This association with symptomology is of interest given the known function of C4A in synaptic pruning, which forms a key part of the neurodevelopmental hypothesis of psychosis. One possibility is that individuals with psychosis who have structural variants associated with increased expression of C4A present with a more severe psychopathology. Future studies should determine gene structure of the C4A locus alongside gene expression and measures of positive symptomology.

5.3 Risperidone and the JAK-STAT1 signature

One potential explanation for the alterations to the JAK-STAT1 signature in relation to illness duration and acuity outlined in Chapter 2 was that treatment with antipsychotic medication, which is known to have immunomodulatory properties, inhibits JAK-STAT1 pathway activity, or alternatively, might account for the increase in the JAK-STAT1 signature observed over the course of the illness.

In the first part of Chapter 3, the JAK-STAT1 composite score was compared between antipsychotic treated participants and untreated participants, with no significant different. As different antipsychotics have been demonstrated to have varying immunomodulatory properties, a subgroup of participants treated with risperidone monotherapy were selected. Risperidone was the most frequently prescribed antipsychotic drug in the study sample, with the remaining prescribed antipsychotics too varied for subgroup analysis. The sample was match on illness duration as this variable was uniquely demonstrated to affect the JAK-STAT1 transcriptional signature in the previous chapter. There was no significant difference in the JAK-STAT1 composite score when comparing untreated participants and this risperidone monotherapy group. However, we found that participants with a longer illness duration who were treated with risperidone monotherapy had significantly elevated JAK-STAT1 composite scores compared to all untreated participants and to risperidone monotherapy treated participants who had a

shorter illness duration. Interestingly, the JAK-STAT1 composite score was positively correlated with illness duration in both the untreated and risperidone monotherapy treated groups.

Next, the THP-1 human monocyte cell line was used to test the effects of risperidone on JAK-STAT1 signature gene expression. We showed that risperidone potentiated the expression of the selected JAK-STAT1 signature genes CXCL10, IRF1 and STAT1 in IFN-γ stimulated THP-1 monocytes. THP-1 monocytes were then differentiated to macrophages and were treated with risperidone under either proinflammatory 'M1' or anti-inflammatory/tissue remodeling 'M2' polarizing conditions. Risperidone again increased the expression of JAK-STAT1 signature genes in both conditions. These data indicate that risperidone skews myeloid cells away from an M2 phenotype and towards an M1 phenotype.

With regards to the clinical findings described in Chapter 2, both the clinical data and cell culture model indicate that antipsychotic treatment does not account for the suppressed JAK-STAT1 transcriptional signature seen earlier in illness. The cell culture model instead demonstrates that risperidone has a potentiating effect on expression of JAK-STAT1 signature genes in monocytes. While causation can't be inferred from the clinical data, the JAK-STAT1 composite score was also increased in risperidone treated participants, but only in those with a longer illness duration. Two possible explanations for these findings are, one, that cumulative effects of long term treatment with risperidone may result in increased JAK-STAT1 signature expression in circulating immune cells, and two, that risperidone treatment in participants with a longer illness duration has a potentiating effect on JAK-STAT1 signature expression that is not seen in participants with a shorter illness duration due to underlying physiological differences.

Interestingly, however, the positive association of the JAK-STAT1 composite score with illness duration seen in both the risperidone treated and untreated participant groups indicates that factors in

addition to antipsychotic treatment contribute to this relationship. Furthermore, the decrease in JAK-STAT1 signature gene expression seen in participants with a shorter illness duration remains unexplained.

5.4 Transcriptional profiling of monocytes in psychosis

Another potential explanation for the alterations observed to the JAK-STAT1 transcriptional signature was that circulating monocytes are skewed from a proinflammatory phenotype (often termed M1) towards an anti-inflammatory and tissue remodeling phenotype (often termed M2) in individuals who are earlier in illness and during periods of increased illness acuity. In Chapter 4, we carried out genome-wide transcriptional profiling of isolated monocytes from an independent cohort of participants with psychosis, all of whom had a DSM-IV diagnosis of schizophrenia. GSEA was used to determine the relative enrichment of selected transcriptional signatures representative of a potentially relevant stimuli associated with specific myeloid cellular phenotypes. Alterations to these signatures were considered primarily in relation to illness duration (medium versus long) in participants with chronic psychosis. We replicated the findings outlined in Chapter 2, demonstrating a suppressed IFN-γ mediated JAK-STAT1 transcriptional signature in participants with the shorter illness duration that reversed in the longer illness duration group. Of the cellular responses measured, suppressed expression earlier in illness was unique to the IFN-γ signature. Additionally, we demonstrated that IRF8 and IRF1 are the primary transcription factors shown to regulate genes within each of the IFN-γ leading edge subsets, which are the genes that drive enrichment of the signature. Additionally, genes regulated by IRF8 an IRF1 were the top 2 enriched sets when the entire medium versus long illness duration ranked list was analyzed in GSEA, but did not withstand correction for multiple testing of the large number of gene sets.

The initial premise for this research was based partly on the well characterized role of IFN-γ mediated JAK-STAT1 signaling in inducing a myeloid cell proinflammatory phenotype. A variety of physiological conditions and ligands have been demonstrated to skew myeloid cells from a phenotype

that is more proinflammatory in nature (M1) towards more anti-inflammatory and tissue remodeling phenotypes (M2). Indeed, in the participants with a shorter illness duration where the IFN-γ signature showed decreased enrichment compared to controls and to the longer illness duration group, there was increased enrichment of the IL-4 and ET signatures compared with controls, and the IL-4 and GC signatures when compared with the longer illness duration group. However, enrichment of these 'M2' signatures was accompanied by enrichment of the LPS acute signature which is considered characteristic of an 'M1' phenotype. Thus, a mixed phenotype was seen in circulating monocytes of individuals with psychosis earlier compared to later in illness, with activation of predominantly anti-inflammatory and tissue remodeling transcriptional programs, but also of LPS responsive genes. In the late illness duration group, the monocyte signature was dominated by an interferon response signature, both IFN-γ and IFN-α.

There are a number of considerations to take into account when interpreting these results. The degree of polarity of these phenotypes in-vivo has been called into question due to the complex nature of stimuli to which cells are exposed. Some studies have recently demonstrated mixed myeloid cell phenotypes, which have characteristics of both proinflammatory and anti-inflammatory/tissue remodeling cells. This is further complicated by the fact that each stimulus can induce transcriptional programs involved in a variety of functions and there are varying degrees of crosstalk between stimuli. An additional consideration is that while we restricted our sampling to isolated monocytes, there is constant turnover of this population, and thus variability in phenotypic signatures may also be due to temporal dynamics of the cellular response to a specific stimulus. Finally, though demographic characteristics were well matched in this cohort, whether these findings are influenced by antipsychotic medications as was suggested in Chapter 3 requires testing in further studies as all but one of the participants with psychosis was treated with antipsychotics.

5.5 Potential relationship of alterations to JAK-STAT1 activity to pathophysiological mechanisms in psychosis

When the IFN-γ mediated JAK-STAT1 signature was selected for investigation we hypothesized elevated activity of this pathway in psychosis based on meta-analyses indicating increased proinflammatory activity in the periphery and CNS in participants with schizophrenia and bipolar disorder (Goldsmith, Rapaport, and Miller 2016). The results presented here, in which we examined in detail the IFN-γ mediated JAK-STAT1 signature in PBMCs, followed by genome-wide analysis of related myeloid cell signatures in isolated monocytes, instead indicates suppression of IFN-γ signatures at earlier illness time points and with greater illness acuity in participants with psychosis. The transcriptomic data demonstrate a corresponding enrichment of opposing M2-like signatures, most consistently the IL-4 signature, in monocytes from the group of participants with psychosis who were earlier in illness.

One of the potential mechanisms linking immune alterations with neurotransmitter changes and therefore symptomology in psychosis are alterations to kynurenine metabolism (B. J. Miller and Goldsmith 2016). Kynurenine is produced following breakdown of tryptophan, and further metabolized to kynurenic acid or quinolinic acid (Müller and Schwarz 2010). Interestingly, a type 2 immune response, in which IL-4 is the primary driving cytokine and an M2 myeloid cell skew is present, has been proposed to play a role in psychosis pathophysiology through inhibition of the metabolism of kynurenine to quinolinic acid in favor of metabolism to kynurenic acid (Müller et al. 2015). Kynurenic acid is the only known endogenous NMDA receptor antagonist, and NMDA receptor hypofunction is strongly implicated in psychosis pathophysiology. Animal studies demonstrate that experimentally increasing kynurenine additionally leads to increased midbrain dopaminergic activity and behavioral changes associated with psychosis including sensorimotor gating impairments, cognitive deficits and decreased social activity (Erhardt, Schwieler, and Imbeault 2017). Furthermore, clinical data demonstrate elevated kynurenic acid in the CSF and post-mortem brain in participants with schizophrenia and bipolar disorder with psychosis. Thus, one

potential consequence of the pattern of immune response seen earlier in illness and with increased illness acuity is elevations in kynurenic acid and subsequent alterations to glutamatergic and dopaminergic signaling. Further studies are required to test this possibility, specifically to determine whether changes to peripheral IFN-γ/IL-4 signatures are associated with elevated central kynurenic acid.

Alternatively, it is possible that the M2-like skew seen in circulating monocytes earlier in illness represents a recruitment of protective leukocytes to the CNS under neuroinflammatory conditions. Such a recruitment of M2-skewed cells is thought to play a protective role following CNS injury and in certain neurological and neurodegenerative disorders via secretion of effectors with anti-inflammatory and tissue repair functions (Ge et al. 2017; Shechter and Schwartz 2013), though this possibility has not yet been explored in psychosis. Disentangling the role of microglia, border-associated macrophages and blood-derived monocyte/macrophages in CNS disorders and associated animal models is a major challenge due to a lack of distinguishing cell markers, though advances are being made using mass cytometry and single-cell RNA sequencing (Sevenich 2018). The application of these techniques and findings to the study of neuropsychiatric illness may shed light on the role of these myeloid cells and other circulating leukocytes in psychosis.

5.6 Overall conclusions and future directions

The data presented in this thesis highlight alterations to immune cell transcriptional signatures in psychosis across different stages of illness, with emphasis on the IFN-γ mediated JAK-STAT1 signature. The primary finding is that there is a decreased IFN-γ-JAK-STAT1 transcriptional signature in circulating PBMCs and monocytes from participants with psychosis that persists during short to medium illness duration time points and is reversed later in illness, indicating a phenotypic shift in these cells. This pattern of suppression and reversal of the IFN-γ mediated JAK-STAT1 signature was seen in two independent cohorts using two different techniques. Based on the results from the first study it is expected that monocyte

genome-wide transcriptional profiling in participants with a shorter illness duration than assessed in the second study would reveal a more pronounced suppression of the IFN-γ signature. This should be tested in future investigations. In the first cohort illness acuity was also assessed and indicated that the IFN-γ-JAK-STAT1 signature is suppressed in participants with high illness acuity requiring hospitalization, even later in illness. However, this was tested using only a small number of participants with high illness acuity who were also later in illness, and participants with an acute exacerbation of illness were not included in the second study. Thus, transcriptional profiling of monocytes in participants with high illness acuity at different illness duration time points should also be carried out.

The increased molecular depth and cellular specificity gained using the genome-wide approach allowed for bioinformatic analyses that highlighted perturbations to several theoretically relevant transcriptional signatures in relation to illness duration in addition to the IFN-γ-JAK-STAT1 signature. The results supported an increased enrichment of signatures associated with anti-inflammatory and tissue remodeling phenotypes in the earlier but not later illness duration group. However, the results also indicated a mixed monocyte phenotype that might reflect the in-vivo complexity of stimuli in the environmental milieu or of the transcriptional programs they elicit, or alternatively, heterogenous activation states in the monocyte population. Testing these possibilities requires improved characterization of signatures underlying specific functional phenotypes as well as the use of single cell transcriptomics. The genome-wide transcriptional profiling data also demonstrated that monocytes in the long illness duration group had an overall enriched interferon signature compared to controls those with a shorter illness duration. The findings from the first study that risperidone treated participants with a longer illness duration had an elevated JAK-STAT1 transcriptional signature and the data from the human monocyte cell culture models showing that risperidone potentiates JAK-STAT1 signature gene expression in a human monocyte cell model suggests that risperidone may contribute to increases in this signature seen later in illness. Interestingly, the positive association of JAK-STAT1 signature gene expression with

illness duration was seen even in untreated participants in this first study, suggesting that medication effects are not sufficient to explain increased expression. However, these findings need to be confirmed in a larger clinical sample and extended to other antipsychotics which may have different effects.

Overall, understanding the cause of these alterations to monocyte phenotype in psychosis and whether and how they influence pathophysiological mechanisms requires further work in a number of converging areas of research. An understanding of monocytes as effectors themselves, and not only as precursors to tissue macrophages, is a relatively recent concept spurred by advances in lineage tracing. Thus, determination of monocyte transcriptional profiles in relation to specific stimuli, functional phenotypes and disease states is ongoing. Additionally, the mechanisms by which peripherally-derived immune cells interface with cells of the CNS and influence neuronal processing and behavior is also an emerging topic of research. The translational potential of rodent models is somewhat hindered by differences between murine and human myeloid cells, though progress is being made in understanding these incongruities.

With regards to clinical research in human psychiatric populations, though serum cytokines have been the norm, more nuanced measures of immune alterations that focus on specific cell types, and genome-wide approaches to investigating cellular activity are needed. Context is also critical. The immune system is dynamic, and these measures should be assessed in relation to genetic background and risk factors, illness stage, symptomology, and treatment. Though access to the CNS is primarily limited to post-mortem tissue, studies that focus on molecular measures of immune activity at the brain borders will likely prove informative. Additionally, it is conceivable that continued advances in brain imagining will allow for the combination of these molecular approaches to immune cell profiling in living subjects with imaging of CNS immune activity, neurotransmitter alterations and neurostructural changes psychosis. As is often the case in medical sciences, alterations that are initially presumed to be deleterious when

discovered in the context of illness or injury may actually serve predominantly protective functions. Whether this is true for some of the changes to monocyte activity highlighted here remains to be determined. Ultimately, the hope is that an understanding of the contribution of immune alterations to pathophysiology over the course of the illness will lead to improved treatment options and outcomes for individuals with psychosis.

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APPENDIX A

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Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

February 13, 2014

Rajiv P. Sharma, MD
Psychiatry
1601 W. Taylor Street
475 P.I., M/C 912
Chicago, IL 60612
Phone: (312) 413-4508 / Fax: (312) 355-1492

RE: Protocol # 2012-0113
"The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain"

Dear Dr. Sharma:

Your Continuing Review was reviewed and approved by the Convened review process on February 11, 2014. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: March 12, 2014 - March 12, 2015
Approved Subject Enrollment #: 240
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC
Sponsor: National Institute of Mental Health
PAF#: 2012-02022
Grant/Contract No: 1 R01 MH094358-01
Grant/Contract Title: The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain
Research Protocol:

a) The H3K9 Histone Switch; 'Levels' In Schizophrenia Blood and Brain Version 4, 11/16/2012

Recruitment Materials:

a) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012
b) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers) Version 3; 02/26/2013
c) (If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 3; 02/26/2013

Informed Consents:

a) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 4 11/16/2012
b) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient Version 4 11/16/2012
c) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 4 - 08/14/2013
d) Waiver of consent for recruitment purposes only was granted under 45 CFR 46.116 (d)

HIPAA Authorizations:

a) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain HIPPA, Version 3, 08/14/2013 – *Continue to use this document as it has not expired.*
b) Waiver of HIPAA Authorization for recruitment purposes only granted under 45 CFR 164.512

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
01/15/2014	Continuing Review	Convened	02/11/2014	Approved

Please remember to:
→ Use your **research protocol number** (2012-0113) on any documents or correspondence with the IRB concerning your research protocol.
→ Review and comply with all requirements on the enclosure,
"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"
(*http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf*)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further

help, please contact OPRS at (312) 996-1711 or me at (312) 355-1609. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Rahab Mwangi, MPH
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

Enclosures:

1. **Informed Consent Documents:**
   a) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 4 11/16/2012
   b) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient Version 4 11/16/2012
   c) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 4 - 08/14/2013
2. **Recruiting Materials:**
   a) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012
   b) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers) Version 3; 02/26/2013
   c) (If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 3; 02/26/2013

cc: Anand Kumar, Psychiatry, M/C 912
OVCR Administration, M/C 672
Privacy Office, Health Information Management Department, M/C 772
IDS, Pharmacy Practice, M/C 883

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review (Response To Modifications)

February 18, 2015

Rajiv P. Sharma, MD
Psychiatry
1601 W. Taylor Street
475 P.I., M/C 912
Chicago, IL 60612
Phone: (312) 413-4508 / Fax: (312) 355-1492

RE: Protocol # 2012-0113
"The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain"

Dear Dr. Sharma:

Your Continuing Review (Response To Modifications) was reviewed and approved by the Expedited review process on February 17, 2015. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: March 12, 2015 - March 11, 2016
Approved Subject Enrollment #: 240
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC
Sponsor: National Institute of Mental Health
PAF#: 2012-02022
Grant/Contract No: 1 R01 MH094358-01
Grant/Contract Title: The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain
Research Protocol:

b) The H3K9 Histone Switch; 'Levels' In Schizophrenia Blood and Brain Version 4,

11/16/2012

Recruitment Materials:

d) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012

e) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015

f) If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015

Informed Consents:

e) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient Version 5, 02/13/2015

f) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015

g) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015

h) Waiver of consent for recruitment purposes only was granted under 45 CFR 46.116 (d)

HIPAA Authorization (Continue to use the previously approved document):

c) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain HIPPA, Version 3, 08/14/2013

d) Waiver of HIPAA Authorization for recruitment purposes only granted under 45 CFR 164.512

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
01/22/2015	Continuing Review	Convened	02/10/2015	Modifications Required
02/13/2015	Response To Modifications	Expedited	02/17/2015	Approved

Please remember to:

→ Use your **research protocol number** (2012-0113) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the right to seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-3788. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Rachel Olech, B.A., CIP
Assistant Director, IRB # 3
Office for the Protection of Research Subjects

Enclosures:

3. Informed Consent Documents:

d) The H3K9 Histone Switch 'Levels'' in Schizophrenia Blood and Brain-Phase 1-Patient Version 5, 02/13/2015
e) The H3K9 Histone Switch 'Levels'' in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015
f) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015

4. Recruiting Materials:

d) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012
e) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015
f) If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015

cc: Anand Kumar, Psychiatry, M/C 912
OVCR Administration, M/C 672
Privacy Office, Health Information Management Department, M/C 772
IDS, Pharmacy Practice, M/C 883

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

February 12, 2016

Rajiv P. Sharma, MD
Psychiatry
1601 W. Taylor Street
475 P.I., M/C 912
Chicago, IL 60612
Phone: (312) 413-4508 / Fax: (312) 355-1492

RE: Protocol # 2012-0113
"The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain"

Dear Dr. Sharma:

Your Continuing Review was reviewed and approved by the Convened review process on February 9, 2016. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: February 9, 2016 - February 8, 2017
Approved Subject Enrollment #: 240
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC
Sponsor: National Institute of Mental Health
PAF#: 2012-02022
Grant/Contract No: 1 R01 MH094358-01
Grant/Contract Title: The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain
Research Protocol:

c) The H3K9 Histone Switch; 'Levels' In Schizophrenia Blood and Brain; Version 5; 03/19/2015

Recruitment Materials:

g) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012

h) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015

i) If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015

j) The H3K9 HISTONE SWITCH: 'LEVELS' IN SCHIZOPHRENIA BLOOD and BRAIN – FEP_Version 2 _03.18.15

Informed Consents:

i) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015

j) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015

k) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient; Version 6; 02/24/2015

l) Waiver of consent for recruitment purposes only was granted under 45 CFR 46.116 (d)

HIPAA Authorizations:

e) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain HIPPA, Version 3, 08/14/2013

f) Waiver of HIPAA Authorization for recruitment purposes only granted under 45 CFR 164.512

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
01/20/2016	Continuing Review	Convened	02/09/2016	Approved

Please remember to:

→ Use your **research protocol number** (2012-0113) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements as explained in the following, which are posted on the OPRS website *(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/index.shtml)*: "**UIC Investigator Responsibilities, Protection of Human Research Subjects**" *(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)*

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-2053. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Laura Litman
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

Enclosures attached electronically:

5. Informed Consent Documents:

g) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015
h) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015
i) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient; Version 6; 02/24/2015

6. Recruiting Materials:

g) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012
h) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015
i) If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015
j) The H3K9 HISTONE SWITCH: 'LEVELS' IN SCHIZOPHRENIA BLOOD and BRAIN – FEP_Version 2 _03.18.15

cc: Anand Kumar, Psychiatry, M/C 912
OVCR Administration, M/C 672
Privacy Office, Health Information Management Department, M/C 772
IDS, Pharmacy Practice, M/C 883

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

January 11, 2017

Rajiv P. Sharma, MD
Psychiatry
1601 W. Taylor Street
475 P.I., M/C 912
Chicago, IL 60612
Phone: (312) 413-4508 / Fax: (312) 355-1492

RE: Protocol # 2012-0113
"The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain"

Dear Dr. Sharma:

Your Continuing Review was reviewed and approved by the Convened review process on January 10, 2017. You may now continue your research.

Please note the following information about your approved research protocol:

Please note that Investigator training credits have lapsed for Kayla Chase (10/16/2016), Alessandro Guidotti (05/19/2016) and Ben Feiner (10/16/2016). These individuals must complete a minimum of two hours each of continuing education on human subject protection prior to further participation as a member of the research team. For further information, please see the OPRS website: http://research.uic.edu/compliance/irb/education-training.

Protocol Approval Period:	February 8, 2017 - February 8, 2018
Approved Subject Enrollment #:	240 (192 enrolled)
Performance Sites:	UIC
Sponsor:	National Institute of Mental Health
PAF#:	00028141
Grant/Contract No:	1 R01 MH094358-01
Grant/Contract Title:	The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain

Research Protocol(s):

d) The H3K9 Histone Switch; 'Levels' In Schizophrenia Blood and Brain; Version 5; 03/19/2015

Recruitment Material(s):

k) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012

l) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015

m) If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015

n) The H3K9 HISTONE SWITCH: 'LEVELS' IN SCHIZOPHRENIA BLOOD and BRAIN – FEP_Version 2 _03.18.15

Informed Consent(s):

m) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015

n) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015

o) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient; Version 6; 02/24/2015

p) Waiver of consent for recruitment purposes only was granted under 45 CFR 46.116 (d)

HIPAA Authorization(s):

g) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain HIPPA, Version 3, 08/14/2013

h) Waiver of HIPAA Authorization for recruitment purposes only granted under 45 CFR 164.512

Additional Determinations for Research Involving Minors:

These determinations have not been made for this study since it has not been approved for enrollment of minors.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
12/12/2016	Continuing Review	Convened	01/10/2017	Approved

Please remember to:

→ Use your **research protocol number** (2012-0113) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the guidance,

"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"
(*http://research.uic.edu/irb/investigators-research-staff/investigator-responsibilities*)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-0241. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,
Ibraheem Oguntade
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

Enclosure(s) sent as attachment to a separate email:

Please note that stamped and approved .pdfs of all recruitment and consent documents will be forwarded as an attachment to a separate email. OPRS/IRB no longer issues paper letters and stamped/approved documents, so it will be necessary to retain these emailed documents for your files for auditing purposes.

7. Informed Consent Document(s):

j) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015
k) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015
l) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient; Version 6; 02/24/2015

8. Recruiting Material(s):

k) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012
l) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015
m)If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015
n) The H3K9 HISTONE SWITCH: 'LEVELS' IN SCHIZOPHRENIA BLOOD and BRAIN –

o) APPENDIX B (continued)

cc: Anand Kumar, Psychiatry, M/C 912
OVCR Administration, M/C 672
Privacy Office, Health Information Management Department, M/C 772
IDS, Pharmacy Practice, M/C 883

Approval Notice
Continuing Review

January 17, 2018

Rajiv P. Sharma, MD
Psychiatry
1601 W. Taylor Street
475 P.I., M/C 912
Chicago, IL 60612
Phone: (312) 413-4508 / Fax: (312) 355-1492

RE: Protocol # 2012-0113
"The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain"

Dear Dr. Sharma:

Your Continuing Review was reviewed and approved by the Convened review process on January 9, 2018. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: January 9, 2018 - January 9, 2019
Approved Subject Enrollment #: 240
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC
Sponsor: National Institute of Mental Health
PAF#: 00028141
Grant/Contract No: 1 R01 MH094358-01
Grant/Contract Title: The H3K9 Histone Switch; 'Levels' in Schizophrenia Blood and Brain
Research Protocol:

e) The H3K9 Histone Switch; 'Levels' In Schizophrenia Blood and Brain; Version 5; 03/19/2015

Recruitment Materials:

o) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012
p) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015
q) If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015
r) The H3K9 HISTONE SWITCH: 'LEVELS' IN SCHIZOPHRENIA BLOOD and

BRAIN – FEP_Version 2 _03.18.15

Informed Consents:

q) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015

r) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015

s) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient; Version 6; 02/24/2015

t) Waiver of consent for recruitment purposes only was granted under 45 CFR 46.116 (d)

HIPAA Authorizations:

i) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain HIPPA, Version 3, 08/14/2013

j) Waiver of HIPAA Authorization for recruitment purposes only granted under 45 CFR 164.512

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
12/19/2017	Continuing Review	Convened	01/09/2018	Approved

Please remember to:

→ Use your **research protocol number** (2012-0113) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the guidance,
"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-2053. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Laura Litman
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

Uploaded documents:

9. Informed Consent Documents:

m) The H3K9 Histone Switch 'Levels' in Schizophrenia Blood and Brain - Phase 2 Version 5, 02/13/2015
n) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Control Version 5, 02/13/2015
o) The H3K9 Histone Switch 'Levels" in Schizophrenia Blood and Brain-Phase 1-Patient; Version 6; 02/24/2015

10. Recruiting Materials:

p) The H3K9 Histone Switch "Levels' in Schizophrenia Blood and Brain-Phone Interview - Version 2, 04/11/2012
q) The H3K9 HISTONE SWITCH "LEVELS IN SCHIZOPHRENIA BLOOD (Healthy Volunteers), Version 6, 02/13/2015
r) If you do suffer) THE H3K9 HISTONE SWITCH LEVELS IN SCHIZOPHRENIA BLOOD AND BRAIN; Version 6, 02/13/2015
s) The H3K9 HISTONE SWITCH: 'LEVELS' IN SCHIZOPHRENIA BLOOD and BRAIN – FEP_Version 2 _03.18.15

cc: Anand Kumar, Psychiatry, M/C 912
OVCR Administration, M/C 672
Privacy Office, Health Information Management Department, M/C 772
IDS, Pharmacy Practice, M/C 883

VITA

NAME: Jennifer Kate Melbourne

EDUCATION: B.S., Biomedical Sciences (Neuroscience), Cardiff University, Cardiff, United Kingdom, 2009

M.S., Mind, Language & Embodied Cognition, University of Edinburgh, Edinburgh, United Kingdom, 2011

Ph.D., Neuroscience, University of Illinois at Chicago, Chicago, Illinois, 2018

HONORS: University Fellowship, Graduate College of the University of Illinois at Chicago, Chicago, Illinois, 2014-2015

Pre-doctoral Education for Clinical & Translational Scientists Fellowship, Center for Clinical and Translational Science at the University of Illinois at Chicago, 2017-2018

PUBLICATIONS:

Melbourne, J.K, Rosen, C., Feiner, B., Sharma, R.P. (2018). C4A mRNA expression in PBMCs predicts the presence and severity of delusions in schizophrenia and bipolar disorder with psychosis. *Schizophrenia Re*search. doi.org/10.1016/j.schres.2018.01.018

Melbourne, J.K., Chase, K.A., Feiner, B., Rosen, C., Sharma, R.P. (2017). Long non-coding and endogenous retroviral RNA levels are associated with proinflammatory cytokine mRNA expression in peripheral blood cells: Implications for schizophrenia. *Psychiatry Research*. 262, 465-468.

Melbourne, J. K., Feiner, B., Rosen, C., Sharma, R.P. (2017). Targeting the Immune System with Pharmacotherapy in Schizophrenia. *Current Treatment Options in Psychiatry,* 4(2), 139-151.

Sharma, R. P., Rosen, C., **Melbourne, J. K.**, Feiner, B., Chase, K. A. (2016). Activated Phosphorylated STAT1 Levels as a Biologically Relevant Immune Signal in Schizophrenia. *Neuroimmunomodulation*, *23*(4), 224–229.

Rosen, C., McCarthy-Jones, S., Chase, K. A., Humpston, C. S., **Melbourne, J. K.,** Kling, L., Sharma, R.P. (2018). The tangled roots of inner speech, voices and delusions. *Psychiatry Research.*

Rosen, C., Jones, N., Longden, E., Chase, K. A., Shattell, M., **Melbourne, J. K.**, Keedy, S., Sharma, R.P. (2017). Exploring the Intersections of Trauma, Structural Adversity and Psychosis Among a Primarily African-American Sample: A Mixed Methods Analysis. *Frontiers in Psychiatry,* 8(57).

Rosen, C., Jones, N., Chase, K. A., **Melbourne, J. K.**, Grossman, L. S., Sharma, R. P. (2017). Immersion in altered experience: An investigation of the relationship between absorption and psychopathology. *Consciousness and Cognition*, *49*, 215–226.

SELECTED RESEARCH POSTERS AND PRESENTATIONS:

Immune alterations in psychosis: focus on the JAK-STAT1 signaling system. Oral presentation at the *Psychiatric Institute & Center for Alcohol Research in Epigenetics Neuroscience Seminar Series*, University of Illinois at Chicago, 2018.

The JAK-STAT1 transcriptional signature is altered in psychosis: relation to illness duration and acuity. Oral presentation at the *Laboratory of Integrative Neuroscience's 17th Annual Spring Symposium*, University of Illinois at Chicago, 2018.

Investigation of the JAK-STAT1 signature in psychosis reveals transcriptional alterations related to illness duration and acuity. Oral presentation at the *Neuroscience Graduate Research Symposium 2018,* University of Illinois at Chicago.

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PROFFESSIONAL MEMBERSHIP:

Society for Neuroscience 2014 - present