INFLAMMATION AND OXIDITATIVE STRESS IN EARLY PHASE PSYCHOSIS – A MULTIMODAL NEUROIMAGING AND MACHINE LEARNING APPROACH

By

ALEX MURRAY

A thesis submitted to

The University of Birmingham

For the degree of

DOCTOR OF PHILOSOPHY

School of Psychology

College of Life and Environmental Sciences

The University of Birmingham

May 2024

Abstract

Inflammation and oxidative stress are two central interlinked processes that are thought to play pivotal roles in the pathophysiology of schizophrenia spectrum disorders. This thesis presents novel findings highlighting the roles of these processes in disorder progression and presenting novel methodological advancements in glutathione quantification and patient stratification. After general introduction in Chapters 1 and 2, Chapter 3 follows presenting a systematic review and meta-analysis of 1H-MRS studies across all psychosis phases identifying glutathione reductions specific to the later stages of the disorder. While MRS acquisition methodologies were not found to significantly affect results, year of study was a confounding factor, perhaps indicating the effect of uncontrolled methodological factors and improved reporting standards. In Chapter 4, work classifying distinct immune-related clusters based on four peripheral inflammatory biomarkers (CRP, IL-6, TNF-a and INF-y) is presented. Employing machine learning clustering models on cytokine profiles, four distinct schizophrenia-specific clusters were identified, each with unique patterns of grey matter volume alterations and clinical symptoms. Proinflammatory cytokine clusters IL-6 and CRP were associated with more severe symptoms and extensive grey matter reductions, providing novel validation of the clustering approach and suggesting potential targets for immune-focused treatments. In Chapter 5, brain-age prediction models are described demonstrating higher brain age gaps in established schizophrenia patients compared to controls, correlating with symptom severity. This accelerated ageing may be linked to poor defence against oxidative stress, as evidenced by correlations with MRS derived glutathione levels, however no such associations were found with peripheral inflammatory biomarkers. Summarised in Chapter 6, this work suggests that inflammation and oxidative stress make excellent candidates for monitoring of disorder progression and future pharmacological interventions in a stratified cohort; however further research is required to elucidate how peripheral markers are related to central mechanisms and downstream symptomatology and potential future directions for this work are suggested.

Acknowledgements

I would like to take a moment to thank everyone who has helped me on my PhD journey on both an academic and personal level, without them none of this would have been possible.

First and foremost, I'd like to thank my supervisors, Professor Rachel Upthegrove, Dr Jack Rogers, Professor Peter Liddle and Dr Zia Katshu for allowing me to undertake this PhD. I would not have made it this far without your support and I have learned so much from you all over the last few years for which I will be forever grateful. Rachel – thank you for everything you have done for me over the course of this PhD, your insight and guidance has been invaluable, and it has been a joy to share my thoughts and findings with you throughout this process. Thank you for always keeping me on track even when I get lost pursuing rabbits down various holes! Jack – it has been an absolute joy working with you, your support on so many technical areas was instrumental in putting together each puzzle piece. Our data analysis sessions were a true highlight of my PhD (even when we spent hours with seemingly no progress!) And our various conference trips really helped build my confidence in my research and expand my network. Peter and Zia – thank you for all your insightful comments, advice and help in shaping my manuscripts and this thesis. I truly believe that I am a better scientific writer thanks to your inputs. Working alongside all of you made my PhD journey a truly enjoyable experience.

In addition to my supervisors, I would like to thank those who have also been involved with the work in this thesis. Dr Martin Wilson, your insights into MRS techniques and analysis have truly been a life saver, thank you for always finding the time to read and comment on my work and help with the many technical difficulties I encountered on the way! Professor Palaniyappan and the whole CEYMH team in Montreal, thank you for taking me in for those four months, my time working in your lab was truly the best experience of my life and I hope we get to continue working together in future.

I have had the pleasure of working alongside many wonderful people in the PRIMED+ lab. Thank you to all my fellow postgrads and research assistants who have made my time studying here so much fun, and for always being around to share the highs and lows of PhD life in the pub. Likewise, to my friends outside of the University thank you for sticking by me through thick and thin. Sam – you have been my rock for many, many years, knowing you are always there to bring joy into my life has helped keep me sane! Maddie – you have always kept me level-headed our late-night chats setting the world to rights will always be something I look forward to, thank you for always being in my corner. James and Ollie, you are my oldest friends and knowing that you will always be there for me waiting with a pint and a laugh really kept me going throughout all of this. Thank you to every one of the "Cov lot" for providing me a safe happy place to turn to when I needed it most.

Finally, I'd like to thank my parents. Without your constant love and support none of this would have been possible. You have always encouraged and supported me (both financially and emotionally) to do whatever I wanted to do and for this I am eternally grateful. Thank

you for always being there, night or day, picking me up when I was down, celebrating my successes. You are truly the best parents anyone could ask for, thank you for everything!

This PhD was funded by the Medical Research Council (MRC) for doctoral training with Rachel Upthegrove, Mohammad Katshu, and Peter Liddle (MR/2434208).

List of Papers and Abstracts

This thesis incorporates the four following papers which correspond to three empirical studies and one narrative synthesis.

Murray, A. J., Rogers, J. C., Katshu, M., Liddle, P. F., & Upthegrove, R. (2021). Oxidative Stress and the Pathophysiology and Symptom Profile of Schizophrenia Spectrum Disorders. *Frontiers in Psychiatry*, *12*. https://www.frontiersin.org/articles/10.3389/fpsyt.2021.703452

Murray, A. J., Humpston, C. S., Wilson, M., Rogers, J. C., Katshu, M., Liddle, P. F., & Upthegrove, R. (2024). Measurement of brain glutathione with magnetic Resonance spectroscopy in Schizophrenia-Spectrum disorders—A systematic review and Meta-Analysis. *Brain, Behavior, and Immunity*, *115*, 3–12. https://doi.org/10.1016/j.bbi.2023.09.017

Murray, A. J., Singh, K., Williams, G., Gregory, C., Conan, S., Rathnaiag, M., Nikkheslat, N., Palaniyappan, L., Koelwijn, L., Walters, J., Katshu, M., Deakin, B., Liddle, P. F., Upthegrove, R., & Rogers, J. (2024). Identification of Detailed Inflammatory Clusters in Patients with Schizophrenia. (*Under review at "Biological Psychiatry"*)

Murray, A. J., Ballester, P. J., Rogers, J. C., Williams, S., Wilson, M., Deakin, B., Katshu, M., Liddle, P. F., Palaniyappan, L. & Upthegrove, R. (2024). Probing the Biological Underpinnings of Advanced Brain Ageing in Schizophrenia. (*Prepared for submission in "JAMA Psychiatry"*)

In addition, the following abstracts arose from presentations of material related to this thesis:

Murray, A. J, Lalousis, P., Rogers, J., Katshu, Z., Liddle, P., Deakin, B., & Upthegrove, R., (2024). Identification of Detailed Inflammatory Clusters in Schizophrenia: A Data

Driven Approach. *Psychoneuroendocrinology, 160, 106779*. https://doi.org/10.1016/j.psyneuen.2023.106779

Murray, A. J, Lalousis, P., Rogers, J., Katshu, Z., Liddle, P., Deakin, B., & Upthegrove, R. Identification of Detailed Inflammatory Clusters in Schizophrenia: A Data Driven Approach. *International Society of Psychoneuroendocrinology Conference, London, UK, 2023.*

Murray, A. J, Lalousis, P., Rogers, J., Katshu, Z., Liddle, P., Khandaker, G., Deakin, B., & Upthegrove, R. Identification of Inflammatory Subtypes Within Schizophrenia: A Machine Learning Approach. *Schizophrenia International Research Symposium, Toronto, Canada, 2023*.

Murray, A. J, Lalousis, P., Rogers, J., Katshu, Z., Liddle, P., Khandaker, G., Deakin, B., & Upthegrove, R. Identification of Inflammatory Subtypes Within Schizophrenia: A Machine Learning Approach. *Mechanisms of Inflammation Symposium, Birmingham, UK, 2023.*

Murray, A. J., Humpston, C. S., Wilson, M., Rogers, J. C., Katshu, M., Liddle, P. F., & Upthegrove, R. Measurement of Brain Glutathione and Its Relationship with Peripheral Oxidative Stress Markers in Schizophrenia-Spectrum Disorders. *Schizophrenia International Research Symposium, Florence, Italy, 2022.*

Contents Listing

Table of Contents

List of Figures

List of Tables

Aims and Hypotheses

The primary objectives of the studies outlined in this thesis are to elucidate the roles played by inflammation and oxidative stress in the pathophysiology of schizophrenia-spectrum disorders. This involves examining changes in brain structure and symptom profiles associated with increases in specific peripheral inflammatory markers and centrally measured oxidative stress. The methodologies used to capture these markers are evaluated with the goal to offer insights for improving patient stratification in future.

Chapters 1 and 2 present a general introduction to the concepts of inflammation and oxidative stress in psychosis-spectrum disorders. Focussing on how they manifest in the brain and a detailed exploration of the pathways involved. Alongside a critique of current acquisition methodologies. With the aim to elucidate areas where further research is required. Chapter 2 has been published in *Frontiers in Psychiatry* (Murray et al., 2021).

In Chapter 3, I conducted a systematic review and meta-analysis of magnetic resonance spectroscopy studies examining glutathione levels in schizophrenia-spectrum disorders. The aim is to clarify how centrally measured oxidative stress may vary across different stages of illness and to understand the potential impact of methodological variations on study outcomes. This paper has been published in *Brain Behavior and Immunity* (Murray et al., 2024).

Chapter 4 aims to identify inflammatory subtypes using a machine-learning clustering algorithm in a diverse sample of individuals with schizophrenia. The hypothesis is that multiple distinct inflammatory clusters will emerge, differing significantly from each other and from matched healthy controls on clinical and neurocognitive measures. Additionally, it

is expected that each cluster will exhibit unique alterations in grey matter volume. This paper is under review at *Biological Psychiatry*.

In Chapter 5, I investigate the associations between peripheral inflammation, central oxidative stress, and accelerated brain ageing. This study aimed to explore whether there is a difference in brain-age gap in people with schizophrenia compared to normal distributions, how this may vary with the stage of illness and its relationship with symptom severity. Furthermore, I hypothesized that cortical antioxidant markers, alongside peripheral inflammatory markers, would correlate with the extent of advanced brain ageing in schizophrenia. This paper has been prepared for submission to *JAMA Psychiatry.*

Finally, in Chapter 6, I discuss the implications of the findings from the preceding chapters, with an emphasis on guiding future research in precision psychiatry.

Chapter 1

The Relevance of Inflammation in Psychosis-Spectrum Disorders

1.1 Psychosis-Spectrum Disorders

Psychosis-spectrum disorders encompass a range of severe mental illnesses, including schizophrenia, schizophreniform disorder, and schizoaffective disorder (American Psychiatric Association, 2013). These disorders are a leading cause of chronic disability among young people worldwide (Vos et al., 2016). According to the National Institute of Mental Health (2016), up to 100,000 adolescents and young adults in the U.S. experience their first episode of psychosis each year, with approximately 3 in 100 people expected to experience psychosis at some point in their lifetime. However, other studies suggest this figure is conservative, indicating a global prevalence of psychotic symptoms as high as 12.5% (Andrade & Wang, 2012). These symptoms are closely linked to poorer overall health.

Psychotic disorders are categorized under the broader label of "schizophrenia spectrum and other psychotic disorders" in the DSM-V (American Psychiatric Association, 2013). The key features of these disorders include positive, negative, and disorganization symptoms. Positive symptoms refer to hallucinations (such as hearing voices or seeing things others cannot) and delusions (such as believing in one's own superiority without evidence). Negative symptoms include a lack of motivation, apathy, and social withdrawal. Disorganization refers to disordered thinking and inappropriate emotional responses. Both negative and disorganization symptoms are often accompanied by cognitive impairments and declines in social and occupational functioning (Nestler et al., 2015; Liddle, 2019).

Psychotic disorders typically emerge between the ages of 18 and 25 in men, and between 25 and 35 in women (Ochoa et al., 2012). Most of these disorders include a pre-

psychotic phase known as the "prodrome," during which individuals experience significant changes in functioning. This phase may involve symptoms like depressed mood, anxiety, and mild psychotic symptoms (Yung et al., 1996). Gross (1989) also observed “basic symptoms” in this phase, such as perceptual and cognitive disturbances. The prodrome generally lasts between 1 and 5 years before developing into a full-blown psychotic disorder (Loebel et al., 1992; Beiser et al., 1993), often resulting in severe psychosocial impairment (Jones et al., 1993). While the exact mechanisms behind the transition from prodrome to psychosis remain unclear (Hodges et al., 1999), this phase offers an opportunity for preventative interventions aimed at halting progression (McFarlane, 2010).

Given the profound impact of psychotic disorders on an individual's life, especially their early onset and potential for lifelong disability, extensive efforts have been dedicated to developing effective treatments. The National Institute for Health and Care Excellence (NICE) (2014) recommends antipsychotic medication as the primary treatment for individuals experiencing psychosis. However, medication is not effective for everyone. Approximately 20% of individuals diagnosed with psychotic disorders are classified as treatment-resistant (Smith et al., 2009). Additionally, antipsychotic medications often cause significant side effects, including sedation, weight gain, and anxiety (Muench & Hamer, 2010; Leucht et al., 2013).

Furthermore, these medications tend to be more effective in addressing positive symptoms than negative symptoms (Lally & MacCabe, 2015). Consequently, new lines of research are focusing on developing novel interventions that may benefit individuals with treatment-resistant schizophrenia and alleviate negative symptoms. Promising developments include anti-inflammatory treatments, such as non-steroidal anti-inflammatory drugs (NSAIDs) and monoclonal antibodies, which have shown some evidence of alleviating the

predominant negative symptoms associated with psychosis (Akhondzadeh et al., 2007; Muller et al., 2010).

1.2 Inflammation

Inflammation, typically understood as the body's natural response to injury or infection, is a complex biological process involving various immune cells, signalling molecules, and tissue reactions (Kirkpatrick & Miller, 2013; Khandaker et al., 2015). The inflammatory response begins with the production and release of chemical agents such as leukocytes by cells in the infected, injured, or diseased tissue. This inflammatory process promotes healing however, if left uncontrolled, can become harmful (Chen et al., 2017).

Broadly speaking, acute inflammation in response to environmental insults presents with warmth, pain and swelling in a localised area. This acute inflammation will usually only last a number of days (Pahwa et al., 2023). Conversely chronic inflammation is a lower-grade response, occurring without the visible signs of its acute counterpart and may be more systemic (Kirkpatrick & Miller, 2013). Chronic inflammation plays a key role in a number of chronic diseases, including cardiovascular disease, diabetes and Alzheimer’s (Henein et al., 2022; Tsalamandris et al., 2019; Kinney et al., 2018). Importantly, chronic inflammation can occur as a result of an unresolved acute response (Chen et al., 2017).

Within the brain, inflammation acts differently from other tissues, it is often termed “neuroinflammation” to differentiate it from peripheral inflammation as it does not present alongside swelling and fever (Jeong et al., 2013; Kirkpatrick & Miller, 2013). However, inflammation may alter the development of specific cells within the brain, presenting a mechanistic link to neurodevelopmental disorders (Ament et al., 2023). Historically, the brain was thought to be “immunologically privileged” with it being shielded from the rest of the

body behind the blood-brain barrier (BBB), a selective semipermeable membrane that regulates the transfer of chemicals from the blood into and out of the brain (Khandaker et al., 2015; Dotiwala et al., 2023). The brain contains relatively few inflammatory cells that are common elsewhere in the body. The primary inflammatory cells in the brain are microglia, constituting about 10% of the brain cell mass, roughly equal to that of neurons (Ochocka & Kaminska, 2021) and with highest concentrations in cortical grey matter. There are numerous terminologies used to describe inflammation, as mentioned previously, neuroinflammation is most commonly used when describing inflammation of the brain, immune active may also be used for when someone is presenting with raised pro-inflammatory biomarker levels and immune dysfunction may describe aberrant inflammatory biomarker levels alongside dysfunctional cellular signalling. Within this thesis the overarching use of inflammation will refer to any immune activity related to brain structure, function or symptomatology within schizophrenia.

Microglia serve similar protective functions to immune cells found in the rest of the body. They also play a role in other brain functions such as pruning and maintenance of synapses, transportation of neurotransmitters and phagocytosing damaged cells (Augusto-Oliveira et al., 2018). Activated microglia produce inflammatory cytokines (molecules that regulate inflammation and play a key role in cell signalling) which provoke an inflammatory response. Microglial activation and subsequent pro-inflammatory cytokine production may disrupt the BBB (Kang et al., 2020). Damage to the BBB can affect its ability to control which inflammatory cells and molecules enter the brain; other substances leak into brain tissue, and the brain is unable to function normally.

In recent years a growing body of evidence has suggested a link between inflammation and various psychiatric disorders, including psychosis, depression, and bipolar disorder. Here I evaluate the evidence behind the role of inflammation in psychosis, explore

the mechanisms behind inflammation, how this inflammation may be crossing from the periphery into the brain, and examine in what way anti-inflammatory interventions may present novel treatment opportunities.

1.3 Inflammation in Psychosis

1.3.1 Cytokines

Cytokines, a group of small proteins, play a crucial role in mediating the inflammatory response in both the body and central nervous system (CNS). This group typically includes interleukins (IL), soluble interleukin receptors (sIL), interferons (IFN), and tumour necrosis factors (TNF). Although not technically a cytokine, C-reactive protein (CRP) is another inflammatory marker commonly measured alongside other cytokines to assess the inflammatory state. CRP contributes significantly to inflammatory processes and host responses to infections, particularly by promoting the production of cytokines such as IL-6 and TNF-α (Sproston et al., 2018). Cytokines and CRP are pivotal in cellular signalling, with various downstream effects that impact neuronal signalling, synaptic plasticity, and overall behaviour or mood. Increases in the concentration of circulating inflammatory cytokines and CRP are indicative of immune activation or inflammation. Moreover, peripheral immune responses, as indicated by elevated cytokine levels, are theorized to reflect the neuroimmune status of the brain (Dunleavy et al., 2022).

Considerable evidence from cross-sectional studies suggests cytokine alterations occur in different phases of schizophrenia. In a recent meta-analysis of 215 studies, Halstead et al. (2023) identified significant elevations, compared to controls, in the concentrations of several cytokines and inflammatory markers in the blood across all disorder stages, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP. Additionally, IL-2 and IFN-γ were significantly

elevated in acute schizophrenia-spectrum disorder (first-episode psychosis or acute exacerbation of chronic schizophrenia), while IL-4, IL-12, and IFN-γ were significantly decreased in chronic schizophrenia-spectrum disorder (patients with schizophrenia on stable medication, including treatment-resistant patients). Research into clinical high-risk cohorts is somewhat limited; however, similar increases in circulating CRP and IL-6 have been noted, Goldsmith et al., 2019 found that increases in IL-6 in CHR at baseline were associated with worsening negative symptoms at a one year follow up. Longitudinal studies have also shown that elevated levels of IL-6 at age 9 double the risk of a psychotic disorder diagnosis by age 18 (Upthegrove et al., 2020). Moreover, higher CRP levels at age 15 are associated with an increased risk of schizophrenia development by age 27 (Metcalf et al., 2017) and linked to anxiety (Morales Munoz, 2022). However, recent meta-analyses have not found differences in these markers between high-risk converters and non-converters to psychsosis (Misiak et al., 2021; Delaney et al., 2019).

These results may indicate separate groups of “trait” or “state” markers of inflammation. Trait markers are seen to be consistently altered in all disorder stages and are typically associated with genetic and developmental factors associated with vulnerability to illness (Dawidowski et al., 2021; Chen et al., 2006), in this case markers such as CRP and IL-6 are hypothesised to be trait markers of inflammation in psychosis. State markers on the other hand are associated with active pathology in symptomatic individuals (Halstead et al., 2023; Chen et al., 2006), these markers present with differing concentrations between illness stages and typically will be higher than controls during an exacerbation of symptoms, but no difference during periods of clinical stability (Miller et al., 2013).

Several studies have observed a link between increased peripheral inflammatory markers and symptom severity in psychosis, alongside advanced brain ageing and impaired cognitive function (Mondelli et al., 2015; Klaus et al., 2022; Morrens et al., 2022). However,

not all patients demonstrate evidence of inflammation. Instead, various machine learning and principal component analysis studies have suggested that inflammation may be relevant to a subgroup of approximately 30-50% of all psychosis patients (Tamminga et al., 2021; Bishop et al., 2022; Enrico et al., 2023). Patients within this group are observed to have a greater degree of cognitive impairment and altered brain structure compared to patients presenting with lower levels of inflammation (Sæther et al., 2023; Lalousis et al., 2023).

1.3.2 Microglia

Microglia, integral cells within the central nervous system (CNS), serve as the frontline of its immune defence system (Laskaris et al., 2016). Originating outside the brain from myeloid lineage progenitors, these cells emerge in the yolk sac and migrate into the CNS via the circulatory system during embryonic development (Bian et al., 2020; Hartmann et al., 2024). Upon entry, they adopt a ramified state, with their branches constantly surveying the surrounding area (Nimmerjahn et al., 2005; Orihuela et al., 2016).

In the brain, microglia orchestrate responses to inflammation and viral or bacterial threats by releasing inflammatory cytokines (Kettenmann et al., 2011; Hartmann et al., 2024). Upon detecting a threat, microglia undergo morphological changes, retracting their branches and swelling their cell bodies to facilitate migration toward the source (Woodburn et al., 2021). Microglial activation mirrors the phenotypic shifts observed in peripheral monocytes, categorized as M1/M2 phenotypes (Mantovani et al., 2005). M1 phenotypes, triggered by inflammation, release IL-6, IL-1B, TNF-α, reactive oxygen species, and glutamate, alongside phagocytosis of cellular debris or pathogens. Conversely, M2 phenotypes work to resolve inflammation, releasing IL-4, IL-13, IL1-receptor agonist, and BDNF (Reus et al., 2015).

However, this binary perspective oversimplifies microglial behaviour, as they exhibit high plasticity and operate along a spectrum depending on environmental cues (Biber et al., 2014).

In schizophrenia, studies have highlighted aberrant microglial activation. Some post-mortem examinations reveal increased numbers of activated microglia in the cortex of schizophrenia patients compared to controls (Radewicz et al., 2000; Fillman et al., 2013; Gober et al., 2021), however, others have found no difference (Steiner et al., 2006; Steiner et al., 2008; Busse et al., 2012), potentially due to differing methods of identifying and counting microglia (Laskaris et al., 2016). PET-imaging studies employing radioligands specific to the 18-kDa translocator protein (TSPO), primarily expressed by microglia, have been instrumental in identifying activated microglia in vivo. Meta-analyses using neuroimaging methods such as radioligand targeting of translocator protein (TSPO) using PET (positron emission tomography) reveal mixed findings for microglia changes in schizophrenia with mostly decreased rather than increased radioligand binding to activated microglia (Plaven-Sigray et al., 2021). Bloomfield et al. (2016) demonstrated elevated TSPO binding in the grey matter of schizophrenia and clinical high-risk patients, indicating increased microglial activation. Subsequent studies have confirmed these findings in both first-episode psychosis and chronic schizophrenia (Collste et al., 2017; Ottoy et al., 2018). However, discrepancies arise when different TSPO radioligands are used, with some studies failing to detect differences in microglial activity among patients (Hafizi et al., 2017; Selvaraj et al., 2018; Conen et al., 2021).

Beyond their immunological role, microglia are pivotal in the formation and maintenance of neuronal circuits during early development (Hartmann et al., 2024). Initially, synapses are formed in excess, with redundant or weak connections selectively pruned via microglial phagocytosis later (Schafer et al., 2013). Within schizophrenia, synaptic loss is believed to contribute to overall symptomatology (Howes and Onwordi, 2023). Microglia can

be "primed" by genetic risk factors or early environmental insults like maternal infection, rendering them more sensitive to inflammatory triggers later in life. These primed microglia may exhibit heightened levels of inflammatory cytokines and mount an exaggerated inflammatory response (Norden et al., 2015). Consequently, increased inflammation in schizophrenia could activate these primed microglia to excessively prune synaptic connections, leading to persistent synaptic deficits possibly linked to the onset of schizophrenia symptoms later in life.

1.4 From Periphery to CNS

The greatest body of research into inflammation and psychotic disorders focus on blood-based biomarkers of inflammation, however, information gathered from the periphery may not necessarily reflect what is going on in the CNS. There are other disorders in which peripheral inflammation appears to impact brain function. Patients with rheumatoid arthritis have up to 40% increased risk of cognitive decline and incident dementia (Ungprasert et al., 2016; Kodishala et al., 2023), animal models of colitis show disruption in the cortex, amygdala and hippocampal regions resulting in anxiety, depression, and cognitive dysfunction (He et al., 2021; Do and Woo, 2018; Riazi et al., 2015), further human studies also find associations between IBD and brain lesions (Dolapcioglu and Dolapcioglu, 2015).

There are a number of mechanisms by which peripheral inflammatory cytokines may enter the brain triggering neuroinflammation with BBB disruption seen as the most fundamental pathway (Sun et al., 2022). High levels of circulating inflammatory mediators such as IL-6, TNF-α and CRP may cause BBB disruption via the interruption of tight junctions, protein complexes that seal the gaps between endothelial cells in the BBB (McKim et al., 2018; Han et al., 2020). Tight junction disruption in turn leads to increased BBB

permeability, thus allowing toxic circulating molecules, inflammatory cytokines and other immune cells access to the brain and can cause further harm to the BBB (Sun et al., 2022).

Other mechanisms by which inflammatory cytokines may enter the brain include the humoral pathway where cytokines utilize endothelial transporter protein channels (Banks, 2005) or transport through “leaky regions” of circumventricular organs in the BBB (Pollak et al., 2018). They may also follow a neural pathway in which the cytokines bind to peripheral nerve fibres which in turn trigger central inflammatory signals (Miller and Goldsmith, 2020). A third pathway may also be involved, termed the cellular pathway, in which activated immune cells are transported to the brain via chemokines produced centrally by microglia (D’Mello et al., 2009).

1.5 Inflammation and Oxidative Stress

Various factors can trigger inflammatory activation, including infection, tissue damage, and exposure to environmental toxins (Megha et al., 2021). Within the CNS, oxidative stress is thought to play a key role in inducing inflammation, representing a critical pathway in the pathophysiology of psychosis-spectrum disorders. Details of oxidative stress and its relevance to psychosis will be explored in detail in Chapter 2, however, in brief, oxidative stress occurs when there's an imbalance between the production of reactive oxygen species (ROS) and the body's inability to neutralize them effectively via endogenous antioxidant defences. While ROS are naturally produced during cellular metabolism, an excess can overwhelm antioxidant systems, resulting in damage to lipids, proteins, and DNA (Pizzino et al., 2017).

Oxidative-related damage and abnormal oxidant metabolism have been demonstrated within schizophrenia, with increased levels of harmful ROS found in the blood and plasma of

individuals with psychosis, alongside decreases in circulating antioxidants such as superoxide dismutase and glutathione peroxidase (Yao et al., 2001; Fendri et al., 2006; Gunes et al., 2017). Magnetic resonance spectroscopy (MRS) studies have also shown reductions in central levels of glutathione, the brain's primary antioxidant, in schizophrenia patients, which correlates with increased symptom severity and cognitive decline (Pavlović et al., 2002; Martínez-Cengotitabengoa et al., 2014). Recent research, however, suggests that the reduction in antioxidant defenses may not be straightforward. Limongi et al. (2021) demonstrated significant increases in glutathione levels in first-episode patients compared to controls, possibly indicating a compensatory response to genetic or environmental factors early in the disorder. Additionally, aberrant antioxidant production may only apply to a subgroup of patients, with those carrying a specific GCLC polymorphism showing significant reductions in glutathione levels compared to controls (Xin et al., 2016).

Inflammation and oxidative stress are closely intertwined, with oxidative stress-induced tissue damage triggering immune responses. ROS can stimulate the expression of genes that code for pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 (Bitanihirwe and Woo, 2011). In the brain, ROS generation can affect synaptic and non-synaptic communication between neurons, leading to neuroinflammation and cell death (Popa-Wagner et al., 2013). Microglia and macrophages also utilize ROS to eliminate pathogens, highlighting the dual role of oxidative stress as both an inducer and a product of inflammation (Koga et al., 2016). These markers may provide alternative routes for antioxidant and anti-inflammatory interventions aimed at reducing symptom burden within the disorder.

1.6 Clinical Implications

1.6.1 Biomarkers of Treatment Response

While not considered a standalone diagnostic criterion, researchers have increasingly turned their attention to blood-based biomarkers of inflammation as a potential tool for predicting treatment response and overall prognosis (Lin et al., 2022). A recent meta-analysis of 12 studies revealed significant reductions in pro-inflammatory markers such as IL-1β, IL-6, IFN-γ, TNF-α, as well as anti-inflammatory markers like IL-4, IL-10, following anti-psychotic interventions (Marcinowicz et al., 2021). Moreover, findings from the OPTiMiSE study, a large-scale trial investigating antipsychotic response in FEP, indicated the existence of a subgroup within FEP patients with a heightened risk of non-response to amisulpride, a dopamine D2 receptor antagonist used in the treatment of acute and chronic schizophrenia. This subgroup exhibited elevated levels of serum pro-inflammatory cytokines and inflammatory markers prior to treatment initiation (Martinuzzi et al., 2019).

Subsequent research has demonstrated that FEP non-responders displayed significantly higher baseline levels of IL-6 and IFN-γ compared to responders. Even after 12 weeks of antipsychotic treatment, these cytokine concentrations remained elevated in non-responders compared to responders (Mondelli et al., 2015). Additionally, elevated levels of CRP at baseline have been shown to predict poorer treatment response one year later. This elevation was associated with more severe negative symptoms at baseline and exacerbated positive symptoms, general psychopathology, and overall symptom severity at the one-year follow-up (Nettis et al., 2019). Therefore, the measurement of certain inflammatory markers in serum may be useful for assessing treatment response and identifying individuals at high risk of being non-responders to antipsychotic medications.

1.6.2 Anti-inflammatory Treatments

To date, results of anti-inflammatory trials linked to schizophrenia have been mixed. While some studies have supported the efficacy of immunomodulatory drugs, such as NSAIDs, minocycline, and COX-inhibitors, on symptoms of schizophrenia (Akhondzadeh et al., 2007; Muller et al., 2010; Liu et al., 2014), these findings were frequently based on small sample sizes. Larger studies have reported no beneficial effects on symptom severity (Deakin et al., 2018).

In their meta-analysis of 70 randomised control trials Jeppesen et al. (2020) found that antipsychotic medication combined with any anti-inflammatory intervention resulted in improvement on scores of the positive and negative syndrome scales (PANSS), with trials on schizophrenia showing greater improvement than other psychotic disorders. However, they included studies looking at both primarily anti-inflammatory drugs such as monoclonal antibodies and antibiotics alongside drugs with potential immunomodulatory properties. They found no difference between either of these interventions. The authors also highlight that the studies with smaller sample sizes have significantly higher effect sizes than those conducted in a larger population.

As previously mentioned, the relevance of anti-inflammatory interventions may also not extend to all individuals with schizophrenia. If only a subgroup, possibly up to 50% of the population, manifests increased circulating inflammatory cytokines, the effects of inflammatory interventions might differ in this subset compared to those lacking evidence of inflammation. For instance, a randomized control trial evaluating infliximab, a TNF-α monoclonal antibody, revealed that antidepressant response correlated with higher CRP levels at baseline (Raison et al., 2013). Despite the absence of published articles investigating patient stratification based on inflammation within schizophrenia, evidence from depression

research suggests that stratifying patients based on inflammation levels could facilitate the identification of responders to anti-inflammatory treatments. In a study examining the effectiveness of minocycline in treatment resistant depression, it was demonstrated that while there were no significant differences in overall depression scores between minocycline and placebo groups, when stratified by CRP levels, patients with CRP levels ≥3 mg/L who received minocycline showed significant improvements compared to other groups (Nettis et al., 2021).

There are proposed studies underway assessing anti-inflammatory interventions within schizophrenia. For example, the ongoing Psychosis Immune Mechanisms Stratified Medicine Study (PIMS) (Foley et al., 2022) aims to test the effects of IL-6 inhibition using the IL-6 uptake inhibitor tocillzumab on patients with psychosis who present with elevated IL-6 levels. In this study patients are screened for high levels of IL-6 at baseline and those meeting criteria of IL-6 >0.7 pg/mL are randomised into a placebo or treatment group. These patients are then followed up over the course of four weeks post-infusion.

1.7 Challenges and Future Directions

When discussing the impact of inflammation, it is crucial to consider several potentially influencing factors. For instance, antipsychotic medication can lead to weight gain and increase the risk of diabetes, both of which are linked to inflammation (Dayabandara et al., 2017; Ouyang et al., 2022). However, low-grade innate inflammation is observed prior to the onset of psychosis and in the immediate relatives of individuals with schizophrenia (Khoury and Nasrallah, 2018) and meta-analyses of patients experiencing their first episode of psychosis indicate that the connection between schizophrenia and inflammation is not solely attributable to antipsychotic medications (Marcinowicz et al., 2021). Moreover,

various other factors like age, gender, smoking, and body mass index can influence levels of inflammatory markers in circulation, potentially affecting research outcomes (Kirkpatrick and Miller, 2013; Kawamoto et al., 2013; Stępień et al., 2014). Yet, the number of studies controlling for these confounders or matching patients with controls on these criteria remains variable.

Inflammation frequently coexists with other physiological abnormalities such as hypertension, diabetes, and oxidative stress, lending support to its role in psychosis but complicating efforts to identify optimal therapeutic targets (Miller et al., 2012). Furthermore, assessing inflammation in the CNS of humans presents challenges. While evidence from peripheral sources holds promise in predicting central inflammation, further research is needed. Examining the brain *in-vivo* remains the best option. Post-mortem studies have yielded mixed evidence regarding increases in activated microglia in the brains of individuals with schizophrenia, highlighting the challenges of working with human tissue. Factors like differences in post-mortem intervals, brain pH levels, and methodological variations in identifying and counting microglial cells can impact study outcomes (Hercher et al., 2014). Additionally, whilst TSPO-PET imaging studies are considered the gold standard for exploring neuroinflammation, their high costs and prerequisites limit widespread use (Yoder et al., 2013; Fond et al., 2020). Another challenge lies in selecting the appropriate radioligand, as each can yield different results (Laskaris et al., 2016).

Looking ahead, the next course of action involves tailoring anti-inflammatory interventions to achieve the optimal response in schizophrenia. These interventions need to focus on specific pathways associated with the pathology of schizophrenia. Currently, the cytokine most consistently seen as disturbed in research studies of schizophrenia is IL-6. Therefore, targeting the IL-6 pathway appears promising, especially considering the widespread use of IL-6 monoclonal antibodies in treating other inflammatory disorders like

rheumatoid arthritis. Additionally, TNF-α and IFN-γ are other potential cytokines of interest, as there is substantial evidence of their disruption in schizophrenia.

In planning these future interventions, it is crucial to identify patients who are most likely to respond successfully to treatment. This involves targeting those who already exhibit chronic levels of low-grade peripheral inflammation. Screening patients at baseline to assess circulating inflammatory markers in their blood is essential. Reliable markers used in previous studies include CRP levels ≥3 mg/L or IL-6 levels ≥0.7 pg/ml. Patients meeting these criteria may be the best candidates for inflammatory treatments.

1.8 Conclusion

Despite the significant diversity within schizophrenia, there exists a substantial body of replicated evidence linking inflammation to various aspects of the disorder, including risk factors, illness progression, and symptoms. Several peripheral inflammatory markers, notably IL-6, CRP, TNF-α, and IFN-γ, are consistently found to be disturbed in at least a subset of patients. Additionally, evidence of neuroinflammation is observed through the assessment of microglia, with increased numbers of activated inflammatory cells found in the brains of individuals with schizophrenia.

While the precise mechanisms by which inflammation traverses from the periphery to the CNS and contributes to symptomatology remain elusive, the most robust theory involves disruption of the BBB impacting on other local inflammatory processes and downstream functional outcomes. Elevated levels of circulating inflammatory cytokines may disrupt tight junctions within the BBB, allowing passage into the CNS and further activating microglia. This activation, potentially primed via maternal immune activation, may lead to excessive pruning of synaptic connections, thereby influencing behaviour.

From a clinical standpoint, the identification of biomarkers holds promise for predicting treatment response and stratifying patients for tailored interventions. Although anti-inflammatory treatments have yielded mixed results, ongoing efforts aim to refine therapeutic strategies by targeting specific cytokine pathways and selecting patients with elevated baseline inflammation levels. Research into the inflammatory aspects of schizophrenia shows promise in enhancing our understanding of the disorder, with potential implications for informing clinical practice and improving outcomes for individuals with chronic low-grade inflammation.

Chapter 2

The following paper has been published in *Frontiers in Psychiatry* and has not been edited from source.

Frontiers in Psychiatry, Volume 12, 22 July 2021

DOI: 10.3389/fpsyt.2021.703452

Oxidative Stress and the Pathophysiology and Symptom Profile of Schizophrenia Spectrum Psychoses

Alex J Murray[1], Jack C Rogers[1], Mohammad Zia Ul Haq Katshu[2,3], Peter F Liddle[2], Rachel Upthegrove[1,4]

[1]Institute for Mental Health, University of Birmingham, Birmingham, United Kingdom

[2]Institute of Mental Health, Division of Mental Health and Neurosciences University of Nottingham, Nottingham, United Kingdom

[3]Nottinghamshire Healthcare NHS Foundation Trust, Nottingham, United Kingdom

[4]Early Intervention Service, Birmingham Women's and Children's NHS Foundation Trust, Centre for Human Brain Health, University of Birmingham

Abstract

Schizophrenia is associated with increased levels of oxidative stress, as reflected by an increase in the concentrations of damaging reactive species and a reduction in anti-oxidant defences to combat them. Evidence has suggested that whilst not the likely primary cause of schizophrenia, increased oxidative stress may contribute to declining course and poor outcomes associated with schizophrenia. Here we discuss how oxidative stress may be implicated in the aetiology of schizophrenia and examine how current understanding relates associations with symptoms, potentially via lipid peroxidation induced neuronal damage. We argue that oxidative stress may be a good target for future pharmacotherapy in schizophrenia and suggest a multi-step model of illness progression with oxidative stress involved at each stage.

2.1 Introduction

Schizophrenia is a severe and debilitating mental disorder that has an estimated life-time prevalence worldwide of 0.75% (Moreno-Kuster, Martin & Pastor, 2018). Long term outcomes of this disorder are often poor, and those diagnosed with schizophrenia are up to three times more likely to die early than the general population in spite of treatment (Oakley et al., 2018). Schizophrenia is characterised by positive, negative and disorganisation symptoms. Positive symptoms include hallucinations (perceptual experiences in the absence of corresponding stimuli, for example: hearing voices or seeing things that are not there) and delusions (unshakeable beliefs arising internally, for example: a delusion of grandeur occurs when a person believes themselves to be superior to others with no evidence for this). Negative symptoms include loss of motivation, apathy and social withdrawal. Disorganization includes disordered form of thought and inappropriate affect. Negative and disorganization symptoms occur alongside impaired cognitive function and deterioration in both social and occupational functioning, (Nestler et al 2015; Liddle, 2019). Schizophrenia has an age of onset of between 18 and 25 years in men and 25 to 35 years in women (Ochoa et al., 2012), with a prodromal phase that can be detected up to 30 months before onset (Addington et al., 2007).

Oxidative stress is caused by an excess of free radicals generated by cellular metabolic stress and an impaired antioxidant defence system and is known to cause membrane dysfunction implicated in the pathophysiology of schizophrenia (Mahadik et al., 2006). However, our understanding of schizophrenia and the involvement of oxidative stress is constantly evolving in the wake of new neurobiological methodologies, such as magnetic resonance spectroscopy, used to assess in vivo metabolites within the brain. The advancement of new technologies and increased understanding will enable us to develop novel treatments

to target clinical symptoms and identify preventative mechanisms to halt transition to schizophrenia in individuals at high risk for mental health disorders.

Despite this, the mechanisms of different psychotic disorders, including schizophrenia, are not fully established, with evidence supporting a number of theories including neuronal maldevelopment (Fatemi & Folsom et al., 2009), hyperactive dopamine transmission (Rampino et al., 2019), hypoactive glutamatergic signalling (Hashimoto 2014) and immune dysfunction (Goldsmith & Rogers., 2008), including microglial dysfunction (Laskaris et al., 2016) and overproduction of inflammatory cytokines via innate immune cells (Hughes & Ashwood, 2020). However, one commonality between many of these theories is altered function of the neuronal membrane, along which is a litany of neurotransmitter receptors and ion channels. The neuronal membrane is the functional site of drug effects and signal transduction (Yao et al., 2001) and, furthermore, represents a point where both genetic and environmental factors related to the aetiology of schizophrenia may interact (Horrobin, Glen & Hudson, 1995).

This review will explore the mechanisms by which oxidative stress may affect the brain and how this may be related to the symptom profile of schizophrenia. Initially we will provide a brief description of oxidative stress, covering free radicals and exploring endogenous antioxidant defences. We will then examine the literature on impaired antioxidant defence mechanisms in schizophrenia, including the methods by which this is assessed, before evaluating how these mechanisms may relate to the symptom profile of schizophrenia, including positive, negative and disorganised symptom severity. The studies were found via PubMed and Google Scholar searches using combinations of key words “schizophrenia”, “oxidative stress”, “antioxidant defence”, “magnetic resonance spectroscopy”, “dopamine”, “glutamate”, “mTOR” “inflammation”, “dysconnectivity” and

"symptoms". The searches yielded original research, meta-analyses and review articles that were peer reviewed and in English.

2.2 Oxidative Stress

Oxidative stress is defined as an imbalance between the production and subsequent build-up of reactive species, or free radicals, and the body's inability to detoxify these reactive products. This in turn can lead to molecular and cellular damage (Pizzino et al., 2017). There are two types of reactive species: reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet -}$) or hydrogen peroxide (H_2O_2), and reactive nitrogen species (RNS), such as the nitroxyl anion (NO^-) and various nitrogen oxides (NO_2, N_2O_4, etc.) (Mirończuk-Chodakowska et al., 2018). Reactive oxygen species are generated as by-products of mitochondrial production of adenosine triphosphate (ATP), a crucial molecule for cellular actions (Ambrosio et al., 1993). The electron transport chain employed in this production consumes roughly 90% of all oxygen absorbed by the cells (Wallace, 2013) with an estimated 0.1-0.5% of this oxygen being converted into superoxide radicals (Servais et al., 2005).

Free radicals, such as the superoxide radical, are known to have some beneficial physiological effects; for example, they can aid the body's innate immune system and provide a key line of defence against pathogens (Rosen et al., 2009). In a healthy state the level of free radicals is controlled to maintain a balance between oxidation and reduction in tissues (Tan et al., 2018). However, when production of these species increases, such as when the body is in a high stress condition or disease state, they begin to negatively affect important structures within cells, such as lipids, proteins, and nucleic acids (Sato et al., 2014). One example of this is when the hydroxyl radical and peroxynitrite are in excess, they can cause lipid peroxidation which in turn damages cell membranes and lipoproteins. This can lead to

the formation of malondialdehyde and conjugated diene, both of which are known to have toxic and mutagenic properties (Frei, 1994). Furthermore, neurons within the central nervous system are at risk of damage from reactive species (Bošković et al., 2011). The brain has high levels of oxygen consumption, around 20% of total basal oxygen consumption and an increased rate of oxidative metabolism. These factors, combined with lower levels of protective antioxidant enzymes and a high proportion of easily oxidised membrane polyunsaturated fatty acids (PUFAs), when compared with the rest of the body, lead to a much greater risk for the negative effects of oxidative stress (Mahadik & Mukherjee, 1996).

To combat excessive accumulation of ROS and RNS there is a complex set of endogenous antioxidant defences, both enzymatic and non-enzymatic. Antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) help to block the initiation of reactive species chain reactions and form the first line of antioxidant defence (Bošković et al., 2011). These enzymes act in conjunction to inactivate the superoxide radical. $O_2^{\bullet-}$ is transferred into H_2O_2 via the addition of an electron in a reaction catalysed by SOD. The hydrogen peroxide produced by this reaction is then decomposed into harmless water and oxygen by CAT and GPx (Lazo-de-la-Vega-Monroy & Fernández-Mejía 2013). As each of these enzymes is critical in different stages of free radical metabolism, change in activity of one without compensation by the others could leave cellular membranes vulnerable to damage (Yao & Keshevan, 2011).

The second line of defence comes from non-enzymatic antioxidant components such as glutathione (GSH), metal binding proteins (MBPs) and uric acid (UA) which rapidly inactivate reactive species and thereby prevent the propagation of chain reactions (Mirończuk-Chodakowska et al., 2018). These non-enzymatic antioxidants work in a number of ways to help neutralize excess free radicals. MBPs inhibit the formation of new reactive species by binding metals such as iron and copper (Battin & Brumaghim, 2009), whereas

GSH is a free radical scavenger; it scavenges reactive species and inactivates them. During the reaction GSH is oxidised into glutathione disulphide GSSG, which can then be reduced back into GSH (Wu et al., 2004). Additionally, dietary antioxidants such as vitamin E, vitamin C and carotenoids can affect the activity of endogenous antioxidants, with vitamin C helping to support the regeneration of GSSG back into GSH (Birben et al., 2012).

To summarize, the human body has to maintain a delicate balance of forming enough reactive species to perform useful physiological roles, whilst breaking down the excess to prevent unnecessary cellular damage. As such oxidative stress is thought to play a key role in many physical disorders such as cardiovascular disease and diabetes (Daiber & Chlopicki, 2020; Zhang et al., 2020), as well as a number of mental disorders such as depression and schizophrenia (Bhatt et al., 2020; Upthegrove & Khandaker, 2019).

2.3 Oxidative Stress and Schizophrenia

Increased levels of reactive species and decreased levels of antioxidant defences are seen to cause oxidative damage to a number of cellular structures. Many studies have now shown that oxidative damage is present in schizophrenia (Yao et al., 2001; Fendri et al., 2006; Gunes et al., 2017). Although this may not be the primary cause of schizophrenia, growing evidence has suggested that it may contribute to the declining course and poor outcome in schizophrenia (Khan et al., 2002).

Examining oxidative stress within the brain is particularly difficult because, until recently, there was no way to assess metabolite concentrations in living human tissue. As such, a variety of methods have previously been employed to assess oxidative stress within schizophrenia. A large number of studies have assessed peripheral biomarkers of oxidative stress such as antioxidant levels. Total antioxidant and glutathione levels have been shown to

be lower within the plasma of non-medicated, medicated, first-episode and chronic schizophrenia patients (Raffa et al., 2011; Reddy et al., 2003; Altuntas et al., 2000; Li et al., 2011). In addition to this, increased levels of reactive oxygen species have been found in the periphery of schizophrenia patients (Sarandol et al., 2015; Miyaoka et al., 2015), in conjunction with reduced levels of SOD and GPx (Dietrich-Muszalska & Kwiatkowska, 2014). Furthermore, redox regulatory findings have been shown to be influenced by illness phase e.g. stable or acute schizophrenia (Solberg et al., 2019). Post-mortem studies also report reduced glutathione levels in the brains of schizophrenic patients, specifically within the prefrontal cortex and the caudate (Gawryluk et al., 2011; Yao et al., 2006), with abnormal protein expression in the anterior cingulate cortex (ACC) a result of increased oxidative stress (Clark et al., 2006).

Results from *in vivo* MRS studies of glutamate/glutamine concentrations in schizophrenia, whilst inconsistent, have highlighted that sub-grouping patients on 'residual schizophrenia' (long-term negative symptoms/impairments) revealed reduced, highly correlated, GSH, glutamate and glutamine concentrations in the ACC (Tayoshi et al., 2009; Kumar et al., 2020). Lower grey matter volume (GMV) in medial frontal and ACC in ultra-high-risk individuals for schizophrenia also predicted poorer long-term functional outcome at follow-up (~9 years later), irrespective of transition to schizophrenia or persistence of at-risk mental state (Reniers, et al., 2017).

It has been suggested that, in schizophrenia, redox dysregulation and subsequent oxidative stress may be limited to a specific subgroup representing approximately one third of patients (Bentsen, 2011; Bentsen, 2013, Bentsen, 2017, Solberg, 2019). This subgroup is characterised by very low levels of polyunsaturated fatty acids (PUFAs) within red blood cells during the acute phase of illness (Bentsen, 2011), when PUFAs were bimodally distributed, as well as deleterious effects of eicosapentanoate (EPA) or vitamin E and C on

mental functioning (Bentsen, 2013, 2017). During a stable phase, PUFA was no longer bimodally distributed, but high 2-amino butyrate in the low PUFA group indicated persistent redox dysregulation (Bentsen, 2019).

2.3.1 Genetic Studies

Genome wide association studies have shown an association between gene polymorphisms for oxidative stress and schizophrenia (Chowdari et al., 2011). Genetic variations have been found in the strands of DNA that code for the rate limiting enzyme, glutathione cysteine ligase and glutathione-S-transferases, involved in the synthesis of glutathione (Gravina et al., 2011). A high-risk genotype for the glutathione cysteine ligase catalytic unit has been linked to impaired capacity to synthesise GSH under conditions of oxidative stress, as well as a reduction in medial prefrontal GSH levels (Gysin et al., 2007; Xin et al., 2016). Genome wide association studies have also identified a "psychiatric susceptibility gene" cacna1c as one of the strongest genetic risk factors for the development of affective disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). This gene has recently been linked to mitochondrial function and subsequent oxidative stress (Michels et al., 2018), suggesting it may play a key role in the aberrant generation of damaging reactive oxygen species seen in schizophrenia.

2.3.2 Animal Models

Higher levels of reactive species within mitochondria have been found in the brains of ketamine-induced rat models of schizophrenia compared to wild-type controls (Faizi et al., 2014). Another rodent model of schizophrenia is the N-methyl-D-aspartate-antagonist MK-801-induced model, and this too has been found to have increased levels of oxidative stress in

the prefrontal cortex (Ozyurt et al., 2014). Glutathione depletion after the administration of 2-cyclohexen-1-one, a chemical which enhances the rapid degradation of GSH has also resulted in schizophrenia-like behaviour in rodents (Iguchi et al., 2014). Knockout mice that lack a subunit of glutamate cysteine ligase have demonstrated a significant reduction of GSH in the anterior cingulate cortex (das Neves Duarte et al., 2012), which has resulted in schizophrenia-like behaviour, including hyperlocomotion and altered social behaviour (Kulak et al., 2012). Furthermore, these knockout mice demonstrate neuronal changes within the hippocampus similar to those seen in schizophrenia, specifically decreasing the numbers of parvalbumin interneurons (Steullet et al., 2017).

Evidence of redox dysregulation is seen in other common neurodevelopmental animal models of schizophrenia. For example, the social isolation rearing model explores the effects of environmental insults via social deprivation on the developing brain after birth, with animals developing specific behaviours and neurobiology akin to schizophrenia (Ferdman et al., 2007; Jones et al., 2011). Within this model increases in SOD activity are seen in conjunction with higher levels of lipid peroxidation in the prefrontal cortex (Moller et al., 2011). Furthermore, mitochondrial dysfunction is also noted, with increased striatal and decreased frontal cortex ATP (Moller et al., 2013). Inflammatory mouse models have also shown evidence of increased oxidative stress with prenatal exposure to the bacterial endotoxin lipopolysaccharide shown to decrease levels of GSH in the hippocampus (Lante et al., 2008). Several studies have demonstrated a significant increase in lipid peroxidation when inflammation is induced postnatally (Zhu et al., 2007; Ribeiro et al., 2013; Macdowell et al., 2013).

2.3.3 Clinical Trials

Several studies report that antioxidant treatment has had positive effects on schizophrenia, although the evidence is inconsistent. Treatment with the antioxidant N-acetylcysteine has been of great interest in recent years, with studies finding that it ameliorated depressive symptoms (Berk et al., 2008; Farokhnia et al., 2013; Zheng et al., 2018) and may reverse oxidative stress induced by mitochondrial dysfunction (Otte et al., 2011). A recent meta-analysis by Yolland et al. (2020) concluded that n-acetylcysteine led to improvement in negative symptom score, total symptom score and working memory. Sulforaphane, another antioxidant, has been shown to have neuroprotective properties (Klomparens & Ding, 2019) and may reduce the risk of transition to schizophrenia from an at-risk state (Hashimoto, 2019). Sedlak et al. (2018) demonstrated that treatment with sulforaphane increased the levels of available GSH in the brains of healthy controls after 7 days. Results from animal trials have suggested that dietary intake of the sulforaphane precursor glucoraphanin prevented cognitive deficits in adult offspring after maternal immune activation (Matsuura et al., 2018). Additionally, a small study of seven human patients with schizophrenia found a significant improvement in a test of working memory after an 8-week treatment with sulforaphane, although the sample size may have been too small to detect any other improvements (Shiina et al., 2015). Due to the positive results seen from these studies clinical trials are now underway to assess the efficacy of sulforaphane in clinical subjects.

Further research into dietary antioxidants such as vitamins and Omega-3 PUFAs have been of interest in recent years (Mitra et al., 2017). Vitamin C and E have been reported to improve patient symptoms (Zhang et al., 2004), however, more recent studies have shown that in high doses, these vitamins may act as pro-oxidants and can increase the levels of oxidative stress, although when combined with ethyl-EPA, vitamin E and C in these high

doses was not deleterious (Bentsen et al., 2013; Brown & Roffman, 2014). Omega-3-PUFAs are shown to be reduced in schizophrenia (Arvindakshan et al., 2003; Khan et al., 2002) and act as an essential building block of eicosanoids which act to regulate inflammation and oxidative stress (van Rensburg et al., 2009). Studies assessing Omega-3 PUFA supplementation have yielded mixed results with some demonstrating a reduction in symptom severity (Emsley et al., 2002; Pawelczyk et al., 2015) and others have found no additional benefit compared to placebo (Fenton et al., 2001; Ross et al., 2007; McGorry et al., 2016). It has been suggested that perhaps the reason for these mixed results is due to illness phase and those with chronic schizophrenia may have progressed too far for supplementation to have a beneficial effect (Mitra et al., 2017). Indeed, one meta-analysis suggested that Omega-3 PUFA supplementation was most effective in earlier phases of illness and reduces the conversion from high risk to first episode (Chen et al., 2015). Furthermore, it has been suggested not only dietary supplementations but elimination of substances which are toxic or not tolerated by some patients may have a beneficial effect in schizophrenia treatment. For example, a recent study showed that the removal of gluten from a patients diet was associated with a reduction in negative symptom severity (Kelly et al., 2019).

It should be noted that there is some contention as to whether peripheral biomarkers can reflect the status of the CNS (Bošković et al., 2011). Traces of oxidative damage may arise from a variety of areas within the body and as such peripheral indicators of oxidative stress may not reflect the conditions within the brain (Michel et al., 2004; Xin et al., 2016). Whilst some studies have shown peripheral antioxidant capacity is consistent with the central nervous system (Siciliano et al., 2007), peripheral status is simply indirect evidence. As a result of this, the next step is to assess oxidative status in vivo. The primary method by which this can occur is the use of magnetic resonance spectroscopy (MRS).

2.3.4 Magnetic Resonance Spectroscopy

MRS is a relatively new tool that is used in conjunction with MRI to non-invasively measure the concentration of metabolites within living tissue (Landheer et al., 2019). It can be used to assess antioxidant concentrations in the brain (Duarte & Xin, 2019). Similar to magnetic resonance imaging (MRI), MRS acquires a signal from hydrogen protons. However, while MRI acquires signal primarily from protons within water and fat, due to their high concentration within the brain, MRS acquires its signal from other molecules, such as GSH. By examining the difference in resonance frequency of hydrogen nuclei in difference chemical environments, MRS can distinguish between hydrogen nuclei in different molecules. Hydrogen protons seen in fat and water are approximately one thousand times more abundant than those detected in molecules by MRS (de Graaf, 2019) and thus MRS employs a method to suppress the water proton signal. Since the molecules of interest within MRS studies are much less abundant than water, larger voxels of acquisition are required, typically 2x2x2 cm^3 (Mikkelsen & Hearshen, 2008). Larger voxels help to improve the signal to noise ratio which is often poor in MRS studies (Tal et al., 2012). However, with a larger voxel comes difficulties in voxel composition, a 2 cm^3 voxel makes it incredibly difficult to get a “pure” white matter location and virtually impossible to obtain a “pure” grey matter placement (Tal et al., 2012).

In light of the evidence for extensive structural and functional brain abnormality in schizophrenia (Karlsgodt et al., 2010), the question of optimum placement of an MRS voxel for identification of relevant abnormalities in antioxidant concentrations in schizophrenia remains unanswered (Fisher et al., 2019). Nonetheless, meta-analyses of convergent GMV loss across diverse psychiatric diagnostic groups (Goodkind et al., 2015), post-mortem studies of tissue from patients and also the evidence from relevant animal models of schizophrenia reviewed above suggest that the prefrontal cortex, anterior cingulate and

medial temporal lobe including hippocampus, are candidate regions of interest. Furthermore, in mice the highest concentrations of GSH are in the cortex followed by the cerebellum, hippocampus and striatum (Kang et al 1999) suggesting differential sensitivity of different brain regions to damage from oxidative stress. There is also the issue of grey matter or white placement, with GSH concentrations shown to be 30% higher in white matter than grey matter (An et al., 2015), however grey matter is seen to be more vulnerable to oxidative stress (Srinivasan et al., 2010). Hence, no 'gold standard' approach has resulted in studies often choosing different areas of the brain to assess antioxidant concentrations resulting in varied findings.

2.4 Interaction of Oxidative Stress and Current Schizophrenia Hypotheses

2.4.1 The Dopamine Hypothesis

The dopamine hypothesis is the most well-known in schizophrenia and has dominated the literature for many years. This theory was proposed after it was discovered that the drug chlorpromazine had antipsychotic properties (Delay et al., 1952), further to this, Carlsson & Waldeck (1958) discovered that dopamine was a neurotransmitter. It was subsequently proposed that the therapeutic effects were the result of selective blockade of dopamine D2 receptors (Van Rossum, 1966). To this day, all antipsychotic drugs act on dopaminergic receptors in the brain (Rampino et al., 2019). It is proposed that D2 receptor neurotransmission is hyperactive within subcortical and limbic brain regions. This hyperactivity is thought to contribute towards positive symptoms in schizophrenia. Alongside D2 hyperactivity, it is also thought that D1 receptor hypoactivity can contribute towards the negative and cognitive symptoms seen in this disorder (Toda & Abi-Dargham, 2007).

Postmortem studies have found an increased density of D2 receptors in the brains of schizophrenia patients (Seeman & Kapur, 2000). Upregulation of D2 receptors within the caudate nucleus is also reported to correlate with cognitive dysfunction (Hirvonen et al., 2004). Additionally, indirect dopamine agonists, such as amphetamine and cocaine, have been shown to induce positive symptoms in the general population (Lieberman et al., 1987) with schizophrenia patients displaying an increased sensitivity to the dopamine-releasing effects of these drugs (Laruelle & Abi-Dargham, 1999; Carlsson et al., 2000; Abi-Dargham, 2004).

However, antipsychotic drugs tend to alleviate positive symptoms more than negative symptoms (Lally & MacCabe, 2015), with some studies showing that they may worsen negative symptoms in patients (Fervaha et al., 2015) and even induce them in healthy controls (Artaloytia et al., 2006). It has been suggested that hypoactivity of the dopamine pathway is a mediator of negative symptoms in schizophrenia, indicating that reduced dopamine activity may be the end difficulty rather than dopamine overactivity (Brisch et al., 2014).

Metabolism of dopamine has been suggested to be a prominent producer of reactive oxygen species in the brain (Mahadik & Mukherjee, 1996). Oxidation of dopamine (both enzymatic and non-enzymatic) results in the generation of H_2O_2 which when in the presence of iron or oxygen can form the more active hydroxyl radical ($^{\cdot}OH$) (Dusek et al., 2016). Additionally, the oxidation of dopamine can form dopamine quinones; these could then react with the sulfhydryl groups of glutathione, thus reducing the levels of GSH and increasing the levels of ROS (Park et al., 2002). One study found that dopamine alone caused a 40% reduction in GSH levels within cortical neurons (Grima et al., 2003).

2.4.2 The Glutamate Hypothesis

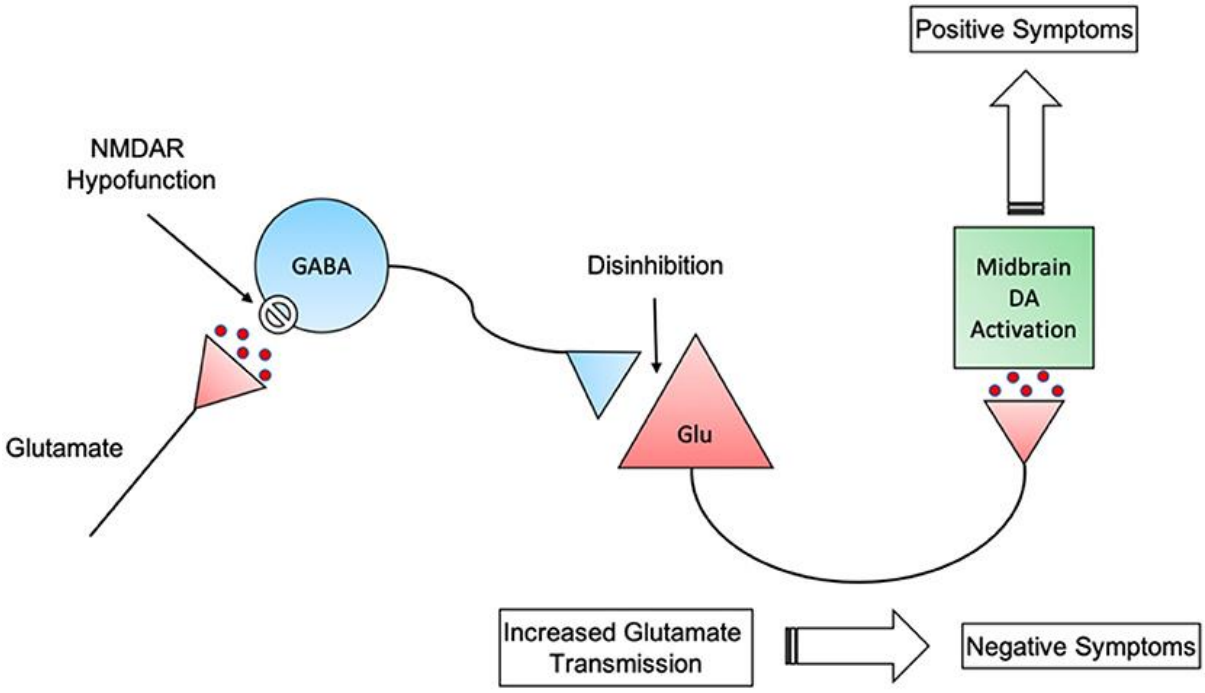
Initially proposed in 1980 after glutamate levels were seen to be lower in the cerebrospinal fluid of schizophrenia patients (Kim et al., 1980), the glutamate hypothesis postulates that the negative symptoms seen in schizophrenia are in part linked to dysfunctional glutamatergic signalling, mediated by NMDA receptors on GABAergic interneurons (Hashimoto, 2014). Similar to the dopamine hypothesis, initial support for this theory came from studies into mind altering drugs. NMDA receptor antagonists, such as ketamine, are seen to induce psychosis in healthy controls (Lin & Lane, 2019), with the induced psychosis caused by NMDA receptor antagonists resembling schizophrenia symptoms more closely than those that act on the dopaminergic system (Krystal et al., 1994).

A number of genes have been seen to influence the function of glutamate receptors (Harrison & Owen, 2003; Moghaddam & Jackson, 2003). Reports from genome wide association studies have found that out of 108 loci related to schizophrenia risk, 6 involve genes implicated in brain glutamate function, with many more thought to affect glutamate function indirectly (Ripke et al., 2014). Bustillo et al. (2017) found that polymorphisms in the glutamate-related genes $CLCN_3$ GRM_3 and SLC_38A_7 were directly correlated with combined glutamate and glutamine signal in the grey matter of younger schizophrenia patients (<36 years). Post-mortem studies have found a reduction in NMDA receptors in the brain tissue of schizophrenia patients (Sokolov, 1998). Moreover, brain imaging studies have demonstrated reduced binding of NMDA receptors in the hippocampus of schizophrenia patients (Pilowsky et al., 2005).

Although the glutamate hypothesis may be closer to the root cause of schizophrenia, it does not rule out the dopamine hypothesis. Recent circuit-based models have implicated both glutamatergic and dopaminergic neurotransmission in the pathogenesis of schizophrenia

(Tsutsui et al., 2017). Dopamine neurons are regulated by glutamatergic inputs to the midbrain dopamine nuclei. As such, dopamine function may be secondary to aberrant glutamate functioning. NMDA receptor dysfunction on GABAergic interneurons leads to disinhibited glutamate transmission which could result in the appearance of negative symptoms. Furthermore, these glutamate neurones project into the midbrain and activate the dopamine pathways that are key to positive symptom appearance (Figure 1) (Nakazowa & Sapkota, 2020).

Figure 1.1: Interactions between GABAergic disinhibition of glutamatergic neurons and subsequent stimulation of midbrain dopaminergic neurons.



Abbreviations: NMDAR, N-methyl-D-aspartate receptor; GABA, Gamma-Aminobutyric acid; Glu, Glutamate; DA, Dopamine.

Results from animal studies show that GSH and glutamate are closely related (Sedlak et al., 2019). Glutathione synthesis is directly related to glutamate uptake in microglia and the

subsequent release of glutamate metabolites (Persson et al., 2006); additionally, glutathione depletion as a result of oxidative stress is strongly related to microglial glutamate release (Barger et al., 2007). Activation of glutamatergic pathways can trigger the generation of free radicals while reducing endogenous protection against free radical damage (Bains & Shaw, 1997). Furthermore, free radicals can trigger the release of glutamate into the synaptic cleft while blocking its reuptake (Volterra et al., 1994).

GSH and NMDA receptor activity are closely linked; an increase in glutathione levels is shown to raise NMDA receptor responsiveness, whereas its depletion has resulted in NMDA receptor hypofunction (Hardingham & Do, 2016; Steullet et al., 2016). Hypofunction of these receptors can result in increased free radical production and subsequent oxidative damage (Papadia et al., 2008). One more recent study has shown that synaptic NMDA receptor activity is intrinsically linked to GSH production, with an increase in synaptic activity triggering a subsequent growth in GSH production and utilisation (Baxter et al., 2015). Taken together these studies demonstrate the close link between the glutamatergic system, NMDA receptor hypofunction and GSH.

2.4.3 mTOR Pathways

More recently a new hypothesis for schizophrenia aetiology has arisen; the mammalian target of rapamycin (mTOR) hypothesis (Gururajan & van den Buuse, 2014). The mammalian target of rapamycin (mTOR) acts as a central regulator of cell metabolism, growth and proliferation via the integration of both intra and extracellular signals (Laplante & Sabatini, 2009). Misregulation of mTOR via upstream proteins phospotidylinositol 3-phosphate kinase (PI3K) and protein kinase B (PKB) is thought to contribute to schizophrenia (Stopkova et al., 2004; Ryskalin et al., 2018). Inhibition of either PI3K/PKB or

mTOR leads to inhibited of neuronal growth and thus might contribute to the aberrant synaptic architecture seen in schizophrenia (Kalkman, 2006). As a result of this inhibited neuronal growth there is a reduction in dendritic branching and subsequent synaptic formation (Jaworski et al., 2005). This may result in the development of negative symptoms due to the lack of neuronal connections (Gururajan & van den Buuse, 2014). Overstimulation of the mTOR system in specific brain areas has been linked with cognitive deficits seen in schizophrenia (Meffre et al., 2012). While inhibition of this pathway causes reduced dendritic branching, overstimulation is thought to increase the number of synaptic connections, thus leading to the generation of positive symptoms (Ibarra-Lecue et al., 2018). The mTOR pathway has been shown to have connections to both serotonin and glutamatergic pathways in the brain, through interactions with the serotonin receptor 5-HT6 (Teng et al., 2018) and glutamate receptors mGluR and NMDA (Tang et al., 2105), thus linking the mTOR pathway to the glutamate hypothesis.

Oxidative stress can also interact with the mTOR pathway resulting in the development of cognitive symptoms within schizophrenia. It has been proposed that cognitive symptoms arise from prefrontal cortex dysconnectivity (Zhou at al., 2015). This dysconnectivity has been related to myelin and oligodendrocyte abnormalities in schizophrenia patients (Uranova et al., 2011). Myelin is produced by mature oligodendrocytes, the precursor of which is particularly susceptible to oxidative stress (Thorburne et al., 1996). It has been proposed that reactive oxygen species can inactivate sections of the mTOR pathway leading to reduced myelination and proliferation of the oligodendrocyte precursors and subsequent disruption of connectivity within the prefrontal cortex (Maas et al., 2017).

2.4.4 The Immune Hypothesis

Long before the development of modern antipsychotics, infections and inflammation were proposed to be the cause of psychosis. During an influenza pandemic in the late 19th century, it was demonstrated that psychiatric conditions could be caused by an infectious agent, in this case the flu, and one specific type of infection can produce a number of different psychiatric syndromes (Kraepelin, 1890). These insights are still valid today (Taquet et al., 2021); however, they have become more generalised, with inflammation thought to play a key role in many psychiatric disorders in the absence of an acute infectious disease (Muller, 2020).

Pro-inflammatory cytokines, astrocytes, microglia and immune cells such as macrophages and T- or B- lymphocytes help to mediate inflammation in the CNS (Muller, 2018). Under normal circumstances these inflammatory mediators play an essential role in combating infection, harmful chemicals and responding to tissue damage (Murphy & Weaver, 2016). However, dysregulation of the inflammatory response, for example via infection, can trigger a cascade which affect central nervous system (CNS) processes and behavioural phenotypes (Comer et al., 2020). This dysregulation is central to the immune hypothesis of schizophrenia. Inflammation could cause significant CNS changes which result in the appearance of positive, negative and disorganised symptoms (Barron et al., 2017).

Genome wide association studies have located key risk genes for schizophrenia within the major histocompatibility complex (MHC) on chromosome 6, a key loci that codes for specific cell surface proteins essential within the immune system (Purcell et al., 2009). Complement component 4 (C4), a gene located within the MHC which affects both synaptic pruning and opsonization of pathogens, is of particular interest. Recent studies have shown that people with schizophrenia overexpress this gene, thus causing a disruption in synaptic

pruning and inflammation related damage (Nimgaonkar et al., 2017). Furthermore, overexpression of C4 may help to explain the developmentally timed nature of schizophrenia (Sekar et al., 2016). Additional polymorphisms on genes coding for inflammatory cytokines have also been implicated in schizophrenia risk (Birnbaum & Weinberger., 2019).

Further clinical studies have found increased biomarkers of neuroinflammation in schizophrenia patients, including greater levels of circulating inflammatory cytokines such as Interleukin-6 (IL-6), Tumour Necrosis Factor Alpha (TNF-α) and Interferon Gamma (IFN-γ) (Mondelli et al., 2015; Baumeister et al., 2016; Goldsmith & Rapaport, 2020; Upthegrove et al., 2014, 2018, 2020). Elevated cytokine levels are seen to arise before the onset of schizophrenia (Khoury & Nasrallah, 2018) and may even predict later transition from an at risk mental state, for example elevated levels of IL-6 at age 9 are shown to double the risk of a psychotic disorder diagnosis at age 18 (Upthegrove et al., 2020). Higher levels of C-reactive protein (CRP) at age 15 are also associated with an increased risk of schizophrenia development by age 27 (Metcalf et al., 2017).

It has been hypothesised that these inflammatory cytokines may result in the appearance of the schizophrenia phenotype via disturbance of key neurotransmitter systems (Khandaker et al., 2014). Evidence has suggested that pro-inflammatory cytokines increase the concentration of kynurenic acid, a naturally occurring NMDA receptor antagonist, triggering hypofunction of the NMDA receptor and thus promoting increased glutamatergic transmission, ultimately resulting in schizophrenia symptoms (Pedraz-Petrozzi et al., 2020).

One potential cause of the neuroinflammation seen in schizophrenia could be maternal immune activation (MIA) (Aguilar-Valles et al., 2020). Studies have shown an association between maternal infection in pregnancy and schizophrenia development in offspring (Brown & Derkits, 2010). Further studies have shown that this is not dependant on

the type of infection the mother has, immune activation alone, and the subsequent cytokine release, is enough to significantly increase schizophrenia risk in offspring (Feigenson et al., 2014). It has been suggested that 14%-21% of all schizophrenia cases could be prevented by the eradication of maternal influenza (Brown, 2006).

Inflammation and oxidative stress are intrinsically linked. Tissue damage caused by oxidative stress can trigger inflammation and an immune response (Lugrin et al., 2014). Furthermore, macrophages and microglia use reactive oxygen species to kill pathogens (Bordt & Polster, 2014). As such oxidative stress can be seen as both an inducer and a product of inflammation (Koga et al., 2016). In addition to this, the imbalance between pro and antioxidants may play a key role in the maternal immune activation model (Lante et al., 2008). In mice it has been demonstrated that MIA resulted in the elevation of a number of oxidative stress markers, including glutathione (Lante et al., 2007). Taken together it appears that inflammation and oxidative stress have a close reciprocal relationship within schizophrenia.

2.4.5 The Dysconnectivity Hypothesis

One of the more prominent schizophrenia hypotheses today is the dysconnectivity hypothesis. First proposed in 1995 by Friston and Frith, it suggests that schizophrenia symptoms may arise from disrupted brain connectivity. This hypothesis was based on findings that schizophrenia patients demonstrated a reduction in the connectivity between the prefrontal cortex (PFC) and temporal brain regions (Frith et al., 1995). Since these initial findings a number of imaging studies have investigated this, each with more descriptive and sensitive techniques (including Dynamic Causal Modelling (DCM), Psycho–Physiological Interaction (PPI) and Independent Component Analysis (ICA)) (Pettersson-Yeo et al., 2011).

A number of white matter abnormalities have been seen in both medicated an unmedicated schizophrenia patients, including a disruption in white matter integrity which is correlated with cognitive impairment (Nazeri et al., 2013; Muetzel et al., 2015; Cohen et al., 2007). Importantly this disruption in white matter integrity occurs before the onset of frank schizophrenia and worsens as symptoms progress (Bloemen et al., 2009; Yao et al., 2013; Holleran et al., 2014). Additionally, diffusor tensor imaging (DTI) has revealed widespread decreases in white matter tracts across a number of long-range pathways within schizophrenia, such as frontal-temporal-limbic, and cortico-cerebellar pathways (Kelly et al., 2018; Nath et al., 2021).

In addition to these white matter abnormalities, meta-analyses have revealed grey matter loss across a number of brain sites, including cortical, subcortical, cerebellar and limbic, with this loss becoming more pronounced as the disorder progresses (Williams, 2008; Birur et al., 2017). Meta-analyses of GMV loss also reveals reduced integrity of anterior insula and dorsal anterior cingulate based neural systems (e.g. the salience network) linked to psychotic disorders and deficits in executive functioning (Goodkind et al., 2015). These deficits are largely attributed to cellular deficits rather than neuronal loss, for example reduced dendritic branching and spine density (Coyle et al., 2016). In spite of these widespread deficits in structural integrity, functional connectivity between brain areas is quite variable. For example, within specific frontal and temporal regions there is a deficit in white matter and subsequent functional connectivity, however in some cases an increase in connectivity is seen (Cocchi et al., 2014; Sun et al., 2017). In addition to this, connectivity patterns may vary based on whether the brain is at rest or performing a task (Godwin et al., 2017; Zhuo et al., 2018). As such it has been proposed that schizophrenia can be characterised by structural brain deficits with irregular functional hypo or hyper-connectivity patterns.

It has been hypothesised that these functional deficits in connectivity are in part due to myelin abnormalities (Lang et al., 2014; Do et al., 2016). As mentioned previously myelin is produced by oligodendrocytes, interrupting the production of myelin can lead to functional dysconnectivity and the appearance of frank psychotic symptoms (Maas et al., 2017). It is here where oxidative stress may play a role, the oligodendrocyte precursors (OPC) are susceptible to oxidative stress, redox dysregulation alongside inflammation and glutamatergic hypofunction to impair the development of OPCs to mature oligodendrocytes, thus impacting neuronal myelination (Rivers et al., 2008; Do et al., 2016). In a series of human and rodent studies, glutathione deficit in the prefrontal cortex was linked to impaired OPC proliferation alongside oligodendrocyte and myelin maturation (Monin et al., 2015). OPCs and oligodendrocytes are known to have up to 6 times more reactive oxygen species within them, perhaps due to the increased metabolic activity required to produce myelin (Juurlink, 1997). As such, these cells are constantly in a state of increased oxidative stress, to which they are already susceptible. It has been indicated that a redox change of as little as 15% can influence the pathways that stimulate oligodendrocyte maturation (Li et al., 2007). Additionally, oxidative stress can trigger downregulation of gene expression related to myelination (Jana & Pahan, 2013). As such the myelin abnormalities and subsequent dysconnectivity of specific brain regions observed within schizophrenia may be due to oxidative-stress induced OPC dysfunction. Furthermore, it has been noted that the impaired development of OPCs to mature oligodendrocytes can be reversed by supplementation with the antioxidant NAC (Monin et al., 2015; Do et al., 2016).

2.5 Oxidative stress and symptom profile

Although oxidative stress has been implicated in the aetiology of schizophrenia many times (Prabakaran et al., 2004; Flatlow et al., 2013; Fraguas et al., 2019), the method by which it may relate to specific symptoms is still unclear (Chien et al., 2020). It has been demonstrated that increased levels of reactive oxygen species and a dysfunction in antioxidant defences can cause significant damage to neuronal architecture. Impairments in oxidative status have been linked to cognitive decline and behavioural abnormalities (Salim, 2017). As such schizophrenia symptoms may be a result of damage to the neuronal lipid membrane in specific regions or networks, caused by excess reactive oxygen species (Solberg et al., 2016).

Studies have shown that an increase in antioxidant enzyme activity in red blood cells, plasma and cerebrospinal fluid are associated with tardive dyskinesia, negative symptoms and poor premorbid dysfunction (Sarandol et al., 2015; Yao et al., 1999, 1998). GSH depletion in particular has been linked to the negative symptoms of schizophrenia (Matsuzawa et al., 2008). Lower glutathione levels have been correlated with worse Positive and Negative Syndrome Scale (PANSS) scores and worse community functioning (Tsai et al., 2013; Ballesteros et al., 2013). Additionally, patients with residual or deficit schizophrenia, two subtypes of schizophrenia with predominantly negative symptoms, exhibited a greater reduction in GSH levels compared to those with stable schizophrenia (Kumar et al., 2020; Maes et al., 2020). It is assumed that the substantial negative symptoms seen in schizophrenia are a result of aberrant glutamatergic transmission mediated by NMDA receptor hypofunction (Howes et al., 2015). As mentioned previously, GSH, the glutamatergic system and NMDA receptor function are closely related. The links between these may suggest why negative symptoms are so strongly correlated with GSH concentration in specific brain regions.

Previous studies have demonstrated that SOD activity is positively associated with both positive and negative symptoms, as well as general psychopathology in chronic schizophrenia patients (Zhang et al., 2009; Bai et al., 2018). However other studies have found no link between overall symptom severity and SOD activity (Gonzalez-Liencres et al., 2014). One study even found that SOD activity was inversely associated with positive symptoms (Wu et al., 2012). A more recent study has found that gender differences play a role in clinical symptoms, with higher SOD activity correlated with negative symptoms in men, but with positive symptoms in women (Wang et al., 2020). A recent study has notably found that platelet lipid peroxidation is associated with the severity of disorganization symptoms (Chien et al., 2020).

A number of reasons have been proposed as to why results are inconsistent, including: antipsychotic medication confound, variable disease severity, number of psychotic episodes and source of test material (blood, plasma, or serum) (Dordevic et al., 2017). Additionally, there are a number of additional confounding factors which may influence results such as, smoking (Zhang et al., 2007) and obesity (An et al., 2018). An alternative argument for this may be that each biomarker for oxidative stress does not work independently, and they could interfere with each other (Guidara et al., 2020). As such it may be the case that each individual biomarker may not have satisfactory diagnostic power (El-Ansary et al., 2017) and it may be more pertinent to combine several markers to improve diagnostic precision in future studies.

2.6 Discussion

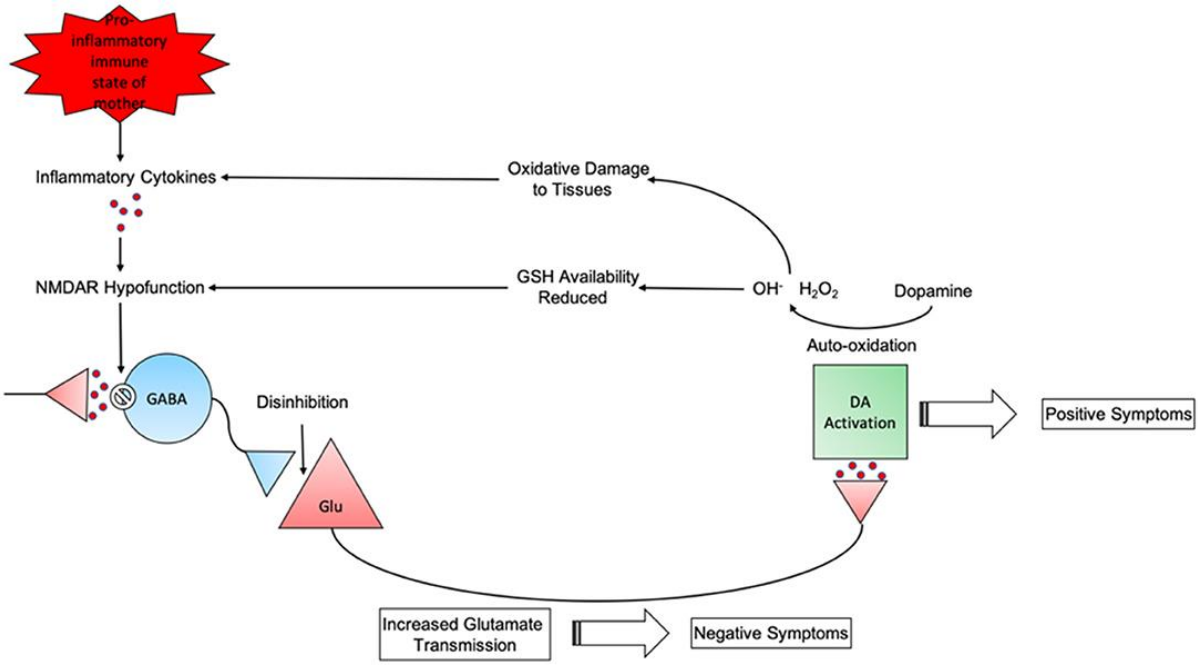
Oxidative stress has been heavily implicated in the pathogenesis of schizophrenia. With a number of studies finding increased levels of reactive species and decreased

concentrations of antioxidant defences alongside significant levels of oxidative damage in schizophrenia (Gunes et al., 2017; Raffa et al., 2011; Sarandol et al., 2015;). Evidence for this has come from a variety of study designs, including post-mortem, genetic, animal and clinical trials (Gawryluk et al., 2011; Michels et al., 2018; Steullet et al., 2017; Hashimoto, 2019). Oxidative stress may be seen within the light of most current biomedical hypotheses of schizophrenia and may play an important role in unifying schizophrenia hypotheses in future. Additionally, a small but increasing number studies have implicated oxidative stress in relation to specific symptoms in schizophrenia, with negative, disorganised and cognitive symptoms most evident (Matsuzawa et al., 2008; Bai et al., 2018). It may be the case that the symptoms more related to neuronal development in schizophrenia are a result of membrane damage via lipid peroxidation.

Taken together these results could present a rough timeline of schizophrenia progression (Figure 2). First, maternal immune activation during pregnancy can cause the release of pro-inflammatory cytokines (Aguilar-Valles et al., 2020). These cytokines can trigger the overproduction of kynurenic acid, an NMDAR antagonist, thus resulting in NMDAR hypofunction on GABAergic interneurons (Pedraz-Petrozzi et al., 2020). The hypofunctioning NMDARs result in disinhibition of glutamate neurons which can lead to negative symptoms of schizophrenia (Snyder & Gao, 2013). These excitatory glutamate neurons project into the midbrain and trigger hyperactivity of dopaminergic pathways which are associated with positive symptoms (Abi-Dargham et al., 2011). Treatment with first generation antipsychotics is seen to alleviate these symptoms (Abou-Setta et al., 2012). Catecholamines such as dopamine can auto-oxidate into free radicals (Stansley & Yamamoto, 2013). Free radicals produced by this will cause tissue damage and increase inflammation (Nakazowa & Sapkota, 2020). In response to increased free radical generation an increase in the antioxidants SOD and GSH are seen to combat this (Ighodaro & Akinloye, 2018). GSH

availability is reduced due to the excessive ROS produced by increased dopamine levels (Bitanihirwe & Woo, 2011). As mentioned previously a reduction in available GSH can lead to NMDA receptor hypofunction within inhibitory GABA interneurons (Hardingham & Do, 2016; Steulett et al., 2016). Thus, generating a cycle of pyramidal glutamatergic neurotransmission and the generation of diverse symptoms seen in acute schizophrenia (Moghaddam & Krystal, 2012).

Figure 1.2: The cyclical nature of schizophrenia progression from maternal pro-inflammatory state to behavioural phenotype.



Abbreviations: NMDAR, N-methyl-D-aspartate receptor; GABA, Gamma-Aminobutyric acid; Glu, Glutamate; DA, Dopamine; H_20_2, Hydrogen Peroxide; $OH^{\bullet}$, Hydroxyl Radical.

A model such as this provides an opportunity for novel therapeutic interventions in schizophrenia. The biomedical underpinnings of schizophrenia are presented here as a multi-step, cyclical, process that ultimately results in the manifestation of positive symptoms,

however such processes are not reflected in current treatments of schizophrenia. Currently antipsychotic medication is prescribed at all stages of the disorder and only effective against positive symptoms. A novel approach would be to use oxidative stress and inflammatory markers as a target for schizophrenia progression and adapting treatment based on the individual, thus moving towards a more personalised approach to schizophrenia treatment (Conus et al., 2018).

Over the last three decades a large number of clinical trials have been performed and the interest within this research area has been increasing. A 2016 review identified 22 clinical studies of antioxidant treatments in schizophrenia (Magalhaes et al, 2016), however, authors noted limited evidence for symptom improvements and under-powered study designs. Indeed, varying results from clinical trials are noted in more recent studies, for example, the antioxidant N-acetylcysteine (NAC) has been shown to improve depressive symptoms in patients (Zheng et al., 2018), however, a separate study found that NAC did not improve clinical symptoms or functional outcomes (Conus et al., 2018). Perhaps these varying results are due to the small sample sizes of the cohorts tested, additionally a recent meta-analysis suggested that longer interventions may be required for antioxidant treatments such as NAC to work, as a significant improvement in symptoms can be seen at 24 weeks or more, but not less than 8 weeks (Yolland et al., 2020). Oxidative stress presents as a good candidate for schizophrenia intervention, future studies should continue to investigate potential treatments over an extended time course. A broad range of interventions from pharmaceuticals to diet and exercise may have potential to be effective, however stratification based on the patient's biochemical and inflammatory are needed (Mitra et al., 2017; Kelly et al., 2019; Sakuma et al., 2018; Roffman et al., 2018; Aucoin et al., 2020). These future studies will not only provide potential new nutraceutical and pharmacological therapies for schizophrenia, but they will also allow us to continually improve the knowledge surrounding this complex disorder.

2.7 Author Contributions

AM researched and drafted the article, revised and edited it. JR critically revised the article, providing support on content and structure. RU supervised the project, critically revised the article, providing support on content and structure. PL and ZK critically revised the article, providing support on content and structure.

2.8 Conclusions from Chapters 1 and 2

The reviewed evidence shows that oxidative stress, plays a significant role in the pathogenesis of schizophrenia, supported by a number of studies employing diverse methodologies and highlighting its association with symptom burden within the disorder. However, in-vivo quantification has been somewhat inconclusive, indicating a need for an interrogation of the effects of GSH acquisition methods in MRS studies. Furthermore, associations between peripheral antioxidant capacity and central oxidative stress remains poorly understood, further research is required to elucidate if these peripheral markers are reflective of the CNS.

Similarly, inflammation emerges as a pivotal player in schizophrenia, with both peripheral and central markers implicated in various features of the disorder including brain morphology and symptomatology. However recent research has indicated that chronic, low-grade inflammation may only be relevant to a subgroup of people with schizophrenia, people within this subgroup may respond more positively to anti-inflammatory interventions. Potentially explaining the disparate findings of recent clinical trials. Further work is required to reliably identify this subgroup or subgroups to aid in improving patient stratification in future.

Overall, both inflammation and oxidative stress may present excellent markers for predicting disorder progression and treatment response, alongside being good targets for future pharmacological interventions within the disorder. However, further research is needed to assess the reliability of these biomarkers at different disorder stages, with an attempt to elucidate the interrelation between both peripheral and central measures and their downstream effects on symptomatology and brain morphology.

Chapter 3

The following paper has been published in *Brain Behavior and Immunity* and has not been edited from source.

Brain, Behavior, and Immunity, Volume 115, Pages 3-12, January 2024

DOI: 10.1016/j.bbi.2023.09.017.

Measurement of Brain Glutathione with Magnetic Resonance Spectroscopy in Schizophrenia-Spectrum Disorders

A Systematic Review and Meta-Analysis

Alex J. Murray[1,2], Clara Humpston[1,3], Martin Wilson[2], Jack C. Rogers[1,2], Mohammad Zia Ul Haq Katshu[4,5], Peter F. Liddle[4] and Rachel Upthegrove[1,2,6]

[1]Institute for Mental Health, University of Birmingham, Birmingham, United Kingdom

[2]Centre for Human Brain Health and School of Psychology, University of Birmingham, Birmingham, United Kingdom

[3]Department of Psychology, University of York, York, United Kingdom

[4] Institute of Mental Health, Division of Mental Health and Clinical Neurosciences University of Nottingham, Nottingham, United Kingdom

[5] Nottinghamshire Healthcare National Health Service Foundation Trust, Nottingham, United Kingdom

[6] Early Intervention Service, Birmingham Women's and Children's National Health Service Foundation Trust, Birmingham, United Kingdom

Abstract

Oxidative stress may contribute to declining course and poor outcomes in psychosis. However, in vivo Magnetic Resonance Spectroscopy studies yield disparate results due to clinical stage, sample demographics, neuroanatomical focus, sample size, and acquisition method variations. We investigated glutathione in brain regions from participants with psychosis, and the relation of glutathione to clinical features and spectroscopy protocols. Meta-analysis comprised 21 studies. Glutathione levels did not differ between total psychosis patients (N = 639) and controls (N = 704) in the Medial Prefrontal region (k = 21, d = -0.09, CI = -0.28 to 0.10, p = 0.37). Patients with stable schizophrenia exhibited a small but significant glutathione reduction compared to controls (k = 14, d = -0.20, CI = -0.40 to −0.00, p = 0.05). Meta-regression showed older studies had greater glutathione reductions, possibly reflecting greater accuracy related to spectroscopy advancements in more recent studies. No significant effects of methodological variables, such as voxel size or echo time were found. Reduced glutathione in patients with stable established schizophrenia may provide novel targets for precision medicine. Standardizing MRS acquisition methods in future studies may help address discrepancies in glutathione levels.

3.1 Introduction

Oxidative stress is defined as a build-up of damaging reactive oxygen species (ROS), and the inability of endogenous antioxidant defences to inactivate these species (Pizzino et al., 2017). When ROS accumulate to excess, they can cause damage to cellular components such as proteins, lipids, and nucleic acids (Sato et al., 2014). Neurons are particularly susceptible to the damaging effects of ROS due to the brain's large consumption of oxygen and reduced levels of protective antioxidant enzymes (Bošković et al., 2011). In patients with schizophrenia, studies have reported increased levels of ROS alongside a reduction in both peripheral and central antioxidants, such as glutathione (GSH) (Gunes et al., 2017, Wang et al., 2019, Kumar et al., 2020), while other reports have noted decreased levels of these antioxidants (Wood et al., 2009, Limongi et al., 2021). Possible causes of these disparate findings may be: substantial heterogeneity in patient profile, including stage of illness e.g. first episode psychosis compared to stable schizophrenia, or those with persistent chronic symptoms. Evidence suggests inflammation, relevant to oxidative stress, and/or glutamatergic function may differ by illness stage. (Murray et al., 2021, Upthegrove and Khandaker, 2019) An additional cause of contrasting findings includes substantial differences in acquisition methods and protocols adopted across studies (Wang et al., 2019, Rowland et al., 2016, Wijtenburg et al., 2017, Hafizi et al., 2018, Dempster et al., 2020).

Measurement of GSH in vivo is captured using magnetic resonance spectroscopy (MRS) acquisition. Due to its comparatively lower concentration relative to other metabolites such as glutamate or N-acetyl aspartate, reliable quantification of GSH presents additional technical challenges (Wilson et al., 2019). These include need for increased voxel size to improve the signal-to-noise ratio, more stringent requirements on magnetic field homogeneity and the use of metabolite specific acquisition methods to suppress unwanted overlapping

metabolite signals (Mikkelsen and Hearshen, 2008). Given the length of acquisition and participant burden, decisions must also be made prior to data acquisition about what regions to focus on (i.e. voxel placement) and what pulse sequence to use (Tal et al., 2012). These additional challenges have subsequently caused large variations in study design that could be contributing to the disparate findings in the field.

Previous reviews of oxidative stress in schizophrenia have limited the region of interest to the anterior cingulate cortex (ACC) (Das et al., 2019), or the scanner strength to 7-Tesla (Sydnor and Roalf, 2020). To date no systematic review has examined clinical stage and demographic characteristics, or the additional effects of methodological variability. To address this evidence gap, we systematically review the current evidence base and present a quantitative meta-analysis of existing MRS studies examining GSH in schizophrenia spectrum psychoses. By consolidating results across a number of studies and assessing how different illness stages, acquisition methodologies and confounds may affect results, this meta-analysis aims to give a definitive answer as to whether GSH is reduced in schizophrenia and provide insight into the methodological improvements that may improve consistency in ongoing research, increasing the potential for mechanistic and pharmacological interventions for schizophrenia in the future.

3.2 Methods

3.2.1 Article Search

The systematic review was registered with PROSPERO under ID 42021226634. Relevant articles were extracted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021) (see Fig. 1 and Supplementary materials). Systematic searches of the literature were performed in the

following databases: PubMed, PsycINFO, Web of Science, MEDLINE, and Embase up to July 23rd, 2023. The search terms used were as follows:

A.

Schizo* OR psychosis OR first episode schizo* OR first episode psychosis OR high-risk for schizo* OR high-risk for psychosis OR prodrome

AND

B.

Oxidative stress OR oxidative defen* OR antioxid* defen* OR Glutathione OR GSH.

AND

C.

Magnetic resonance spectroscopy OR 1H-MRS OR MRS.

Additionally, a manual search was conducted within the reference lists of review articles and full-text articles that met the eligibility criteria for this analysis. A supplementary search using Google Scholar was also performed to identify articles not indexed in the aforementioned databases. The systematic search was carried out by AM, and both AM and CH independently assessed articles for inclusion and exclusion criteria.

3.2.2 Inclusion and exclusion Criteria

Articles written in English, either full-length or short, were included in the review if they met the following criteria: (1) The study was conducted in a cohort of individuals diagnosed with schizophrenia, first-episode psychosis, schizoaffective disorder, or clinical high-risk for psychosis; (2) The study included a healthy control comparison group; (3)

Glutathione levels were measured using 1H-MRS; (4) Sufficient data were provided or could be obtained to calculate standardized mean differences between the groups. Studies reporting glutathione levels measured with chemical shift imaging or 13C-MRS were excluded, as well as studies where the patient sample completely overlapped. Studies that did not meet the criteria for the meta-analysis but fulfilled the original search terms were still included in the narrative synthesis.

The quality assessment of individual studies was conducted using a modified version of the checklist introduced by Das et al. (2019). This checklist was employed to evaluate both the methodology utilized in magnetic resonance spectroscopy (MRS) acquisition and analysis, as well as the overall quality of study demographics and reporting. The modifications to the checklist were based on the "Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations" proposed by Lin et al. (2021).

By employing this assessment, each study was rated on a scale from 0 to 18, providing an indication of the potential reliability of the reported results. Supplementary Table 1 provides the details of the modified checklist used for the quality evaluation of the individual studies. If a study scored below 75 % on the data quality measures, then it was excluded from the analysis. Further analysis was conducted to assess if study quality could explain any of the variance seen in the primary meta-analysis.

3.2.3 Data Extraction

Studies included in this review involved in vivo measurement of GSH using 1H-MRS in individuals with a clinical diagnosis of a schizophrenia spectrum disorder (including first episode and/or stable cases) or individuals at clinical high-risk for psychosis. Data extracted

from each study included mean and standard deviation of GSH levels for both the patient and control groups, along with various demographic and methodological variables (see Table 1). In instances where group-specific mean and standard deviation values were not reported, authors were contacted twice over the course of one month. Risk of bias was assessed by AM using Egger's test and leave-one-out analysis was used to assess study weighting.

3.2.4 Analysis

The meta-analysis was performed using the metafor package (version 4.2) (Viechtbauer, 2010) in R (version 4.3.0) (R Core Team, 2023). GSH concentration differences between psychosis patients and healthy controls were standardized using Hedge's g effect sizes. Hedge's g accounts for potential bias in small sample sizes, providing a more robust and reliable estimation of the treatment effect. It is calculated as the difference between the two raw mean scores divided by the pooled standard deviation, adjusted by sample size.

Initially, separate meta-analyses were conducted to assess GSH levels in specific brain regions. However, for further subgroup and meta-regression analysis, studies where the voxel was placed in the medial-prefrontal cortex (mPFC), or ACC were combined into a single group. These regions were selected due to their substantial overlap and the large number of studies reporting GSH levels in these areas.

Subgroup analyses were performed based on the phase of illness, including stable schizophrenia (symptoms present for more than 2 years, stably medicated), first episode psychosis (within 2 years of symptom onset, minimally medicated), and clinical high-risk. Additionally, further groupings were based on magnetic field strength and MRS pulse sequence. In the subgroup analysis, Cramer-Rao lower bounds (CRLB) were also assessed,

using a cut-off of < 20 %. However, meta-regressions could not be conducted on CRLB data due to the limited number of studies reporting raw CRLB statistics.

For the meta-regressions, various factors were assessed to determine their effect on the results. These factors included age, sex, medication status, sample size, symptom severity, echo time, and voxel size. To ensure standardized scores across different symptom measures, the percentage of maximum possible symptom scores (POMP) was calculated. This standardization method allows for comparisons across scoring methods, overcoming the issues associated with alternative standardization approaches (e.g., z-scores) that do not facilitate comparisons of scores across studies and samples. The use of POMP ensures that symptom scores are in a standardized format for analysis (Cohen et al., 1999).

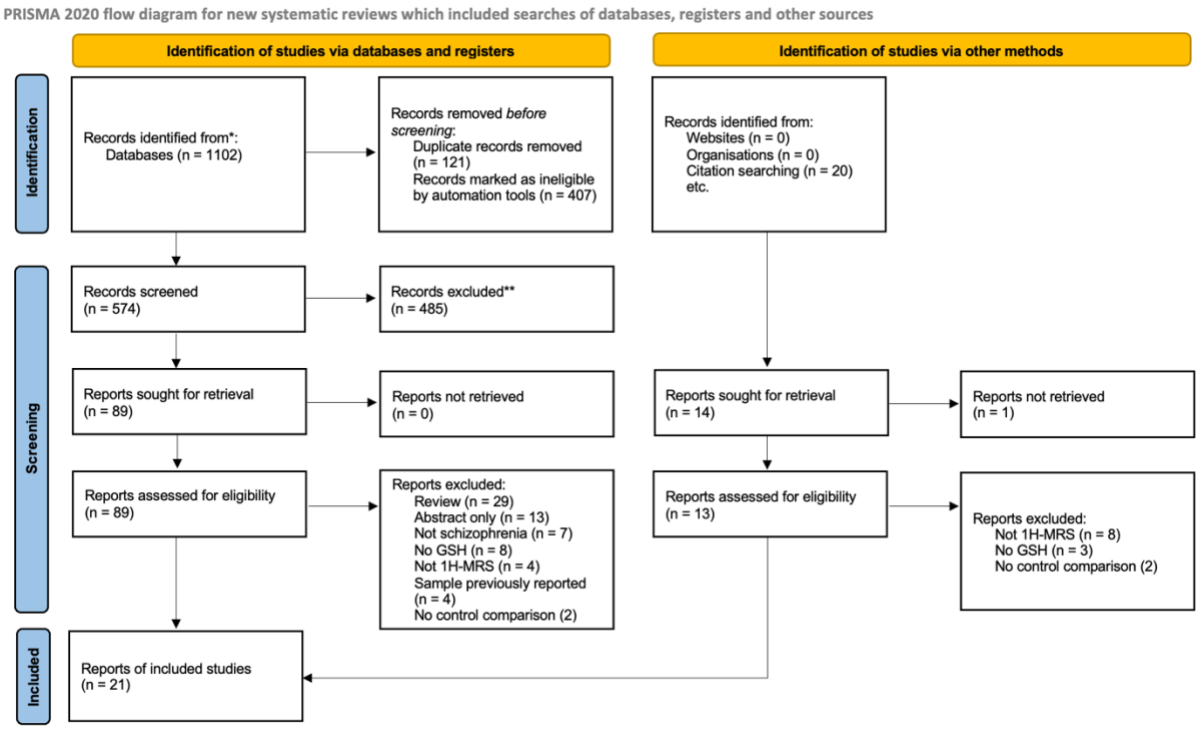
3.3 Results

3.3.1 Search Results

We identified 21 case-control studies with a healthy control (HC) comparison group, providing group-specific mean and standard deviation values for meta-analysis (Wang et al., 2019, Kumar et al., 2020, Rowland et al., 2016, Wijtenburg et al., 2017, Hafizi et al., 2018, Brandt et al., 2016, Coughlin et al., 2020, Do et al., 2000, Girgis et al., 2019, Iwata et al., 2021, Lesh et al., 2019, Matsuzawa et al., 2008, Ravanfar et al., 2022, Rowland et al., 2016, Taylor et al., 2017, Terpstra et al., 2005, Godlewska et al., 2021, MacKinley et al., 2022, Reid et al., 2019, Xin et al., 2016, Da Silva et al., 2018). Additionally, one study (Wijtenburg et al., 2017) presented comparisons across two patient-control groups – old and young schizophrenia, with age-matched controls. In this case, both comparisons were included as separate data points since there was no overlap between the groups. All studies were published between December 2001 and October 2022. Two additional studies (Reyes-

Madrigal et al., 2019, Wood et al., 2009) met the inclusion criteria; however, these studies used unique voxel locations (caudate and temporal lobe, respectively), preventing meta-comparisons.

Figure 3.1: PRISMA Flow Diagram Highlighting the Literature Search Process



Abbreviations: 1H-MRS, Magnetic Resonance Spectroscopy; GSH, Glutathione.

This meta-analysis included data from 672 psychosis patients and 641 healthy controls. The psychosis group's age ranged from 19.4 to 49.5 years (M = 30.18, SD = 8.27), comprising 70.43 % male participants, with 60.72 % medicated with anti-psychotics. Among the studies, 14 involved participants with stable schizophrenia/schizoaffective disorder (i.e. stably medicated with an illness duration > 2.5 years) (Kumar et al., 2020, Rowland et al., 2016, Wijtenburg et al., 2017, Brandt et al., 2016, Coughlin et al., 2020, Do et al., 2000, Girgis et al., 2019, Iwata et al., 2021, Lesh et al., 2019, Matsuzawa et al., 2008, Ravanfar et

al., 2022, Rowland et al., 2016, Taylor et al., 2017, Terpstra et al., 2005), while 5 studies (224 SZ, 227 HC) were conducted in individuals experiencing first-episode psychosis (i.e., within 2 years of symptom onset) (Wang et al., 2019, Godlewska et al., 2021, MacKinley et al., 2022, Reid et al., 2019, Xin et al., 2016). Only 2 studies included a clinical high-risk sample (Hafizi et al., 2018, Da Silva et al., 2018). Five studies reported concentrations across multiple voxels (Wang et al., 2019, Kumar et al., 2020, Lesh et al., 2019, Taylor et al., 2017, Godlewska et al., 2021), and one study had two eligible contrasts (Wijtenburg et al., 2017), resulting in a total of 28 datasets. For the initial analysis, studies were separated by voxel location (ACC, mPFC, DLPFC, etc.). Subsequent analysis focused solely on a combined grouping of the ACC and mPFC voxels, termed “medial frontal” (Merritt et al., 2021) to avoid including assessments of the same participant cohorts multiple times.

Table 3.1: Characteristics of Studies Included in this Review.

Author	Sample (PSY/HC)	PSY Type	% on Antipsychotics	Mean Age (PSY)	Mean Age (HC)	PSY % Male	HC % Male	ROIs	Field Strength (t)	MRS Sequence	Echo Time (ms)	Quality Score
Brandt et al., 2016	48 (24/24)	SZ	100.00	37.50	36.60	83.33	79.17	ACC	7	STEAM	28.00	19
Coughlin et al., 2020	26 (16/10)	SZ	86.96	34.20	32.10	73.91	68.00	ACC	3	MEGA-PRESS	35.00	19
Da Silva et al., 2018	56 (30/26)	CHR	13.33	20.30	22.80	50.00	38.46	mPFC	3	MEGA-PRESS	68.00	18
Do et al., 2001	28 (14/14)	SZ	35.71	32.20	35.60	100.00	100.00	mPFC	1.5	PRESS	75.00	16
Girgis et al., 2019	39 (19/20)	SZ	0.00	38.20	37.60	73.68	70.00	ACC	3	PRESS	68.00	19
Godlewska et al., 2021	34 (16/18)	FEP	88.23	25.69	27.10	100.00	100.00	ACC DLPFC Put	7	STEAM	11.00	18
Hafizi et al., 2018	28 (27/21)	CHR	0.00	20.30	22.86	51.85	47.62	mPFC	3	MEGA-PRESS	68.00	18
Iwata et al., 2021	92 (67/25)	SZ	100.00	43.71	40.80	75.13	73.08	ACC	3	MEGA-PRESS	68.00	18
Kumar et al., 2020	72 (27/45)	SZ	73.68	27.18	27.89	71.43	64.44	ACC Insula VC	7	STEAM	17.00	19
Lesh et al., 2019	70 (33/37)	FEP	N/A	21.40	21.90	69.44	67.50	DLPFC VC	3	MEGA-PRESS	131.00	19
MacKinley et al., 2023	87 (57/30)	FEP	52.60	22.75	21.57	84.21	63.33	ACC	7	Semi-LASER	70.00	18
Matsuzawa et al.,	36 (16/20)	SZ	100.00	30.70	30.00	60.00	75.00	mPFC	3	MEGA-	94.00	17

2008										PRESS		
Ravanfar et al., 2022	26 (12/14)	SZ	100.00	36.20	32.60	58.33	42.86	ACC	7	STEAM	6.00	20
Reid et al., 2019	42 (21/21)	FEP	95.24	23.20	23.50	76.19	76.19	ACC	7	STEAM	5.00	20
Rowland et al., 2016 (a)	56 (27/29)	SZ	81.48	34.40	29.70	62.96	48.28	ACC	7	STEAM	14.00	19
Rowland et al., 2016 (b)	98 (45/53)	SZ	91.10	37.70	37.10	64.40	60.38	ACC	3	STEAM	6.50	19
Taylor et al., 2017	34 (16/18)	SZ	87.50	22.70	23.90	81.25	61.11	ACC Thal	7	STEAM	10.00	19
Terpstra et al., 2005	22 (13/9)	SZ	100.00	26.00	25.00	61.53	44.44	ACC	4	STEAM	5.00	15
Wang et al., 2019	162 (74/88)	FEP	N/A	22.30	23.30	70.37	46.15	ACC CSO DLPFC OFR Thal	7	STEAM	14.00	19
Wijtenburg et al., 2016 (Old)	86 (47/39)	SZ	91.67	49.50	51.20	55.32	64.10	mPFC	3	STEAM	6.50	19
Wijtenburg et al., 2016 (Young)	99 (48/51)	SZ	95.74	25.20	25.20	70.83	46.30	mPFC	3	STEAM	6.50	19
Xin et al., 2016	58 (25/33)	FEP	80.00	24.80	25.40	72.00	54.55	mPFC	3	SPECIAL	6.00	19

PSY Type abbreviations: SZ – schizophrenia, CHR – clinical high risk, FEP – first episode psychosis.

ROIs abbreviations: ROI – region of interest, ACC – anterior cingulate cortex, mPFC – medial prefrontal cortex, VC – visual cortex, DLPFC – dorsolateral prefrontal cortex, Thal – thalamus, CSO – centrum semiovale, OFR – orbitofrontal region, Put - putamen.

MRS Sequence abbreviations: STEAM - stimulated echo acquisition mode, MEGA-PRESS - Mescher-Garwood point resolved spectroscopy, semi-LASER - semi-localized by adiabatic selective refocusing, SPECIAL - spin-echo full-intensity acquired localized spectroscopy.

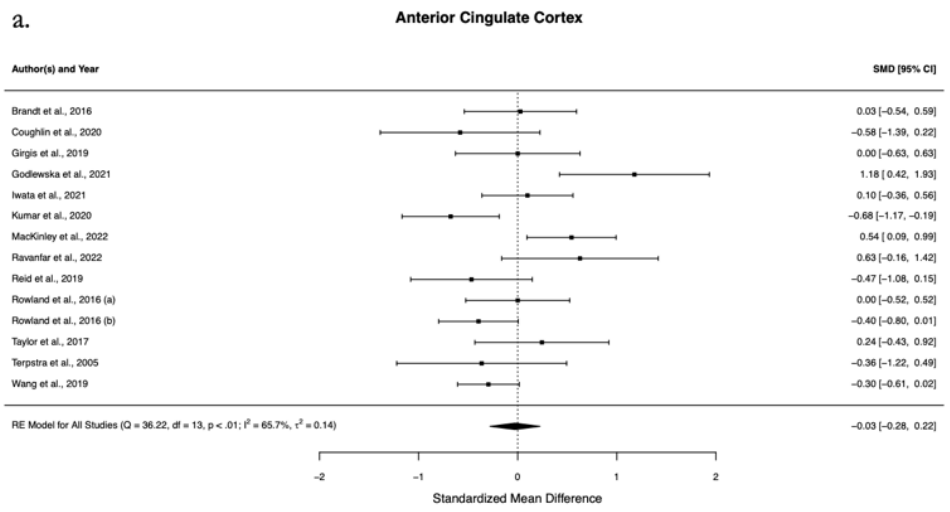
3.3.2 Meta-Analyses

14 studies positioned the voxel in the ACC, 6 in the mPFC, 3 in the DLPFC, 2 in the thalamus, and 2 in the VC. The application of random effects analysis revealed no significant difference in GSH levels between patients with psychosis and healthy controls in any of these investigated brain regions (see Fig. 2).

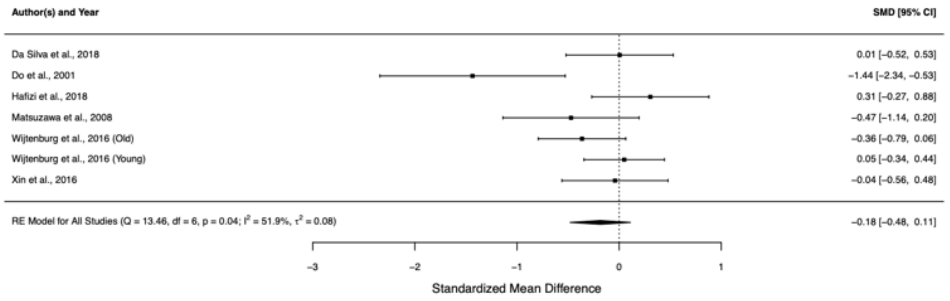
Voxel placement in either the mPFC or ACC was observed in 20 out of 21 studies, encompassing a total of 639 patients and 704 controls. These studies were combined into a group labelled “medial frontal” voxels (Merritt et al., 2021). Once more, the analysis demonstrated no significant difference in GSH levels between patients and controls within this grouping ($k = 21$, $d = -0.09$, CI = -0.28 to 0.10, $p = 0.37$).

Figure 3.2: Forest Plot of Standard Mean Difference in GSH Between PSY and HC Groups in Different Voxel Locations.

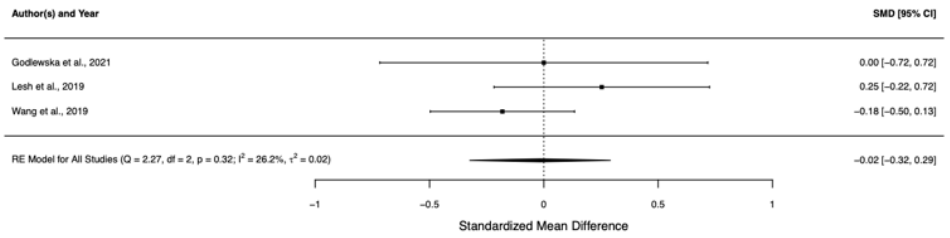
a.


Anterior Cingulate Cortex
Author(s) and Year
SMD [95% CI]
Brandt et al., 2016
0.03 [−0.54, 0.59]
Coughlin et al., 2020
−0.58 [−1.39, 0.22]
Girgis et al., 2019
0.00 [−0.63, 0.63]
Godlewska et al., 2021
1.18 [0.42, 1.93]
Iwata et al., 2021
0.10 [−0.36, 0.56]
Kumar et al., 2020
−0.68 [−1.17, −0.19]
MacKinley et al., 2022
0.54 [0.09, 0.99]
Ravanfar et al., 2022
0.63 [−0.16, 1.42]
Reid et al., 2019
−0.47 [−1.08, 0.15]
Rowland et al., 2016 (a)
0.00 [−0.52, 0.52]
Rowland et al., 2016 (b)
−0.40 [−0.80, 0.01]
Taylor et al., 2017
0.24 [−0.43, 0.92]
Terpstra et al., 2005
−0.36 [−1.22, 0.49]
Wang et al., 2019
−0.30 [−0.61, 0.02]
RE Model for All Studies (Q = 36.22, df = 13, p < .01; I² = 65.7%, τ² = 0.14)
−0.03 [−0.28, 0.22]
−2
−1
0
1
2
Standardized Mean Difference

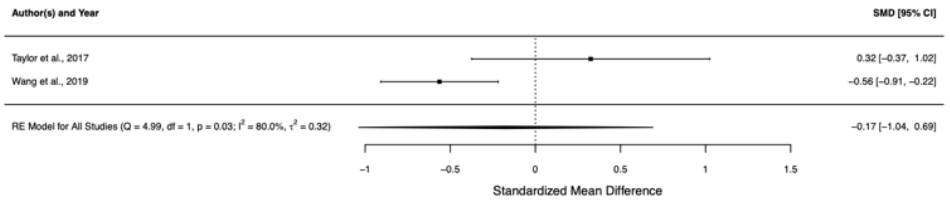
b.


Medial Prefrontal Cortex
Author(s) and Year
SMD [95% CI]
Da Silva et al., 2018
0.01 [−0.52, 0.53]
Do et al., 2001
−1.44 [−2.34, −0.53]
Hafizi et al., 2018
0.31 [−0.27, 0.88]
Matsuzawa et al., 2008
−0.47 [−1.14, 0.20]
Wijtenburg et al., 2016 (Old)
−0.36 [−0.79, 0.06]
Wijtenburg et al., 2016 (Young)
0.05 [−0.34, 0.44]
Xin et al., 2016
−0.04 [−0.56, 0.48]
RE Model for All Studies (Q = 13.46, df = 6, p = 0.04; I² = 51.9%, τ² = 0.08)
−0.18 [−0.48, 0.11]
−3
−2
−1
0
1
Standardized Mean Difference

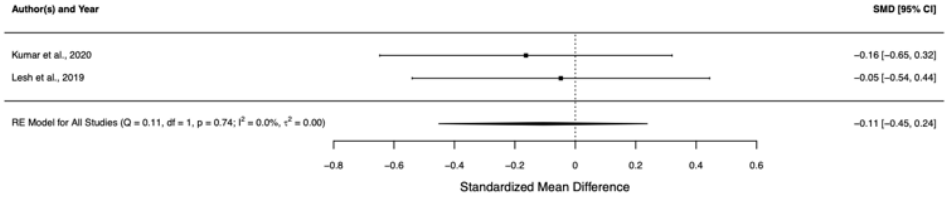
c.


Dorsolateral Prefrontal Cortex
Author(s) and Year
SMD [95% CI]
Godlewska et al., 2021
0.00 [−0.72, 0.72]
Lesh et al., 2019
0.25 [−0.22, 0.72]
Wang et al., 2019
−0.18 [−0.50, 0.13]
RE Model for All Studies (Q = 2.27, df = 2, p = 0.32; I² = 26.2%, τ² = 0.02)
−0.02 [−0.32, 0.29]
−1
−0.5
0
0.5
1
Standardized Mean Difference

d.


Thalamus
Author(s) and Year
SMD [95% CI]
Taylor et al., 2017
0.32 [−0.37, 1.02]
Wang et al., 2019
−0.56 [−0.91, −0.22]
RE Model for All Studies (Q = 4.99, df = 1, p = 0.03; I² = 80.0%, τ² = 0.32)
−0.17 [−1.04, 0.69]
−1
−0.5
0
0.5
1
1.5
Standardized Mean Difference

e.

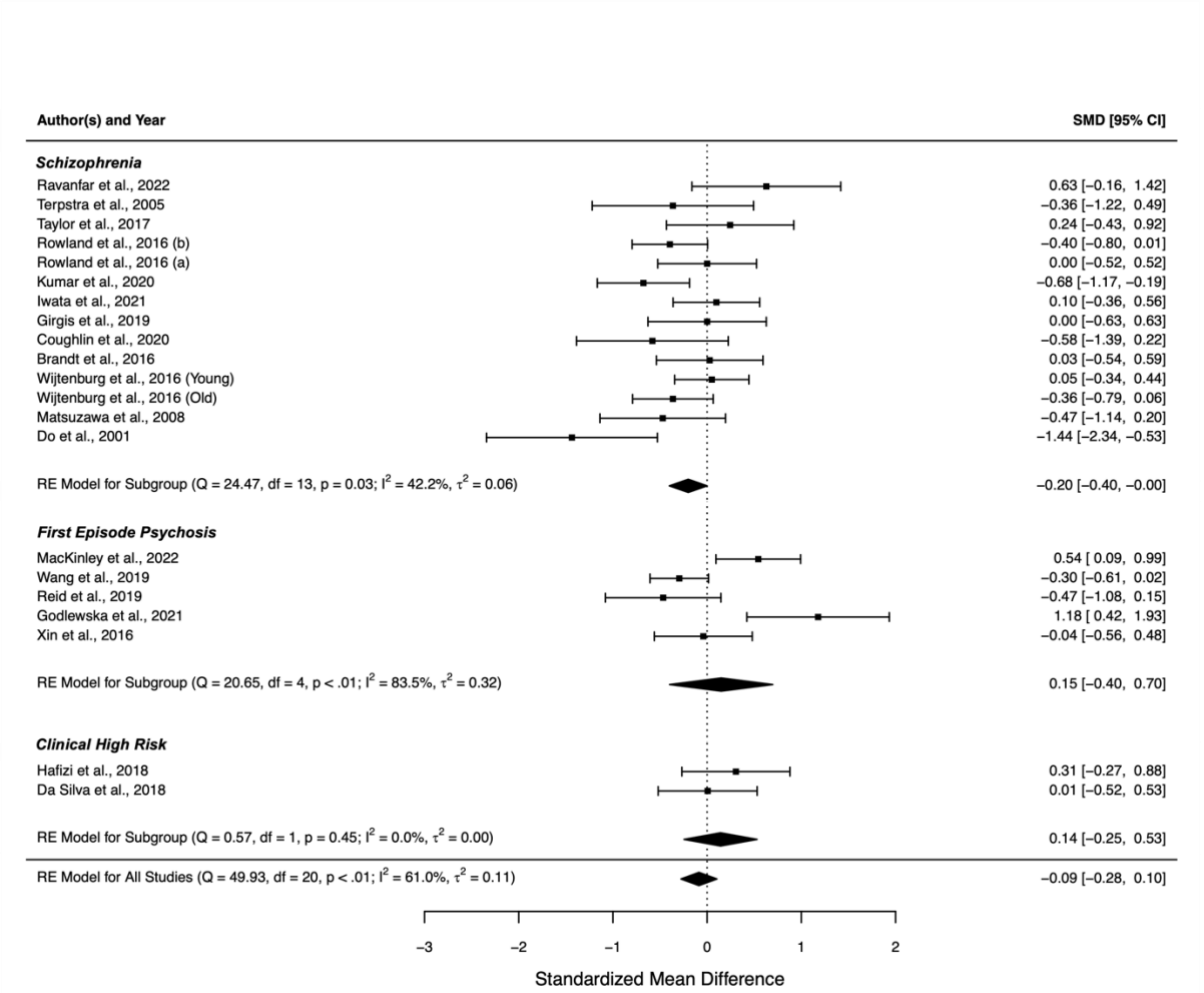

Visual Cortex
Author(s) and Year
SMD [95% CI]
Kumar et al., 2020
−0.16 [−0.65, 0.32]
Lesh et al., 2019
−0.05 [−0.54, 0.44]
RE Model for All Studies (Q = 0.11, df = 1, p = 0.74; I² = 0.0%, τ² = 0.00)
−0.11 [−0.45, 0.24]
−0.8
−0.6
−0.4
−0.2
0
0.2
0.4
0.6
Standardized Mean Difference

3.3.3 Subgroup Analysis

Subgroup analysis showed a significant reduction in GSH levels among individuals with stable schizophrenia (constituting 58 % of the sample) (k = 14, d = -0.20, CI = -0.40 to −0.00, p = 0.05). However, this was not replicated in the FEP subgroup (34 % of the sample) (k = 5, d = 0.15, CI = -0.40 to 0.70, p = 0.59) or the clinical high-risk subgroup (8 % of the sample) (k = 2, d = 0.14, CI = -0.25 to 0.53, p = 0.47). Heterogeneity was notable in both the FEP and stable schizophrenia subgroups (Tau^2 = 0.32, X^2 = 20.65, df = 4, p < 0.001, I2 = 84 %; Tau^2 = 0.06, X^2 = 24.47, df = 13, p = 0.03, I2 = 42 % respectively). Fig. 3.

Furthermore, no significant alterations in GSH levels were observed in patients compared to controls when studies were stratified by magnetic field strength: 3 T (k = 10, d = -0.12, CI = -0.29 to 0.05, p = 0.39) or 7 T (k = 9, d = 0.09, CI = -0.28 to 0.45, p = 0.64). Additionally, when grouped by pulse sequence, no specific sequence yielded significant results. Notably, only one study reported utilizing the semi-LASER sequence, and within this study, GSH levels were found to be significantly higher in FEP compared to HC. Studies who report a CRLB cutoff of < 20 % also demonstrated no difference in GSH levels (k = 16, d = -0.05, CI = -0.27 to 0.16, p = 0.63).

Figure 3.3: Forest Plot of Standard Mean Difference in GSH Between PSY and HC Grouped by Illness Phase

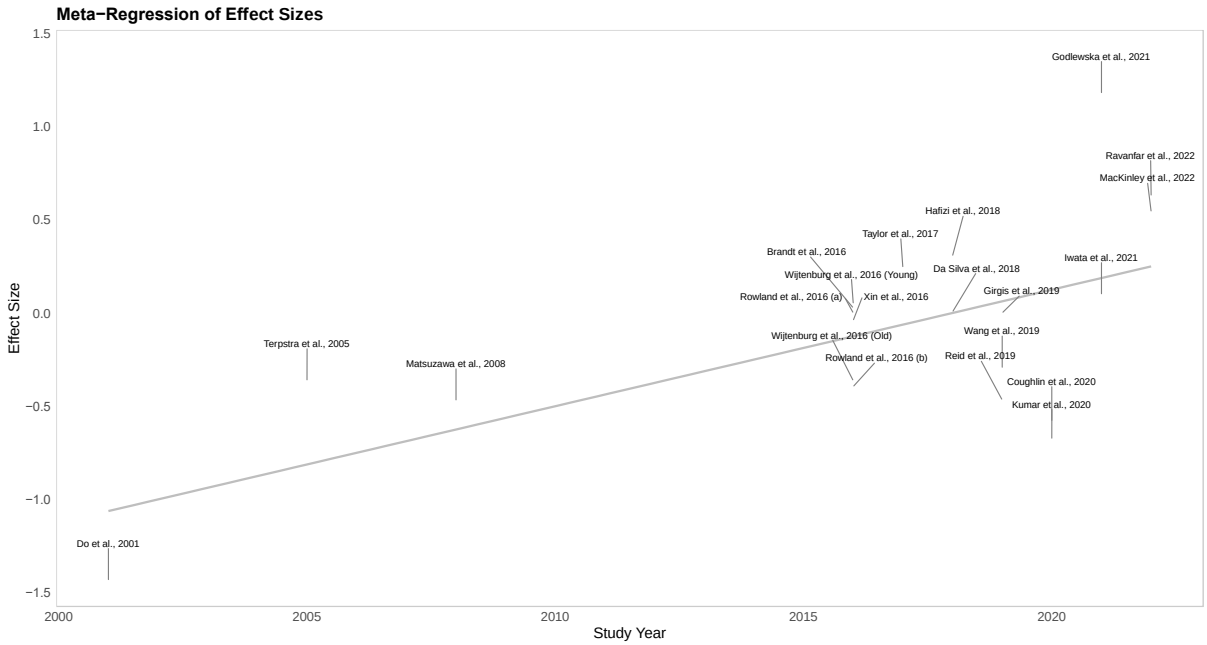


3.3.4 Meta-Regression Analysis

No statistically significant moderator effects were found across the whole PSY group for patient age (Z = -0.86p = 0.39), proportion of medicated patients (Z = -0.78, p = 0.44) or patient gender (Z = 0.50, p = 0.62). Furthermore, no significant effects of methodological moderators were found such as echo time (Z = -0.02, p = 0.98), voxel size (Z = -0.73, p = 0.47), and sample size (Z = -1.29, p = 0.20), or study quality score (Z = -0.01, p = 0.94). Regression analyses did not find a significant association of GSH with negative symptoms (Z = -0.55, p = 0.58) or positive symptoms (Z = -0.59, p = 0.56). These results persisted when looking at either the FEP or the stable schizophrenia subgroups.

There was, however, a significant association with study year – with older studies demonstrating a greater GSH reduction than their more recent counterparts (Z = 3.04, p < 0.01). This was also demonstrated in the stable schizophrenia subgroup (Z = 2.29, p = 0.02), but not the FEP subgroup (Z = 1.32, p = 0.19). Fig. 4.

Figure 3.4: Meta-Regression of Year of Study Effect on Effect Sizes

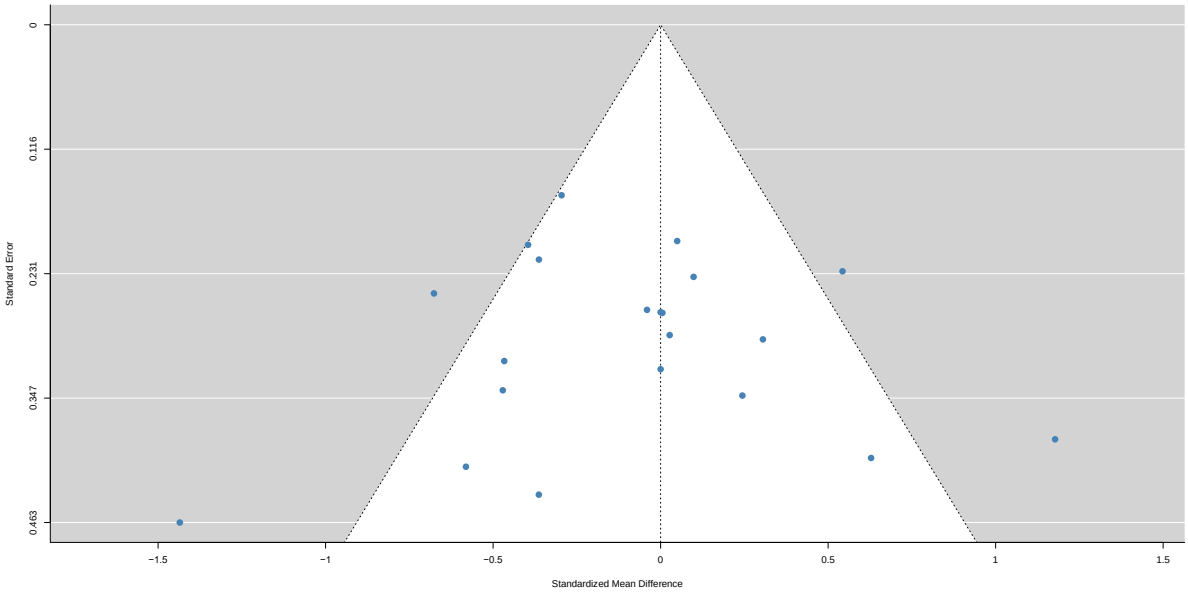


3.3.5 Risk of Bias

Inspection of funnel plots indicated no clear evidence of publication bias in the "medial frontal" group (Fig. 5). Egger's test was not significant (T = 0.33, p = 0.74) indicating that results were likely representative of the field. This finding was replicated in both the FEP and stable schizophrenia subgroups (T = 1.12, p = 0.34; T = -0.44, p = 0.66 respectively). All iterations of leave one out (LOO) analysis, resulted in consistent findings.

LOO sensitivity analysis within the stable schizophrenia grouping showed that the removal of any one of seven separate studies (Kumar et al., 2020, Wijtenburg et al., 2017, Coughlin et al., 2020, Do et al., 2000, Matsuzawa et al., 2008, Rowland et al., 2016, Terpstra et al., 2005) would result in a non-significant finding, with Rosenberg's failsafe N indicating 11 non-significant studies would need to be included to give an overall non-significant finding (see supplement).

Figure 3.5: Funnel Plot of All "Medial Frontal" Studies



3.4 Discussion

The current study represents the largest meta-analysis of in vivo central GSH levels within patients with psychosis to date. Our findings reveal a significant reduction in GSH among patients with stable schizophrenia but not in patients with broader defined psychosis, those with first episode or clinical high-risk, when compared to healthy controls. In the meta-regression analysis, we found that symptom severity, demographic factors, and MRS methods did not significantly influence the effect sizes. However, a significant association between the year of study and the extent of GSH reductions within the psychosis group was found.

Specifically, older studies demonstrated greater reductions in GSH compared to more recent studies. This effect was also evident in the stable schizophrenia subgroup, suggesting that it is not solely due to an increase in FEP-focused studies over time. Assessment of publication bias indicated that the data included in this analysis are likely representative of the field.

Our findings of no significant reduction in GSH across all clinical stages of psychosis is consistent with a number of studies (Wijtenburg et al., 2017, Coughlin et al., 2020, Matsuzawa et al., 2008). However, these findings run counter to two recent meta-analyses of schizophrenia which demonstrate significant reductions in GSH in the ACC region and in studies at 7 T respectively (Das et al., 2019, Sydnor and Roalf, 2020). Evidence for a reduction of GSH converges from a variety of study designs, including post-mortem, genetic, animal and clinical trials (Gawryluk et al., 2011, Michels et al., 2018, Steullet et al., 2017, Hashimoto, 2019). Our differing results could also be due to the inclusion of MRS studies across all clinical stages of psychosis, reflecting an increase in heterogeneity. Inconsistency of 1H-MRS studies has been suggested to result from a lack of a "gold standard" acquisition method (Fisher et al., 2020); however, we note that no significant methodological variables had a moderating effect. Inconsistency of results may be indicative of a specific subgroup of patients who have reduction in GSH; those with or on a path to stable schizophrenia.

It has previously been suggested that this subgroup may represent up to one third of patients and is characterised by very low levels of polyunsaturated fatty acids within red blood cells during the acute phase of illness, signifying persistent redox dysregulation (Bentsen et al., 2011, Solberg et al., 2019). Furthermore, those with a specific glutamate cysteine ligase catalytic (GCLC) subunit polymorphism may have lower levels of GSH in the brain, thus suggesting there may be a genetic component to this subgroup (Xin et al., 2016). It may be that absence of GSH reductions in the early stages of the disorder is indicative of the substantial heterogeneity within the FEP population, indeed we found that heterogeneity was

much higher in the FEP cohort than the stable cohort. First episode patients who present with significant GSH reductions may be predisposed to progress to more persistent illnesses.

To our knowledge this is the first meta-analysis to demonstrate that GSH reductions may be limited to patients with stable schizophrenia regardless of other clinical and demographic variables. It has been proposed that the abnormalities in GSH levels may arise due to damage caused by glutamatergic hyperactivity in the early phase of the disorder (Kumar et al., 2020). As such in first episode patients GSH levels may be similar to those seen in healthy controls or slightly increased to compensate for the excess ROS generated by the ongoing damage (Limongi et al., 2021). Furthermore, an increase in GSH in the early phase of illness is associated with more favourable long-term outcomes (Wijtenburg et al., 2017), and thus a greater number of first episode patients who may be part of the oxidative stress vulnerable subgroup would progress to an established phase of the disorder.

This finding could be influenced by the inclusion of older studies. We demonstrate a significant interaction between the year of study and differences in GSH levels between patients and controls. The three studies conducted before 2008 (Do et al., 2000, Matsuzawa et al., 2008, Terpstra et al., 2005) had notably lower GSH levels in stable schizophrenia compared to healthy controls. The removal of any one of these three studies led to the overall finding falling below the significance threshold. This relationship might be attributed to advancements in MRS methodologies, more stringent reporting criteria, and technological improvements in brain imaging. Older studies likely employed less sophisticated techniques, resulting in reduced accuracy and greater variation in reported GSH findings. Moreover, researchers have become more diligent in ensuring consistency and accuracy of data collection and analysis, with a focus on improving data quality (Lin et al., 2021). While we found no association with methodologies, other unmeasured or unaccounted factors could also be contributing to this observation.

The present study has several strengths including a large sample, the largest to-date to our knowledge, with little influence of publication bias. However, some limitations need to be considered. Firstly, the data did not allow for control of additional confounding factors such smoking, BMI and food intake. Studies have demonstrated that these can influence GSH levels (Manna and Jain, 2015, Young et al., 2007; Zhang et al., 2007).

Secondly, the combined group of mPFC and ACC may have been too diverse. It has been noted that glutamatergic alterations may be more apparent in the rostral area of the ACC compared to the dorsal area (Jeon et al., 2021). A large medial frontal grouping of voxels would be unable to detect these small changes in metabolite concentrations across neighbouring regions.

Thirdly, it has been noted that both schizophrenia and antipsychotics can affect the relaxation rates of metabolites (Bracken et al., 2013), therefore it is likely that MRS acquisition methods and medication status will affect the ability to detect changes in concentration between PSY and HC, however we found no confounding influence of medication exposure in our analysis. Whilst we included several acquisition variables in our meta-regression, given the small number of studies within certain subgroups we suggest cautious interpretation of these analyses.

While several studies reported CRLB cutoffs below 20 %, the most widely used cutoff for GSH quantification (Kreis, 2015), only two studies provided raw CRLB scores (Ravanfar et al., 2022, Reid et al., 2019). The absence of raw CRLB scores from multiple studies limited our ability to comprehensively evaluate the impact of data quality on the quantification of GSH levels in our meta-analysis. Poor data quality could potentially lead to differential effects on the accurate determination of GSH concentrations, affecting the

reliability of the results across studies, as has been demonstrated for glutamate (Smucny et al., 2021) and NAA and choline (Yang et al., 2023).

Despite the significant advances in MRS acquisition and analysis in recent years, alongside a significant effort by the research community to unify reporting criteria (Lin et al., 2021), comparing across studies remains challenging as researchers have not settled on a best practice for metabolite quantification. For GSH quantification, a J-edited pulse sequence, with longer echo times (~130 ms) for higher editing efficiency (Nezhad et al., 2017) is recommended. While some authors have noted that this may not offer advantages over other common methods e.g. STEAM or PRESS (Ravanfar et al., 2022, Duffy et al., 2014), these methods may overestimate GSH levels, particularly in concentrations less than 3 mM (Wijtenburg et al., 2014), furthermore, these studies represent the "gold standard" of PRESS acquisition, reporting the use of short echo times and experienced MRS operators. This is not always reflective of the field as typically longer echo times are employed for PRESS acquisition as GSH is rarely the metabolite of interest. To combat the potential deleterious effects of the inclusion of these 3 T PRESS studies a sensitivity analysis was conducted with these studies removed. The results of which can be found in the Supplementary materials (Supplementary Figure S5).

Furthermore, methods of metabolite reporting frequently change from study to study, Hoch et al. (2017) suggested that reporting metabolite ratios e.g. GSH: creatine, offers multiple advantages compared to raw metabolite levels such as reduced sample size and an increase in statistical significance. MRS studies in schizophrenia will often report metabolite levels in relation to creatine, in these cases creatine is used as a reference point as it is assumed that creatine levels will not vary across subgroups. Some studies have demonstrated significant alterations in creatine levels in the DLPFC and ACC in schizophrenia (Wood et al., 2003, Öngür et al., 2009) however two separate meta-analyses found no significant

differences between schizophrenia and healthy controls (Yang et al., 2023; Kraguljac et al., 2012). Additionally, if the researcher is employing a J-edited pulse sequence, which is recommended, a second sequence will also be required to acquire spectra for the reference metabolite.

Although progress has been achieved in improving Magnetic Resonance Spectroscopy (MRS) techniques and establishing consistent reporting methods, the varying ways studies are conducted alongside challenges in accurately measuring metabolite levels highlight the intricate nature of understanding and contrasting results in MRS-related research. It is essential to carefully acknowledge these methodological intricacies to ensure the strength and accuracy of MRS studies when examining conditions such as schizophrenia.

This meta-analysis indicates that reduced GSH and oxidative stress may be specific to people with stable schizophrenia, however, this may have been influenced by unmeasured variation in methodology – as demonstrated by the significant interaction with study year. Future work should focus on patient stratification and examining how GSH levels may differ between illness phases. From our meta-analysis, the results appear to be unaffected by variations in MRS acquisition, however further consistency is still warranted to improve individual study reporting and future pooling of data.

3.5 Author Contributions

RU and JR conceived the initial research project. AM conducted the article search, extracted all relevant data, and performed all statistical analyses. AM also conducted research and drafted the article. Both AM and CH independently assessed articles for inclusion and exclusion criteria. All authors critically revised the article, offering support on content and structure.

Chapter 4

The following paper is currently under review at *Biological Psychiatry* 25/05/2024

Identification of Detailed Inflammatory Clusters in Patients with Schizophrenia

Alex J. Murray[1], Bill Deakin[2], Peter F Liddle[3], Paola Dazzan[4], Eileen Joyce[5], Nicholas M. Barnes[1], Carmine Pariante[4], Krish D. Singh[6], Gemma Williams[6,7], Catherine J. Gregory[2], Silke Conen[9], Richard Smallman[2], Mohanbabu Rathnaiah[3,9], Naghmeh Nikkheslat[4], Lena Palaniyappan[10,11], Loes Charlton[8], James T. R. Walters[12], John Suckling[13], Mohammad Zia Katshu[3,9], Rachel Upthegrove[1,14], Jack Rogers[1,15]

1. Institute for Mental Health, University of Birmingham, Birmingham, UK.
2. Division of Psychology and Mental Health, School of Biological Sciences, University of Manchester, Manchester, UK.
3. Institute of Mental Health, University of Nottingham, Nottingham, UK.
4. Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK.
5. Institute of Neurology, University College London, London, UK.
6. Cardiff University's Brain Research Imaging Centre (CUBRIC), School of Psychology, Cardiff University, Cardiff, UK.
7. Aneurin Bevan University Health Board, Newport, UK.
8. Division of Medical Education, School of Medical Science, University of Manchester, Manchester, UK.
9. Nottinghamshire Healthcare National Health Service Foundation Trust, Nottingham, UK.
10. Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montreal, Quebec, Canada.
11. Robarts Research Institute, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada.
12. Centre for Neuropsychiatric Genetics and Genomics, Division of Psychological Medicine and Clinical Neurosciences, School of Medicine, Cardiff University, Cardiff, UK.
13. Department of Psychiatry, University of Cambridge, Cambridge, UK.
14. Early Intervention Service, Birmingham Women's and Children's National Health Service Foundation Trust, University of Birmingham, Birmingham, UK.
15. Centre for Human Brain Health, University of Birmingham, Birmingham, UK.

Abstract:

Background:

Inflammation has been linked to reduced grey matter volume, impaired global cognitive function, and heightened symptom severity in individuals with schizophrenia. However, one or more immune-active groups may exist within schizophrenia that may differ in cognitive and brain structural patterns. Identification of these subgroups using readily available cytokine profiles could enable more targeted interventions linked to inflammatory response.

Methods:

We employed a semi-supervised machine learning clustering model, using a combined dataset from the Benefit of Minocycline Study (207 First episode patients) and The Study of Psychosis and the Role of Inflammation and GABA/Glutamate (58 first episode, 76 established psychosis and 73 controls). We aimed to establish illness-related clusters based on four cytokines of interest (IL-6, CRP, TNF-α, and IFN-γ). We explored differences in GMV, neurocognitive and clinical features among the clusters.

Results:

Our optimal clustering solution identified four distinct, schizophrenia-specific clusters, separable from controls: Increased CRP (N=29); IL-6 (N=52); TNF-α (N=39); and Non-inflamed (N=109), with an Adjusted Rand Index of 0.83 indicating a high degree of cluster stability. Each cluster exhibited unique patterns of GMV alterations, neurocognitive and clinical symptoms. GMV reductions, compared to HCs, were seen in the non-inflamed, IL-6 and CRP clusters, but not in the TNF-α cluster. The CRP and IL-6 clusters showed the most extensive reductions with 13 and 9 distinct voxel clusters identified respectively. When comparing across clusters on neurocognitive and clinical measures, the CRP subgroup demonstrated significantly worse scores on most cognitive scales, whereas the IL-6 subgroup scored highest on the anxio-depressive PANSS scales.

Conclusions:

We identified four schizophrenia clusters characterized by distinct immune signatures, associated with different GMV reductions and neurocognitive/clinical symptoms. These findings support the notion of multiple inflammatory signatures in schizophrenia, with proinflammatory IL-6 and CRP potentially signalling a more impaired cluster. This holds potential for the development for better targeting of immune-focused treatments for individuals with schizophrenia.

4.1 Introduction

It has long been suggested that immune dysfunction may play a significant role in the development and progression of schizophrenia. Studies have observed elevated levels of peripheral inflammatory cytokines, such as interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ), alongside increases in c-reactive protein (CRP), in individuals with schizophrenia, before the onset of illness and in first episode psychosis (FEP) (Upthegrove et al., 2014). Inflammatory cytokines may enter the brain via disruption of blood brain barrier function, among other mechanisms (Pape et al., 2019; Sun et al., 2022). This implies the relevance of elevated peripheral inflammatory cytokine levels to structural and functional abnormalities in the brain seen in schizophrenia (Frodl & Amico, 2014; Goldsmith et al., 2023).

In-vivo studies have shown increased levels of circulating IL-6, CRP, and IFN-γ are associated with reduced cortical thickness and grey matter volume in regions such as Broca's area, temporal gyrus, frontal cortex, and cingulate cortex (Kalmady et al., 2014; Fillman et al., 2016; Jacomb et al., 2018; Wu et al., 2019; Lizano et al., 2020; Williams et al., 2022). CRP may also affect blood-brain barrier permeability by disrupting the tight junctions through modification of the cytoskeletal structure (Ullah et al., 2021). Once CRP enters the central nervous system (CNS), it is thought to trigger neuroinflammation, which is further associated with cognitive deficits (Fourrier et al., 2019). Moreover, IL-6 may impact microglia activity and/or astrocytic function, leading to aberrant synaptic pruning and altered excitatory-inhibitory synaptic firing, thereby affecting brain structure, particularly reduced cortical thickness in the superior frontal region and reduced GMV in the frontal, middle and inferior cortex (Tu et al., 2017; Murray et al., 2021; Williams et al., 2022). On the other hand, the effects of TNF-α can be either neurodegenerative or neuroprotective, depending on receptor activation. TNF-α signalling occurs through two major receptors: TNF-α RI and

TNF-α RII. TNF-α RI activation has been associated with neurodegenerative effects, while TNF-α RII may have neuroprotective properties (Probert et al., 2015; Lin et al., 2021).

In addition to structural associations, peripheral levels of inflammatory cytokines may be related to aberrant cognitive functioning in schizophrenia. Secondary analysis of the Benefit of Minocycline (BeneMin) dataset revealed that patients with lower premorbid and current IQ had greater circulating concentrations of peripheral CRP and reductions in total intracranial brain volume compared to those with IQ within the normal range (Watson et al., 2022). Furthermore, patients with higher CRP levels performed significantly worse on cognitive tests assessing general intellectual ability, abstract reasoning, and working memory (Misiak et al., 2018). Additionally, increased IL-6 and its impact on neuronal/synaptic function have been associated with disruptions in learning, memory, and overall cognitive function (Penades et al., 2015; Fourrier et al., 2019). Conversely, participants with increased levels of TNF-α showed improvements in cognitive function, suggesting a potential increase in TNF-α and downstream neuroprotective effects (Misiak et al., 2018). Recent meta-analyses have cast doubt on the impact of inflammation on cognitive function, Morrens et al., 2022 found only weak associations between blood-based immune markers and cognitive performance within psychotic disorders. They cite high levels of publication bias, alongside substantial heterogeneity, as likely to inflate the observed associations. As such further research is required to elucidate if and how inflammation may be linked to cognitive function.

In recent years, it has been proposed that inflammation may only be relevant to a subset of schizophrenia patients, potentially accounting for up to 40% of the schizophrenia population (Fillman et al., 2014; Lizano et al., 2020). Based on the diverse range of cytokine evidence presented above, it is also possible that multiple subgroups exist, each with distinct inflammatory profiles. For example, in their recent paper, Lalousis et al., 2023 identified 5

separate inflammation-related clusters, including “low inflammation”, “elevated CRP”, “classic inflammation”, “elevated IFN-γ” and “anti-inflammatory”. These individual inflammatory clusters may exhibit differential responses to anti-inflammatory treatments (Girgis et al., 2018).

Most studies that have investigated an inflammatory cluster of schizophrenia to date have examined only one specific inflammatory marker or included a small sample size and only identified broad differences in inflammation e.g., low, moderate, and high. Furthermore, traditional clustering techniques employed in most studies e.g., two-step or hierarchical, may be affected by underlying variance in age, gender, thus producing artefactual clusters (Dinga et al., 2019). Novel, semi-supervised machine learning approaches can reduce said variance. A semi-supervised approach offers improved candidates for predictive modelling and facilitates the identification of patients with poor outcomes, allowing for more accurate prediction of immune-active candidates.

Identifying the existence of multiple inflammatory clusters in psychosis and understanding their relationship with differences in brain structure and clinical presentation, could enhance our knowledge of underlying mechanistic pathways and guide the targeting of stratified treatment trials that employ anti-inflammatory interventions e.g. tocilizumab or infliximab. In this study, we employed a semi-supervised machine learning approach to identify inflammatory clusters within a large and diverse sample of schizophrenia patients. Our hypothesis is that distinct clusters of patients, distinguishable from healthy controls, can be identified based on their inflammatory profiles, these clusters will align with the pro-inflammatory clusters identified in Lalousis et al. (2023). Furthermore, we predict that our identified clusters will exhibit divergent clinical, neurocognitive, and neuroanatomical characteristics. Specifically, we expect clusters with increased IL-6 and CRP levels to demonstrate the most significant neurocognitive deficits and functional impairments

associated with grey matter volume loss in the superior frontal regions and middle temporal gyri. Increased CRP levels in schizophrenia have been associated with poor performance on cognitive tests (Misiak et al., 2018) and IL-6 has been associated with disruptions in learning, memory, and overall cognitive function (Penades et al., 2015; Fourrier et al., 2019), disruptions of these cognitive processes have also been found in conjunction with reduced GMV in the superior frontal regions in schizophrenia (Fan et al., 2022; Alagapan et al., 2019; Constantinidis & Klingberg, 2016).

4.2 Methods

4.2.1 Sample

Data was combined for secondary analysis from two study populations: The Benefit of Minocycline Study (BeneMin) and The Study of Psychosis and the Role of Inflammation and GABA/Glutamate (SPRING). These studies were combined to increase the sample size while maintaining a diverse schizophrenia sample thus improving reliability and introducing a healthy control group necessary to run HYDRA clustering. These two studies were selected due to their similarities in study design, specifically the symptom and cognitive assessments used, MRI acquisition and acquisition methods of blood-based inflammatory markers. Methods for these studies have been reported elsewhere (Deakin et al., 2018; Gascoyne et al., 2021) but in brief; BeneMin recruited 207 participants, aged 16-35, across 6 sites (Manchester, Edinburgh, Cambridge, King's College London, University College London and Birmingham). All participants were within 5 years of symptom onset, and deemed to meet diagnostic criteria for first episode schizophrenia, schizophreniform, or schizoaffective

disorder; SPRING recruited 207 participants across three sites, 58 minimally medicated (no more than 12 weeks since the commencement of antipsychotic medication), first episode patients aged 18-41, within 5 years of symptom onset; 76 established (EST) psychosis patients aged 27-55, with symptoms present for greater than 10 years; and 73 age and sex matched healthy controls across two sites (Manchester and Cardiff).

Participants were excluded from this analysis if they did not have both blood-based inflammatory markers and a good quality (> 75% image quality rating) 3T T1-weighted structural MRI scan at baseline. Participants with CRP levels > 2 standard deviations from the mean (M=2.63) were also excluded as this may be indicative of an acute physical illness (e.g., infection) influencing immune function (Jacomb et al., 2018). Sample consisted of a total of N=267; 229 schizophrenia patients (combined 196 FEP and 33 EST) (64 Female) and 38 HC (8 Female) (see Table 1).

4.2.2 Clinical, Neurocognitive and Blood Based Biomarker Assessment

Participants from both studies were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Scores for 5 PANSS dimensions: the positive dimension, negative dimension, anxio-depressive dimension, excitement dimension and disorganisation/other dimension were all calculated via summing of relevant symptom scores (Yazaji et al., 2002). Current IQ was estimated using the Wechsler Adult Intelligence Scale III developed for patients with schizophrenia , which included the subtests Information, Arithmetic, Block Design and the Digit Symbol test of processing speed (Blyler et al, 2000). The Wechsler Test of Adult Reading (WTAR) was used to estimate premorbid IQ (Kreutzer et al., 2011).

For both BeneMin and SPRING study, blood samples were collected, and plasma supernatant were separated for the measurements of inflammatory markers including hs-CRP, IL-6, TNF-α, and IFN-γ at King's College London centre. Meso Scale Discovery (MSD) V-plex immunoassays (human) kits (MSD, Rockville, USA) (Nettis et al., 2023, Zajkowska et al., 2023) were used to measure inflammatory cytokines for both studies using Pro-inflammatory Panel 1. MSD Vascular Injury Panel 2 human kit were used to measure CRP levels for SPRING study and the Cormay anti-CRP antibody (PZ Cormay SA, Lomianki, Poland) for BeneMin study.

4.2.3 MRI Acquisition and Pre-processing

Structural magnetic resonance imaging (sMRI) was carried out with 3 Tesla GE or Siemens scanners using a standard acquisition protocol (see data supplement). Image pre-processing was carried out via the open-source CAT-12 toolbox (r1830; http://dbm.neuro.uni.jena.de/cat12/) following a standard voxel-based morphometry pipeline (see data supplement). Images were corrected for bias field, global intensity was normalized with images segmented into grey matter, white matter, and cerebrospinal fluid. Image quality rating was assessed and images with a quality rating of >75% were smoothed at 6mm FWHM for use in further VBM analysis.

4.2.4 Group Identification

A multivariate classification and clustering technique was employed to identify inflammatory clusters in the total sample using Heterogeneity Through Discriminative Analysis (HYDRA) (https://github.com/evarol/HYDRA; Varol et al., 2017; Lalousis et al., 2023). This fits a convex polytope classifier, formed by multiple linear max-margin

classifiers, between the patient and control groups to identify k-number of disease specific clusters.

Inflammatory marker data from all participants was used to classify patients from HCs and identify patient clusters based on disease-related heterogeneity. HYDRA was run with 0.25 c-regularisation, 1000 iterations and 20 consensus clustering steps with 2 to 8 clustering solutions set to obtain a range of possible solutions and the adjusted rand index (ARI) was computed using 10-fold cross-validation to assess cluster stability. The identified clusters were then compared post-hoc in terms of demographic information (age, gender, BMI etc.) before further cluster characterisation.

4.2.5 Inflammatory Cluster Characterization

Participants were stratified according to the inflammatory clusters to which they were assigned, and then compared on their respective inflammatory biomarker levels, neurocognitive performance, PANSS scores and demographic information using one-way ANOVA models (see Table 2). Due to the healthy control cohort coming exclusively from the SPRING study (2 sites) site correction was not possible, furthermore, neither dataset contained travelling head data as such, potential scanner differences across sites could not be addressed.

Examinations of voxel wise GMV differences between participants in different clusters were carried out using voxel-based morphometry within CAT-12 and statistical parametric toolbox 12 (SPM12) (http://dbm.neuro.uni.jena.de/cat12; Wellcome Trust Centre for Neuroimaging; https://www.fil.ion.ucl.ac.uk/spm/software/spm12) (see supplemental materials for analytical pipeline). Age, sex, and total intracranial volume were added as covariates within all models. Voxel level differences between participants assigned to

inflammatory clusters and healthy participants were thresholded at $p < 0.05$, using family-wise error (FWE) whole-brain corrected. We report clusters >10 contiguous suprathreshold voxels.

4.3 Results

Table 4.1: Demographic and Clinical Characteristics of the Sample

	SCZ (N=229)	**HC (N=38)**	**t/X²**	***p***
Demographics				
Age	27.98 (8.44)	35.04 (12.18)	-4.45	<.001***
Sex [1]	64 Female	8 Female	0.79	0.38
BMI	27.44 (6.03)	25.33 (4.19)	2.08	0.03*
Inflammatory Markers				
IFN-γ (pg/ml)	4.59 (5.36)	3.33 (2.24)	1.43	0.14
IL-6 (pg/ml)	0.70 (0.46)	0.50 (0.24)	2.44	<.001***
TNF-α (pg/ml)	2.56 (0.75)	2.29 (0.43)	2.09	0.03*
CRP (mg/L)	2.68 (2.93)	2.34 (3.28)	0.64	0.84
Cognitive Scores				
WAIS Digit Symbol	6.85 (2.53)	10.42 (3.26)	-7.65	0.01*
WAIS Arithmetic	8.36 (3.19)	10.00 (3.21)	-2.93	0.78
WAIS Information	10.12 (3.15)	11.92 (3.06)	-3.27	0.34
WAIS Block Design	9.62 (3.09)	11.47 (3.19)	-3.40	0.84
WAIS Sum of scales Scores	33.89 (9.36)	43.82 (9.84)	-6.01	0.96
WAIS Current IQ	91.79 (14.84)	105.09 (20.88)	-4.79	0.04*
WTAR Premorbid IQ	98.29 (18.11)	106.89 (15.03)	-2.77	0.01*

SCZ schizophrenia, *HC* healthy controls, *BMI* body mass index, *pg/ml* picograms per millilitre, *mg/L* milligrams per litre, *IFN-γ* interferon gamma, *IL-6* interleukin 6, *TNF-α* tumour necrosis factor alpha, *CRP* C-reactive protein, *PANSS* positive and negative syndrome scale, *WAIS* Wechsler Adult Intelligence Scale, *WTAR* Wechsler Test of Adult Reading. *t* t-test statistic, X^2 Chi-Square test statistic.

* p = <0.05, ** p = <0.01, *** p = <0.001

[1] Chi-square tests

4.3.1 Identification of Inflammatory Clusters

The optimal clustering solution in HYDRA resulted in 4 patient clusters that were separable from Healthy Controls (Cluster 1: CRP, N = 29; Cluster 2: IL-6, N = 52; Cluster 3: TNF- a, N = 39; Cluster 4: Non-Inflamed, N = 109; ARI = 0.83). One-way ANOVA revealed that clusters did not differ on age (p = 0.14), sex (p = 0.51) or proportion of first episode vs established patients (p = 0.62). Furthermore, study participants were equally distributed across each cluster (supplementary table 5). The clusters differed significantly on BMI (p <0.001), post-hoc analysis using Tukey's post-hoc test identified that cluster 4 scored significantly lower on the BMI scale than other clusters (Mean = 24.92, p <0.001) but no significant difference between the other three clusters was identified. Site effects were found to be present (p = 0.05). Clinical and inflammatory biomarker profiles of the clusters can be found in Table 2.

Table 4.2: Demographic and Clinical Characteristics of the Clusters

	CRP (N=29)	**IL-6 (N=52)**	**TNF-α (N=39)**	**Non-inflamed (N=109)**	**Healthy Control (N=38)**	**F/X²**	***p***
	Demographics						
Age	28.64 (7.80) [b]	29.59 (8.23) [b]	28.32 (8.43) [b]	26.92 (8.66) [b]	35.04 (12.18) [a]	5.80	<0.001***
Sex [1]	8 Female	18 Female	9 Female	29 Female	8 Female	2.53	0.64
BMI	29.00 (6.49) [a]	31.06 (7.23) [a]	28.25 (6.18) [ab]	25.00 (3.80) [b]	25.33 (4.19) [b]	13.71	<0.001***
	Cytokines						
IFN-γ (pg/ml)	3.34 (1.99) [b]	5.94 (5.46) [a]	6.86 (10.21) [a]	3.46 (2.11) [b]	3.33 (2.24) [b]	5.56	<0.001**
IL-6 (pg/ml)	0.70 (0.27) [b]	1.31 (0.46) [a]	0.77 (0.28) [b]	0.37 (0.14) [c]	0.50 (0.24) [c]	106.51	<0.001***
TNF-α (pg/ml)	2.57 (0.48) [b]	2.53 (0.57) [b]	3.57 (0.89) [a]	2.19 (0.44) [c]	2.29 (0.43) [b]	46.12	<0.001***
CRP (mg/L)	7.82 (3.10) [a]	3.53 (2.63) [bc]	2.25 (2.03) [cd]	1.06 (0.88) [e]	2.34 (3.28) [d]	57.22	<0.001***
	Clinical Symptoms						
PANSS Total Positive	15.83 (5.64)	15.94 (4.63)	15.77 (5.66)	15.97 (5.42)	-	0.02	1.00
PANSS Total Negative	16.97 (6.28)	16.02 (5.83)	16.77 (6.11)	15.13 (6.11)	-	1.15	0.33
PANSS Total General	32.79 (9.23)	32.50 (7.59)	31.85 (8.13)	30.71 (7.55)	-	0.92	0.43
PANSS Total	65.59 (19.62)	64.46 (13.58)	64.38 (16.30)	61.89 (15.00)	-	0.66	0.58

Negative Dimension	22.03 (8.69)	20.94 (7.36)	22.03 (7.71)	19.83 (7.51)	-	1.17	0.32
Positive Dimension	13.24 (5.03)	13.02 (4.47)	12.97 (4.24)	13.18 (4.87)	-	0.03	0.99
Anxiodepressive Dimension	9.93 (3.99) [b]	10.37 (3.42) [ab]	8.74 (3.93) [b]	8.52 (3.52) [bc]	-	3.63	0.01*
Excitement Dimension	4.34 (1.54)	4.75 (2.00)	4.69 (2.07)	4.97 (2.12)	-	0.80	0.49
Disorganisation Dimension	10.52 (3.97)	10.56 (3.51)	10.87 (3.64)	10.61 (3.41)	-	0.08	0.97
	Cognitive Tests						
WAIS Digit Symbol	5.52 (1.85) [c]	6.82 (2.56) [bc]	6.83 (2.05) [bc]	7.23 (2.73) [b]	10.42 (3.26) [a]	17.66	<0.001***
WAIS Arithmetic	7.34 (3.53) [bc]	8.27 (3.16) [b]	8.57 (3.28) [b]	8.61 (3.06) [b]	10.00 (3.21) [ab]	3.11	0.02*
WAIS Information	9.14 (2.93) [b]	10.35 (3.13) [b]	10.08 (3.36) [b]	10.28 (3.13) [b]	11.92 (3.06) [a]	3.54	0.01*
WAIS Block Design	8.67 (2.79) [b]	9.55 (3.18) [b]	9.81 (3.40) [b]	9.82 (3.01) [b]	11.47 (3.19) [a]	3.75	0.01*
WAIS Sum of Scaled Scores	29.38 (8.92) [c]	33.69 (9.96) [bc]	33.31 (10.30) [bc]	35.39 (8.50) [b]	43.82 (9.84) [a]	11.73	<0.001***
WAIS Current IQ	84.14 (13.28) [c]	91.80 (15.04) [bc]	93.21 (16.29) [bc]	93.32 (14.17) [b]	105.09 (20.88) [a]	7.93	<0.001***
WTAR Premorbid IQ	87.28 (22.20) [b]	99.16 (15.75) [a]	100.28 (18.36) [a]	100.18 (16.98) [a]	106.89 (15.03) [a]	5.47	<0.001***

BMI body mass index, *pg/ml* picograms per millilitre, *mg/L* milligrams per litre, *IFN-γ* interferon gamma, *IL-6* interleukin 6, *TNF-α* tumour necrosis factor alpha, *CRP* C-reactive protein, *PANSS* positive and negative syndrome scale, *WAIS* Wechsler Adult Intelligence Scale, *WTAR* Wechsler Test of Adult Reading, *F* ANOVA F test statistic, X^2 Chi-Square test statistic, *IQ* intelligence quotient.

[a-b] Means in a row without a common superscript letter differ ($P < 0.05$), as analysed by Tukey's HSD.

* $p = <0.05$, ** $p = <0.01$, *** $p = <0.001$

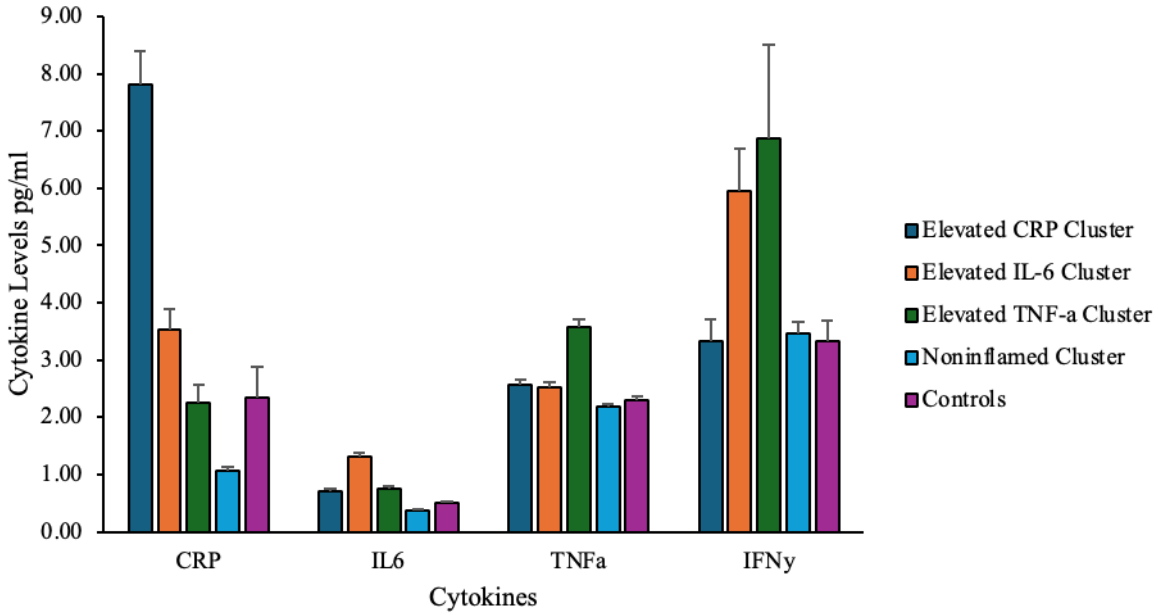
[1] Chi-square test

4.3.2 Inflammatory characteristics of Clusters

Cluster one (N = 29), is characterised by significant increases in CRP compared to the three other clusters and healthy controls (referred to as the CRP cluster hereafter). Cluster two (N = 52) presented significantly higher IL-6 levels than all other clusters and the HCs (referred to as the IL-6 cluster). Cluster three (N = 39) demonstrated significantly higher TNF-α than all other clusters and controls (referred to as the TNF-α cluster). IFN-γ was also

significantly higher in this cluster compared to all others, however there was one single outlier with extremely high IFN-γ levels (>5 standard deviations). Removal of this outlier reduced mean IFN-γ levels to 5.49 pg/ml (SD = 5.58) and had no effect on overall clustering when removed prior to running HYDRA. Cluster four (N = 109) represented significantly lower levels of all inflammatory biomarkers compared to the other three clusters and did not differ from healthy controls (referred to as the non-inflamed cluster). See table 2 and figure 1. (see data supplement for post-hoc comparisons)

Figure 4.1: Cytokine Levels in Each Cluster



IFN-γ interferon gamma, *IL-6* interleukin 6, *TNF-α* tumour necrosis factor alpha, *CRP* C-reactive protein.

4.3.3 Clinical and Neurocognitive Differences

The non-inflamed cluster demonstrated the lowest average scores on the PANSS subscale or factor scores (4 of 8), alongside lowest PANSS total scores. The IL-6 cluster

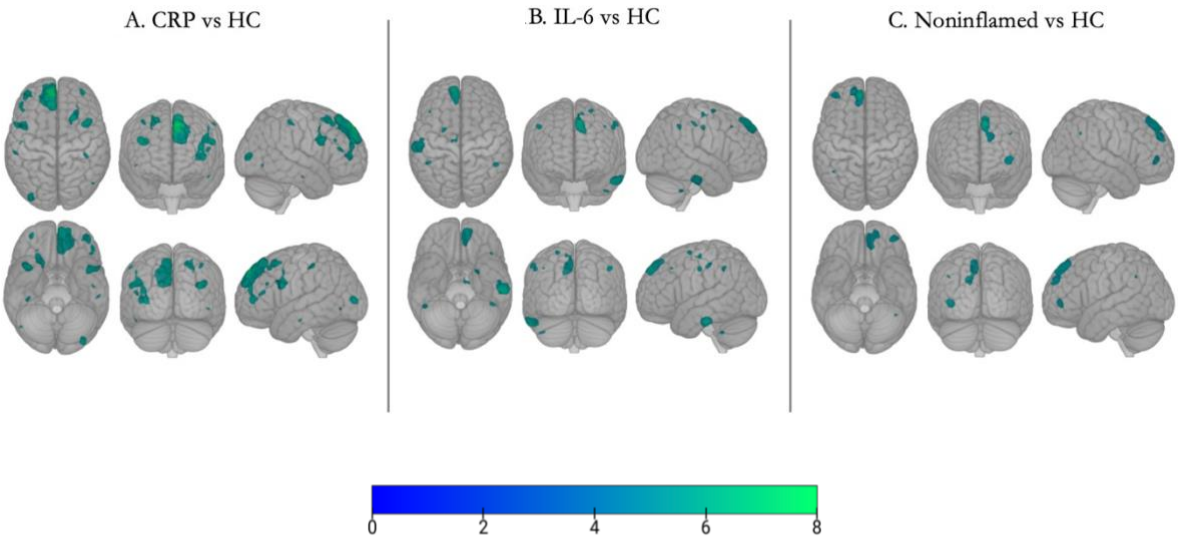
scored significantly higher on the anxio-depressive PANSS scale compared to the non-inflamed cluster ($p = 0.01$).

The CRP cluster showed the lowest average scores across cognitive tasks. Specifically, WAIS digit symbol, sum of scaled scores, WAIS III current IQ and WTAR premorbid IQ reaching significance (all $p < 0.03$). Post-hoc analysis with Tukey's test showed that the CRP cluster scored significantly lower than all other clusters on these measures (see data supplement).

4.3.4 GMV Differences

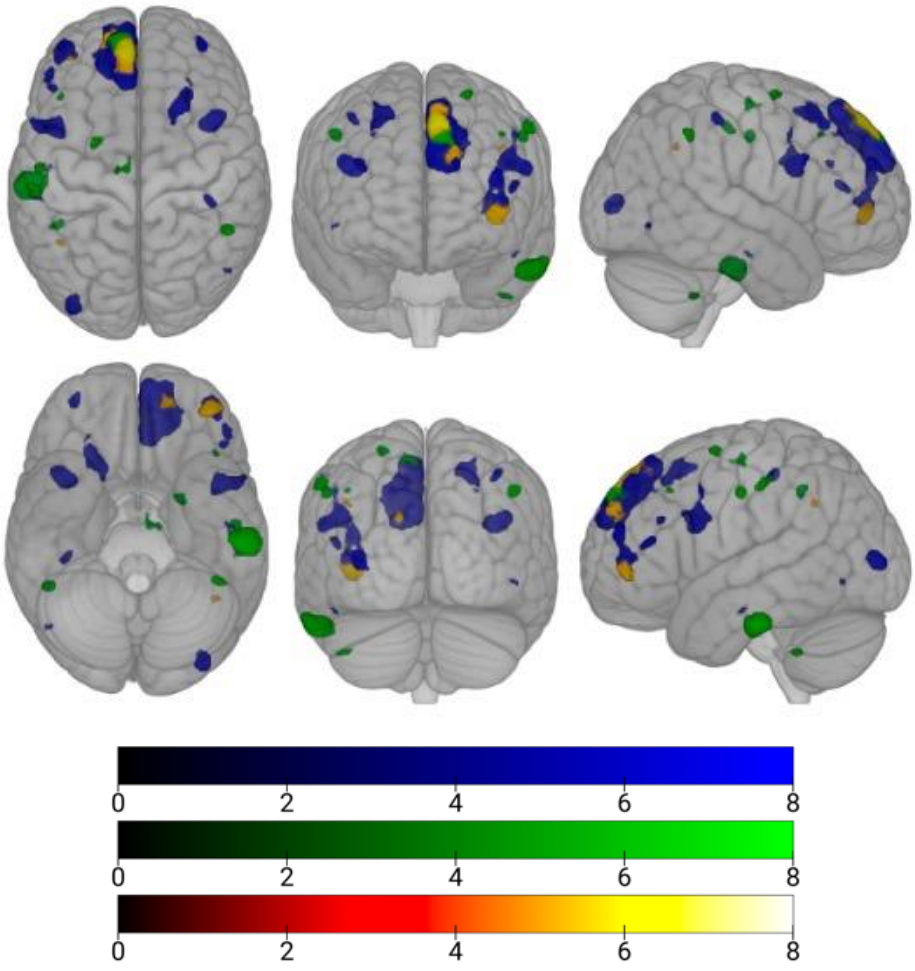
The CRP cluster compared to HCs demonstrated widespread GMV reductions with sites of greatest reduction in the superior frontal gyrus, middle frontal gyrus and inferior frontal gyrus (see Figure 2a). The IL-6 cluster compared to HCs revealed reduced GMV within the superior frontal gyrus, inferior temporal gyrus and postcentral gyrus (Figure 2b). The non-inflamed cluster compared to HCs demonstrated GMV reduction in the superior frontal and middle frontal gyri (Figure 2c), These three clusters all revealed reductions within the superior frontal gyrus (see Figure 2B for overlay). The TNF-α cluster did not exhibit any significant changes in GMV compared to HC.

Figure 4.2: GMV Deficits in Clusters Compared to Controls



T - Values

D. All Comparisons



T - Values

A. C-reactive protein cluster versus healthy controls: 13 distinct voxel clusters >10 voxels. Largest peaks in the left superior frontal (-6, 50, 46; 1896 voxels; $p<0.001$); left inferior frontal (-45, 9, 30; 362 voxels; $p<0.001$) and left middle frontal (-39, 51, 21; 310 voxels; $p<0.005$).

B. Interleukin-6 cluster versus healthy controls: 9 distinct voxel clusters >10 voxels. Largest peaks in the left superior frontal (-8, 52, 44; 772 voxels; $p<0.001$); left inferior temporal (-54, -22, -28; 637 voxels; $p<0.001$) and left postcentral (-56, -27, 48; 76 voxels; $p<0.005$).

C. Noninflamed cluster versus healthy controls: 3 distinct voxel clusters >10 voxels. Peaks in the left superior frontal (-6, 48, 48; 965 voxels; $p<0.001$); left middle frontal (-39, 46, 0; 182 voxels; $p<0.005$) and left supramarginal (-40, -52, 36; 14 voxels; $p<0.05$).

D. Overlay of 1a/b/c onto one brain to demonstrate overlapping voxels. Blue: CRP vs HC; Green: IL-6 vs HC; Yellow: Noninflamed vs HC.

All comparisons were family-wise error corrected $p<0.05$ as shown in colour scales.

Abbreviations: *IFN-γ* interferon gamma, *IL-6* interleukin 6, *TNF-α* tumour necrosis factor alpha, *CRP* C-reactive protein, *HC* healthy controls.

No GMV differences survived FWE correction when comparing across clusters. However, at an uncorrected threshold ($p<.001$, unc), similar patterns of GMV reductions were identified in the CRP and IL-6 clusters when compared to both the TNF-α cluster, and the non-inflamed cluster with peak deficits in the SFG for both CRP and IL-6 and the ITG for IL-6 specifically when comparing across groups. Further group comparisons demonstrated no differences between the CRP and IL-6 clusters, or the TNF-α and noninflamed clusters. For more detail on the between-cluster analysis please see supplementary figure 2.

4.4 Discussion

Using an advanced semi-supervised machine learning algorithm, we identified four distinct inflammatory clusters among patients with schizophrenia, which were separable from healthy controls. These clusters were characterized as elevated CRP (12.6% of the total sample), elevated IL-6 (22.7%), elevated TNF-α (17.0%), and noninflamed (47.7%). As predicted, these clusters exhibited differences in cognitive test scores, anxio-depressive measures, and BMI, in line with our hypothesis that inflammatory profiles would correlate with divergent clinical outcomes. Specifically, clusters with elevated IL-6 and CRP levels demonstrated the most significant cognitive deficits and functional impairments, as hypothesized, aligning with previous findings (e.g., Misiak et al., 2018; Penades et al., 2015). These impairments were associated with grey matter volume (GMV) reductions in the superior frontal and middle temporal gyri, which was also consistent with our expectations. While cognitive and clinical differences were evident, age, sex, and illness stage (FEP/established) did not differ significantly among the clusters. Subsequent analysis using voxel-based morphometry revealed distinct patterns of GMV deficits in overlapping and distinct regions of the superior and middle frontal, temporal, and motor regions for each inflammatory cluster compared to healthy controls, further supporting the hypothesis that inflammatory clusters exhibit unique neuroanatomical characteristics.

Specifically, the CRP cluster exhibited more pronounced impairments in WAIS full-scale IQ, total sum of scaled scores, and WTAR premorbid IQ, and 13 GMV voxel cluster reductions compared to healthy controls, particularly in the left prefrontal cortex, including the left superior frontal gyrus (SFG). These pronounced cognitive impairments were only found in the CRP cluster. In their meta-analysis examining peripheral inflammatory markers and associations with cognitive deficits in psychotic disorders, Misiak et al., 2018 found the strongest associations between high CRP levels and poor cognitive performance in

schizophrenia cohorts. Furthermore, a secondary analysis of data from the BeneMin study by Watson et al. (2022) identified three IQ subtypes (preserved, deteriorated, and compromised IQ) using k-means clustering. The compromised IQ group, characterized by low premorbid IQ and cognitive decline and the greatest reduction in brain volume, exhibited significantly higher CRP levels compared to the preserved IQ group. This finding underscores a potential link between CRP, brain volume, and cognitive performance, supporting our own results. The association suggests that heightened CRP levels and GMV reductions may contribute to inferior cognitive performance, possibly stemming from neurodevelopmental abnormalities triggered by early inflammatory insults, such as maternal immune activation (Lalousis et al., 2023; Meyer 2019).

CRP levels may serve as a potential marker reflecting early environmental insults, exerting lasting effects on brain development. The CRP cluster, with more pronounced cognitive impairments and specific GMV reductions, aligns with the hypothesis that elevated CRP might indicate a constitutional vulnerability, influencing the early neurodevelopmental trajectory in individuals with schizophrenia. Notably, deficits in constitutional IQ measures (e.g., WTAR) within the CRP cluster suggest a potential link between early inflammatory insults, cognitive performance, and neuroanatomical alterations. This concept aligns with previous literature associating maternal immune activation with neurodevelopmental abnormalities in offspring (Lalousis et al., 2023; Meyer 2019). Early-life infection or inflammation may set the stage for increased CRP secretion in later life, contributing to the observed cognitive deficits and GMV. Alternatively, an explanation of reverse causality may explain our findings. Those presenting with pronounced cognitive deficits may have elevated CRP due to other factors, for example those with lower general cognitive ability were found to be prone to make fewer healthy lifestyle choices such as an increased tendency to smoke, this could lead to increased levels of inflammation (Davies et al., 2019; Xie et al., 2024).

The SFG plays a crucial role in both the executive control network and the default mode network (Wang et al., 2019). Abnormalities in the SFG have been hypothesized to impact the communication of information between these networks, leading to the production of abnormal experiences and various clinical symptoms (Fan et al., 2022). Furthermore, the SFG is involved in working memory function, and direct stimulation of the SFG, via direct electrical stimulation, or TMS, has been shown to enhance working memory performance (Alagapan et al., 2019; Constantinidis & Klingberg, 2016). Based on these findings, we propose that the association between increased CRP, cognitive deficits, GMV reductions, and potential hypofunction of the SFG may be mediated through these mechanisms.

In contrast, the IL-6 cluster did not exhibit significant cognitive impairments compared to noninflamed participants. However, they did display marked increases in scores on the PANSS anxio-depressive dimension and widespread GMV reductions compared to healthy controls, with observed reductions in the left superior frontal gyrus, fusiform gyrus, and middle frontal gyrus, suggesting potential illness-related decline. Elevated IL-6 levels are commonly observed in patients with psychosis and depression, implicating the involvement of shared mechanisms in both disorders (Khandaker et al., 2018). Notably, elevated IL-6 levels have been predictive of negative symptoms and depressive symptom severity in clinical high-risk cohorts (Goldsmith et al., 2019). Comorbidity between recent-onset depression and recent-onset psychosis populations has also been observed, indicating substantial overlap of transdiagnostic symptoms (Upthegrove et al., 2017; Lalousis et al., 2021).

A considerable portion of the GMV deficits identified in the IL-6 cluster overlapped with those found in the CRP cluster. This could be attributed to the strong interrelation between the two inflammatory biomarkers; circulating IL-6 can induce hepatic cells to produce CRP, this can further lead to the induction of IL-6 by macrophages (Sproston et al.,

2018). However, the IL-6 cluster exhibited higher scores on anxio-depressive measures without the advanced cognitive deficits observed in the CRP cluster. IL-6 operates through two distinct signalling pathways: "classic" signalling and "trans"-signalling (Del Giudice et al., 2018). In classic signalling, IL-6 binds to membrane-bound IL-6 receptor (IL-6R), primarily found in hepatocytes, initiating a signalling cascade that modulates cellular function and stimulates the production of acute phase proteins like CRP. During an inflammatory insult or chronic inflammation, neutrophils expressing IL-6R are recruited. Subsequently, neutrophils will begin to shed their soluble IL-6R resulting in trans-signalling and the recruitment of macrophages and monocytes (Rose-John et al., 2017). These signalling pathways exhibit a bell-shaped dose-response curve with moderate IL-6 levels favouring classic signalling and excess IL-6 promoting trans-signalling (Del Giudice et al., 2018). The trans-signalling pathway is thought to be implicated in psychotic disorders, with the IL-6/sIL-6R complex believed to hinder kynurenine metabolism and synaptic plasticity (Garcia-Juarez et al., 2022). Additionally, studies have shown a decrease in classic signalling in major depressive disorder, whereas an increase in trans-signalling was linked to reduced CRP levels and more pronounced depressive traits (Kelly et al., 2021). These results may be relevant to schizophrenia, explaining why the IL-6 cluster exhibited higher scores on anxio-depressive subscales and lower CRP levels compared to the CRP cluster, despite both being part of the same pathway.

Distinct GMV deficits in the left inferior temporal gyrus (ITG) were observed exclusively in the increased IL-6 cluster. This might suggest they relate to the greater anxio-depressive ratings specific to IL-6 cluster. Indeed, several structural and functional brain imaging studies have suggested that ITG has an important role in the pathogenesis of depression aligning with its presumed functions in social cognition in social cognition, self-referential processing, and processing of affective stimuli (Gallagher et al., 2003; Herold et

al., 2016; Hu et al., 2017). For example, a recent meta-analysis identified the left ITG as one of three regions of reduced function in major depressive disorder (Gray et al., 2020). Furthermore, within early onset psychosis patients, reduced functional connectivity of the left inferior temporal gyrus with the posterior cingulate cortex, a key region of the default mode network, has been noted (Peng et al., 2021). ITG volume was positively correlated with scores on the Hamilton Depression Rating Scale in a definitive imaging study (Li et al., 2020). Many studies in schizophrenia have reported GMV reduction in the ITG (Ontisuka et al., 2004; Kuroki et al., 2006; Zhao et al., 2018; Mennigen et al., 2019) and have linked this to increased PANSS scores of delusions, suspiciousness, and anxiety (Mennigen et al., 2019). Therefore, it is possible that that ITG GMV reductions in the IL-6 cluster may give rise to a more anxio-depressive schizophrenia phenotype.

The noninflamed cluster exhibited better overall cognitive performance compared to the other clusters, along with lower scores on PANSS negative scales, although these results did not reach the significance threshold. They also displayed higher positive symptom scores than the CRP and IL-6 clusters. It has been suggested that low levels of inflammation may be associated with less severe disease status and better long-term outcomes (Lalousis et al., 2023). Antipsychotic medications tend to alleviate positive symptoms more effectively than negative symptoms (Blanchard et al., 2011; Leucht et al., 2017), and residual schizophrenia predominantly manifests with negative symptoms (World Health Organization, 1992). Therefore, participants in the noninflamed cluster, characterized by greater positive symptoms and fewer negative symptoms may see improved long-term outcomes with antipsychotic medication. Alternatively, since most cases, including FEP, have been treated with antipsychotics, the high positive symptom score might indicate non-response to antipsychotics in this subgroup. As such, further research is necessary to follow up on medication response within this patient cluster.

In contrast, the TNF-α cluster did not exhibit significant differences from the noninflamed cluster in terms of PANSS or cognitive measures. Furthermore, when compared to healthy controls, no significant differences in GMV were detected. TNF-α plays a vital role in neuro-immune regulation and autoimmunity, with its signalling pathways found to be both neuroprotective and neurodegenerative depending on receptor activation (Probert 2015). Therefore, disentangling the downstream effects of elevated TNF-α from peripheral levels alone can be challenging. Meta-analyses have shown that increased TNF-α is associated with better cognitive performance, while other studies have found associations between TNF-α and increased levels of negative symptoms in clinical high-risk and first-episode drug-naïve cohorts (Goldsmith et al., 2019; Lin et al., 2021). Based on the findings presented here, it can be hypothesized that TNF-α in this cohort likely activated the TNF-α RII receptor, exerting neuroprotective effects. However, further exploration in experimental conditions is necessary to validate this hypothesis.

It is important to acknowledge several limitations of the current study. Firstly, the healthy control cohort solely came from the SPRING study, resulting in a small sample size, and controlling for site effects was challenging due to data analysed from two studies with separate designs across 8 total sites. Additionally, healthy controls were age matched to patients in the SPRING study, which included cases with long-established illness, leading to a greater mean age relative to patient in BENEMIN study and in all inflammatory clusters which included cases from both studies. This might be relevant to grey matter differences but in fact could be predicted to reduce the GMV deficits in patients relative to controls. We included age as a covariate in the analysis of GMV. Secondly, medication status was not controlled for due to variations in reporting strategies across the two studies. Cytokines such as TNF-α and IL-6 are sensitive to antipsychotic dosage and may be differentially affected (Lin et al., 2021; Feng et al., 2020). The two datasets lacked data from traveling heads,

preventing the examination of potential scanner differences across sites. Furthermore, while the study yielded significant results, it is essential to consider the potential impact of sampling errors introduced by the clustering algorithm, the clusters defined here appear to be relevant to only one inflammatory biomarker rather than a combination of them. It is possible that the machine learning algorithm was selecting biomarker extremes as the basis of the clusters due to their highly correlated nature. However, clusters identified here broadly match those identified in previous studies employing similar methods (Lalousis et al., 2023), though they included a greater number of biomarkers in their analysis. Sampling errors may also be relevant to the VBM findings, with the relatively small number of participants included in each cluster it may be that the GMV reductions demonstrated in each group compared to HC are sampling errors of the overall main effect (supplementary figure 3).

The identification of inflammatory clusters can enhance our understanding of the pathogenesis of schizophrenia and provide potential avenues for stratifying samples in the context of novel treatments. Immune-focused treatments in schizophrenia have yielded mixed results (Jeppesen et al., 2020). Findings such as those presented in this study may offer a solution by identifying valid and reproducible clusters of patients with altered inflammatory levels, who may respond differently to anti-inflammatory treatment. Targeted anti-inflammatory medication, in conjunction with antipsychotic treatment, may improve cognitive function.

In summary, this study identified four distinct inflammatory clusters within a diverse schizophrenia cohort. Each cluster exhibited a unique inflammatory profile, distinct patterns of grey matter volume, and variations in neurocognitive and clinical symptoms. These results contribute to the identification of inflammatory clusters, which can potentially pave the way for targeted anti-inflammatory treatment approaches in schizophrenia.

4.5 Author Contributions

RU conceived the initial research project. Data was collected and processed prior to this analysis by the BeneMin and SPRING teams (BD, PFL, PD, EJ, NMB, CP, KDS, GW, CJG, SC, RS, MR, NN, LP, LC, JTRW, JS, MZK and RU). AM combined the existing data, conducted all statistical analysis, researched, and drafted the article. JR aided AM in processing of the VBM data. All authors critically revised the article, offering support on content and structure.

Chapter 5

Probing the Biological Underpinnings of Advanced Brain Ageing in Schizophrenia

Alex J. Murray,[1] Pedro L. Ballester[2], Jack C. Rogers[1,3], Stephen Williams[4], Martin Wilson[3], Bill Deakin[4], Mohammad Katshu[5,6], Peter Liddle[5], Lena Palaniyappan[7], Rachel Upthegrove[1,9]

1. Institute for Mental Health, University of Birmingham, Birmingham, UK.
2. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada.
3. Centre for Human Brain Health, University of Birmingham, Birmingham, UK.
4. Division of Psychology and Mental Health, School of Biological Sciences, University of Manchester, Manchester, UK.
5. Institute of Mental Health, University of Nottingham, Nottingham, UK.
6. Nottinghamshire Healthcare National Health Service Foundation Trust, Nottingham, UK.
7. Early Intervention Service, Birmingham Women's and Children's National Health Service Foundation Trust, University of Birmingham, Birmingham, UK.
8. Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montreal, Quebec, Canada.
9. Robarts Research Institute, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada.

Background: Recent evidence suggests that patients with schizophrenia may show advanced brain ageing, particularly evident after the first year of onset. However, it is unclear if accelerated ageing persists or increases over time and the underlying causes are poorly understood. Theories suggest disruptions in glutamate function, oxidative stress, and inflammation may contribute to progressive brain changes in people with schizophrenia. This study examines whether brain ageing differs between early and established stages of schizophrenia, the correlation with symptom severity and relationship to markers of brain function, antioxidants, inflammation.

Methods: Two brain-age prediction models assessed 112 participants (34 recent onset psychosis (ROP), 36 established schizophrenia (EST), 42 controls (HC)). Brain age gap (BAG) was calculated by subtracting chronological age from predicted age. Shapley's additive explanations (SHAP) identified influential structural magnetic resonance imaging (MRI) features driving brain-age prediction. Linear regression models and partial correlations, adjusting for age, explored associations between BAG and neurometabolites, inflammatory markers, medication exposure and clinical scores in the whole sample.

Results: The EST group showed higher BAG (Mean = 6.21, SD = 7.30) compared to HC (Mean = -0.01, SD = 9.10), while ROP (Mean = 4.23, SD = 9.25) did not differ significantly from HC. The top 10 SHAP features diving the BAG included ventricular enlargement and total grey matter volume, which was similar in psychosis to HC. In a combined psychosis group (EST + ROP), higher BAG correlated with more severe symptoms (PANSS total, general, and anxiodepressive subscales). BAG positively associated with magnetic resonance spectroscopy (MRS) measured glutathione (GSH) and negatively with n-acetyl aspartate (NAA).

Discussion: Accelerated brain age in schizophrenia may be related to illness severity and poor defence against oxidative stress. The lack of differences in SHAP features between schizophrenia and HC suggests that the pattern of brain ageing is in keeping with advanced normal ageing. Findings suggest potential treatment targets to improve brain health in schizophrenia, warranting further research.

5.1 Introduction

Advanced brain-ageing (ABA) has been observed across various psychiatric disorders, with some evidence that these are present even in early stages (Koutsouleris et al., 2014; Schnack et al., 2016; Ballester et al., 2021). The quantification of ABA involves assessing the deviation between an individual's actual age and a prediction derived from models trained on a large datasets of structural neuroimaging features from healthy individuals (Franke and Gaser, 2019; Sanford et al., 2022). Specifically in schizophrenia, the acceleration in ageing may be driven by reduced grey matter tissue (Ballester et al., 2023).

Schnack et al. (2016) observed an increase in brain ageing in schizophrenia after the first year of onset, particularly in the subsequent two years, but with a slowdown five years later. Recent findings, however, challenge this proposed relationship between advanced brain ageing and illness duration, especially in samples including more extended periods of illness. Constantinides et al. (2022) did not find any associations between ABA and age of onset or illness duration, suggesting that ABA in schizophrenia may not be primarily driven by disease progression. These findings raise questions about whether the observed gap closes or persists over many years of illness, particularly in medicated patients with established schizophrenia. While a longitudinal study with repeated imaging could provide conclusive answers, as a preliminary step should be assessing groups of patients with first episode or established schizophrenia in an accelerated ageing design.

To date no study has examined the associations between peripheral inflammatory biomarkers, central neurometabolites and advanced brain ageing in schizophrenia. Understanding these potential neurobiological determinants is of importance as potential for targeted therapies. Theories of progressive brain changes in schizophrenia suggest the

involvement of the glutamate system, oxidative stress, and inflammatory pathways, yet the specific contribution of each to ABA have not yet been explored. Magnetic resonance spectroscopy (MRS) measures of glutamate and glutathione (GSH) from the anterior cingulate cortex (ACC) have emerged as reliable indicators of disruptions in glutamatergic signalling and oxidative stress dysfunction in schizophrenia (Egerton et al., 2020, Merritt et al 2021; Das et al., 2019; Dempster et al., 2020; Murray et al., 2023). Additionally, certain inflammatory markers such as interleukin-6 (IL-6) and c-reactive protein (CRP) have demonstrated reliability in indicating the involvement of inflammatory processes in the pathophysiology of schizophrenia and have been linked to brain structural variations within the disorder (Williams et al., 2022; Lalousis et al., 2023; Murray et al., 2024 unpublished).

However, relating model-based estimates of ABA to other mechanistic markers, is challenging and it is important to study the associations based on participant-level contributors for the estimated brain-age. One approach towards extracting participant level features that contribute to a model is to employ the SHapley Additive exPlanations (SHAP) approach (Lundberg & Lee, 2017; Ballester et al., 2023) and measure the marginal contribution of each structural feature to a given prediction. This approach allows the identification of key contributors within patient groups and relate them to other biological markers of interest, providing insights into the potential mechanisms influencing ABA in patients.

In this study, our primary objective was to investigate whether cortical glutamatergic and antioxidant markers, along with peripheral inflammatory markers, are related to the degree of advanced brain-ageing in schizophrenia. We hypothesise that MRS markers of GSH will negatively correlate with advanced brain ageing as reduced antioxidant capacity will be indicative of a pro-oxidative state of the brain thus leading to structural variations associated with the ageing brain. Furthermore, increases in pro-inflammatory markers such as IL-6 and

CRP will be positively correlated with ABA due to their associations with reduced GMV in the frontal regions identified in Chapter 4. No previous studies relate MRS and inflammatory markers to ABA in schizophrenia and we propose that in identifying potential mechanistic contributors, we lay the groundwork targeted interventions that promote brain health in individuals with schizophrenia.

5.2 Methods

5.2.1 Participants

Participants were recruited as part of the "The Study of Psychosis and the Role of Inflammation and GABA/Glutamate" (SPRING). In total, 207 participants were recruited across three sites: Cardiff, Manchester, and Nottingham. The study included 58 first episode psychosis (FEP) patients who were minimally medicated (<12 weeks), aged 18-41, and within 5 years of symptom onset, 76 established psychosis patients (EST) with symptoms present for more than 10 years and aged 27-55. 73 healthy controls (HC) were age and sex matched to the participants. For this analysis, only participants recruited to the Cardiff and Manchester sites were included due to variation in magnet strength (3T GE/Siemens in Cardiff and Manchester, 7T Phillips in Nottingham). Participants were excluded from the analysis if they did not have a combination of blood-based inflammatory markers, 1H-MRS, and 3T structural MRI at baseline with an image quality rating >75% (assessed by CAT-12 toolbox version r1830; http://dbm.neuro.uni.jena.de/cat12/). The resulting sample consisted of 112 participants, including 34 FEP (10 Female), 36 EST (10 Female), and 41 HC (10 Female) (Table 1). Additional measures for patients' current antipsychotic usage were collected, including defined daily dose, and an 11- point rating of lifetime antipsychotic

exposure measure reflecting both dose and duration of medication with fixed criteria for each level.

5.2.2 Data Acquisition

Participant symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Scores for 5 PANSS dimensions: the positive dimension, negative dimension, anxio-depressive dimension, excitement dimension and disorganisation/other dimension were all calculated via summing of relevant symptom scores (Yazaji et al., 2002). Neurocognitive function was evaluated using the Wechsler Adult Intelligence Scale III for patients with schizophrenia, which included subtests such as "Information," "Arithmetic," "Block Design," and "Digit Symbol Test" for processing speed. Premorbid IQ was assessed using the Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001).

Venous blood was collected and centrifuged within one hour at 1300–2000g for 10 min. In total 11 inflammatory markers were collected – IL-1B, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, CRP, IFN-y and TNF-a. Cytokines were measured in duplicate using Meso Scale Discovery (MSD) V-plex immunoassays, Pro-inflammatory Panel 1 (human) kit (MSD, Maryland, USA), and MSD Vascular Injury Panel 2 human kit weas used to measure CRP levels.

T1-weighted structural neuroimaging was conducted using a standard acquisition protocol. The Cardiff site collected data with a 3T GE MRI scanner, and the Manchester site collected data on a 3T Siemens MRI scanner. Further 1H-MRS neuro-metabolite data was acquired at each site following a standardised sequence (see supplement). A single voxel was placed in the bilateral dorsal anterior cingulate cortex (dACC) superior to the genu of the

corpus collosum (supplementary figure s1). A point resolved spectroscopy (PRESS) 1H-MRS sequence (TE/TR = 35/2000 ms, bandwidth = 2000 Hz, Navg = 1 Voxel size = 35 x 40 x 20 mm^3) was used to acquire water-suppressed spectra as well as a water-unsuppressed spectrum for spectral editing and quantification. Additional Mescher-Garwood (MEGA)-edited PRESS data for glutathione was collected from the same voxel (TE/TR = 130/2000 ms, bandwidth = 2000 Hz, Navg = 64 Voxel size = 35 x 40 x 20 mm^3).

5.2.3 Pre-processing and Site Correction

Raw T1 structural MRI data was assessed for data quality using the open-source CAT-12 toolbox (version r1830; http://dbm.neuro.uni.jena.de/cat12/), and participants with a raw image quality rating <75% were excluded from further analysis (see supplementary methods). Images were pre-processed and segmented using the *recon-all* command in Freesurfer 7.2.0. Relevant features were extracted using *asegstats2table* within Freesurfer and the HCP multimodal atlas (Glasser et al., 2019). In total, 1,118 features were extracted, covering volume, area, and thickness measurements.

PRESS and MEGA-PRESS data were pre-processed and quantified using the SPANT software package in R (https://github.com/martin3141/spant). SPANT pre-processing included coil combination, removal of bad averages, and frequency and phase correction. Data were quantified using ABFit model fitting (Wilson 2021), and basis sets were simulated within SPANT for the metabolites: n-acetylaspartate (NAA), n-acetylaspartylglutamate (NAAG), glutamate, glutamine, creatine, phosphocreatine, glycerophosphocholine, phosphocholine, myo-inositol (Ins), scyllo-inositol, GABA, aspartame, taurine, glucose, glutathione (GSH), and glycine. Some metabolite concentrations were then combined to estimate "total" (t) concentrations: tNAA (NAA+NAAG), tCr (creatine + phosphocreatine),

Glx (glutamate + glutamine). Of the metabolites measured, 5 were selected for further analysis: GSH, Glx, tNAA, GABA and Ins. These were selected due to their high data quality (Cramer Rao Lower Bounds <20% in most participants) and their relevance to schizophrenia.

ComBat harmonization was employed to correct for MRI scanner variances, batch effects, and site effects. ComBat was run separately for the T1 structural, MRS, and blood-based biomarkers data. Correction was implemented in r. using the neuroCombat package (https://github.com/Jfortin1/neuroCombat_Rpackage). Covariates of age, sex, and disease status were protected during the removal of site/scanner effects. Empirical Bayes was set to false for the MRS and blood-based biomarkers due to the number of features being substantially smaller than the number of participants.

Principal component analysis was conducted in order to reduce the dimensionality of the inflammatory marker data. The kaiser criterion was employed to select the optimal number of components while explaining the greatest amount of variance in the data.

5.2.4 Brain Age Prediction

The publicly available "brain-age" prediction model developed by Kaufmann et al. (2019) was used to assess advanced brain ageing in our dataset. Pre-trained gradient-boosted trees generated by the XGBoost method, provided by the authors, are available online (https://github.com/tobias-kaufmann/brainage). Full details of the model training can be found in (Kaufmann et al., 2019), however in brief, two separate models were trained (one for males and one for females) on a total of 35,474 individuals aged 3-89 years. The average chronological ages from the pretrained models were 48.01 for males and 46.63 for females. Model performance was assessed by the mean absolute error (MAE) between predicted brain

age and chronological age and the Pearson correlation coefficients (r) between predicted brain age and chronological age.

The resulting XGBoost models were compared to the performance of a separate pretrained model, BrainAgeR (https://github.com/james-cole/brainageR), to ensure prediction accuracy. BrainAgeR employs Gaussian Process regression to estimate brain age using raw T1 scans in nifti format, potentially reducing the confounding effects of atypical brain morphology and removing variance introduced by differences in pre-processing (Cole et al., 2017). The original BrainAgeR model was trained on 3377 healthy individuals aged 18-92 (mean age = 40.6 years).

As the accuracy of predicted brain age varies with sample age, an age-correction procedure proposed by Beheshti et al. (2019) was employed. This procedure fits a linear model between predicted age and age of healthy participants in order to adjust for age-related confounds. Then, an age-corrected predicted age is derived for both HC and SCZ samples based on the slope, intercept, and predicted age extracted from the HC group. This method ensures that the model has a consistent level of error across the whole age range of healthy participants which can then be applied to the schizophrenia sample. This corrected prediction was used to compare brain age gap BAG across the groups. Subsequent analyses of neurometabolic and inflammatory marker associations were conducted using the raw, uncorrected, brain-age predictions generated from the XGBoost model with age and age^2 included as covariates in the models to reduce the likelihood of over-correction.

5.2.5 Shapley's Additive Explanations (SHAP)

To better understand the contribution and importance of individual structural brain measures for making brain age predictions, Shapley's Additive Explanations (SHAP) method

was used. SHAP is a method based on game theory that extracts marginal contributions of features from predictions. The SHAP value for a specific feature for an individual prediction represents the difference in the prediction when that feature is left out of the decision tree (marginal contribution). The SHAP for XGBoost package in R (https://cran.r-project.org/web/packages/SHAPforxgboost/index.html) was used in this case (Ballester et al., 2023). SHAP values represent the contribution of each feature to the deviation of the prediction from the mean age of the database (the starting point of the XGBoost model).

5.2.6 Statistical Analysis

Brain age gap (BAG) was calculated as brain-predicted age minus chronological age. This was computed for each SCZ participant and used as the outcome variable. Although sex-specific prediction models were built, the generated BAG values were pooled across males and females for subsequent statistical analyses within each cohort. This was done to improve statistical power as previous work has shown similar correlations with chronological age in each sex (Bacas et al., 2023). BAG was assessed to identify significant brain age differences between the two stages of schizophrenia (FEP and EST) and age and sex-matched healthy controls.

The EST and FEP schizophrenia groups were then combined into one cohort to improve statistical power in analysis of the relationship with biomarkers of interest. Partial correlation matrices were then generated for this combined schizophrenia cohort and healthy controls to assess the associations between uncorrected BAG, MRS-derived neurometabolites, blood-based biomarkers of inflammation, and clinical/neurocognitive characteristics. In each matrix, both age and age-squared were included as covariates to correct for the systemic age-related bias in brain age prediction. Similar matrices were

generated for the top 10 performing SHAP features in each model to assess the association between these features of interest and brain-derived neurometabolites or blood-based inflammatory markers. This method can help delineate the underlying mechanisms of association between these features (supplementary results).

5.3 Results

5.3.1 PCA Dimensionality Reduction

Principal component analysis of all inflammatory markers was conducted in order to reduce the dimensionality of the dataset. Based on the Kaiser criterion (eigenvalues >1), five principal components were retained for further analysis. These five principal components capture approximately 67% of the total variance.

Table 5.1: Factor loadings for each component.

	PC1	PC2	PC3	PC4	PC5
IFN-y	**0.36**	0.35	-0.33	0.08	0.27
IL-10	0.27	-0.15	**0.49**	-0.30	0.39
IL-12	0.04	0.07	-0.06	0.42	**0.45**
IL-13	0.07	**0.41**	0.24	0.32	-0.20
IL-1B	0.05	0.44	**-0.39**	-0.22	0.36
IL-2	0.16	0.37	0.29	**0.43**	-0.13
IL-4	0.04	**0.33**	-0.14	**-0.54**	-0.22
IL-6	**0.56**	-0.21	-0.12	0.04	-0.18
IL-8	0.13	**0.38**	0.36	-0.25	**-0.29**
TNF-a	**0.47**	**-0.11**	0.29	-0.14	0.26
CRP	**0.46**	**-0.21**	-0.34	0.10	-0.38

PC1: Pro-inflammatory Factor

Key Features: ↑ IL-6, ↑ TNF-a, ↑ CRP

PC2: Anti-inflammatory Factor

Key Features: ↑ IL-13, ↑ IL-4, ↓ CRP, ↓ IL-6

PC3: Immuno-modulation Factor

Key Features: ↑ IL-10, ↓ IL-1B

PC4: T-Cell Modulation Factor

Key Features: ↑ IL-2, ↓ IL-4

PC5: Th1 Immune Response Factor

Key Features: ↑ IL-12, ↓ IL-8

Abbreviations: *PC* principal component, *IFN-γ* interferon gamma, *IL* interleukin (1-13), *TNF-α* tumour necrosis factor alpha, *CRP* C-reactive protein.

Table 5.2: Demographic Information and Markers of Interest in participant groups.

	FEP (n=34)	EST (n=36)	HC (n=42)	F/t/X^2	P Value
Sex (M/F)	24/10	26/10	32/10	0.46	0.927
Antipsychotic DDD	0.89 (0.76)	1.56 (0.88)	-	-3.33	<0.001***
Lifetime Antipsychotic Exposure	1.85 (0.70)	8.11 (1.30)	-	-24.78	<0.001***
Mean Age	24.12 (4.72)	41.98 (7.90)	34.85 (11.66)	36.13	<0.001***
XGBoost BAG	4.23 (9.25)	6.21 (7.30)	-0.01 (9.10)	5.35	0.006**
BrainageR BAG	0.07 (5.39)	2.76 (6.17)	-2.46 (8.00)	5.85	0.004***
Biomarkers of Interest					
ACC GSH	1.88 (3.37)	1.63 (1.75)	1.62 (1.95)	0.16	0.849
ACC tNAA	1.61 (0.20)	1.48 (0.25)	1.55 (0.27)	2.13	0.124
ACC Glx	0.95 (0.24)	0.90 (0.15)	0.98 (0.40)	0.64	0.530
ACC GABA	0.49 (0.22)	0.38 (0.19)	0.44 (0.34)	1.55	0.216
ACC Ins	0.13 (0.20)	0.19 (0.24)	0.16 (0.28)	0.57	0.566
Proinflammatory Factor	-0.34 (1.02)	0.82 (1.90)	-0.43 (1.06)	9.55	<0.001***
Anti-inflammatory Factor	0.18 (1.29)	-0.04 (1.61)	-0.11 (1.06)	0.48	0.628
Immuno-modulation Factor	-0.20 (0.99)	0.22 (1.42)	-0.03 (0.87)	1.24	0.293
T-Cell Modulation Factor	-0.19 (1.27)	-0.07 (1.00)	0.02 (0.95)	1.39	0.253
Th1 Immune Response Factor	0.01 (1.08)	-0.04 (1.00)	0.02 (1.00)	0.04	0.961
Neurocognitive/Clinical Measures					
PANSS Total	53.32 (14.94)	52.14 (13.82)	-	0.33	0.952
WAIS Total	36.47 (10.12)	36.50 (8.34)	-	-0.01	0.102
WTAR	98.00 (30.28)	103.28 (15.69)	-	-1.23	0.083
Full Scale IQ	94.21 (17.25)	94.00 (15.54)	-	0.06	0.109

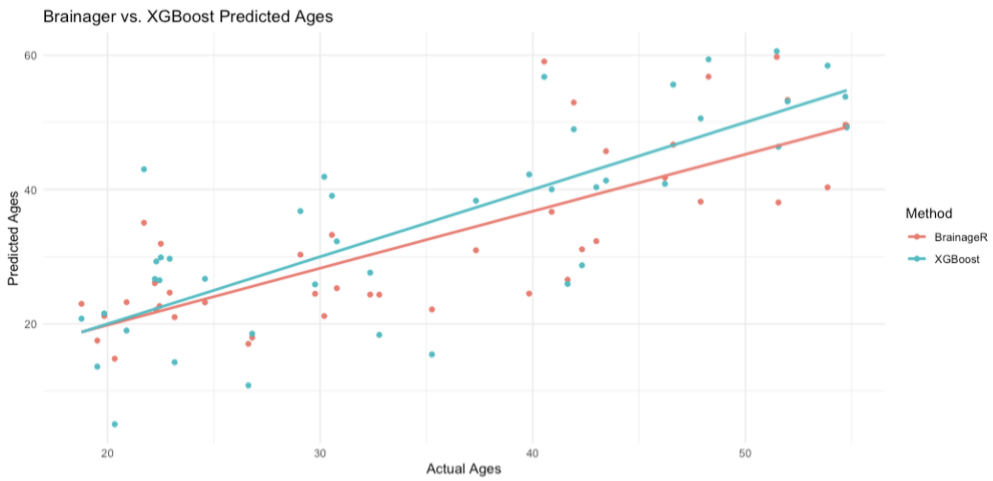
FEP first episode psychosis, *EST* established schizophrenia, *HC* healthy controls, M*/F* Male/Female, *DDD* defined daily dose of antipsychotics, *BAG* brain age gap, *ACC* anterior cingulate cortex, *GSH* glutathione, *tNAA* total n-acetyl aspartate, *Glx* glutamate + glutamine,

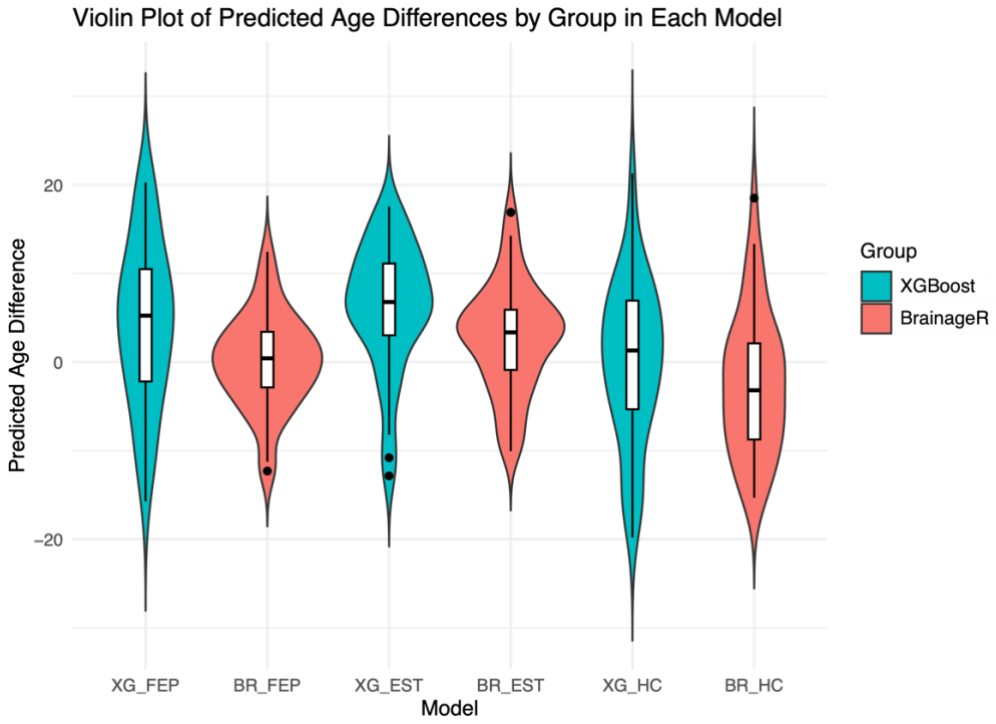
Ins inostitol, *Th1* t-helper 1, *PANSS* positive and negative syndrome scale, *WAIS* Wechsler Adult Intelligence Scale, *WTAR* Wechsler Test of Adult Reading, *F* ANOVA F test statistic, *t* t-test statistic, X^2 Chi-Square test statistic, *IQ* intelligence quotient.

5.3.2 Model Comparisons

Overall BrainageR marginally outperformed the XGBoost model on typical measures of model performance. It had a lower mean absolute error (6.75 years vs 7.17 years), but R^2 association between brain predicted age and chronological age was the same for both models. As such further analysis was conducted on the XGBoost model outputs as Shapley's additive explanations is more informative with gradient-boosted tree models.

Figure 5.1: Performance of XGBoost and BrainageR Models.





Top: Comparison of model performances: chronological age vs model predicted age of HCs.

BrainageR $R^2 = 0.62$; XGBoost $R^2 = 0.62$

Bottom: Violin plots displaying predicted age difference (brain predicted age – chronological age) for each group in each model.

Mean absolute error for healthy controls BrainageR = 6.75 years; XGBoost = 7.17 years

XG XGBoost algorithm, *BR* BrainageR algorithm, *FEP* first episode psychosis, *EST* established schizophrenia, *HC* healthy control.

5.3.3 Brain-Age Predictions

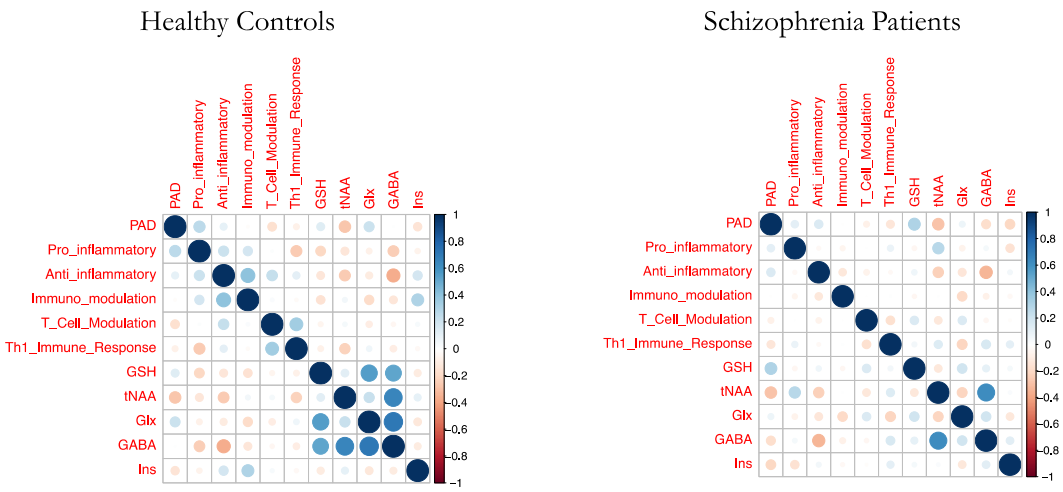
Overall, using the XGBoost model predictions, brain-ageing was seen to be accelerated in the EST group (M = 6.21, SD = 7.30) compared to HC (M = -0.01, SD = 9.10) ($p = 0.005$), but the FEP group (M = 4.23, SD = 9.25) was not significantly different from either the EST or HC groups ($p = 0.605$, $p = 0.088$ respectively). The same result was found for the BrainageR model (Figure 2).

5.3.4 Biomarker Associations with Brain age Gap (BAG)

The EST and FEP schizophrenia groups were then combined into one cohort to improve statistical power in analysis of the relationship with biomarkers of interest resulting in a cohort of 70 psychosis patients termed SCZ. This group did not differ on mean age (M = 33.31, SD = 10.79) from healthy controls (M = 34.85, SD =11.66) (p = 0.48), the SCZ group did differ significantly from the HC group on BAG (M = 5.25, SD = 8.30 and M = -0.01, SD = 9.10 respectively) (p = 0.002).

Partial Pearson correlation matrices, controlling for the effects of age and age^2, were generated for each group to assess associations between BAG difference and biomarkers. Within the combined group the biomarkers presenting with the greatest correlation with predicted age difference were total n-acetylaspartate (r(66) = -0.287, p = 0.018) and glutathione (r(66) = 0.301, p = 0.013). In contrast, the HC group demonstrated no significant associations with any biomarker of interest. No blood-based biomarker factor was significantly correlated with BAG in either SCZ or HC, however when looking at the whole dataset (SCZ+HC), the pro-inflammatory factor was significantly positively associated with BAG (r(108) = 0.227, p = 0.017) (see supplementary results). Furthermore, predicted-age difference was not associated with defined daily dose of antipsychotic medication (r(68) = 0.034, p = 0.786) nor lifetime anti-psychotic exposure (r(66) = -0.004, p = 0.976).

Figure 5.2: Correlation Matrices Demonstrating Associations Between BAG and Biomarkers of Interest



Blue dot indicates positive correlation, orange indicates negative.

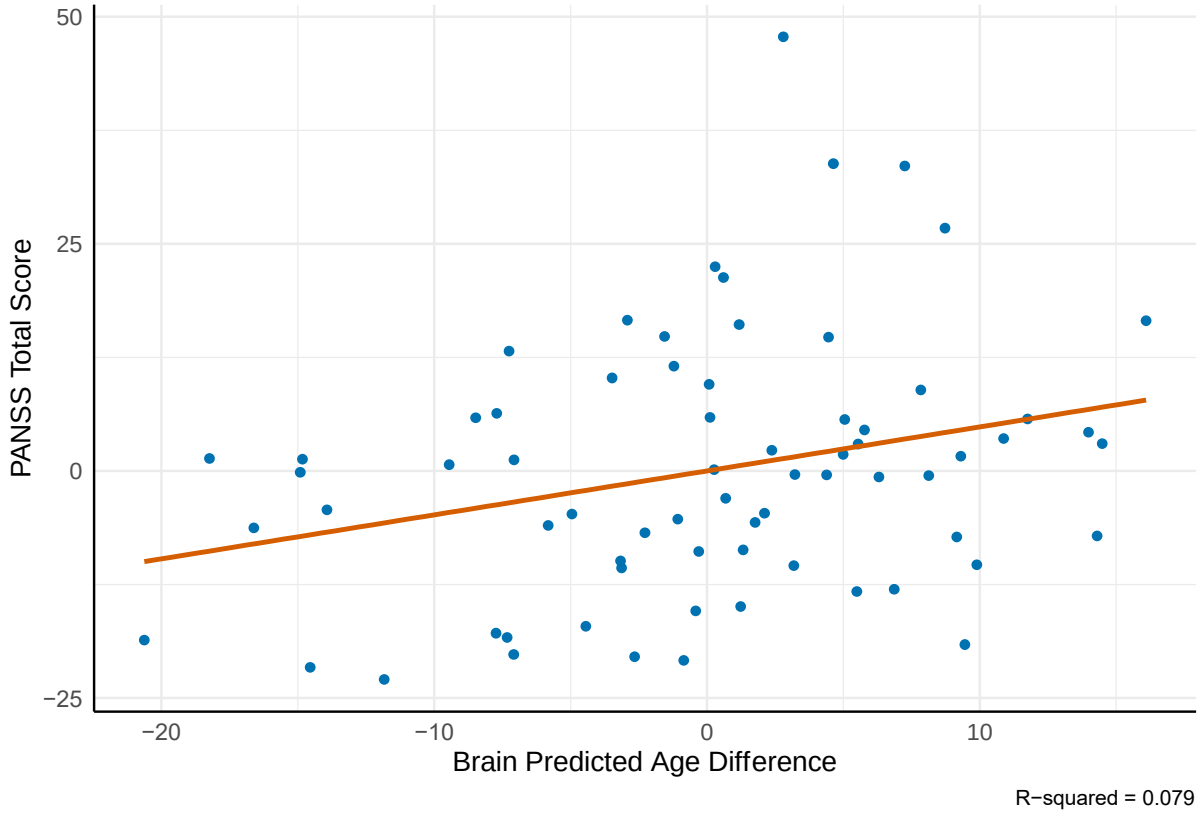
Dot size represents strength of correlation.

GSH glutathione, *tNAA* total n-acetyl aspartate, *Glx* glutamate + glutamine, *GABA* gamma-aminobutyric acid, *Ins* inostitol, *Th1* t-helper 1.

5.3.5 Neurocognitive Associations with Brain Age Gap

Neurocognitive scores, including PANSS subscales, WAIS scores, IQ and WTAR, were included in a partial correlation matrix alongside BAG. The effects of age and age^2 were controlled for. Overall, no significant associations were found between BAG and any of the cognitive scale scores within the combined-schizophrenia group. However, significant positive associations were found with PANSS general (r(66) =0.299, p = 0.013), total (r(66) = 0.281, p = 0.020) and anxio-depressive scores (r(66) = 0.238, p = 0.050).

Figure 5.3: Relationship Between BAG and PANSS Total Scores.



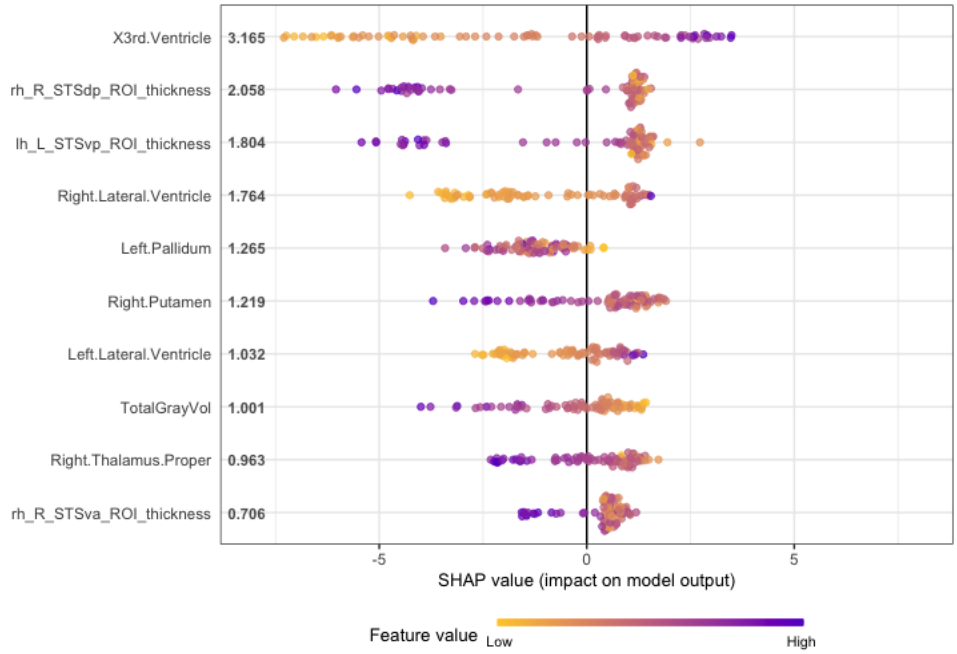
BAG brain age gap, *PANSS* positive and negative syndrome scale.

5.3.6 Shapley's Additive Explanations

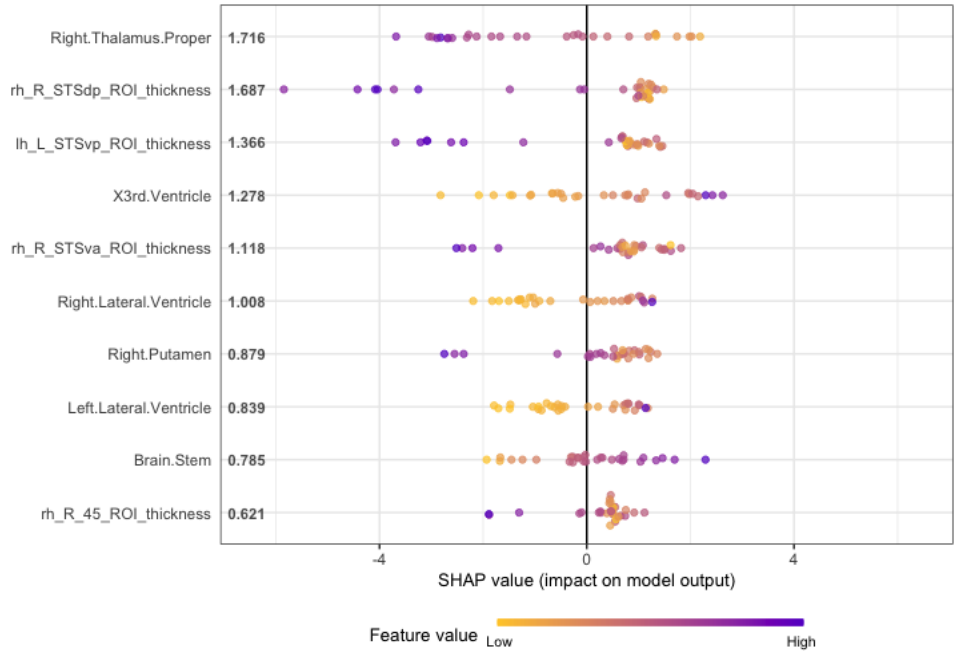
To better understand the contribution of individual features to brain-predicted age, irrespective of diagnosis, SHAP values were calculated for each of the 1,118 features and averaged across all participants, regardless of group. The top ten most relevant features based on mean absolute contribution to the prediction were extracted from the male and female models. In total 12 unique features were identified (9/10 features overlapped between models). One-way ANCOVA, with sex, age and age^2 as covariates, was conducted to assess how these features differed between groups. None of the individual features differed significantly between groups i.e. no individual feature from the top 12 was significantly differently to brain age estimation in the schizophrenia groups than the healthy controls, indicating that the same morphological feature were driving the brain-predicted age measure in both groups.

Figure 5.4: Top 10 SHAP features for each model.

A. Males:



B. Females:



Rh Right hemisphere; *lh* left hemisphere; *STS* superior temporal sulcus; *dp* dorsal posterior; *vp* ventral posterior; *va* ventral anterior; *ROI* region of interest.

5.4 Discussion

The present study aimed to identify if advanced brain ageing was evident across different phases of psychosis. Overall, it was found that the established schizophrenia group showed more advanced brain ageing compared to controls, while the first episode psychosis group did not differ significantly from controls. The top features related to advanced brain age, including ventricular enlargement, subcortical thalamic and total grey matter volume showed no significant differences between patients and controls suggesting cumulative minor alterations similar to 'normal ageing' may contribute to advanced brain ageing in psychosis. Counter to our original hypothesis that MRS markers of GSH would negatively correlate with advanced brain ageing, we found that in the combined psychosis group, more advanced brain ageing was positively associated with increased glutathione, alongside more severe symptoms and decreased n-acetyl aspartate. Furthermore, we hypothesised that pro-inflammatory markers of CRP and IL-6 would be positively associated with advanced brain ageing, however our results found no evidence of any relationship with individual peripheral markers of inflammation, including CRP and IL-6.

Our findings are consistent with prior research findings (Koutsouleris et al., 2015; Ballester et al., 2021) however challenge some studies that focused on the early phases of psychosis, which reported an average advancement in brain ageing by 2-3 years (Schnack et al., 2016; Hajek et al., 2017; Kolenic et al., 2018). Our results do indicate a trend towards a higher brain-age gap in the FEP group compared to HC, indicating that the effect size of accelerated brain ageing in FEP is smaller than in established cases, thus requiring a larger sample size for it to be detected. In fact, the absence of a significant difference in brain-age gap between first episode and established patients also supports the presence of subtle but small effect of ABA in FEP in a sample of this size. One reason for this small effect could be

the presence of more neurobiologically heterogeneous subgroups in the FEP sample, compared to the established cases.

Unlike previous work identifying total grey matter volume as the primary underlying feature driving increased BAG in schizophrenia (Ballester et al., 2023). We did not find any individual SHAP feature to differ between schizophrenia and controls. This may indicate that, at least in the dataset presented, the cumulative impact of subtle brain alterations contributes to the advanced brain ageing seen in schizophrenia. Indeed, many studies have implicated a number of widespread variations in brain volume, area, and thickness in schizophrenia. Madre et al., 2020 identified extensive cortical volume and thickness reductions across the whole brain in schizophrenia, alongside surface area reductions in the superior frontal cortex. Similarly, in their study of 4,474 individuals with schizophrenia, the ENIGMA consortium identified cortical thinning in all Desikan-Killiany (DK) atlas regions bar the bilateral pericalcarine region and smaller cortical area in all DK atlas regions bar the bilateral isthmus cingulate region (Van Erp et al., 2018).

To our knowledge this is the first study to relate peripheral inflammatory markers and MRS-derived neurometabolites to advanced brain ageing. We identified positive associations with ACC GSH and negative associations with ACC tNAA in the combined-schizophrenia group. GSH is the brain's dominant antioxidant, measurement of which is the primary method used to assess oxidative stress within the brain (Palaniyappan et al., 2021). Past research has suggested that there is a reduction in brain GSH within psychosis and therefore, people with schizophrenia are more vulnerable to the damaging effects of free radicals (Do et al., 2000; Kumar et al., 2020). In our recent meta-analysis, we identified significant GSH reductions in the ACC of individuals with established schizophrenia but not first episode, indicating potential dysregulation in oxidative stress pathways in the later stages of the disorder. Though we did find a significant association with study year – when older studies,

likely employing outdated methodologies, were removed, GSH levels in the established group did not differ from controls (Murray et al., 2024).

The positive association of GSH with brain age gap identified here may be counter to some previous findings. Notably, certain studies have reported GSH increases in individuals experiencing first episode psychosis (Limongi et al., 2021; MacKinley et al., 2022). The rise in GSH has been explained as a compensatory reaction to heightened oxidative stress within the brain. Consequently, individuals with schizophrenia in whom processes that promote accelerated brain ageing are active (e.g. neurotoxic oxidative stress), a trigger for a compensatory rise in GSH may operate. Given the cross-sectional nature of our study with two stage specific groups, we are not able to conclude if the elevated GSH levels indeed confer any clinical or functional advantage in patients, though prior studies (Dempster et al., 2020; Mackinley et al., 2022) provide some support to this notion.

N-acetyl-aspartate serves as a commonly utilized indicator of neuronal health (Castellano et al., 2012). Numerous studies have reported diminished levels of tNAA in conjunction with neuronal loss or impaired neuronal metabolism (Hardy et al., 2011; Malaspina et al., 2016; DeMayo et al., 2023). As such, the observed decrease in tNAA linked to the elevated predicted-age difference may implicate ongoing neuronal degradation. Additionally, tNAA is known to exhibit a substantial decline with advancing age. Even though age was controlled for in all our analyses, it remains plausible that this reduction may still mirror the impacts of ageing on brain-predicted age difference.

We did not identify any association between BAG and peripherally measured inflammatory markers in either cohort. Suggesting that peripheral inflammation may not be associated with advanced brain ageing. To date several studies have identified associations between brain structure and blood-based inflammatory markers though findings have been

disparate. In one study, patients presenting with “classic inflammation” demonstrated widespread grey matter volume reductions in a number of brain regions including the anterior cingulate and frontal gyri (Lalousis et al., 2023), alternatively grey matter thickening was seen in patients within a high “inflammatory proband” compared to those in the low inflammatory group (Lizano et al., 2021). Further studies have found no association between peripheral inflammatory markers and brain structure but did find an association with monocyte gene expression and cortical thickness (Cui et al., 2023). These results bring into question the validity of using blood-based inflammatory markers to identify structural and functional differences in the brain and highlights the need for improved methods for non-invasively assessing neuro-inflammation.

The positive associations between BAG and PANSS total, general, and anxiodepressive subscales mirror findings from previous work, with a recent meta-analysis highlighting PANSS scores on all subscales to be increased with advanced brain ageing (Blake et al., 2023), thus highlighting the clinical relevance of this phenomena. Similar to previous studies (Koutsouleris et al., 2014; Kolenic et al., 2018; Wang et al., 2021), we found no association with defined daily dose of antipsychotic medication also suggests that this advancement in brain-age is likely not a result of medicinal intervention. Taken together this highlights the utility of BAG as a neurobiological marker reflecting the severity of psychiatric symptoms in schizophrenia. Clinically targeting advanced brain ageing may offer a novel avenue for interventions aimed at improving overall brain health and mitigating symptom burden in individuals with schizophrenia.

While this study provides valuable insights, certain limitations are worth consideration. The cross-sectional nature of the study limits our ability to establish causal relationships. Longitudinal studies with extended follow-up periods are crucial to elucidate the trajectory of advanced brain ageing over the course of schizophrenia. Furthermore, our

sample size may be underpowered to detect group-specific associations between biomarkers and BAG. Additionally, the study's focus on neurometabolites in the anterior cingulate cortex calls for future investigations into other brain regions to comprehensively understand the neurobiological basis of accelerated ageing in schizophrenia.

In conclusion, this study advances our understanding of advanced brain ageing in schizophrenia, shedding light on its neurobiological underpinnings and clinical implications. The associations with oxidative stress function and symptom burden open avenues for targeted interventions, paving the way for future research aimed at unravelling the complexities of this phenomenon in psychotic disorders.

5.5 Author Contributions

AM conceived the initial research project and collated existing data. Data collection and processing of inflammatory markers were conducted by the SPRING team (BD, PFL, MZK, and SW) prior to analysis. AM conducted all statistical analyses, research, and drafted the article. RU and JR provided supervision, oversight and detailed comments on interpretation and manuscript drafts, PB assisted AM in producing SHAP values, while MW aided in extracting MRS data. All authors critically revised the article.

Chapter 6

General Discussion

6.1 Summary of Chapter Findings

Chapters 1 and 2 provide a comprehensive overview of the topics addressed in this thesis. Chapter 1 focuses on the examination of inflammation in schizophrenia, focusing on its manifestation in the brain and potential mechanisms for peripheral inflammation to reach the CNS. Alongside a brief introduction to inflammation and oxidative stress. The discussion extends to the potential utility of measuring inflammation and oxidative stress in psychotic disorders for informing clinical interventions. Chapter 2 (Murray et al., 2021) consists of a narrative review of schizophrenia and oxidative stress, followed by a detailed exploration of oxidative stress pathways, measurement methods, and their relevance to prevailing theories of schizophrenia pathogenesis. This chapter presents evidence linking oxidative stress to all schizophrenia hypotheses and proposes a theoretical model depicting schizophrenia progression from a primed pro-inflammatory state to symptom manifestation.

Chapter 3 (Murray et al., 2024) presents a systematic review with meta-analysis focusing on 1h-MRS studies investigating GSH levels in schizophrenia-spectrum disorders. The results suggest elevated oxidative stress levels in the brains of stable schizophrenia patients but not in those with first episode psychosis. Meta-regression analysis indicates that variations in acquisition modality did not significantly influence these findings. However, there was a significant association with study year, with the removal of studies predating 2008 causing the observed effect to fall below the significance threshold. Consequently, caution is advised when interpreting the reduced GSH levels observed in later psychosis phases. These findings underscore the importance of patient stratification in schizophrenia

research and advocates for further exploration into how GSH levels may vary across different illness phases.

Chapter 4 examines the identification of detailed inflammatory clusters within a diverse sample of individuals with schizophrenia. A semi-supervised machine learning clustering model was employed, using a combined dataset taken from the BeneMin and SPRING studies to refine previous subgroup classifications and establish illness-related clusters based on four key inflammatory biomarkers: IL-6, CRP, TNF-α, and IFN-γ. The results suggest that a 4-cluster solution is optimal, with three clusters showing unique elevations in CRP, IL-6, or TNF-α levels, while the fourth cluster exhibits biomarker levels similar to those of controls. These clusters demonstrate distinct variations in neurocognitive and clinical scores relative to each other, along with significant reductions in GMV compared to controls. Notably, the IL-6 and CRP clusters exhibit more pronounced impairment in anxiodepressive PANSS domains and neurocognitive tests respectively, as well as the most substantial GMV reductions relative to controls. These findings demonstrate the existence of multiple inflammatory signatures in schizophrenia, with elevated IL-6 and CRP potentially indicating greater impairment. This has implications for developing more targeted immune-focused treatments for individuals with schizophrenia. However, further research is needed to elucidate the precise mechanisms underlying this impairment, as each biomarker can signal through various pathways such as downstream IL-6 or TNF-α signalling resulting pro- or anti-inflammatory effects.

Chapter 5 assesses advanced brain ageing across schizophrenia stages using data taken from the SPRING study. Aiming to elucidate the biological underpinnings of this phenomenon through examination of the associations with peripheral inflammatory markers and central neurometabolites and oxidative stress measures. Two brain-age prediction algorithms were implemented to ensure best accuracy of the results. Shapley's Additive

Explanations was further employed to allow examination of the primary features driving brain-age prediction. The influence of inflammatory markers, neurometabolites and oxidative stress on SHAP values and brain-predicted age difference was assessed using linear regression models. Overall, brain ageing was found to be advanced in the established psychosis group, compared to healthy controls, but not the first episode group. The combined psychosis group (FEP + EST) demonstrated associations between advanced brain ageing, symptom severity and glutathione, but not peripheral inflammatory markers, implicating redox dysregulation in the underlying processes. No difference was found in the top 10 SHAP featured between patients and controls suggesting a cumulative effect of minor brain alterations is contributing to advanced brain ageing.

6.2 Oxidative Stress and Schizophrenia

Numerous studies have presented evidence of increased levels of oxidative stress alongside reduced antioxidant capacity in individuals with schizophrenia. Signs of oxidative-related damage are found in the blood of people with schizophrenia alongside significant reductions in total antioxidant levels within the plasma of non-medicated, medicated, first-episode and chronic schizophrenia patients (Altuntas et al., 2000; Reddy et al., 2003; Yao et al., 2001; Fendri et al., 2006; Raffa et al., 2011; Li et al., 2011; Gunes et al., 2017). Regarding central measures of oxidative stress, two recent meta-analyses of in-vivo 1h-MRS studies have revealed significant reductions in GSH in the ACC region and in studies at 7T respectively (Das et al., 2019, Sydnor and Roalf, 2020).

Data presented in the above chapters refutes these findings to some extent. Chapter 3 could not replicate the outcomes presented in previous meta-analyses, finding no significant difference in GSH between patients and controls when considering a broad schizophrenia

grouping across all clinical stages, including subgroup analysis of 7T or ACC studies. We did however find evidence of a reduction in GSH specific to the later stages of the disorder, though this finding was significantly influenced by study year. Conversely, results from chapter 5 did not find evidence of GSH alterations, compared to matched controls in any disorder stage. Furthermore, an increase in GSH in the ACC was associated with more advanced brain ageing in a combined psychosis group, indicating GSH could be a marker of brain health. Further supplementary analysis of the data presented in chapter 5 revealed no significant direct associations between GSH and neurocognitive or symptom scores (Appendix C).

Increased levels of GSH may serve as a compensatory mechanism in response to oxidative stress insults. However, prolonged exposure to excessive ROS generated by continuous damage can diminish GSH availability over time (Limongi et al., 2021; Palaniyappan et al., 2021a). This observation aligns with the findings in Chapter 3, where reductions in GSH were primarily evident in the later stages of the disorder. Moreover, while oxidative stress is often associated with advanced brain ageing, it may not be the primary causal factor but rather a consequence of ongoing neurodegenerative processes. These processes could trigger a surge in GSH levels as a compensatory mechanism to counteract the surplus of free radicals generated by ongoing damage. However, this hypothesis would imply a reduction in GSH during the later stages of the disorder, where brain ageing is further accelerated – a result not supported by our research. Indeed, in both of the longitudinal studies assessing GSH levels in psychosis, conducted over six months and four years respectively, GSH levels remained consistently stable (Jeon et al., 2021; Wang et al., 2020).

An alternative hypothesis suggests that GSH deficits may only be relevant to a specific subgroup of individuals with schizophrenia (Palaniyappan et al., 2021a). Genetic research has identified specific polymorphisms in the glutamate cysteine ligase catalytic

subunit that are linked to reductions in GSH levels (Xin et al., 2016). These polymorphisms are present in approximately 30% of schizophrenia patients, providing evidence for a subgroup within schizophrenia characterised by GSH deficits. Additionally, significant variability in glutathione levels is observed within psychosis, particularly in the early stages (Palaniyappan et al., 2021b), a finding supported by chapter 3. This variability may indicate that people who belong to this GSH-deficit subgroup are more widely distributed among FEP patients. Individuals in this deficit subgroup could be more susceptible to the harmful effects of oxidative stress, potentially leading to the progression of their condition into more persistent illnesses. It is possible that our study on advanced brain ageing lacked sufficient power to detect this subgroup hence why only positive associations were found between GSH and brain ageing.

6.3 Inflammation and Oxidative Stress

Chronic low-level inflammation has been linked to various clinical and neurocognitive effects in schizophrenia. Elevated levels of circulating CRP are often associated with poorer performance on cognitive tests and lower premorbid IQ in both first episode and chronic patients (Misiak et al., 2018; Watson et al., 2023). Furthermore, increases in IL-6 are believed to be correlated with heightened negative and depressive symptoms, with raised IL-6 commonly found in patients with comorbid depression and psychosis (Khandaker at al., 2018).

Chapter 4 replicates these findings, elucidating specific neurocognitive deficits and increased anxiodepressive symptoms associated with pro-inflammatory CRP or IL-6 cluster characterisation, respectively. However, analysis in Chapter 5 reveals associations between advanced brain ageing, symptom burden, and centrally measured oxidative stress, rather than

peripheral inflammation, in a broader schizophrenia population. This discrepancy might be attributed to the relatively small cluster sizes of these immune-active subgroups, with raised CRP representing 13% and raised IL-6 representing 23% of the total sample in Chapter 4. Such small group sizes may not exert a significant impact on the overall findings, furthermore they are likely more heavily influenced by variations in sample selection.

Inflammation and oxidative stress are often considered intrinsically linked, with damage caused by excessive free radicals thought to trigger inflammatory processes (Lugrin et al., 2014). Additionally, macrophages and microglia utilise reactive oxygen species to destroy invading pathogens (Bordt & Polster, 2014). As such, oxidative stress has been classified as both an inducer and a product of inflammation (Koga et al., 2016).

However, supplementary analysis of the data presented in Chapter 5, finds no association between peripherally measured inflammatory markers and central glutathione levels (see Appendix C). This suggests that this link may not extend beyond the blood-brain barrier. Although some studies have shown that circulating inflammatory cytokines can affect blood-brain barrier permeability, allowing inflammation to reach the brain (McKim et al., 2018; Han et al., 2020), measuring peripheral inflammatory markers does not provide insight into their specific signalling pathways. Many cytokines signal via multiple pathways, leading to diverse downstream effects. For instance, "classic" IL-6 signalling is generally considered anti-inflammatory and contributes to blood-brain barrier maintenance, while "trans" IL-6 signalling is pro-inflammatory and can cause damage to the blood-brain barrier (Del Giudice et al., 2018). Similarly, TNF-α can either have neuro-protective or degenerative effects depending on downstream receptor activation (Probert et al., 2015; Lin et al., 2021). Therefore, peripheral measures of inflammation and oxidative stress may not always accurately reflect central processes.

This is further supported by a supplementary qualitative analysis presented in Chapter 3 (Appendix A). Among the limited number of studies examining both peripheral oxidative stress markers and brain glutathione levels, none found significant associations (Xin et al., 2016; Conus et al., 2018; Da Silva et al., 2018; Bryll et al., 2020; Coughlin et al., 2020; Fisher et al., 2020a). It has been suggested that there might not be a significant transport system for glutathione to cross the blood-brain barrier. Additionally, blood measurements of glutathione are prone to oxidation artifacts during sample preparation and long-term storage, potentially impacting the true redox status (Fisher et al., 2020b).

While inflammation and oxidative stress may be closely related, the evidence presented in this thesis indicates that this relationship might not extend to the blood-brain barrier. In other words, peripheral markers of inflammation and/or oxidative stress may not accurately reflect the brain's condition. Consequently, future studies should prioritise assessing these markers directly within the brain.

6.4 Acquisition Methods

There are a limited number of modalities that allow inflammation and oxidative stress to be measured centrally. Post-mortem studies can offer valuable insight in this regard; however, they do not allow for exploration of the temporal aspects of schizophrenia, often being limited to analysis of the late-stage consequences of schizophrenia. PET studies may allow for assessment of inflammation within the brain by employing TSPO markers to identify the total number of activated microglia (Collste et al., 2017; Ottoy et al., 2018). However, recent works have cast doubt on their ability to do so, with different radioligands providing different results (Hafizi et al., 2017; Selvaraj et al., 2018; Conen et al., 2021). On the other hand, glutathione levels can be assessed in the brain with 1h-MRS relatively easily,

however, due to this method of assessment being in its relative infancy, there has been no consensus on optimal methodology to employ. In conducting an MRS study, researchers must decide on the size and placement of a single voxel in the brain, select which pulse sequence to employ and the length of echo time.

In Chapter 3, our goal was to investigate how these methodological variations might affect results. While we found no significant impact of MRS methodologies, the study year had a notable effect, suggesting potential improvements in data quality control over time. Several confounding factors, such as gender bias, smoking status, and marijuana usage, can influence glutathione levels (Sarafian et al., 1999; Mandal et al., 2012; Chitty et al., 2015). Similar factors exist for inflammatory markers, with smoking, age and BMI known to elevate CRP and IL-6 levels (Ferrucci et al., 2005; Aldaham et al., 2015; Menezes et al., 2019). Additionally, different antipsychotic medications can have varying effects on inflammation and oxidative stress markers (Fisher et al., 2020b; Patlola et al., 2023). However, controlling for all these factors is not feasible in many studies, and even fewer report on them. Future research studies should take care in ensuring the control of external effects on these markers.

6.5 Clinical Implications

The pharmacological treatment of schizophrenia-spectrum disorders has remained largely unchanged for the past 2-3 decades, with all current antipsychotics acting on the dopamine pathway in the brain (Rampino et al., 2019). However, these interventions have significant limitations, often addressing positive symptoms more effectively than negative symptoms and frequently causing adverse side effects such as sedation, weight gain, and cognitive impairment. Chapters 3-5 present data that underscore the involvement of inflammation and oxidative stress in at least a subset of patients with psychosis, suggesting that these factors

could be promising targets for future pharmacological interventions or for monitoring disorder progression.

While some studies have suggested the efficacy of immunomodulatory drugs in alleviating schizophrenia symptoms (Akhondzadeh et al., 2007; Muller et al., 2010; Liu et al., 2014), these findings have often been based on small sample sizes. Larger studies have failed to demonstrate beneficial effects on symptom severity or brain-related changes, such as reductions in grey matter volume (Deakin et al., 2018; Jeppesen et al., 2020). Similarly, antioxidant interventions have yielded mixed results, with studies reporting varying efficacy of antioxidant drugs like N-acetylcysteine (Zheng et al., 2018; Conus et al., 2018; Yolland et al., 2020).

Multiple studies have proposed the existence of an immune active subgroup within schizophrenia, comprising up to 40% of the total population. In their 2023 paper, Lalousis et al. identified five inflammation-related subgroups, with the elevated IL-6/IL-8 group showing the most widespread reductions in grey matter volume. Results from chapter 4 support the existence of distinct inflammatory clusters within schizophrenia, corroborating the findings of previous work in a reduced set of cytokines. The presence of these subgroups may explain the varied results of clinical trials of anti-inflammatories. Patients without underlying inflammation may not benefit from these treatments. Stratifying patients based on inflammation levels could help identify anti-inflammatory responders in future studies. Evidence from depression research supports this approach; patients with higher CRP levels showed significant improvements when treated with minocycline (Nettis et al., 2021).

Likewise, there is likely to be a subgroup within schizophrenia with a deficit in GSH. Antioxidant therapies may be more beneficial for patients with pre-existing deficits in central antioxidants. However, clinical trials of antioxidants have not attempted to stratify patients based on their GSH levels. There may be overlap between the immune active and GSH-

deficit subgroups, as some antioxidants have anti-inflammatory effects, for example NAC can suppress the activity of nuclear factor kappa B (NF-κB), thereby reducing the levels of TNF-α, IL-6 and IL-1β (Tenorio et al., 2021). Further research is needed to explore the extent of this overlap and the potential for combined anti-inflammatory and antioxidant interventions.

Glutathione may also serve as a marker of brain health. MRS studies have demonstrated a decrease in GSH in the precuneus, cingulate, and occipital regions associated with advancing age (Detcheverry et al., 2023). ageing is often associated with impairments in CNS function, resulting from loss of neurons and leading to diminished cognitive performance (Iskusnykh, et al., 2022). Advanced brain ageing has been observed in schizophrenia and is linked to increased symptom severity. Chapter 5 reveals significant positive associations between ACC levels of glutathione and brain-predicted age difference within schizophrenia patients but not healthy controls. This association suggests that individuals with poorer outcomes in the disorder may exhibit significant variations in central GSH levels, indicating a potential need for additional interventions. This study represents the first attempt to relate MRS-derived GSH to advanced brain ageing, providing insight into brain health as the disorder progresses.

6.6 Strengths and Limitations

This thesis has notable strengths, including utilising multi-site data from BeneMin and SPRING. The meta-analysis in Chapter 3 represents the largest cohort of GSH 1h-MRS studies to date and demonstrates minimal influence from publication bias. Robust data-analysis techniques were applied for the classification of inflammatory clusters. The semi-supervised machine learning approach in Chapter 4 offers several advantages over traditional

hierarchical clustering methods, allowing for the identification of schizophrenia-specific subgroups that are distinguishable from healthy controls and less susceptible to underlying variance in age and gender. Additionally, for the analysis of accelerated brain ageing in Chapter 5, two separate models were employed to ensure prediction accuracy, with further analysis of contributing features via SHAP values aiding in the understanding of the machine learning tools' predictions. The results are also linked to clinical practice, aiming to enhance tools for patient stratification and precision medicine.

However, certain limitations must be acknowledged. Due to the nature of the datasets, controlling for certain confounding factors in all analyses was not feasible. For instance, medication status could not be adequately controlled for in Chapter 4 due to variations in reporting between the two studies, and controlling for site effects was challenging since the entire healthy control cohort came from the SPRING study. Sampling errors associated with the clustering algorithm may have been introduced, as the clusters defined were primarily relevant to individual inflammatory biomarkers rather than a combination of them. It is possible that the machine learning algorithm selected biomarker extremes as the basis for clusters due to their highly correlated nature.

Furthermore, given the substantial heterogeneity observed in previous inflammation and oxidative stress research, it is likely that both Chapters 4 and 5 were underpowered. The relatively small sizes of the inflammatory clusters may have limited the statistical power to detect meaningful differences in brain morphology in Chapter 4, and in Chapter 5, the sample size may have been insufficient to detect group-specific associations between biomarkers and BAG. Additionally, all data presented is cross-sectional in nature, restricting the ability to establish causal relationships between inflammation, oxidative stress, and brain morphology. Longitudinal studies with extended follow-up periods are essential to elucidate the trajectory

of these biomarkers and their relationship to clinical symptoms, neurocognitive scores, and changes in brain structure over the course of schizophrenia.

6.7 Concluding Remarks

In conclusion, the research presented in this thesis offers a nuanced understanding of the complex interplay between oxidative stress, inflammation, and schizophrenia. While previous studies have consistently pointed to the involvement of oxidative stress in schizophrenia, our findings suggest that the relationship between oxidative stress markers, particularly GSH, and the disorder's progression is more intricate than previously thought. Contrary to some existing meta-analyses, our systematic review in Chapter 3 could not replicate the consistent reductions observed in GSH across all stages of schizophrenia, highlighting potential variations in illness phase, supporting the notion of distinct GSH-deficit subgroups within the disorder. Furthermore, Chapter 5 finds evidence of a potential compensatory increase in GSH levels associated with advanced brain ageing, as opposed to the relative decreases in GSH noted by other MRS studies. These results emphasize the need for a more detailed understanding of oxidative stress dynamics in schizophrenia.

Our research also sheds light on the role of inflammation in schizophrenia, demonstrating its association with specific brain-related variations and neurocognitive deficits. However, our analysis suggests a disconnect between peripheral markers of inflammation and centrally measured oxidative stress, demonstrating the limitations of relying solely on peripheral measures as a proxy of central processes in schizophrenia. This highlights the importance of direct assessment of markers within the brain.

Furthermore, our findings emphasize the substantial heterogeneity within schizophrenia, particularly regarding immune activation and GSH deficits. Identifying

subgroups based on these factors could pave the way for more targeted interventions, potentially improving treatment efficacy and patient outcomes. Furthermore, the association between GSH levels and brain ageing highlights the potential utility of GSH as a marker of brain health and disease progression in schizophrenia, though the directionality of this association remains unclear.

Overall, this thesis contributes to a deeper understanding of the molecular mechanisms underlying schizophrenia, offering insights that could inform the development of novel therapeutic approaches tailored to individual patient profiles. However, further research is warranted to elucidate the precise mechanisms driving oxidative stress and inflammation in schizophrenia and to validate the potential utility of GSH and immune profiling in clinical practice.

References

7.1 The Relevance of Inflammation in Psychosis-Spectrum Disorders - References

Akhondzadeh, S., Tabatabaee, M., Amini, H., Ahmadi Abhari, S. A., Abbasi, S. H., & Behnam, B. (2007). Celecoxib as adjunctive therapy in schizophrenia: A double-blind, randomized and placebo-controlled trial. *Schizophrenia Research*, *90*(1), 179–185. https://doi.org/10.1016/j.schres.2006.11.016

Alexandros Lalousis, P., Schmaal, L., Wood, S. J., L.E.P Reniers, R., Cropley, V. L., Watson, A., Pantelis, C., Suckling, J., Barnes, N. M., Pariante, C., Jones, P. B., Joyce, E., Barnes, T. R. E., Lawrie, S. M., Husain, N., Dazzan, P., Deakin, B., Shannon Weickert, C., & Upthegrove, R. (2023). Inflammatory subgroups of schizophrenia and their association with brain structure: A semi-supervised machine learning examination of heterogeneity. *Brain, Behavior, and Immunity*, *113*, 166–175. https://doi.org/10.1016/j.bbi.2023.06.023

Ament, S. A., Cortes-Gutierrez, M., Herb, B. R., Mocci, E., Colantuoni, C., & McCarthy, M. M. (2023). A single-cell genomic atlas for maturation of the human cerebellum during early childhood. Science Translational Medicine, 15(721), eade1283. https://doi.org/10.1126/scitranslmed.ade1283

Andrade, L. H., & Wang, Y.-P. (2012). Prevalence of psychotic symptoms in the general population varies across 52 countries. *Evidence Based Mental Health*, *15*(4), 105–105. https://doi.org/10.1136/ebmental-2012-100849

Augusto-Oliveira, M., Arrifano, G. P., Lopes-Araújo, A., Santos-Sacramento, L., Takeda, P. Y., Anthony, D. C., Malva, J. O., & Crespo-Lopez, M. E. (2019). What Do Microglia Really Do in Healthy Adult Brain? *Cells*, *8*(10). https://doi.org/10.3390/cells8101293

Banks, W. A. (2005). Blood-brain barrier transport of cytokines: A mechanism for neuropathology. *Current Pharmaceutical Design*, *11*(8), 973–984. https://doi.org/10.2174/1381612053381684

Beiser, M., Erikson, D., Fleming, J. A., & Iacono, W. G. (1993). Establishing the onset of psychotic illness. *American Journal of Psychiatry*, *150*(9), 1349–1354. https://doi.org/10.1176/ajp.150.9.1349

Bian, Z., Gong, Y., Huang, T., Lee, C. Z. W., Bian, L., Bai, Z., Shi, H., Zeng, Y., Liu, C., He, J., Zhou, J., Li, X., Li, Z., Ni, Y., Ma, C., Cui, L., Zhang, R., Chan, J. K. Y., Ng, L. G., … Liu, B. (2020). Deciphering human macrophage development at single-cell resolution. *Nature*, *582*(7813), 571–576. https://doi.org/10.1038/s41586-020-2316-7

Biber, K., Owens, T., & Boddeke, E. (2014). What is microglia neurotoxicity (Not)? *Glia*, *62*(6), 841–854. https://doi.org/10.1002/glia.22654

Bishop, J. R., Zhang, L., & Lizano, P. (2022). Inflammation Subtypes and Translating Inflammation-Related Genetic Findings in Schizophrenia and Related Psychoses: A

Perspective on Pathways for Treatment Stratification and Novel Therapies. *Harvard Review of Psychiatry*, *30*(1), 59–70. https://doi.org/10.1097/HRP.0000000000000321

Bloomfield, P. S., Selvaraj, S., Veronese, M., Rizzo, G., Bertoldo, A., Owen, D. R., Bloomfield, M. A., Bonoldi, I., Kalk, N., Turkheimer, F., McGuire, P., de Paola, V., & Howes, O. D. (2016). Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [(11)C]PBR28 PET Brain Imaging Study. *The American Journal of Psychiatry*, *173*(1), 44–52. https://doi.org/10.1176/appi.ajp.2015.14101358

Blyler, C. R., Gold, J. M., Iannone, V. N., & Buchanan, R. W. (2000). Short form of the WAIS-III for use with patients with schizophrenia. *Schizophrenia Research*, *46*(2), 209–215. https://doi.org/10.1016/S0920-9964(00)00017-7

Busse, S., Busse, M., Schiltz, K., Bielau, H., Gos, T., Brisch, R., Mawrin, C., Schmitt, A., Jordan, W., Müller, U. J., Bernstein, H.-G., Bogerts, B., & Steiner, J. (2012). Different distribution patterns of lymphocytes and microglia in the hippocampus of patients with residual versus paranoid schizophrenia: Further evidence for disease course-related immune alterations? *Brain, Behavior, and Immunity*, *26*(8), 1273–1279. https://doi.org/10.1016/j.bbi.2012.08.005

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208

Chen, Y., Cinnamon Bidwell, L., & Norton, D. (2006). Trait vs. State Markers for Schizophrenia: Identification and Characterization Through Visual Processes. *Current Psychiatry Reviews*, *2*(4), 431–438. https://doi.org/10.2174/157340006778699729

Collste, K., Plavén-Sigray, P., Fatouros-Bergman, H., Victorsson, P., Schain, M., Forsberg, A., Amini, N., Aeinehband, S., Karolinska Schizophrenia Project (KaSP) consortium, Erhardt, S., Halldin, C., Flyckt, L., Farde, L., & Cervenka, S. (2017). Lower levels of the glial cell marker TSPO in drug-naive first-episode psychosis patients as measured using PET and [11C]PBR28. *Molecular Psychiatry*, *22*(6), 850–856. https://doi.org/10.1038/mp.2016.247

Conen, S., Gregory, C. J., Hinz, R., Smallman, R., Corsi-Zuelli, F., Deakin, B., & Talbot, P. S. (2021). Neuroinflammation as measured by positron emission tomography in patients with recent onset and established schizophrenia: Implications for immune pathogenesis. *Molecular Psychiatry*, *26*(9), 5398–5406. https://doi.org/10.1038/s41380-020-0829-y

D'Mello, C., Le, T., & Swain, M. G. (2009). Cerebral Microglia Recruit Monocytes into the Brain in Response to Tumor Necrosis Factorα Signaling during Peripheral Organ Inflammation. *The Journal of Neuroscience*, *29*(7), 2089–2102. https://doi.org/10.1523/JNEUROSCI.3567-08.2009

Dawidowski, B., Górniak, A., Podwalski, P., Lebiecka, Z., Misiak, B., & Samochowiec, J. (2021). The Role of Cytokines in the Pathogenesis of Schizophrenia. *Journal of Clinical Medicine*, *10*(17), Article 17. https://doi.org/10.3390/jcm10173849

Dayabandara, M., Hanwella, R., Ratnatunga, S., Seneviratne, S., Suraweera, C., & de Silva, V. A. (2017). Antipsychotic-associated weight gain: Management strategies and impact on treatment adherence. *Neuropsychiatric Disease and Treatment*, *13*, 2231–2241. https://doi.org/10.2147/NDT.S113099

Deakin, B., Suckling, J., Barnes, T. R. E., Byrne, K., Chaudhry, I. B., Dazzan, P., Drake, R. J., Giordano, A., Husain, N., Jones, P. B., Joyce, E., Knox, E., Krynicki, C., Lawrie, S. M., Lewis, S., Lisiecka-Ford, D. M., Nikkheslat, N., Pariante, C. M., Smallman, R., … Dunn, G. (2018). The benefit of minocycline on negative symptoms of schizophrenia in patients with recent-onset psychosis (BeneMin): A randomised, double-blind, placebo-controlled trial. *The Lancet Psychiatry*, *5*(11), 885–894. https://doi.org/10.1016/S2215-0366(18)30345-6

Delaney, S., Fallon, B., Alaedini, A., Yolken, R., Indart, A., Feng, T., Wang, Y., & Javitt, D. (2019). Inflammatory biomarkers in psychosis and clinical high risk populations. *Schizophrenia Research*, *206*, 440–443. https://doi.org/10.1016/j.schres.2018.10.017

Do, J., & Woo, J. (2018). From Gut to Brain: Alteration in Inflammation Markers in the Brain of Dextran Sodium Sulfate-induced Colitis Model Mice. *Clinical Psychopharmacology and Neuroscience: The Official Scientific Journal of the Korean College of Neuropsychopharmacology*, *16*(4), 422–433. https://doi.org/10.9758/cpn.2018.16.4.422

Dolapcioglu, C., & Dolapcioglu, H. (2015). Structural brain lesions in inflammatory bowel disease. *World Journal of Gastrointestinal Pathophysiology*, *6*(4), 124–130. https://doi.org/10.4291/wjgp.v6.i4.124

Dotiwala, A. K., McCausland, C., & Samra, N. S. (2024). Anatomy, Head and Neck: Blood Brain Barrier. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK519556/

Dunleavy, C., Elsworthy, R. J., Upthegrove, R., Wood, S. J., & Aldred, S. (2022). Inflammation in first-episode psychosis: The contribution of inflammatory biomarkers to the emergence of negative symptoms, a systematic review and meta-analysis. *Acta Psychiatrica Scandinavica*, *146*(1), 6–20. https://doi.org/10.1111/acps.13416

Enrico, P., Delvecchio, G., Turtulici, N., Aronica, R., Pigoni, A., Squarcina, L., Villa, F. M., Perlini, C., Rossetti, M. G., Bellani, M., Lasalvia, A., Bonetto, C., Scocco, P., D'Agostino, A., Torresani, S., Imbesi, M., Bellini, F., Veronese, A., Bocchio-Chiavetto, L., … GET UP Group. (2023). A machine learning approach on whole blood immunomarkers to identify an inflammation-associated psychosis onset subgroup. *Molecular Psychiatry*, *28*(3), 1190–1200. https://doi.org/10.1038/s41380-022-01911-1

Fendri, C., Mechri, A., Khiari, G., Othman, A., Kerkeni, A., & Gaha, L. (2006). Oxidative stress involvement in schizophrenia pathophysiology: A review. *L'Encephale*, *32*(2 Pt 1), 244–252. https://doi.org/10.1016/s0013-7006(06)76151-6

Fillman, S. G., Cloonan, N., Catts, V. S., Miller, L. C., Wong, J., McCrossin, T., Cairns, M., & Weickert, C. S. (2013). Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Molecular Psychiatry*, *18*(2), 206–214. https://doi.org/10.1038/mp.2012.110

Foley, É. M., Griffiths, S. L., Murray, A., Rogers, J., Corsi-Zuelli, F., Hickinbotham, H., Warwick, E., Wilson, M., Kaser, M., Murray, G. K., Deakin, B., Jadon, D., Suckling, J., Barnes, N. M., Upthegrove, R., Khandaker, G. M., & PIMS Collaboration. (2023). Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A randomised double-blind placebo-controlled trial of single-dose tocilizumab in patients with psychosis. *BMJ Open*, *13*(3), e067944. https://doi.org/10.1136/bmjopen-2022-067944

Fond, G., Lançon, C., Korchia, T., Auquier, P., & Boyer, L. (2020). The Role of Inflammation in the Treatment of Schizophrenia. *Frontiers in Psychiatry*, *11*, 160. https://doi.org/10.3389/fpsyt.2020.00160

Gober, R., Ardalan, M., Shiadeh, S. M. J., Duque, L., Garamszegi, S. P., Ascona, M., Barreda, A., Sun, X., Mallard, C., & Vontell, R. T. (2022). Microglia activation in postmortem brains with schizophrenia demonstrates distinct morphological changes between brain regions. *Brain Pathology*, *32*(1), e13003. https://doi.org/10.1111/bpa.13003

Gross, G. (1989). The 'Basic' Symptoms of Schizophrenia. *The British Journal of Psychiatry*, *155*(S7), 21–25. https://doi.org/10.1192/S0007125000291423

Gunes, M., Altindag, A., Bulut, M., Demir, S., Ibiloglu, A. O., Kaya, M. C., Atli, A., & Aksoy, N. (2017). Oxidative metabolism may be associated with negative symptoms in schizophrenia. *Psychiatry and Clinical Psychopharmacology*, *27*(1), 54–61. https://doi.org/10.1080/24750573.2017.1293243

Hafizi, S., Tseng, H.-H., Rao, N., Selvanathan, T., Kenk, M., Bazinet, R. P., Suridjan, I., Wilson, A. A., Meyer, J. H., Remington, G., Houle, S., Rusjan, P. M., & Mizrahi, R. (2017). Imaging Microglial Activation in Untreated First-Episode Psychosis: A PET Study With [18F]FEPPA. *The American Journal of Psychiatry*, *174*(2), 118–124. https://doi.org/10.1176/appi.ajp.2016.16020171

Halstead, S., Siskind, D., Amft, M., Wagner, E., Yakimov, V., Shih-Jung Liu, Z., Walder, K., & Warren, N. (2023). Alteration patterns of peripheral concentrations of cytokines and associated inflammatory proteins in acute and chronic stages of schizophrenia: A systematic review and network meta-analysis. *The Lancet Psychiatry*, *10*(4), 260–271. https://doi.org/10.1016/S2215-0366(23)00025-1

Han, Y., Ding, L., Cheng, X., Zhao, M., Zhao, T., Guo, L., Li, X., Geng, Y., Fan, M., Liao, H., & Zhu, L. (2020). Hypoxia Augments Cerebral Inflammation in a Dextran Sulfate Sodium-Induced Colitis Mouse Model. *Frontiers in Cellular Neuroscience*, *14*, 611764. https://doi.org/10.3389/fncel.2020.611764

Hartmann, S.-M., Heider, J., Wüst, R., Fallgatter, A. J., & Volkmer, H. (2024). Microglia-neuron interactions in schizophrenia. *Frontiers in Cellular Neuroscience*, *18*. https://doi.org/10.3389/fncel.2024.1345349

He, X.-F., Li, L.-L., Xian, W.-B., Li, M.-Y., Zhang, L.-Y., Xu, J.-H., Pei, Z., Zheng, H.-Q., & Hu, X.-Q. (2021). Chronic colitis exacerbates NLRP3-dependent neuroinflammation and cognitive impairment in middle-aged brain. *Journal of Neuroinflammation*, *18*(1), 153. https://doi.org/10.1186/s12974-021-02199-8

Henein, M. Y., Vancheri, S., Longo, G., & Vancheri, F. (2022). The Role of Inflammation in Cardiovascular Disease. *International Journal of Molecular Sciences*, *23*(21), 12906. https://doi.org/10.3390/ijms232112906

Hercher, C., Chopra, V., & Beasley, C. L. (2014). Evidence for morphological alterations in prefrontal white matter glia in schizophrenia and bipolar disorder. *Journal of Psychiatry & Neuroscience : JPN*, *39*(6), 376–385. https://doi.org/10.1503/jpn.130277

Hodges, A., Byrne, M., Grant, E., & Johnstone, E. (1999). People at risk of schizophrenia: Sample characteristics of the first 100 cases in the Edinburgh High-Risk Study. *British Journal of Psychiatry*, *174*(6), 547–553. https://doi.org/10.1192/bjp.174.6.547

Howes, O. D., & Onwordi, E. C. (2023). The synaptic hypothesis of schizophrenia version III: A master mechanism. Molecular Psychiatry, 28(5), 1843–1856. https://doi.org/10.1038/s41380-023-02043-w

Jeong, H.-K., Ji, K., Min, K., & Joe, E.-H. (2013). Brain Inflammation and Microglia: Facts and Misconceptions. *Experimental Neurobiology*, *22*(2), 59–67. https://doi.org/10.5607/en.2013.22.2.59

Jeppesen, R., Christensen, R. H. B., Pedersen, E. M. J., Nordentoft, M., Hjorthøj, C., Köhler-Forsberg, O., & Benros, M. E. (2020). Efficacy and safety of anti-inflammatory agents in treatment of psychotic disorders – A comprehensive systematic review and meta-analysis. *Brain, Behavior, and Immunity*, *90*, 364–380. https://doi.org/10.1016/j.bbi.2020.08.028

Jones, P. B., Bebbington, P., Foerster, A. D. I., Lewis, S. W., Murray, R. M., Russell, A. P. G., … Wilkins, S. (1993). Premorbid social underachievement in schizophrenia. Results from the Camberwell Collaborative Psychosis Study. *The British Journal of Psychiatry : The Journal of Mental Science*, *162*, 65–71. https://doi.org/10.1192/bjp.162.1.65

Kang, R., Gamdzyk, M., Lenahan, C., Tang, J., Tan, S., & Zhang, J. H. (2020). The Dual Role of Microglia in Blood-Brain Barrier Dysfunction after Stroke. *Current Neuropharmacology*, *18*(12), 1237–1249. https://doi.org/10.2174/1570159X18666200529150907

Kawamoto, R., Kusunoki, T., Abe, M., Kohara, K., & Miki, T. (2013). An association between body mass index and high-sensitivity C-reactive protein concentrations is influenced by age in community-dwelling persons. Annals of Clinical Biochemistry, 50(5), 457–464. https://doi.org/10.1177/0004563212473445

Kelly, K. M., Smith, J. A., & Mezuk, B. (2021). Depression and interleukin-6 signaling: A Mendelian Randomization study. *Brain, Behavior, and Immunity*, *95*, 106–114. https://doi.org/10.1016/j.bbi.2021.02.019

Kettenmann, H., Hanisch, U.-K., Noda, M., & Verkhratsky, A. (2011). Physiology of microglia. *Physiological Reviews*, *91*(2), 461–553. https://doi.org/10.1152/physrev.00011.2010

Khandaker, G. M., Cousins, L., Deakin, J., Lennox, B. R., Yolken, R., & Jones, P. B. (2015). Inflammation and immunity in schizophrenia: Implications for pathophysiology and treatment. *The Lancet. Psychiatry*, *2*(3), 258–270. https://doi.org/10.1016/S2215-0366(14)00122-9

Khoury, R., & Nasrallah, H. A. (2018). Inflammatory biomarkers in individuals at clinical high risk for psychosis (CHR-P): State or trait? *Schizophrenia Research*, *199*, 31–38. https://doi.org/10.1016/j.schres.2018.04.017

Kinney, J. W., Bemiller, S. M., Murtishaw, A. S., Leisgang, A. M., Salazar, A. M., & Lamb, B. T. (2018). Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's & Dementia : Translational Research & Clinical Interventions*, *4*, 575–590. https://doi.org/10.1016/j.trci.2018.06.014

Kirkpatrick, B., & Miller, B. J. (2013). Inflammation and Schizophrenia. *Schizophrenia Bulletin*, *39*(6), 1174–1179. https://doi.org/10.1093/schbul/sbt141

Klaus, F., Nguyen, T. T., Thomas, M. L., Liou, S. C., Soontornniyomkij, B., Mitchell, K., Daly, R., Sutherland, A. N., Jeste, D. V., & Eyler, L. T. (2022). Peripheral inflammation levels associated with degree of advanced brain ageing in schizophrenia. *Frontiers in Psychiatry*, *13*, 966439. https://doi.org/10.3389/fpsyt.2022.966439

Kodishala, C., Hulshizer, C. A., Kronzer, V. L., Davis, J. M., Ramanan, V. K., Vassilaki, M., Mielke, M. M., Crowson, C. S., & Myasoedova, E. (2023). Risk Factors for Dementia in Patients With Incident Rheumatoid Arthritis: A Population-Based Cohort Study. *The Journal of Rheumatology*, *50*(1), 48–55. https://doi.org/10.3899/jrheum.220200

Laskaris, L. E., Di Biase, M. A., Everall, I., Chana, G., Christopoulos, A., Skafidas, E., Cropley, V. L., & Pantelis, C. (2016). Microglial activation and progressive brain changes in schizophrenia. *British Journal of Pharmacology*, *173*(4), 666–680. https://doi.org/10.1111/bph.13364

Leucht, S., Cipriani, A., Spineli, L., Mavridis, D., Orey, D., Richter, F., … Davis, J. M. (2013). Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: A multiple-treatments meta-analysis. *Lancet (London, England)*, *382*(9896), 951–962. https://doi.org/10.1016/S0140-6736(13)60733-3

Lin, P., Sun, J., Lou, X., Li, D., Shi, Y., Li, Z., Ma, P., Li, P., Chen, S., Jin, W., Liu, S., Chen, Q., Gao, Q., Zhu, L., Xu, J., Zhu, M., Wang, M., Liang, K., Zhao, L., … Guo, X. (2022). Consensus on potential biomarkers developed for use in clinical tests for schizophrenia. *General Psychiatry*, *35*(1), e100685. https://doi.org/10.1136/gpsych-2021-100685

Liu, F., Guo, X., Wu, R., Ou, J., Zheng, Y., Zhang, B., Xie, L., Zhang, L., Yang, L., Yang, S., Yang, J., Ruan, Y., Zeng, Y., Xu, X., & Zhao, J. (2014). Minocycline supplementation for treatment of negative symptoms in early-phase schizophrenia: A double blind, randomized, controlled trial. *Schizophrenia Research*, *153*(1), 169–176. https://doi.org/10.1016/j.schres.2014.01.011

Mantovani, A., Sica, A., & Locati, M. (2005). Macrophage polarization comes of age. *Immunity*, *23*(4), 344–346. https://doi.org/10.1016/j.immuni.2005.10.001

Marcinowicz, P., Więdłocha, M., Zborowska, N., Dębowska, W., Podwalski, P., Misiak, B., Tyburski, E., & Szulc, A. (2021). A Meta-Analysis of the Influence of Antipsychotics on Cytokines Levels in First Episode Psychosis. *Journal of Clinical Medicine*, *10*(11), 2488. https://doi.org/10.3390/jcm10112488

Martínez-Cengotitabengoa, M., Micó, J. A., Arango, C., Castro-Fornieles, J., Graell, M., Payá, B., Leza, J. C., Zorrilla, I., Parellada, M., López, M. P., Baeza, I., Moreno, C., Rapado-Castro, M., & González-Pinto, A. (2014). Basal low antioxidant capacity correlates with cognitive deficits in early onset psychosis. A 2-year follow-up study. Schizophrenia Research, 156(1), 23–29. https://doi.org/10.1016/j.schres.2014.03.025

Martinuzzi, E., Barbosa, S., Daoudlarian, D., Bel Haj Ali, W., Gilet, C., Fillatre, L., Khalfallah, O., Troudet, R., Jamain, S., Fond, G., Sommer, I., Leucht, S., Dazzan, P., McGuire, P., Arango, C., Diaz-Caneja, C. M., Fleischhacker, W., Rujescu, D., Glenthøj, B., … Glaichenhaus, N. (2019). Stratification and prediction of remission in first-episode psychosis patients: The OPTiMiSE cohort study. *Translational Psychiatry*, *9*, 20. https://doi.org/10.1038/s41398-018-0366-5

McFarlane, W. R. (2011). Prevention of the First Episode of Psychosis. *The Psychiatric Clinics of North America*, *34*(1), 95–107. https://doi.org/10.1016/j.psc.2010.11.012

McKim, D. B., Weber, M. D., Niraula, A., Sawicki, C. M., Liu, X., Jarrett, B. L., Ramirez-Chan, K., Wang, Y., Roeth, R. M., Sucaldito, A. D., Sobol, C. G., Quan, N., Sheridan, J. F., & Godbout, J. P. (2018). Microglial recruitment of IL-1β-producing monocytes to brain endothelium causes stress-induced anxiety. *Molecular Psychiatry*, *23*(6), 1421–1431. https://doi.org/10.1038/mp.2017.64

Megha, KB., Joseph, X., Akhil, V., & Mohanan, PV. (2021). Cascade of immune mechanism and consequences of inflammatory disorders. Phytomedicine, 91, 153712. https://doi.org/10.1016/j.phymed.2021.153712

Metcalf, S. A., Jones, P. B., Nordstrom, T., Timonen, M., Mäki, P., Miettunen, J., Jääskeläinen, E., Järvelin, M.-R., Stochl, J., Murray, G. K., Veijola, J., & Khandaker, G. M. (2017). Serum C-reactive protein in adolescence and risk of schizophrenia in adulthood: A prospective birth cohort study. *Brain, Behavior, and Immunity*, *59*, 253–259. https://doi.org/10.1016/j.bbi.2016.09.008

Miller, B. J., & Goldsmith, D. R. (2020). Evaluating the Hypothesis That Schizophrenia Is an Inflammatory Disorder. *Focus: Journal of Life Long Learning in Psychiatry*, *18*(4), 391–401. https://doi.org/10.1176/appi.focus.20200015

Miller, B. J., Culpepper, N., Rapaport, M. H., & Buckley, P. (2013). Prenatal inflammation and neurodevelopment in schizophrenia: A review of human studies. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *42*, 92–100. https://doi.org/10.1016/j.pnpbp.2012.03.010

Miller, B. J., Gassama, B., Sebastian, D., Buckley, P., & Mellor, A. (2013). Meta-analysis of lymphocytes in schizophrenia: Clinical status and antipsychotic effects. *Biological Psychiatry*, *73*(10), 993–999. https://doi.org/10.1016/j.biopsych.2012.09.007

Misiak, B., Bartoli, F., Carrà, G., Stańczykiewicz, B., Gładka, A., Frydecka, D., Samochowiec, J., Jarosz, K., Hadryś, T., & Miller, B. J. (2021). Immune-inflammatory markers and psychosis risk: A systematic review and meta-analysis.

Psychoneuroendocrinology, *127*, 105200. https://doi.org/10.1016/j.psyneuen.2021.105200

Mondelli, V., Ciufolini, S., Belvederi Murri, M., Bonaccorso, S., Di Forti, M., Giordano, A., Marques, T. R., Zunszain, P. A., Morgan, C., Murray, R. M., Pariante, C. M., & Dazzan, P. (2015). Cortisol and Inflammatory Biomarkers Predict Poor Treatment Response in First Episode Psychosis. *Schizophrenia Bulletin*, *41*(5), 1162–1170. https://doi.org/10.1093/schbul/sbv028

Morales-Muñoz, I., Palmer, E. R., Marwaha, S., Mallikarjun, P. K., & Upthegrove, R. (2022). Persistent Childhood and Adolescent Anxiety and Risk for Psychosis: A Longitudinal Birth Cohort Study. Biological Psychiatry, 92(4), 275–282. https://doi.org/10.1016/j.biopsych.2021.12.003

Morrens, M., Overloop, C., Coppens, V., Loots, E., Van Den Noortgate, M., Vandenameele, S., Leboyer, M., & De Picker, L. (2022). The relationship between immune and cognitive dysfunction in mood and psychotic disorder: A systematic review and a meta-analysis. *Molecular Psychiatry*, *27*(8), 3237–3246. https://doi.org/10.1038/s41380-022-01582-y

Muench, J., & Hamer, A. M. (2010). Adverse effects of antipsychotic medications. *American Family Physician*, *81*(5), 617–622.

Müller, N., Krause, D., Dehning, S., Musil, R., Schennach-Wolff, R., Obermeier, M., Möller, H.-J., Klauss, V., Schwarz, M. J., & Riedel, M. (2010). Celecoxib treatment in an early stage of schizophrenia: Results of a randomized, double-blind, placebo-controlled trial of celecoxib augmentation of amisulpride treatment. *Schizophrenia Research*, *121*(1), 118–124. https://doi.org/10.1016/j.schres.2010.04.015

National Institute for Health and Care Excellence. (2014). *Psychosis and schizophrenia in adults: prevention and management* (NICE Clinical guideline [CG178]). Retrieved from https://www.nice.org.uk/guidance/cg178

National Intitute of Mental Health (2016) *Fact Sheet: First Episode Psychosis* (NIH Publication No. OM 16-4306). Retrieved from https://www.nimh.nih.gov/health/publications/raise-fact-sheet-first-episode-psychosis

Nettis, M. A., Lombardo, G., Hastings, C., Zajkowska, Z., Mariani, N., Nikkheslat, N., Worrell, C., Enache, D., McLaughlin, A., Kose, M., Sforzini, L., Bogdanova, A., Cleare, A., Young, A. H., Pariante, C. M., & Mondelli, V. (2021). Augmentation therapy with minocycline in treatment-resistant depression patients with low-grade peripheral inflammation: Results from a double-blind randomised clinical trial. *Neuropsychopharmacology*, *46*(5), 939–948. https://doi.org/10.1038/s41386-020-00948-6

Nettis, M. A., Pergola, G., Kolliakou, A., O'Connor, J., Bonaccorso, S., David, A., Gaughran, F., Di Forti, M., Murray, R. M., Marques, T. R., Blasi, G., Bertolino, A., Pariante, C. M., Dazzan, P., & Mondelli, V. (2019). Metabolic-inflammatory status as predictor of clinical outcome at 1-year follow-up in patients with first episode psychosis. *Psychoneuroendocrinology*, *99*, 145–153. https://doi.org/10.1016/j.psyneuen.2018.09.005

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo. *Science*, *308*(5726), 1314–1318. https://doi.org/10.1126/science.1110647

Norden, D. M., Muccigrosso, M. M., & Godbout, J. P. (2015). Microglial priming and enhanced reactivity to secondary insult in ageing, and traumatic CNS injury, and neurodegenerative disease. *Neuropharmacology*, *96*, 29–41. https://doi.org/10.1016/j.neuropharm.2014.10.028

Ochoa, S., Usall, J., Cobo, J., Labad, X., & Kulkarni, J. (2012). Gender differences in schizophrenia and first-episode psychosis: A comprehensive literature review. *Schizophrenia Research and Treatment*, *2012*, 916198. https://doi.org/10.1155/2012/916198

Ochocka, N., & Kaminska, B. (2021). Microglia Diversity in Healthy and Diseased Brain: Insights from Single-Cell Omics. *International Journal of Molecular Sciences*, *22*(6), 3027. https://doi.org/10.3390/ijms22063027

Orihuela, R., McPherson, C. A., & Harry, G. J. (2016). Microglial M1/M2 polarization and metabolic states. *British Journal of Pharmacology*, *173*(4), 649–665. https://doi.org/10.1111/bph.13139

Ottoy, J., De Picker, L., Verhaeghe, J., Deleye, S., Wyffels, L., Kosten, L., Sabbe, B., Coppens, V., Timmers, M., van Nueten, L., Ceyssens, S., Stroobants, S., Morrens, M., & Staelens, S. (2018). 18F-PBR111 PET Imaging in Healthy Controls and Schizophrenia: Test-Retest Reproducibility and Quantification of Neuroinflammation. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *59*(8), 1267–1274. https://doi.org/10.2967/jnumed.117.203315

Ouyang, F., He, J., Cheng, X., Zhou, W., Xiao, S., & Fang, J. (2022). Antipsychotic-Related Risks of Type 2 Diabetes Mellitus in Enrollees With Schizophrenia in the National Basic Public Health Service Program in Hunan Province, China. *Frontiers in Psychiatry*, *13*, 754775. https://doi.org/10.3389/fpsyt.2022.754775

Pahwa, R., Goyal, A., & Jialal, I. (2024). Chronic Inflammation. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK493173/

Pavlovic, D., Tamburic, V., Stojanovic, I., Kocic, G., Jetovic, T., Dordevic, V. (2002). Oxidative Stress as a Marker of Positive Symptoms in Schizophrenia. *Medicine and Biology*, *9*(2), 157-161.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. Oxidative Medicine and Cellular Longevity, 2017. https://doi.org/10.1155/2017/8416763

Plavén-Sigray, P., Matheson, G. J., Coughlin, J. M., Hafizi, S., Laurikainen, H., Ottoy, J., De Picker, L., Rusjan, P., Hietala, J., Howes, O. D., Mizrahi, R., Morrens, M., Pomper, M. G., & Cervenka, S. (2021). Meta-analysis of the Glial Marker TSPO in Psychosis Revisited: Reconciling Inconclusive Findings of Patient–Control Differences. Biological Psychiatry, 89(3), e5–e8. https://doi.org/10.1016/j.biopsych.2020.05.028

Pollak, T. A., Drndarski, S., Stone, J. M., David, A. S., McGuire, P., & Abbott, N. J. (2018). The blood-brain barrier in psychosis. *The Lancet. Psychiatry*, *5*(1), 79–92. https://doi.org/10.1016/S2215-0366(17)30293-6

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., & Buga, A.-M. (2013). ROS and Brain Diseases: The Good, the Bad, and the Ugly. Oxidative Medicine and Cellular Longevity, 2013, 963520. https://doi.org/10.1155/2013/963520

Radewicz, K., Garey, L. J., Gentleman, S. M., & Reynolds, R. (2000). Increase in HLA-DR Immunoreactive Microglia in Frontal and Temporal Cortex of Chronic Schizophrenics. *Journal of Neuropathology & Experimental Neurology*, *59*(2), 137–150. https://doi.org/10.1093/jnen/59.2.137

Raison, C. L., Rutherford, R. E., Woolwine, B. J., Shuo, C., Schettler, P., Drake, D. F., Haroon, E., & Miller, A. H. (2013). A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: The role of baseline inflammatory biomarkers. *JAMA Psychiatry*, *70*(1), 31–41. https://doi.org/10.1001/2013.jamapsychiatry.4

Réus, G. Z., Fries, G. R., Stertz, L., Badawy, M., Passos, I. C., Barichello, T., Kapczinski, F., & Quevedo, J. (2015). The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience*, *300*, 141–154. https://doi.org/10.1016/j.neuroscience.2015.05.018

Riazi, K., Galic, M. A., Kentner, A. C., Reid, A. Y., Sharkey, K. A., & Pittman, Q. J. (2015). Microglia-dependent alteration of glutamatergic synaptic transmission and plasticity in the hippocampus during peripheral inflammation. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *35*(12), 4942–4952. https://doi.org/10.1523/JNEUROSCI.4485-14.2015

Sæther, L. S., Ueland, T., Haatveit, B., Maglanoc, L. A., Szabo, A., Djurovic, S., Aukrust, P., Roelfs, D., Mohn, C., Ormerod, M. B. E. G., Lagerberg, T. V., Steen, N. E., Melle, I., Andreassen, O. A., & Ueland, T. (2023). Inflammation and cognition in severe mental illness: Patterns of covariation and subgroups. *Molecular Psychiatry*, *28*(3), 1284–1292. https://doi.org/10.1038/s41380-022-01924-w

Schafer, D. P., Lehrman, E. K., & Stevens, B. (2013). The "Quad-partite" Synapse: Microglia-Synapse Interactions in the Developing and Mature CNS. Glia, 61(1), 24–36. https://doi.org/10.1002/glia.22389

Selvaraj, S., Bloomfield, P. S., Cao, B., Veronese, M., Turkheimer, F., & Howes, O. D. (2018). Brain TSPO imaging and gray matter volume in schizophrenia patients and in people at ultra high risk of psychosis: An [11C]PBR28 study. *Schizophrenia Research*, *195*, 206–214. https://doi.org/10.1016/j.schres.2017.08.063

Smith, T. E., Weston, C. A., & Lieberman, J. A. (2009). Schizophrenia (maintenance treatment). *BMJ Clinical Evidence*, *2009*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2907810/

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in Immunology*, *9*, 754. https://doi.org/10.3389/fimmu.2018.00754

Steiner, J., Bielau, H., Brisch, R., Danos, P., Ullrich, O., Mawrin, C., Bernstein, H.-G., & Bogerts, B. (2008). Immunological aspects in the neurobiology of suicide: Elevated microglial density in schizophrenia and depression is associated with suicide. *Journal of Psychiatric Research*, *42*(2), 151–157. https://doi.org/10.1016/j.jpsychires.2006.10.013

Steiner, J., Mawrin, C., Ziegeler, A., Bielau, H., Ullrich, O., Bernstein, H.-G., & Bogerts, B. (2006). Distribution of HLA-DR-positive microglia in schizophrenia reflects impaired cerebral lateralization. *Acta Neuropathologica*, *112*(3), 305–316. https://doi.org/10.1007/s00401-006-0090-8

Sun, Y., Koyama, Y., & Shimada, S. (2022). Inflammation From Peripheral Organs to the Brain: How Does Systemic Inflammation Cause Neuroinflammation? *Frontiers in ageing Neuroscience*, *14*, 903455. https://doi.org/10.3389/fnagi.2022.903455

Tamminga, C. A., Clementz, B. A., Pearlson, G., Keshavan, M., Gershon, E. S., Ivleva, E. I., McDowell, J., Meda, S. A., Keedy, S., Calhoun, V. D., Lizano, P., Bishop, J. R., Hudgens-Haney, M., Alliey-Rodriguez, N., Asif, H., & Gibbons, R. (2021). Biotyping in psychosis: Using multiple computational approaches with one data set. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *46*(1), 143–155. https://doi.org/10.1038/s41386-020-00849-8

Tsalamandris, S., Antonopoulos, A. S., Oikonomou, E., Papamikroulis, G.-A., Vogiatzi, G., Papaioannou, S., Deftereos, S., & Tousoulis, D. (2019). The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *European Cardiology Review*, *14*(1), 50–59. https://doi.org/10.15420/ecr.2018.33.1

Ungprasert, P., Wijarnpreecha, K., & Thongprayoon, C. (2016). Rheumatoid arthritis and the risk of dementia: A systematic review and meta-analysis. *Neurology India*, *64*(1), 56. https://doi.org/10.4103/0028-3886.173623

Upthegrove, R., & Khandaker, G. M. (2020). Cytokines, Oxidative Stress and Cellular Markers of Inflammation in Schizophrenia. In G. M. Khandaker, U. Meyer, & P. B. Jones (Eds.), *Neuroinflammation and Schizophrenia* (pp. 49–66). Springer International Publishing. https://doi.org/10.1007/7854_2018_88

Vos, T., Abajobir, A. A., Abate, K. H., Abbafati, C., Abbas, K. M., Abd-Allah, F., … Murray, C. J. L. (2017). Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet*, *390*(10100), 1211–1259. https://doi.org/10.1016/S0140-6736(17)32154-2

Woodburn, S. C., Bollinger, J. L., & Wohleb, E. S. (2021). The semantics of microglia activation: Neuroinflammation, homeostasis, and stress. *Journal of Neuroinflammation*, *18*(1), 258. https://doi.org/10.1186/s12974-021-02309-6

Xin L, Mekle R, Fournier M, et al. Genetic Polymorphism Associated Prefrontal Glutathione and Its Coupling With Brain Glutamate and Peripheral Redox Status in Early Psychosis. Schizophrenia Bulletin. 2016;42(5):1185-1196. doi:10.1093/schbul/sbw038

Yao, J. K., Reddy, R. D., & van Kammen, D. P. (2001). Oxidative Damage and Schizophrenia. CNS Drugs, 15(4), 287–310. https://doi.org/10.2165/00023210-200115040-00004

Yoder, K. K., Nho, K., Risacher, S. L., Kim, S., Shen, L., & Saykin, A. J. (2013). Influence of TSPO genotype on 11C-PBR28 standardized uptake values. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *54*(8), 1320–1322. https://doi.org/10.2967/jnumed.112.118885

Yung, A. R., & McGorry, P. D. (1996). The Prodromal Phase of First-episode Psychosis: Past and Current Conceptualizations. *Schizophrenia Bulletin*, *22*(2), 353–370. https://doi.org/10.1093/schbul/22.2.353

7.2 Oxidative Stress and the Pathophysiology and Symptom Profile of Schizophrenia Spectrum Psychoses – References

Abbott, A. (2010). Schizophrenia: The drug deadlock. *Nature*, *468*(7321), 158–159. https://doi.org/10.1038/468158a

Abi-Dargham, A. (2004). Do we still believe in the dopamine hypothesis? New data bring new evidence. *The International Journal of Neuropsychopharmacology*, *7*(5), S1–S5. https://doi.org/10.1017/S1461145704004110

Abou-Setta, A. M., Mousavi, S. S., Spooner, C., Schouten, J. R., Pasichnyk, D., Armijo-Olivo, S., Beaith, A., Seida, J. C., Dursun, S., Newton, A. S., & Hartling, L. (2012). First-Generation Versus Second-Generation Antipsychotics in Adults: Comparative Effectiveness. In *First-Generation Versus Second-Generation Antipsychotics in Adults: Comparative Effectiveness [Internet]*. Agency for Healthcare Research and Quality (US). https://www.ncbi.nlm.nih.gov/books/NBK107237/

Addington, J., Penn, D., Woods, S. W., Addington, D., & Perkins, D. O. (2008). Social Functioning in Individuals at Clinical High Risk for Psychosis. *Schizophrenia Research*, *99*(1–3), 119–124. https://doi.org/10.1016/j.schres.2007.10.001

Aguilar-Valles, A., Rodrigue, B., & Matta-Camacho, E. (2020). Maternal Immune Activation and the Development of Dopaminergic Neurotransmission of the Offspring: Relevance for Schizophrenia and Other Psychoses. *Frontiers in Psychiatry, 11*. https://doi.org/10.3389/fpsyt.2020.00852

Altuntas, I., Aksoy, H., Coskun, I., Çayköylü, A., & Akçay, F. (2000). Erythrocyte Superoxide Dismutase and Glutathione Peroxidase Activities, and Malondialdehyde and Reduced Glutathione Levels in Schizophrenic Patients. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *38*(12), 1277–1281. https://doi.org/10.1515/CCLM.2000.201

Ambrosio, G., Zweier, J. L., Duilio, C., Kuppusamy, P., Santoro, G., Elia, P. P., Tritto, I., Cirillo, P., Condorelli, M., & Chiariello, M. (1993). Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. *Journal of Biological Chemistry*, *268*(25), 18532–18541.

An, H., Du, X., Huang, X., Qi, L., Jia, Q., Yin, G., Xiao, C., Huang, X.-F., Ning, Y., Cassidy, R. M., Wang, L., Soares, J. C., & Zhang, X. Y. (2018). Obesity, altered oxidative stress, and clinical correlates in chronic schizophrenia patients. *Translational Psychiatry*, *8*(1), 1–7. https://doi.org/10.1038/s41398-018-0303-7

An, L., Li, S., Murdoch, J. B., Araneta, M. F., Johnson, C., & Shen, J. (2015). Detection of glutamate, glutamine, and glutathione by radiofrequency suppression and echo time optimization at 7 tesla. *Magnetic Resonance in Medicine*, *73*(2), 451–458. https://doi.org/10.1002/mrm.25150

Artaloytia, J. F., Arango, C., Lahti, A., Sanz, J., Pascual, A., Cubero, P., Prieto, D., & Palomo, T. (2006). Negative signs and symptoms secondary to antipsychotics: A double-blind, randomized trial of a single dose of placebo, haloperidol, and risperidone in healthy

volunteers. *The American Journal of Psychiatry*, *163*(3), 488–493. https://doi.org/10.1176/appi.ajp.163.3.488

Aucoin M, LaChance L, Cooley K, Kidd S. (2020). Diet and psychosis: a Scoping review. *Neuropsychobiology. 79* 20–42. doi: 10.1159/000493399

Bai, Z.-L., Li, X.-S., Chen, G.-Y., Du, Y., Wei, Z.-X., Chen, X., Zheng, G.-E., Deng, W., & Cheng, Y. (2018). Serum Oxidative Stress Marker Levels in Unmedicated and Medicated Patients with Schizophrenia. *Journal of Molecular Neuroscience*, *66*(3), 428–436. https://doi.org/10.1007/s12031-018-1165-4

Bains, J. S., & Shaw, C. A. (1997). Neurodegenerative disorders in humans: The role of glutathione in oxidative stress-mediated neuronal death. *Brain Research. Brain Research Reviews*, *25*(3), 335–358. https://doi.org/10.1016/s0165-0173(97)00045-3

Ballesteros, A., Summerfelt, A., Du, X., Jiang, P., Chiappelli, J., Tagamets, M., O'Donnell, P., Kochunov, P., & Hong, L. E. (2013). Electrophysiological intermediate biomarkers for oxidative stress in schizophrenia. Clinical Neurophysiology, 124(11), 2209–2215. https://doi.org/10.1016/j.clinph.2013.05.021

Barger, S. W., Goodwin, M. E., Porter, M. M., & Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *Journal of Neurochemistry*, *101*(5), 1205–1213. https://doi.org/10.1111/j.1471-4159.2007.04487.x

Barron, H., Hafizi, S., Andreazza, A. C., & Mizrahi, R. (2017). Neuroinflammation and Oxidative Stress in Psychosis and Psychosis Risk. *International Journal of Molecular Sciences, 18*(3). https://doi.org/10.3390/ijms18030651

Battin, E. E., & Brumaghim, J. L. (2009). Antioxidant activity of sulfur and selenium: A review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell Biochemistry and Biophysics*, *55*(1), 1–23. https://doi.org/10.1007/s12013-009-9054-7

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C. M., & Mondelli, V. (2016). Childhood trauma and adulthood inflammation: A meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Molecular Psychiatry, 21*(5), 642–649. https://doi.org/10.1038/mp.2015.67

Baxter, P. S., Bell, K. F. S., Hasel, P., Kaindl, A. M., Fricker, M., Thomson, D., Cregan, S. P., Gillingwater, T. H., & Hardingham, G. E. (2015). Synaptic NMDA receptor activity is coupled to the transcriptional control of the glutathione system. *Nature Communications*, *6*, 6761. https://doi.org/10.1038/ncomms7761

Bentsen, H., Osnes, K., Refsum, H., Solberg, D. K., & Bøhmer, T. (2013). A randomized placebo-controlled trial of an omega-3 fatty acid and vitamins E+C in schizophrenia. *Translational Psychiatry*, *3*(12), e335–e335. https://doi.org/10.1038/tp.2013.110

Berk, M., Copolov, D., Dean, O., Lu, K., Jeavons, S., Schapkaitz, I., Anderson-Hunt, M., Judd, F., Katz, F., Katz, P., Ording-Jespersen, S., Little, J., Conus, P., Cuenod, M., Do, K. Q., & Bush, A. I. (2008). N-acetyl cysteine as a glutathione precursor for schizophrenia—A double-blind, randomized, placebo-controlled trial. *Biological Psychiatry*, *64*(5), 361–368. https://doi.org/10.1016/j.biopsych.2008.03.004

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative Stress and Antioxidant Defense. *The World Allergy Organization Journal*, *5*(1), 9–19. https://doi.org/10.1097/WOX.0b013e3182439613

Birnbaum, R., & Weinberger, D. R. (2020). A Genetics Perspective on the Role of the (Neuro)Immune System in Schizophrenia. *Schizophrenia Research*, *217*, 105–113. https://doi.org/10.1016/j.schres.2019.02.005

Birur, B., Kraguljac, N. V., Shelton, R. C., & Lahti, A. C. (2017). Brain structure, function, and neurochemistry in schizophrenia and bipolar disorder—A systematic review of the magnetic resonance neuroimaging literature. Npj Schizophrenia, 3(1), 1–15. https://doi.org/10.1038/s41537-017-0013-9

Bitanihirwe, B. K. Y., & Woo, T.-U. W. (2011). Oxidative Stress in Schizophrenia: An Integrated Approach. *Neuroscience and Biobehavioral Reviews*, *35*(3), 878–893. https://doi.org/10.1016/j.neubiorev.2010.10.008

Bloemen, O., de Koning, M., Schmitz, N., Nieman, D., Becker, H. E., de Haan, L., Dingemans, P., Linszen, D., & Van amelsvoort, T. (2009). White-matter markers for psychosis in a prospective ultra-high-risk cohort. Psychological Medicine, 40, 1297–1304. https://doi.org/10.1017/S0033291709991711

Bošković, M., Vovk, T., Kores Plesničar, B., & Grabnar, I. (2011). Oxidative Stress in Schizophrenia. *Current Neuropharmacology*, *9*(2), 301–312. https://doi.org/10.2174/157015911795596595

Bordt, E. A., & Polster, B. M. (2014). NADPH oxidase- and mitochondria-derived reactive oxygen species in proinflammatory microglial activation: A bipartisan affair? *Free Radical Biology & Medicine*, *76*, 34–46. https://doi.org/10.1016/j.freeradbiomed.2014.07.033

Brisch, R., Saniotis, A., Wolf, R., Bielau, H., Bernstein, H.-G., Steiner, J., Bogerts, B., Braun, K., Jankowski, Z., Kumaratilake, J., Henneberg, M., & Gos, T. (2014). The Role of Dopamine in Schizophrenia from a Neurobiological and Evolutionary Perspective: Old Fashioned, but Still in Vogue. *Frontiers in Psychiatry*, *5*. https://doi.org/10.3389/fpsyt.2014.00047

Brown, A. S. (2006). Prenatal Infection as a Risk Factor for Schizophrenia. *Schizophrenia Bulletin*, *32(*2), 200–202. https://doi.org/10.1093/schbul/sbj052

Brown, H. E., & Roffman, J. L. (2014). Vitamin Supplementation in the Treatment of Schizophrenia. *CNS Drugs*, *28*(7), 611–622. https://doi.org/10.1007/s40263-014-0172-4

Bustillo, J. R., Patel, V., Jones, T., Jung, R., Payaknait, N., Qualls, C., Canive, J. M., Liu, J., Perrone-Bizzozero, N. I., Calhoun, V. D., Turner, J. A., & Gasparovic, C. (2017). Risk-Conferring Glutamatergic Genes and Brain Glutamate Plus Glutamine in Schizophrenia. *Frontiers in Psychiatry*, *8*. https://doi.org/10.3389/fpsyt.2017.00079

Carlsson, A., & Waldeck, B. (1958). A Fluorimetric Method for the Determination of Dopamine (3-Hydroxytyramine.). Acta Physiologica Scandinavica, 44(3–4), 293–298. https://doi.org/10.1111/j.1748-1716.1958.tb01628.x

Carlsson, A., Waters, N., Waters, S., & Carlsson, M. L. (2000). Network interactions in schizophrenia—Therapeutic implications. *Brain Research Reviews*, *31*(2), 342–349. https://doi.org/10.1016/S0165-0173(99)00050-8

Chien, Y.-L., Hwu, H.-G., Hwang, T.-J., Hsieh, M. H., Liu, C.-C., Lin-Shiau, S.-Y., & Liu, C.-M. (2020). Clinical implications of oxidative stress in schizophrenia: Acute relapse and chronic stable phase. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *99*, 109868. https://doi.org/10.1016/j.pnpbp.2020.109868

Chowdari, K. V., Bamne, M. N., & Nimgaonkar, V. L. (2011). Genetic Association Studies of Antioxidant Pathway Genes and Schizophrenia. *Antioxidants & Redox Signaling*, *15*(7), 2037–2045. https://doi.org/10.1089/ars.2010.3508

Clark, D., Dedova, I., Cordwell, S., & Matsumoto, I. (2006). A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia. *Molecular Psychiatry*, *11*(5), 459–470. https://doi.org/10.1038/sj.mp.4001806

Cocchi, L., Harding, I. H., Lord, A., Pantelis, C., Yucel, M., & Zalesky, A. (2014). Disruption of structure–function coupling in the schizophrenia connectome. NeuroImage: Clinical, 4, 779–787. https://doi.org/10.1016/j.nicl.2014.05.004

Cohen, A. S., Saperstein, A. M., Gold, J. M., Kirkpatrick, B., Carpenter, W. T., Jr, & Buchanan, R. W. (2007). Neuropsychology of the Deficit Syndrome: New Data and Meta-analysis of Findings To Date. Schizophrenia Bulletin, 33(5), 1201–1212. https://doi.org/10.1093/schbul/sbl066

Comer, A. L., Carrier, M., Tremblay, M.-È., & Cruz-Martín, A. (2020). The Inflamed Brain in Schizophrenia: The Convergence of Genetic and Environmental Risk Factors That Lead to Uncontrolled Neuroinflammation. *Frontiers in Cellular Neuroscience*, *14*. https://doi.org/10.3389/fncel.2020.00274

Conus, P., Seidman, L. J., Fournier, M., Xin, L., Cleusix, M., Baumann, P. S., Ferrari, C., Cousins, A., Alameda, L., Gholam-Rezaee, M., Golay, P., Jenni, R., Woo, T.-U. W., Keshavan, M. S., Eap, C. B., Wojcik, J., Cuenod, M., Buclin, T., Gruetter, R., & Do, K. Q. (2018). N-acetylcysteine in a Double-Blind Randomized Placebo-Controlled Trial: Toward Biomarker-Guided Treatment in Early Psychosis. Schizophrenia Bulletin, 44(2), 317–327. https://doi.org/10.1093/schbul/sbx093

Conway, F., & Brown, A. S. (2019). Maternal Immune Activation and Related Factors in the Risk of Offspring Psychiatric Disorders. *Frontiers in Psychiatry*, *10*, 430. https://doi.org/10.3389/fpsyt.2019.00430

Coyle, J. T., Balu, D., Puhl, M., & Konopaske, G. (2016). A Perspective on the history of the concept of "disconnectivity" in schizophrenia. Harvard Review of Psychiatry, 24(2), 80–86. https://doi.org/10.1097/HRP.0000000000000102

Cross-Disorder Group of the Psychiatric Genomics Consortium. (2013). Identification of risk loci with shared effects on five major psychiatric disorders: A genome-wide analysis. *Lancet*, *381*(9875), 1371–1379. https://doi.org/10.1016/S0140-6736(12)62129-1

das Neves Duarte, J. M., Kulak, A., Gholam-Razaee, M. M., Cuenod, M., Gruetter, R., & Do, K. Q. (2012). N-acetylcysteine normalizes neurochemical changes in the glutathione-deficient schizophrenia mouse model during development. *Biological Psychiatry*, *71*(11), 1006–1014. https://doi.org/10.1016/j.biopsych.2011.07.035

de Graaf, R. A. (2019). *In Vivo NMR Spectroscopy: Principles and Techniques*. John Wiley & Sons.

Delay, J., Deniker, P., & Harl, J. M. (1952). [Therapeutic method derived from hiberno-therapy in excitation and agitation states]. *Annales Medico-Psychologiques*, *110*(2 2), 267–273.

Dietrich-Muszalska, A., & Kwiatkowska, A. (2014). Generation of superoxide anion radicals and platelet glutathione peroxidase activity in patients with schizophrenia. *Neuropsychiatric Disease and Treatment*, *10*, 703–709. https://doi.org/10.2147/NDT.S60034

Đorđević, V. V., Lazarević, D., Ćosić, V., Knežević, M. Z., & Đorđević, V. B. (2017). Age-related changes of superoxide dismutase activity in patients with schizophrenia. *Vojnosanitetski Pregled*, *74*(1), 31–37.

Duarte, J. M. N., & Xin, L. (2019). Magnetic Resonance Spectroscopy in Schizophrenia: Evidence for Glutamatergic Dysfunction and Impaired Energy Metabolism. *Neurochemical Research*, *44*(1), 102–116. https://doi.org/10.1007/s11064-018-2521-z

Dusek, P., Schneider, S. A., & Aaseth, J. (2016). Iron chelation in the treatment of neurodegenerative diseases. Journal of Trace Elements in Medicine and Biology, 38, 81-92. doi:10.1016/j.jtemb.2016.03.010

El-Ansary, A., Bjørklund, G., Chirumbolo, S., & Alnakhli, O. M. (2017). Predictive value of selected biomarkers related to metabolism and oxidative stress in children with autism spectrum disorder. Metabolic Brain Disease, 32(4), 1209–1221. https://doi.org/10.1007/s11011-017-0029-x

Faizi, M., Salimi, A., Rasoulzadeh, M., Naserzadeh, P., & Pourahmad, J. (2014). Schizophrenia Induces Oxidative Stress and Cytochrome C Release in Isolated Rat Brain Mitochondria: A Possible Pathway for Induction of Apoptosis and Neurodegeneration. *Iranian Journal of Pharmaceutical Research : IJPR*, *13*(Suppl), 93–100.

Farde, L., Hall, H., Ehrin, E., & Sedvall, G. (1986). Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. *Science*, *231*(4735), 258–261. https://doi.org/10.1126/science.2867601

Farokhnia, M., Azarkolah, A., Adinehfar, F., Khodaie-Ardakani, M.-R., Hosseini, S.-M.-R., Yekehtaz, H., Tabrizi, M., Rezaei, F., Salehi, B., Sadeghi, S.-M.-H., Moghadam, M., Gharibi, F., Mirshafiee, O., & Akhondzadeh, S. (2013). N-acetylcysteine as an adjunct to risperidone for treatment of negative symptoms in patients with chronic schizophrenia: A randomized, double-blind, placebo-controlled study. *Clinical Neuropharmacology*, *36*(6), 185–192. https://doi.org/10.1097/WNF.0000000000000001

Fatemi, H. S., & Folsom, T. D. (n.d.). *Neurodevelopmental Hypothesis of Schizophrenia, Revisited | Schizophrenia Bulletin | Oxford Academic*. Retrieved 22 October 2020, from https://academic.oup.com/schizophreniabulletin/article/35/3/528/1871490

Feigenson, K. A., Kusnecov, A. W., & Silverstein, S. M. (2014). Inflammation and the Two-Hit Hypothesis of Schizophrenia. *Neuroscience and Biobehavioral Reviews*, *38*, 72–93. https://doi.org/10.1016/j.neubiorev.2013.11.006

Fendri, C., Mechri, A., Khiari, G., Othman, A., Kerkeni, A., & Gaha, L. (2006). [Oxidative stress involvement in schizophrenia pathophysiology: A review]. *L'Encephale*, *32*(2 Pt 1), 244–252. https://doi.org/10.1016/s0013-7006(06)76151-6

Fervaha, G., Takeuchi, H., Lee, J., Foussias, G., Fletcher, P. J., Agid, O., & Remington, G. (2015). Antipsychotics and Amotivation. *Neuropsychopharmacology*, *40*(6), 1539–1548. https://doi.org/10.1038/npp.2015.3

Fisher, E., Gillam, J., Upthegrove, R., Aldred, S., & Wood, S. J. (2020). Role of magnetic resonance spectroscopy in cerebral glutathione quantification for youth mental health: A systematic review. *Early Intervention in Psychiatry*, *14*(2), 147–162. https://doi.org/10.1111/eip.12833

Flatow, J., Buckley, P., & Miller, B. J. (2013). Meta-Analysis of Oxidative Stress in Schizophrenia. *Biological Psychiatry*, *74*(6), 400–409. https://doi.org/10.1016/j.biopsych.2013.03.018

Fraguas, D., Díaz-Caneja, C. M., Ayora, M., Hernández-Álvarez, F., Rodríguez-Quiroga, A., Recio, S., Leza, J. C., & Arango, C. (2019). Oxidative Stress and Inflammation in First-Episode Psychosis: A Systematic Review and Meta-analysis. *Schizophrenia Bulletin*, *45*(4), 742–751. https://doi.org/10.1093/schbul/sby125

Frei, B. (1994). Reactive oxygen species and antioxidant vitamins: Mechanisms of action. *The American Journal of Medicine*, *97*(3, Supplement 1), S5–S13. https://doi.org/10.1016/0002-9343(94)90292-5

Friston, K., & Frith, C. (1995). Schizophrenia: A disconnection syndrome? Clinical Neuroscience (New York, N.Y.), 3, 89–97.

Frith, C. D., Friston, K. J., Herold, S., Silbersweig, D., Fletcher, P., Cahill, C., Dolan, R. J., Frackowiak, R. S. J., & Liddle, P. F. (1995). Regional Brain Activity in Chronic Schizophrenic Patients during the Performance of a Verbal Fluency Task. The British Journal of Psychiatry, 167(3), 343–349. https://doi.org/10.1192/bjp.167.3.343

Gawryluk, J. W., Wang, J.-F., Andreazza, A. C., Shao, L., & Young, L. T. (2011). Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *The International Journal of Neuropsychopharmacology*, *14*(1), 123–130. https://doi.org/10.1017/S1461145710000805

Godwin, D., Ji, A., Kandala, S., & Mamah, D. (2017). Functional Connectivity of Cognitive Brain Networks in Schizophrenia during a Working Memory Task. Frontiers in Psychiatry, 8. https://doi.org/10.3389/fpsyt.2017.00294

Goldsmith, C.-A. W., & Rogers, D. P. (2008). The Case for Autoimmunity in the Etiology of Schizophrenia. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *28*(6), 730–741. https://doi.org/10.1592/phco.28.6.730

Goldsmith, D. R., & Rapaport, M. H. (2020). Inflammation and Negative Symptoms of Schizophrenia: Implications for Reward Processing and Motivational Deficits. *Frontiers in Psychiatry, 11*. https://doi.org/10.3389/fpsyt.2020.00046

Gonzalez-Liencres, C., Tas, C., Brown, E. C., Erdin, S., Onur, E., Cubukcoglu, Z., Aydemir, O., Esen-Danaci, A., & Brüne, M. (2014). Oxidative stress in schizophrenia: A case-

control study on the effects on social cognition and neurocognition. *BMC Psychiatry*, *14*(1), 268. https://doi.org/10.1186/s12888-014-0268-x

Goodkind, M., Eickhoff, S. B., Oathes, D. J., Jiang, Y., Chang, A., Jones-Hagata, L. B., Ortega, B. N., Zaiko, Y. V., Roach, E. L., Korgaonkar, M. S., Grieve, S. M., Galatzer-Levy, I., Fox, P. T., & Etkin, A. (2015). Identification of a common neurobiological substrate for mental illness. *JAMA Psychiatry, 72*(4), 305–315. https://doi.org/10.1001/jamapsychiatry.2014.2206

Gravina, P., Spoletini, I., Masini, S., Valentini, A., Vanni, D., Paladini, E., Bossù, P., Caltagirone, C., Federici, G., Spalletta, G., & Bernardini, S. (2011). Genetic polymorphisms of glutathione S-transferases GSTM1, GSTT1, GSTP1 and GSTA1 as risk factors for schizophrenia. *Psychiatry Research*, *187*(3), 454–456. https://doi.org/10.1016/j.psychres.2010.10.008

Guidara, W., Messedi, M., Naifar, M., Maalej, M., Grayaa, S., Omri, S., Ben Thabet, J., Maalej, M., Charfi, N., & Ayadi, F. (2020). Predictive value of oxidative stress biomarkers in drug-free patients with schizophrenia and schizo-affective disorder. Psychiatry Research, 293, 113467. https://doi.org/10.1016/j.psychres.2020.113467

Gunes, M., Altindag, A., Bulut, M., Demir, S., Ibiloglu, A. O., Kaya, M. C., Atli, A., & Aksoy, N. (2017). Oxidative metabolism may be associated with negative symptoms in schizophrenia. *Psychiatry and Clinical Psychopharmacology*, *27*(1), 54–61. https://doi.org/10.1080/24750573.2017.1293243

Gururajan, A., & van den Buuse, M. (2014). Is the mTOR-signalling cascade disrupted in Schizophrenia? *Journal of Neurochemistry*, *129*(3), 377–387. https://doi.org/10.1111/jnc.12622

Hardingham, G. E., & Do, K. Q. (2016). Linking early-life NMDAR hypofunction and oxidative stress in schizophrenia pathogenesis. *Nature Reviews Neuroscience, 17*(2), 125–134. https://doi.org/10.1038/nrn.2015.19

Harrison, P. J., & Owen, M. J. (2003). Genes for schizophrenia? Recent findings and their pathophysiological implications. *The Lancet*, *361*(9355), 417–419. https://doi.org/10.1016/S0140-6736(03)12379-3

Hashimoto, K. (2014). Targeting of NMDA receptors in new treatments for schizophrenia. *Expert Opinion on Therapeutic Targets*, *18*(9), 1049–1063. https://doi.org/10.1517/14728222.2014.934225

Hashimoto, K. (2019). Recent Advances in the Early Intervention in Schizophrenia: Future Direction from Preclinical Findings. *Current Psychiatry Reports*, *21*(8), 75. https://doi.org/10.1007/s11920-019-1063-7

Hirvonen, M., Laakso, A., Någren, K., Rinne, J. O., Pohjalainen, T., & Hietala, J. (2004). C957T polymorphism of the dopamine D2 receptor (DRD2) gene affects striatal DRD2 availability in vivo. *Molecular Psychiatry*, *9*(12), 1060–1061. https://doi.org/10.1038/sj.mp.4001561

Holleran, L., Ahmed, M., Anderson-Schmidt, H., McFarland, J., Emsell, L., Leemans, A., Scanlon, C., Dockery, P., McCarthy, P., Barker, G. J., McDonald, C., & Cannon, D. M. (2014). Altered interhemispheric and temporal lobe white matter microstructural organization in severe chronic schizophrenia. Neuropsychopharmacology: Official

Publication of the American College of Neuropsychopharmacology, 39(4), 944–954. https://doi.org/10.1038/npp.2013.294

Horrobin, D. F., Glen, A. I. M., & Hudson, C. J. (1995). Possible relevance of phospholipid abnormalities and genetic interactions in psychiatric disorders: The relationship between dyslexia and schizophrenia. *Medical Hypotheses*, *45*(6), 605–613. https://doi.org/10.1016/0306-9877(95)90246-5

Howes, O., McCutcheon, R., & Stone, J. (2015). Glutamate and dopamine in schizophrenia: An update for the 21st century. *Journal of Psychopharmacology (Oxford, England)*, *29*(2), 97–115. https://doi.org/10.1177/0269881114563634

Hughes, H. K., & Ashwood, P. (2020). Overlapping evidence of innate immune dysfunction in psychotic and affective disorders. Brain, Behavior, & Immunity - Health, 2, 100038. https://doi.org/10.1016/j.bbih.2020.100038

Ibarra-Lecue, I., Mollinedo-Gajate, I., Meana, J. J., Callado, L. F., Diez-Alarcia, R., & Urigüen, L. (2018). Chronic cannabis promotes pro-hallucinogenic signaling of 5-HT2A receptors through Akt/mTOR pathway. *Neuropsychopharmacology*, *43*(10), 2028–2035. https://doi.org/10.1038/s41386-018-0076-y

Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, *54*(4), 287–293. https://doi.org/10.1016/j.ajme.2017.09.001

Iguchi, Y., Kosugi, S., Nishikawa, H., Lin, Z., Minabe, Y., & Toda, S. (2014). Repeated Exposure of Adult Rats to Transient Oxidative Stress Induces Various Long-Lasting Alterations in Cognitive and Behavioral Functions. *PLOS ONE*, *9*(12), e114024. https://doi.org/10.1371/journal.pone.0114024

Jana, M., & Pahan, K. (2013). Down-regulation of Myelin Gene Expression in Human Oligodendrocytes by Nitric Oxide: Implications for Demyelination in Multiple Sclerosis. Journal of Clinical & Cellular Immunology, 4. https://doi.org/10.4172/2155-9899.1000157

Juurlink, B. H. J. (1997). Response of Glial Cells to Ischemia: Roles of Reactive Oxygen Species and Glutathione. Neuroscience & Biobehavioral Reviews, 21(2), 151–166. https://doi.org/10.1016/S0149-7634(96)00005-X

Kalkman, H. O. (2006). The role of the phosphatidylinositide 3-kinase-protein kinase B pathway in schizophrenia. *Pharmacology & Therapeutics*, *110*(1), 117–134. https://doi.org/10.1016/j.pharmthera.2005.10.014

Kang, Y., Viswanath, V., Jha, N., Qiao, X., Mo, J. Q., & Andersen, J. K. (1999). Brain γ-glutamyl cysteine synthetase (GCS) mRNA expression patterns correlate with regional-specific enzyme activities and glutathione levels. *Journal of Neuroscience Research*, *58*(3), 436–441. https://doi.org/10.1002/(SICI)1097-4547(19991101)58:3<436::AID-JNR9>3.0.CO;2-B

Karlsgodt, K. H., Sun, D., & Cannon, T. D. (2010). Structural and Functional Brain Abnormalities in Schizophrenia. *Current Directions in Psychological Science*, *19*(4), 226–231. https://doi.org/10.1177/0963721410377601

Kelly, S., Jahanshad, N., Zalesky, A., Kochunov, P., Agartz, I., Alloza, C., Andreassen, O. A., Arango, C., Banaj, N., Bouix, S., Bousman, C. A., Brouwer, R. M., Bruggemann, J., Bustillo, J., Cahn, W., Calhoun, V., Cannon, D., Carr, V., Catts, S., … Donohoe, G. (2018). Widespread white matter microstructural differences in schizophrenia across 4322 individuals: Results from the ENIGMA Schizophrenia DTI Working Group. Molecular Psychiatry, 23(5), 1261–1269. https://doi.org/10.1038/mp.2017.170

Khan, M. M., Evans, D. R., Gunna, V., Scheffer, R. E., Parikh, V. V., & Mahadik, S. P. (2002). Reduced erythrocyte membrane essential fatty acids and increased lipid peroxides in schizophrenia at the never-medicated first-episode of psychosis and after years of treatment with antipsychotics. *Schizophrenia Research*, *58*(1), 1–10. https://doi.org/10.1016/s0920-9964(01)00334-6

Khandaker, G. M., Pearson, R. M., Zammit, S., Lewis, G., & Jones, P. B. (2014). Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: A population-based longitudinal study. *JAMA Psychiatry, 71*(10), 1121–1128. https://doi.org/10.1001/jamapsychiatry.2014.1332

Khoury, R., & Nasrallah, H. A. (2018). Inflammatory biomarkers in individuals at clinical high risk for psychosis (CHR-P): State or trait? *Schizophrenia Research, 199*, 31–38. https://doi.org/10.1016/j.schres.2018.04.017

Kim, J. S., Kornhuber, H. H., Schmid-Burgk, W., & Holzmüller, B. (1980). Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neuroscience Letters*, *20*(3), 379–382. https://doi.org/10.1016/0304-3940(80)90178-0

Klomparens, E. A., & Ding, Y. (2019). The neuroprotective mechanisms and effects of sulforaphane. *Brain Circulation*, *5*(2), 74–83. https://doi.org/10.4103/bc.bc_7_19

Koga, M., Serritella, A. V., Sawa, A., & Sedlak, T. W. (2016). Implications for reactive oxygen species in schizophrenia pathogenesis. *Schizophrenia Research, 176*(1), 52–71. https://doi.org/10.1016/j.schres.2015.06.022

Kraepelin, E. (1890). Ueber Psychosen nach Influenza. *DMW - Deutsche Medizinische Wochenschrift, 16*(11), 209–212. https://doi.org/10.1055/s-0029-1207083

Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., Heninger, G. R., Bowers, M. B., & Charney, D. S. (1994). Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. *Archives of General Psychiatry*, *51*(3), 199–214. https://doi.org/10.1001/archpsyc.1994.03950030035004

Kulak, A., Cuenod, M., & Do, K. Q. (2012). Behavioral phenotyping of glutathione-deficient mice: Relevance to schizophrenia and bipolar disorder. *Behavioural Brain Research*, *226*(2), 563–570. https://doi.org/10.1016/j.bbr.2011.10.020

Kumar, J., Liddle, E. B., Fernandes, C. C., Palaniyappan, L., Hall, E. L., Robson, S. E., Simmonite, M., Fiesal, J., Katshu, M. Z., Qureshi, A., Skelton, M., Christodoulou, N. G., Brookes, M. J., Morris, P. G., & Liddle, P. F. (2020). Glutathione and glutamate in schizophrenia: A 7T MRS study. *Molecular Psychiatry*, *25*(4), 873–882. https://doi.org/10.1038/s41380-018-0104-7

Lally, J., & MacCabe, J. H. (2015). Antipsychotic medication in schizophrenia: A review. *British Medical Bulletin, 114*(1), 169–179. https://doi.org/10.1093/bmb/ldv017

Landheer, K., Schulte, R. F., Treacy, M. S., Swanberg, K. M., & Juchem, C. (2020). Theoretical description of modern 1H in Vivo magnetic resonance spectroscopic pulse sequences. *Journal of Magnetic Resonance Imaging*, *51*(4), 1008–1029. https://doi.org/10.1002/jmri.26846

Lane, H.-Y., Lin, C.-H., Green, M. F., Hellemann, G., Huang, C.-C., Chen, P.-W., Tun, R., Chang, Y.-C., & Tsai, G. E. (2013). Add-on Treatment of Benzoate for Schizophrenia: A Randomized, Double-blind, Placebo-Controlled Trial of D -Amino Acid Oxidase Inhibitor. *JAMA Psychiatry*, *70*(12), 1267. https://doi.org/10.1001/jamapsychiatry.2013.2159

Lang, D. J. M., Yip, E., MacKay, A. L., Thornton, A. E., Vila-Rodriguez, F., MacEwan, G. W., Kopala, L. C., Smith, G. N., Laule, C., MacRae, C. B., & Honer, W. G. (2014). 48 echo T2 myelin imaging of white matter in first-episode schizophrenia: Evidence for aberrant myelination. NeuroImage: Clinical, 6, 408–414. https://doi.org/10.1016/j.nicl.2014.10.006

Lanté, F., Meunier, J., Guiramand, J., Maurice, T., Cavalier, M., de Jesus Ferreira, M.-C., Aimar, R., Cohen-Solal, C., Vignes, M., & Barbanel, G. (2007). Neurodevelopmental damage after prenatal infection: Role of oxidative stress in the fetal brain. *Free Radical Biology & Medicine*, *42*(8), 1231–1245. https://doi.org/10.1016/j.freeradbiomed.2007.01.027

Lanté, F., Meunier, J., Guiramand, J., De Jesus Ferreira, M.-C., Cambonie, G., Aimar, R., Cohen-Solal, C., Maurice, T., Vignes, M., & Barbanel, G. (2008). Late N-acetylcysteine treatment prevents the deficits induced in the offspring of dams exposed to an immune stress during gestation. *Hippocampus*, *18*(6), 602–609. https://doi.org/10.1002/hipo.20421

Laplante, M., & Sabatini, D. M. (2009). MTOR signaling at a glance. *Journal of Cell Science*, *122*(20), 3589–3594. https://doi.org/10.1242/jcs.051011

Laruelle, M., & Abi-Dargham, A. (1999). Dopamine as the wind of the psychotic fire: New evidence from brain imaging studies. *Journal of Psychopharmacology*, *13*(4), 358–371. https://doi.org/10.1177/026988119901300405

Laskaris, L. E., Di Biase, M. A., Everall, I., Chana, G., Christopoulos, A., Skafidas, E., Cropley, V. L., & Pantelis, C. (2016). Microglial activation and progressive brain changes in schizophrenia. British Journal of Pharmacology, 173(4), 666–680. https://doi.org/10.1111/bph.13364

Laursen, T. M., Nordentoft, M., & Mortensen, P. B. (2014). Excess early mortality in schizophrenia. *Annual Review of Clinical Psychology*, *10*, 425–448. https://doi.org/10.1146/annurev-clinpsy-032813-153657

Lazo-de-la-Vega-Monroy, M.-L., & Fernández-Mejía, C. (2013). Oxidative Stress in Diabetes Mellitus and the Role Of Vitamins with Antioxidant Actions. *Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants*. https://doi.org/10.5772/51788

Li, X. F., Zheng, Y. L., Xiu, M. H., Chen, D. C., Kosten, T. R., & Zhang, X. Y. (2011). Reduced plasma total antioxidant status in first-episode drug-naive patients with schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *35*(4), 1064–1067. https://doi.org/10.1016/j.pnpbp.2011.03.001

Li, Z., Dong, T., Pröschel, C., & Noble, M. (2007). Chemically Diverse Toxicants Converge on Fyn and c-Cbl to Disrupt Precursor Cell Function. PLOS Biology, 5(2), e35. https://doi.org/10.1371/journal.pbio.0050035

Lieberman, J. A., Kane, J. M., & Alvir, J. (1987). Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacology*, *91*(4), 415–433. https://doi.org/10.1007/BF00216006

Liddle, P. F. (2019). The Core Deficit of Classical Schizophrenia: Implications for Predicting the Functional Outcome of Psychotic Illness and Developing Effective Treatments. *Canadian Journal of Psychiatry. Revue Canadienne De Psychiatrie, 64*(10), 680–685. https://doi.org/10.1177/0706743719870515

Lin, C.-H., & Lane, H.-Y. (2019). Early Identification and Intervention of Schizophrenia: Insight From Hypotheses of Glutamate Dysfunction and Oxidative Stress. *Frontiers in Psychiatry*, *10*. https://doi.org/10.3389/fpsyt.2019.00093

Lin, C.-H., Lin, C.-H., Chang, Y.-C., Huang, Y.-J., Chen, P.-W., Yang, H.-T., & Lane, H.-Y. (2018). Sodium Benzoate, a D-Amino Acid Oxidase Inhibitor, Added to Clozapine for the Treatment of Schizophrenia: A Randomized, Double-Blind, Placebo-Controlled Trial. *Biological Psychiatry*, *84*(6), 422–432. https://doi.org/10.1016/j.biopsych.2017.12.006

Lugrin, J., Rosenblatt-Velin, N., Parapanov, R., & Liaudet, L. (2014). The role of oxidative stress during inflammatory processes. *Biological Chemistry, 395*(2), 203–230. https://doi.org/10.1515/hsz-2013-0241

Maas, D. A., Vallès, A., & Martens, G. J. M. (2017). Oxidative stress, prefrontal cortex hypomyelination and cognitive symptoms in schizophrenia. *Translational Psychiatry*, *7*(7), e1171–e1171. https://doi.org/10.1038/tp.2017.138

Maes, M., Sirivichayakul, S., Matsumoto, A. K., Michelin, A. P., de Oliveira Semeão, L., de Lima Pedrão, J. V., Moreira, E. G., Barbosa, D. S., Carvalho, A. F., Solmi, M., & Kanchanatawan, B. (2020). Lowered Antioxidant Defenses and Increased Oxidative Toxicity Are Hallmarks of Deficit Schizophrenia: A Nomothetic Network Psychiatry Approach. *Molecular Neurobiology*, *57*(11), 4578–4597. https://doi.org/10.1007/s12035-020-02047-5

Mahadik, S. P., & Mukherjee, S. (1996). Free radical pathology and antioxidant defense in schizophrenia: A review. *Schizophrenia Research*, *19*(1), 1–17. https://doi.org/10.1016/0920-9964(95)00049-6

Mahadik, S. P., Pillai, A., Joshi, S., & Foster, A. (2006). Prevention of oxidative stress-mediated neuropathology and improved clinical outcome by adjunctive use of a combination of antioxidants and omega-3 fatty acids in schizophrenia. *International Review of Psychiatry*, *18*(2), 119–131. https://doi.org/10.1080/09540260600581993

Matsuura, A., Ishima, T., Fujita, Y., Iwayama, Y., Hasegawa, S., Kawahara-Miki, R., Maekawa, M., Toyoshima, M., Ushida, Y., Suganuma, H., Kida, S., Yoshikawa, T., Iyo, M., & Hashimoto, K. (2018). Dietary glucoraphanin prevents the onset of psychosis in the adult offspring after maternal immune activation. *Scientific Reports, 8*(1), 2158. https://doi.org/10.1038/s41598-018-20538-3

Matsuzawa, D., Obata, T., Shirayama, Y., Nonaka, H., Kanazawa, Y., Yoshitome, E., Takanashi, J., Matsuda, T., Shimizu, E., Ikehira, H., Iyo, M., & Hashimoto, K. (2008).

Negative Correlation between Brain Glutathione Level and Negative Symptoms in Schizophrenia: A 3T 1H-MRS Study. *PLOS ONE*, *3*(4), e1944. https://doi.org/10.1371/journal.pone.0001944

Meffre, J., Chaumont-Dubel, S., Mannoury la Cour, C., Loiseau, F., Watson, D. J. G., Dekeyne, A., Séveno, M., Rivet, J.-M., Gaven, F., Déléris, P., Hervé, D., Fone, K. C. F., Bockaert, J., Millan, M. J., & Marin, P. (2012). 5-HT(6) receptor recruitment of mTOR as a mechanism for perturbed cognition in schizophrenia. *EMBO Molecular Medicine*, *4*(10), 1043–1056. https://doi.org/10.1002/emmm.201201410

Metcalf, S. A., Jones, P. B., Nordstrom, T., Timonen, M., Mäki, P., Miettunen, J., Jääskeläinen, E., Järvelin, M.-R., Stochl, J., Murray, G. K., Veijola, J., & Khandaker, G. M. (2017). Serum C-reactive protein in adolescence and risk of schizophrenia in adulthood: A prospective birth cohort study. *Brain, Behavior, and Immunity, 59*, 253–259. https://doi.org/10.1016/j.bbi.2016.09.008

Michel, T. M., Thome, J., Martin, D., Nara, K., Zwerina, S., Tatschner, T., Weijers, H. G., & Koutsilieri, E. (2004). Cu, Zn- and Mn-superoxide dismutase levels in brains of patients with schizophrenic psychosis. *Journal of Neural Transmission (Vienna, Austria: 1996)*, *111*(9), 1191–1201. https://doi.org/10.1007/s00702-004-0160-9

Michels, S., Wöhr, M., Schwarting, R. K., & Culmsee, C. (2018). Psychiatric risk gene Cacna1c determines mitochondrial resilience against oxidative stress in neurons. *Cell Death & Disease*, *9*(6). https://doi.org/10.1038/s41419-018-0676-9

Mikkelsen, T., & Hearshen, D. (2008). CHAPTER 28—Magnetic Resonance Spectroscopy. In H. B. Newton & F. A. Jolesz (Eds.), Handbook of Neuro-Oncology NeuroImaging (pp. 257–263). Academic Press. https://doi.org/10.1016/B978-012370863-2.50030-0

Mirończuk-Chodakowska, I., Witkowska, A. M., & Zujko, M. E. (2018). Endogenous non-enzymatic antioxidants in the human body. *Advances in Medical Sciences*, *63*(1), 68–78. https://doi.org/10.1016/j.advms.2017.05.005

Miyaoka, T., Ieda, M., Hashioka, S., Wake, R., Furuya, M., Liaury, K., Hayashida, M., Tsuchie, K., Arauchi, R., Araki, T., Shioji, I., Ezoe, S., Inoue, K., Yamaguchi, T., & Horiguchi, J. (2015). Analysis of oxidative stress expressed by urinary level of biopyrrins and 8-hydroxydeoxyguanosine in patients with chronic schizophrenia. *Psychiatry and Clinical Neurosciences*, *69*(11), 693–698. https://doi.org/10.1111/pcn.12319

Moghaddam, B., & Jackson, M. E. (2003). Glutamatergic animal models of schizophrenia. *Annals of the New York Academy of Sciences*, *1003*, 131–137. https://doi.org/10.1196/annals.1300.065

Moghaddam, B., & Krystal, J. H. (2012). Capturing the angel in 'angel dust': Twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. *Schizophrenia Bulletin*, *38*(5), 942–949. https://doi.org/10.1093/schbul/sbs075

Mondelli, V., Ciufolini, S., Belvederi Murri, M., Bonaccorso, S., Di Forti, M., Giordano, A., Marques, T. R., Zunszain, P. A., Morgan, C., Murray, R. M., Pariante, C. M., & Dazzan, P. (2015). Cortisol and Inflammatory Biomarkers Predict Poor Treatment Response in First Episode Psychosis. *Schizophrenia Bulletin*, *41*(5), 1162–1170. https://doi.org/10.1093/schbul/sbv028

Moreno-Küstner, B., Martín, C., & Pastor, L. (2018). Prevalence of psychotic disorders and its association with methodological issues. A systematic review and meta-analyses. *PloS One*, *13*(4), e0195687. https://doi.org/10.1371/journal.pone.0195687

Muetzel, R. L., Mous, S. E., van der Ende, J., Blanken, L. M. E., van der Lugt, A., Jaddoe, V. W. V., Verhulst, F. C., Tiemeier, H., & White, T. (2015). White matter integrity and cognitive performance in school-age children: A population-based neuroimaging study. NeuroImage, 119, 119–128. https://doi.org/10.1016/j.neuroimage.2015.06.014

Müller, N. (2018). Inflammation in Schizophrenia: Pathogenetic Aspects and Therapeutic Considerations. *Schizophrenia Bulletin*, *44*(5), 973–982. https://doi.org/10.1093/schbul/sby024

Müller, N. (2020). A Brief History of Immunological Research into Psychosis and Pathways for Immune Influence of the Brain. *Current Topics in Behavioral Neurosciences*, *44*, 1–8. https://doi.org/10.1007/7854_2018_82

Murphy, M., Kenneth, & Weaver, C. (2016). *Janeway's Immunobiology: Ninth International Student Edition. W.W. Norton & Company.*

Nakazawa, K., & Sapkota, K. (2020). The origin of NMDA receptor hypofunction in schizophrenia. *Pharmacology & Therapeutics*, *205*, 107426. https://doi.org/10.1016/j.pharmthera.2019.107426

Nath, M., Wong, T. P., & Srivastava, L. K. (2021). Neurodevelopmental insights into circuit dysconnectivity in schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 104, 110047. https://doi.org/10.1016/j.pnpbp.2020.110047

Nazeri, A., Chakravarty, M. M., Felsky, D., Lobaugh, N. J., Rajji, T. K., Mulsant, B. H., & Voineskos, A. N. (2013). Alterations of superficial white matter in schizophrenia and relationship to cognitive performance. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 38(10), 1954–1962. https://doi.org/10.1038/npp.2013.93

Nestler, E. J., Peña, C. J., Kundakovic, M., Mitchell, A., & Akbarian, S. (2016). Epigenetic Basis of Mental Illness. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *22*(5), 447–463. https://doi.org/10.1177/1073858415608147

Nimgaonkar, V. L., Prasad, K. M., Chowdari, K. V., Severance, E. G., & Yolken, R. H. (2017). The complement system: A gateway to gene-environment interactions in schizophrenia pathogenesis. *Molecular Psychiatry*, *22*(11), 1554–1561. https://doi.org/10.1038/mp.2017.151

Nutt, D. J., & Need, A. C. (2014). Where now for schizophrenia research? *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, *24*(8), 1181–1187. https://doi.org/10.1016/j.euroneuro.2014.05.012

Oakley, P., Kisely, S., Baxter, A., Harris, M., Desoe, J., Dziouba, A., & Siskind, D. (2018). Increased mortality among people with schizophrenia and other non-affective psychotic disorders in the community: A systematic review and meta-analysis. *Journal of Psychiatric Research*, *102*, 245–253. https://doi.org/10.1016/j.jpsychires.2018.04.019

Ochoa, S., Usall, J., Cobo, J., Labad, X., & Kulkarni, J. (2012). Gender differences in schizophrenia and first-episode psychosis: A comprehensive literature review. *Schizophrenia Research and Treatment*, *2012*, 916198. https://doi.org/10.1155/2012/916198

Otte, D.-M., Sommersberg, B., Kudin, A., Guerrero, C., Albayram, Ö., Filiou, M. D., Frisch, P., Yilmaz, Ö., Drews, E., Turck, C. W., Bilkei-Gorzó, A., Kunz, W. S., Beck, H., & Zimmer, A. (2011). N-acetyl Cysteine Treatment Rescues Cognitive Deficits Induced by Mitochondrial Dysfunction in G72/G30 Transgenic Mice. *Neuropsychopharmacology*, *36*(11), 2233–2243. https://doi.org/10.1038/npp.2011.109

Ozyurt, H., Ozyurt, B., Sarsilmaz, M., Kus, I., Songur, A., & Akyol, O. (2014). Potential role of some oxidant/antioxidant status parameters in prefrontal cortex of rat brain in an experimental psychosis model and the protective effects of melatonin. *European Review for Medical and Pharmacological Sciences*, *18*(15), 2137–2144.

Papadia, S., Soriano, F. X., Léveillé, F., Martel, M.-A., Dakin, K. A., Hansen, H. H., Kaindl, A., Sifringer, M., Fowler, J., Stefovska, V., McKenzie, G., Craigon, M., Corriveau, R., Ghazal, P., Horsburgh, K., Yankner, B. A., Wyllie, D. J. A., Ikonomidou, C., & Hardingham, G. E. (2008). Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nature Neuroscience*, *11*(4), 476–487. https://doi.org/10.1038/nn2071

Park, S. U., Ferrer, J. V., Javitch, J. A., & Kuhn, D. M. (2002). Peroxynitrite Inactivates the Human Dopamine Transporter by Modification of Cysteine 342: Potential Mechanism of Neurotoxicity in Dopamine Neurons. Journal of Neuroscience, 22(11), 4399–4405. https://doi.org/10.1523/JNEUROSCI.22-11-04399.2002

Pedraz-Petrozzi, B., Elyamany, O., Rummel, C., & Mulert, C. (2020). Effects of inflammation on the kynurenine pathway in schizophrenia—A systematic review. *Journal of Neuroinflammation*, *17*(1), 56. https://doi.org/10.1186/s12974-020-1721-z

Perez, S. M., & Lodge, D. J. (2014). New approaches to the management of schizophrenia: Focus on aberrant hippocampal drive of dopamine pathways. *Drug Design, Development and Therapy*, *8*, 887–896. https://doi.org/10.2147/DDDT.S42708

Persson, M., Sandberg, M., Hansson, E., & Rönnbäck, L. (2006). Microglial glutamate uptake is coupled to glutathione synthesis and glutamate release. *The European Journal of Neuroscience*, *24*(4), 1063–1070. https://doi.org/10.1111/j.1460-9568.2006.04974.x

Pettersson-Yeo, W., Allen, P., Benetti, S., McGuire, P., & Mechelli, A. (2011). Dysconnectivity in schizophrenia: Where are we now? Neuroscience & Biobehavioral Reviews, 35(5), 1110–1124. https://doi.org/10.1016/j.neubiorev.2010.11.004

Pilowsky, L. S., Bressan, R. A., Stone, J. M., Erlandsson, K., Mulligan, R. S., Krystal, J. H., & Ell, P. J. (2006). First in vivo evidence of an NMDA receptor deficit in medication-free schizophrenic patients. *Molecular Psychiatry*, *11*(2), 118–119. https://doi.org/10.1038/sj.mp.4001751

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, *2017*. https://doi.org/10.1155/2017/8416763

Pomarol-Clotet, E., Canales-Rodríguez, E. J., Salvador, R., Sarró, S., Gomar, J. J., Vila, F., Ortiz-Gil, J., Iturria-Medina, Y., Capdevila, A., & McKenna, P. J. (2010). Medial prefrontal cortex pathology in schizophrenia as revealed by convergent findings from multimodal imaging. *Molecular Psychiatry*, *15*(8), 823–830. https://doi.org/10.1038/mp.2009.146

Prabakaran, S., Swatton, J. E., Ryan, M. M., Huffaker, S. J., Huang, J.-J., Griffin, J. L., Wayland, M., Freeman, T., Dudbridge, F., Lilley, K. S., Karp, N. A., Hester, S., Tkachev, D., Mimmack, M. L., Yolken, R. H., Webster, M. J., Torrey, E. F., & Bahn, S. (2004). Mitochondrial dysfunction in schizophrenia: Evidence for compromised brain metabolism and oxidative stress. *Molecular Psychiatry*, *9*(7), 684–697. https://doi.org/10.1038/sj.mp.4001511

Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., Sullivan, P. F., Sklar, P., Purcell (Leader), S. M., Stone, J. L., Sullivan, P. F., Ruderfer, D. M., McQuillin, A., Morris, D. W., O'Dushlaine, C. T., Corvin, A., Holmans, P. A., O'Donovan, M. C., Sklar, P., Wray, N. R., … Stanley Center for Psychiatric Research and Broad Institute of MIT and Harvard. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*, *460*(7256), 748–752. https://doi.org/10.1038/nature08185

Raffa, M., Atig, F., Mhalla, A., Kerkeni, A., & Mechri, A. (2011). Decreased glutathione levels and impaired antioxidant enzyme activities in drug-naive first-episode schizophrenic patients. *BMC Psychiatry*, *11*, 124. https://doi.org/10.1186/1471-244X-11-124

Rampino, A., Marakhovskaia, A., Soares-Silva, T., Torretta, S., Veneziani, F., & Beaulieu, J. M. (2019). Antipsychotic Drug Responsiveness and Dopamine Receptor Signaling; Old Players and New Prospects. *Frontiers in Psychiatry*, *9*. https://doi.org/10.3389/fpsyt.2018.00702

Reddy, R., Keshavan, M., & Yao, J. K. (2003). Reduced plasma antioxidants in first-episode patients with schizophrenia. *Schizophrenia Research*, *62*(3), 205–212. https://doi.org/10.1016/S0920-9964(02)00407-3

Ripke, S., Neale, B. M., Corvin, A., Walters, J. T. R., Farh, K.-H., Holmans, P. A., Lee, P., Bulik-Sullivan, B., Collier, D. A., Huang, H., Pers, T. H., Agartz, I., Agerbo, E., Albus, M., Alexander, M., Amin, F., Bacanu, S. A., Begemann, M., Belliveau Jr, R. A., … Psychosis Endophenotypes International Consortium. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, *511*(7510), 421–427. https://doi.org/10.1038/nature13595

Rivers, L. E., Young, K. M., Rizzi, M., Jamen, F., Psachoulia, K., Wade, A., Kessaris, N., & Richardson, W. D. (2008). PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nature Neuroscience, 11(12), 1392–1401. https://doi.org/10.1038/nn.2220

Roiser, J. P., Howes, O. D., Chaddock, C. A., Joyce, E. M., & McGuire, P. (2013). Neural and Behavioral Correlates of Aberrant Salience in Individuals at Risk for Psychosis. *Schizophrenia Bulletin*, *39*(6), 1328–1336. https://doi.org/10.1093/schbul/sbs147

Rosen, B. M., & Percec, V. (2009). Single-Electron Transfer and Single-Electron Transfer Degenerative Chain Transfer Living Radical Polymerization. *Chemical Reviews*, *109*(11), 5069–5119. https://doi.org/10.1021/cr900024j

Sarandol, A., Sarandol, E., Acikgoz, H. E., Eker, S. S., Akkaya, C., & Dirican, M. (2015). First-episode psychosis is associated with oxidative stress: Effects of short-term antipsychotic treatment. *Psychiatry and Clinical Neurosciences*, *69*(11), 699–707. https://doi.org/10.1111/pcn.12333

Sato, A., Okada, M., Shibuya, K., Watanabe, E., Seino, S., Narita, Y., Shibui, S., Kayama, T., & Kitanaka, C. (2014). Pivotal role for ROS activation of p38 MAPK in the control of differentiation and tumor-initiating capacity of glioma-initiating cells. *Stem Cell Research*, *12*(1), 119–131. https://doi.org/10.1016/j.scr.2013.09.012

Sedlak, T. W., Nucifora, L. G., Koga, M., Shaffer, L. S., Higgs, C., Tanaka, T., Wang, A. M., Coughlin, J. M., Barker, P. B., Fahey, J. W., & Sawa, A. (2018). Sulforaphane Augments Glutathione and Influences Brain Metabolites in Human Subjects: A Clinical Pilot Study. *Molecular Neuropsychiatry*, *3*(4), 214–222. https://doi.org/10.1159/000487639

Sedlak, T. W., Paul, B. D., Parker, G. M., Hester, L. D., Snowman, A. M., Taniguchi, Y., Kamiya, A., Snyder, S. H., & Sawa, A. (2019). The glutathione cycle shapes synaptic glutamate activity. *Proceedings of the National Academy of Sciences*, *116*(7), 2701–2706. https://doi.org/10.1073/pnas.1817885116

Seeman, P., & Kapur, S. (2000). Schizophrenia: More dopamine, more D2 receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(14), 7673–7675.

Sekar, A., Bialas, A. R., de Rivera, H., Davis, A., Hammond, T. R., Kamitaki, N., Tooley, K., Presumey, J., Baum, M., Van Doren, V., Genovese, G., Rose, S. A., Handsaker, R. E., Daly, M. J., Carroll, M. C., Stevens, B., & McCarroll, S. A. (2016). Schizophrenia risk from complex variation of complement component 4. *Nature*, *530*(7589), 177–183. https://doi.org/10.1038/nature16549

Servais, S., Boussouar, A., Molnar, A., Douki, T., Pequignot, J. M., & Favier, R. (2005). Age-related sensitivity to lung oxidative stress during ozone exposure. *Free Radical Research*, *39*(3), 305–316. https://doi.org/10.1080/10715760400011098

Siciliano, G., Volpi, L., Piazza, S., Ricci, G., Mancuso, M., & Murri, L. (2007). Functional diagnostics in mitochondrial diseases. *Bioscience Reports*, *27*(1–3), 53–67. https://doi.org/10.1007/s10540-007-9037-0

Snyder, M. A., & Gao, W.-J. (2013). NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia. *Frontiers in Cellular Neuroscience*, 7. https://doi.org/10.3389/fncel.2013.00031

Sokolov, B. P. (1998). Expression of NMDAR1, GluR1, GluR7, and KA1 Glutamate Receptor mRNAs Is Decreased in Frontal Cortex of "Neuroleptic-Free" Schizophrenics: Evidence on Reversible Up-Regulation by Typical Neuroleptics. *Journal of Neurochemistry*, *71*(6), 2454–2464. https://doi.org/10.1046/j.1471-4159.1998.71062454.x

Srinivasan, R., Ratiney, H., Hammond-Rosenbluth, K. E., Pelletier, D., & Nelson, S. J. (2010). MR spectroscopic imaging of glutathione in the white and gray matter at 7 T with an application to multiple sclerosis. *Magnetic Resonance Imaging*, *28*(2), 163–170. https://doi.org/10.1016/j.mri.2009.06.008

Stansley, B. J., & Yamamoto, B. K. (2013). L-dopa-induced dopamine synthesis and oxidative stress in serotonergic cells. *Neuropharmacology*, *67*, 243–251. https://doi.org/10.1016/j.neuropharm.2012.11.010

Steullet, P., Cabungcal, J.-H., Coyle, J., Didriksen, M., Gill, K., Grace, A. A., Hensch, T. K., LaMantia, A.-S., Lindemann, L., Maynard, T. M., Meyer, U., Morishita, H., O'Donnell, P., Puhl, M., Cuenod, M., & Do, K. Q. (2017). Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. *Molecular Psychiatry*, *22*(7), 936–943. https://doi.org/10.1038/mp.2017.47

Sun, Y., Dai, Z., Li, J., Collinson, S. L., & Sim, K. (2017). Modular-level alterations of structure-function coupling in schizophrenia connectome. Human Brain Mapping, 38(4), 2008–2025. https://doi.org/10.1002/hbm.23501

Tal, A., Kirov, I. I., Grossman, R. I., & Gonen, O. (2012). The Role of Gray and White Matter Segmentation in Quantitative Proton MR Spectroscopic Imaging. NMR in Biomedicine, 25(12), 1392–1400. https://doi.org/10.1002/nbm.2812

Tan, B. L., Norhaizan, M. E., Liew, W.-P.-P., & Sulaiman Rahman, H. (2018). Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. *Frontiers in Pharmacology*, *9*. https://doi.org/10.3389/fphar.2018.01162

Tang, J., Xue, W., Xia, B., Ren, L., Tao, W., Chen, C., Zhang, H., Wu, R., Wang, Q., Wu, H., Duan, J., & Chen, G. (2015). Involvement of normalized NMDA receptor and mTOR-related signaling in rapid antidepressant effects of Yueju and ketamine on chronically stressed mice. *Scientific Reports*, *5*(1), 13573. https://doi.org/10.1038/srep13573

Taquet, M., Luciano, S., Geddes, J. R., & Harrison, P. J. (2021). Bidirectional associations between COVID-19 and psychiatric disorder: Retrospective cohort studies of 62 354 COVID-19 cases in the USA. *The Lancet Psychiatry, 8*(2), 130–140. https://doi.org/10.1016/S2215-0366(20)30462-4

Teng, L.-L., Lu, G.-L., Chiou, L.-C., Lin, W.-S., Cheng, Y.-Y., Hsueh, T.-E., Huang, Y.-C., Hwang, N.-H., Yeh, J.-W., Liao, R.-M., Fan, S.-Z., Yen, J.-H., Fu, T.-F., Tsai, T.-F., Wu, M.-S., & Wang, P.-Y. (2019). Serotonin receptor HTR6-mediated mTORC1 signaling regulates dietary restriction–induced memory enhancement. *PLOS Biology*, *17*(3), e2007097. https://doi.org/10.1371/journal.pbio.2007097

Thorburne, S. K., & Juurlink, B. H. J. (1996). Low Glutathione and High Iron Govern the Susceptibility of Oligodendroglial Precursors to Oxidative Stress. *Journal of Neurochemistry*, *67*(3), 1014–1022. https://doi.org/10.1046/j.1471-4159.1996.67031014.x

Toda, M., & Abi-Dargham, A. (2007). Dopamine hypothesis of schizophrenia: Making sense of it all. *Current Psychiatry Reports*, *9*(4), 329–336. https://doi.org/10.1007/s11920-007-0041-7

Tsai, M.-C., Liou, C.-W., Lin, T.-K., Lin, I.-M., & Huang, T.-L. (2013). Changes in oxidative stress markers in patients with schizophrenia: The effect of antipsychotic drugs. *Psychiatry Research*, *209*(3), 284–290. https://doi.org/10.1016/j.psychres.2013.01.023

Tsutsui, K., Kanbayashi, T., Takaki, M., Omori, Y., Imai, Y., Nishino, S., Tanaka, K., & Shimizu, T. (2017). N-Methyl-D-aspartate receptor antibody could be a cause of catatonic symptoms in psychiatric patients: Case reports and methods for detection.

Neuropsychiatric Disease and Treatment, *13*, 339–345. https://doi.org/10.2147/NDT.S125800

Upthegrove, R., & Khandaker, G. M. (2020). Cytokines, Oxidative Stress and Cellular Markers of Inflammation in Schizophrenia. *Current Topics in Behavioral Neurosciences*, *44*, 49–66. https://doi.org/10.1007/7854_2018_88

Upthegrove, R., Manzanares-Teson, N., & Barnes, N. M. (2014). Cytokine function in medication-naive first episode psychosis: A systematic review and meta-analysis. *Schizophrenia Research*, *155*(1–3), 101–108. https://doi.org/10.1016/j.schres.2014.03.005

Uranova, N. A., Vikhreva, O. V., Rachmanova, V. I., & Orlovskaya, D. D. (2011, June 28). *Ultrastructural Alterations of Myelinated Fibers and Oligodendrocytes in the Prefrontal Cortex in Schizophrenia: A Postmortem Morphometric Study* [Research Article]. Schizophrenia Research and Treatment; Hindawi. https://doi.org/10.1155/2011/325789

Van Rossum, J. M. (1967). The significance of dopamine-receptor blockade for the action of neuroleptic drugs. Neuropsychopharmacology. *Proceedings 5th Collegium Internationale Neruropsychopharmacologicum, 1967*. https://ci.nii.ac.jp/naid/10018363770/

Volterra, A., Trotti, D., Tromba, C., Floridi, S., & Racagni, G. (1994). Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *14*(5 Pt 1), 2924–2932.

Wallace, D. C. (2013). A mitochondrial bioenergetic etiology of disease. *The Journal of Clinical Investigation*, *123*(4), 1405–1412. https://doi.org/10.1172/JCI61398

Wang, D. M., Chen, D. C., Wang, L., & Zhang, X. Y. (2020). Sex differences in the association between symptoms and superoxide dismutase in patients with never-treated first-episode schizophrenia. *The World Journal of Biological Psychiatry*, *0*(0), 1–10. https://doi.org/10.1080/15622975.2020.1805510

Williams, L. M. (2008). Voxel-based morphometry in schizophrenia: Implications for neurodevelopmental connectivity models, cognition and affect. Expert Review of Neurotherapeutics, 8(7), 1049–1065. https://doi.org/10.1586/14737175.8.7.1049

Wood, S. J., Berger, G. E., Wellard, R. M., Proffitt, T.-M., McConchie, M., Berk, M., McGorry, P. D., & Pantelis, C. (2009). Medial temporal lobe glutathione concentration in first episode psychosis: A 1H-MRS investigation. *Neurobiology of Disease*, *33*(3), 354–357. https://doi.org/10.1016/j.nbd.2008.11.018

Wu, G., Fang, Y.-Z., Yang, S., Lupton, J. R., & Turner, N. D. (2004). Glutathione metabolism and its implications for health. *The Journal of Nutrition*, *134*(3), 489–492. https://doi.org/10.1093/jn/134.3.489

Wu, Z., Zhang, X. Y., Wang, H., Tang, W., Xia, Y., Zhang, F., Liu, J., Fu, Y., Hu, J., Chen, Y., Liu, L., Chen, D. C., Xiu, M. H., Kosten, T. R., & He, J. (2012). Elevated plasma superoxide dismutase in first-episode and drug naive patients with schizophrenia: Inverse association with positive symptoms. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *36*(1), 34–38. https://doi.org/10.1016/j.pnpbp.2011.08.018

Yao, J. K., & Keshavan, M. S. (2011). Antioxidants, Redox Signaling, and Pathophysiology in Schizophrenia: An Integrative View. *Antioxidants & Redox Signaling*, *15*(7), 2011–2035. https://doi.org/10.1089/ars.2010.3603

Yao, J. K., Leonard, S., & Reddy, R. (2006). Altered Glutathione Redox State in Schizophrenia. *Disease Markers*, *22(*1–2), 83–93. https://doi.org/10.1155/2006/248387

Yao, J. K., Reddy, R., McElhinny, L. G., & van Kammen, D. P. (1998). Reduced status of plasma total antioxidant capacity in schizophrenia. Schizophrenia Research, 32(1), 1–8. https://doi.org/10.1016/S0920-9964(98)00030-9

Yao, J. K., Reddy, R. D., & van Kammen, D. P. (1999). Human plasma glutathione peroxidase and symptom severity in schizophrenia. Biological Psychiatry, 45(11), 1512–1515. https://doi.org/10.1016/S0006-3223(98)00184-X

Yao, J. K., Reddy, R. D., & van Kammen, D. P. (2001). Oxidative Damage and Schizophrenia. *CNS Drugs*, *15*(4), 287–310. https://doi.org/10.2165/00023210-200115040-00004

Yao, L., Lui, S., Liao, Y., Du, M.-Y., Hu, N., Thomas, J. A., & Gong, Q.-Y. (2013). White matter deficits in first episode schizophrenia: An activation likelihood estimation meta-analysis. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 45, 100–106. https://doi.org/10.1016/j.pnpbp.2013.04.019

Yolland, C. O., Hanratty, D., Neill, E., Rossell, S. L., Berk, M., Dean, O. M., Castle, D. J., Tan, E. J., Phillipou, A., Harris, A. W., Barreiros, A. R., Hansen, A., & Siskind, D. (2020). Meta-analysis of randomised controlled trials with N-acetylcysteine in the treatment of schizophrenia. Australian & New Zealand Journal of Psychiatry, 54(5), 453–466. https://doi.org/10.1177/0004867419893439

Zhang, X. Y., Tan, Y. L., Cao, L. Y., Wu, G. Y., Xu, Q., Shen, Y., & Zhou, D. F. (2006). Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. *Schizophrenia Research*, *81*(2), 291–300. https://doi.org/10.1016/j.schres.2005.10.011

Zhang, X. Y., Tan, Y. L., Zhou, D. F., Haile, C. N., Wu, G. Y., Cao, L. Y., Kosten, T. A., & Kosten, T. R. (2007). Nicotine Dependence, Symptoms and Oxidative Stress in Male Patients with Schizophrenia. *Neuropsychopharmacology*, *32*(9), 2020–2024. https://doi.org/10.1038/sj.npp.1301317

Zhang, X. Y., Zhou, D. F., Cao, L. Y., Xu, C. Q., Chen, D. C., & Wu, G. Y. (2004). The Effect of Vitamin E Treatment on Tardive Dyskinesia and Blood Superoxide Dismutase: A Double-Blind Placebo-Controlled Trial. *Journal of Clinical Psychopharmacology*, *24*(1), 83–86. https://doi.org/10.1097/01.jcp.0000104912.75206.2b

Zhang, X. Y., Zhou, D. F., Shen, Y. C., Zhang, P. Y., Zhang, W. F., Liang, J., Chen, D. C., Xiu, M. H., Kosten, T. A., & Kosten, T. R. (2012). Effects of risperidone and haloperidol on superoxide dismutase and nitric oxide in schizophrenia. *Neuropharmacology*, *62*(5), 1928–1934. https://doi.org/10.1016/j.neuropharm.2011.12.014

Zheng, W., Zhang, Q.-E., Cai, D.-B., Yang, X.-H., Qiu, Y., Ungvari, G. S., Ng, C. H., Berk, M., Ning, Y.-P., & Xiang, Y.-T. (2018). N-acetylcysteine for major mental disorders: A systematic review and meta-analysis of randomized controlled trials. *Acta Psychiatrica Scandinavica*, *137*(5), 391–400. https://doi.org/10.1111/acps.12862

Zhou, Y., Fan, L., Qiu, C., & Jiang, T. (2015). Prefrontal cortex and the dysconnectivity hypothesis of schizophrenia. *Neuroscience Bulletin*, *31*(2), 207–219. https://doi.org/10.1007/s12264-014-1502-8

Zhuo, C., Wang, C., Wang, L., Guo, X., Xu, Q., Liu, Y., & Zhu, J. (2018). Altered resting-state functional connectivity of the cerebellum in schizophrenia. Brain Imaging and Behavior, 12(2), 383–389. https://doi.org/10.1007/s11682-017-9704-0

7.3 Measurement of Brain Glutathione with Magnetic Resonance Spectroscopy in Schizophrenia-Spectrum Disorders – References

Bentsen H, Solberg DK, Refsum H, et al. Bimodal distribution of polyunsaturated fatty acids in schizophrenia suggests two endophenotypes of the disorder. *Biol Psychiatry*. 2011;70(1):97-105. doi:10.1016/j.biopsych.2011.02.011

Bošković M, Vovk T, Kores Plesničar B, Grabnar I. Oxidative stress in schizophrenia. *Curr Neuropharmacol*. 2011;9(2):301-312. doi:10.2174/157015911795596595

Bracken BK, Rouse ED, Renshaw PF, Olson DP. T2 relaxation effects on apparent N-acetylaspartate concentration in proton magnetic resonance studies of schizophrenia. *Psychiatry Research: Neuroimaging*. 2013;213(2):142-153. doi:10.1016/j.pscychresns.2013.03.005

Brandt AS, Unschuld PG, Pradhan S, et al. Age-related changes in anterior cingulate cortex glutamate in schizophrenia: A H-1 MRS Study at 7 Tesla. *Schizophrenia Research*. 2016;172(1-3):101-105. doi:10.1016/j.schres.2016.02.017

Cohen P, Cohen J, Aiken LS, West SG. The Problem of Units and the Circumstance for POMP. *Multivariate Behavioral Research*. 1999;34(3):315-346. doi:10.1207/S15327906MBR3403_2

Coughlin JM, Yang K, Marsman A, et al. A multimodal approach to studying the relationship between peripheral glutathione, brain glutamate, and cognition in health and in schizophrenia. *Molecular Psychiatry*. Published online 2020. doi:10.1038/s41380-020-00901-5

Da Silva T, Hafizi S, Andreazza AC, et al. Glutathione, the Major Redox Regulator, in the Prefrontal Cortex of Individuals at Clinical High Risk for Psychosis. *International Journal of Neuropsychopharmacology*. 2018;21(4):311-318. doi:10.1093/ijnp/pyx094

Das TK, Javadzadeh A, Dey A, et al. Antioxidant defense in schizophrenia and bipolar disorder: A meta-analysis of MRS studies of anterior cingulate glutathione. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;91:94-102. doi:10.1016/j.pnpbp.2018.08.006

Dempster K, Jeon P, MacKinley M, Williamson P, Theberge J, Palaniyappan L. Early treatment response in first episode psychosis: a 7-T magnetic resonance spectroscopic study of glutathione and glutamate. *Molecular Psychiatry*. 2020;25(8):1640-1650. doi:10.1038/s41380-020-0704-x

Do KQ, Trabesinger AH, Kirsten-Krüger M, et al. Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *Eur J Neurosci*. 2000;12(10):3721-3728. doi:10.1046/j.1460-9568.2000.00229.x

Duffy SL, Lagopoulos J, Hickie IB, et al. Glutathione relates to neuropsychological functioning in mild cognitive impairment. *Alzheimer's & Dementia*. 2014;10(1):67-75. doi:10.1016/j.jalz.2013.01.005

Fisher E, Wood SJ, Elsworthy RJ, Upthegrove R, Aldred S. Exercise as a protective mechanism against the negative effects of oxidative stress in first-episode psychosis: a biomarker-led study. *Transl Psychiatry*. 2020;10(1):1-11. doi:10.1038/s41398-020-00927-x

Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *Int J Neuropsychopharmacol*. 2011;14(1):123- 130. doi:10.1017/S1461145710000805

Girgis RR, Baker S, Mao X, et al. Effects of acute N-acetylcysteine challenge on cortical glutathione and glutamate in schizophrenia: A pilot in vivo proton magnetic resonance spectroscopy study. *Psychiatry Research*. 2019;275:78-85. doi:10.1016/j.psychres.2019.03.018

Godlewska BR, Minichino A, Emir U, et al. Brain glutamate concentration in men with early psychosis: A magnetic resonance spectroscopy case–control study at 7 T. *Translational Psychiatry*. 2021;11(1). doi:10.1038/s41398-021-01477-6

Gunes M, Altindag A, Bulut M, et al. Oxidative metabolism may be associated with negative symptoms in schizophrenia. *Psychiatry and Clinical Psychopharmacology*. 2017;27(1):54-61. doi:10.1080/24750573.2017.1293243

Hafizi S, Da Silva T, Meyer JH, et al. Interaction between TSPO-a neuroimmune marker- and redox status in clinical high risk for psychosis: a PET-MRS study. *Neuropsychopharmacology*. 2018;43(8):1700-1705. doi:10.1038/s41386-018-0061-5

Hashimoto K. Recent Advances in the Early Intervention in Schizophrenia: Future Direction from Preclinical Findings. *Curr Psychiatry Rep*. 2019;21(8):75. doi:10.1007/s11920-019-1063-7

Hoch SE, Kirov II, Tal A. When Are Metabolic Ratios Superior to Absolute Quantification? A Statistical Analysis. *NMR Biomed*. 2017;30(7):10.1002/nbm.3710. doi:10.1002/nbm.3710

Iwata Y, Nakajima S, Plitman E, et al. Glutathione Levels and Glutathione-Glutamate Correlation in Patients With Treatment-Resistant Schizophrenia. *Schizophr Bull Open*. 2021;2(1):sgab006. doi:10.1093/schizbullopen/sgab006

Jeon P, Limongi R, Ford SD, et al. Progressive Changes in Glutamate Concentration in Early Stages of Schizophrenia: A Longitudinal 7-Tesla MRS Study. *Schizophrenia Bulletin Open*. 2021;2(sgaa072). doi:10.1093/schizbullopen/sgaa072

Kraguljac NV, Reid M, White D, et al. Neurometabolites in schizophrenia and bipolar disorder - a systematic review and meta-analysis. *Psychiatry Res*. 2012;203(2-3):111-125. doi:10.1016/j.pscychresns.2012.02.003

Kreis R. The trouble with quality filtering based on relative cramér-rao lower bounds. *Magnetic Resonance in Medicine*. 2015;75(1):15-18. doi:10.1002/mrm.25568

Kumar J, Liddle EB, Fernandes CC, et al. Glutathione and glutamate in schizophrenia: a 7T MRS study. *Molecular Psychiatry*. 2020;25(4):873-882. doi:10.1038/s41380-018-0104-7

Lesh T.A., Maddock R.J., Howell A., et al. Extracellular free water and glutathione in first-episode psychosis-a multimodal investigation of an inflammatory model for psychosis. *Mol Psychiatry*. 2019;((Lesh, Maddock, Howell, Wang, Tanase, Daniel Ragland, Niendam, Carter) Department of Psychiatry, University of California, Davis, United States). doi:10.1038/s41380-019-0428-y

Limongi R, Jeon P, Theberge J, Palaniyappan L. Counteracting Effects of Glutathione on the Glutamate-Driven Excitation/Inhibition Imbalance in First-Episode Schizophrenia: A 7T MRS and Dynamic Causal Modeling Study. *Antioxidants (Basel, Switzerland)*. 2021;10(1). doi:10.3390/antiox10010075

Lin A, Andronesi O, Bogner W, et al. Minimum reporting standards for in vivo magnetic resonance spectroscopy (mrsinmrs): Experts' consensus recommendations. *NMR in Biomedicine*. 2021;34(5). doi:10.1002/nbm.4484

MacKinley M, Ford SD, Jeon P, Théberge J, Palaniyappan L. Central oxidative stress and early vocational outcomes in first episode psychosis: A 7-tesla magnetic resonance spectroscopy study of glutathione. *Schizophrenia Bulletin*. 2022;48(4):921-930. doi:10.1093/schbul/sbac012

Manna P, Jain SK. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metab Syndr Relat Disord*. 2015;13(10):423-444. doi:10.1089/met.2015.0095

Matsuzawa D, Obata T, Shirayama Y, et al. Negative correlation between brain glutathione level and negative symptoms in schizophrenia: a 3T 1H-MRS study. *PLoS One*. 2008;3(4):e1944. doi:10.1371/journal.pone.0001944

Merritt K, McGuire PK, Egerton A, et al. Association of Age, Antipsychotic Medication, and Symptom Severity in Schizophrenia With Proton Magnetic Resonance Spectroscopy Brain Glutamate Level: A Mega-analysis of Individual Participant-Level Data. *JAMA Psychiatry*. 2021;78(6):667. doi:10.1001/jamapsychiatry.2021.0380

Michels S, Wöhr M, Schwarting RK, Culmsee C. Psychiatric risk gene Cacna1c determines mitochondrial resilience against oxidative stress in neurons. *Cell Death & Disease*. 2018;9(6):1-3. doi:10.1038/s41419-018-0676-9

Mikkelsen T, Hearshen D. CHAPTER 28 - Magnetic Resonance Spectroscopy. In: Newton HB, Jolesz FA, eds. *Handbook of Neuro-Oncology NeuroImaging*. Academic Press; 2008:257-263. doi:10.1016/B978-012370863-2.50030-0

Murray AJ, Rogers JC, Katshu MZ, Liddle PF, Upthegrove R. Oxidative stress and the pathophysiology and symptom profile of Schizophrenia Spectrum Disorders. *Frontiers in Psychiatry*. 2021;12. doi:10.3389/fpsyt.2021.703452

Nezhad FS, Anton A, Parkes LM, Deakin B, Williams SR. Quantification of glutathione in the human brain by MR spectroscopy at 3 Tesla: Comparison of PRESS and MEGA-PRESS. *Magnetic Resonance in Medicine*. 2017;78(4):1257-1266. doi:10.1002/mrm.26532

Öngür D, Prescot AP, Jensen JE, Cohen BM, Renshaw PF. Creatine abnormalities in schizophrenia and bipolar disorder. *Psychiatry Research: Neuroimaging*. 2009;172(1):44-48. doi:10.1016/j.pscychresns.2008.06.002

Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71

Pizzino G, Irrera N, Cucinotta M, et al. Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*. 2017;2017:e8416763. doi:10.1155/2017/8416763

R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ravanfar P, Syeda WT, Jayaram M, et al. In vivo 7-Tesla MRI investigation of brain iron and its metabolic correlates in chronic schizophrenia. *Schizophrenia*. 2022;8(1). doi:10.1038/s41537-022-00293-1

Reid M.A., Salibi N., White D.M., Gawne T.J., Denney T.S., Lahti A.C. 7T Proton Magnetic Resonance Spectroscopy of the Anterior Cingulate Cortex in First-Episode Schizophrenia. *Schizophr Bull*. 2019;45(1):180-189. doi:10.1093/schbul/sbx190

Reyes-Madrigal F, Leon-Ortiz P, Mao X, Mora-Duran R, Shungu DC, De la Fuente-Sandoyal C. Striatal Glutathione in First-episode Psychosis Patients Measured In Vivo with Proton Magnetic Resonance Spectroscopy. *Archives of Medical Research*. 2019;50(4):207-213. doi:10.1016/j.arcmed.2019.08.003

Rowland LM, Pradhan S, Korenic S, et al. Elevated brain lactate in schizophrenia: a 7T magnetic resonance spectroscopy study. *Translational Psychiatry*. 2016;6:e967. doi:10.1038/tp.2016.239

Rowland LM, Summerfelt A, Wijtenburg SA, et al. Frontal Glutamate and γ- Aminobutyric Acid Levels and Their Associations With Mismatch Negativity and Digit Sequencing Task Performance in Schizophrenia. *JAMA Psychiatry*. 2016;73(2):166. doi:10.1001/jamapsychiatry.2015.2680

Sato A, Okada M, Shibuya K, et al. Pivotal role for ROS activation of p38 MAPK in the control of differentiation and tumor-initiating capacity of glioma-initiating cells. *Stem Cell Research*. 2014;12(1):119-131. doi:10.1016/j.scr.2013.09.012

Solberg DK, Refsum H, Andreassen OA, Bentsen H. A five-year follow-up study of antioxidants, oxidative stress and polyunsaturated fatty acids in schizophrenia. *Acta Neuropsychiatrica*. 2019;31(4):202-212. doi:10.1017/neu.2019.14

Steullet P, Cabungcal JH, Coyle J, et al. Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. *Mol Psychiatry*. 2017;22(7):936-943. doi:10.1038/mp.2017.47

Sydnor VJ, Roalf DR. A meta-analysis of ultra-high field glutamate, glutamine, GABA and glutathione 1HMRS in psychosis: Implications for studies of psychosis risk. *Schizophr Res*. 2020;226:61-69. doi:10.1016/j.schres.2020.06.028

Tal A, Kirov II, Grossman RI, Gonen O. The role of gray and white matter segmentation in quantitative proton MR spectroscopic imaging. *NMR Biomed*. 2012;25(12):1392-1400. doi:10.1002/nbm.2812

Taylor R., Osuch E.A., Schaefer B., et al. Neurometabolic abnormalities in schizophrenia and depression observed with magnetic resonance spectroscopy at 7 T. *BJPsych Open*. 2017;3(1):6-11. doi:10.1192/bjpo.bp.116.003756

Terpstra M, Vaughan TJ, Ugurbil K, Lim KO, Schulz SC, Gruetter R. Validation of glutathione quantitation from STEAM spectra against edited H-1 NMR spectroscopy at 4T: application to schizophrenia. *Magnetic Resonance Materials in Physics Biology and Medicine*. 2005;18(5):276-282. doi:10.1007/s10334-005-0012-0

Upthegrove R, Khandaker GM. Cytokines, oxidative stress and cellular markers of inflammation in schizophrenia. *Neuroinflammation and Schizophrenia*. 2019:49-66. doi:10.1007/7854_2018_88

Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software*. 2010;36(3). doi:10.18637/jss.v036.i03

Wang AM, Pradhan S, Coughlin JM, et al. Assessing Brain Metabolism With 7-T Proton Magnetic Resonance Spectroscopy in Patients With First-Episode Psychosis. *Jama Psychiatry*. 2019;76(3):314-323. doi:10.1001/jamapsychiatry.2018.3637

Wijtenburg SA, Gaston FE, Spieker EA, et al. Reproducibility of Phase Rotation STEAM at 3T: Focus on Glutathione. *Magnetic Resonance in Medicine*. 2014;72(3):603-609. doi:10.1002/mrm.24959

Wijtenburg SA, Wright SN, Korenic SA, et al. Altered Glutamate and Regional Cerebral Blood Flow Levels in Schizophrenia: A 1H-MRS and pCASL study. *Neuropsychopharmacol*. 2017;42(2):562-571. doi:10.1038/npp.2016.172

Wilson M, Andronesi O, Barker PB, et al. Methodological consensus on clinical proton MRS of the brain: Review and recommendations. *Magn Reson Med*. 2019;82(2):527-550. doi:10.1002/mrm.27742

Wood SJ, Berger G, Velakoulis D, et al. Proton Magnetic Resonance Spectroscopy in First Episode Psychosis and Ultra High-Risk Individuals. *Schizophrenia Bulletin*. 2003;29(4):831-843. doi:10.1093/oxfordjournals.schbul.a007049

Wood SJ, Berger GE, Wellard RM, et al. Medial temporal lobe glutathione concentration in first episode psychosis: A H-1-MRS investigation. *Neurobiology of Disease*. 2009;33(3):354-357. doi:10.1016/j.nbd.2008.11.018

Xin L, Mekle R, Fournier M, et al. Genetic Polymorphism Associated Prefrontal Glutathione and Its Coupling With Brain Glutamate and Peripheral Redox Status in Early Psychosis. *Schizophrenia Bulletin*. 2016;42(5):1185-1196. doi:10.1093/schbul/sbw038

Young J, McKinney SB, Ross BM, Wahle KWJ, Boyle SP. Biomarkers of oxidative stress in schizophrenic and control subjects. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2007;76(2):73-85. doi:10.1016/j.plefa.2006.11.003

Zhang XY, Tan YL, Zhou DF, et al. Nicotine Dependence, Symptoms and Oxidative Stress in Male Patients with Schizophrenia. *Neuropsychopharmacol*. 2007;32(9):2020-2024. doi:10.1038/sj.npp.1301317

7.4 Identification of Detailed Inflammatory Clusters in Patients with Schizophrenia – References

Alagapan, S., Lustenberger, C., Hadar, E., Shin, H. W., & Fröhlich, F. (2019). Low-frequency direct cortical stimulation of left superior frontal gyrus enhances working memory performance. *NeuroImage*, *184*, 697–706. https://doi.org/10.1016/j.neuroimage.2018.09.064

Alexandros Lalousis, P., Schmaal, L., Wood, S. J., L.E.P Reniers, R., Cropley, V. L., Watson, A., Pantelis, C., Suckling, J., Barnes, N. M., Pariante, C., Jones, P. B., Joyce, E., Barnes, T. R. E., Lawrie, S. M., Husain, N., Dazzan, P., Deakin, B., Shannon Weickert, C., & Upthegrove, R. (2023). Inflammatory subgroups of schizophrenia and their association with brain structure: A semi-supervised machine learning examination of heterogeneity. *Brain, Behavior, and Immunity*, *113*, 166–175. https://doi.org/10.1016/j.bbi.2023.06.023

Blanchard, J. J., Kring, A. M., Horan, W. P., & Gur, R. (2011). Toward the Next Generation of Negative Symptom Assessments: The Collaboration to Advance Negative Symptom Assessment in Schizophrenia. *Schizophrenia Bulletin*, *37*(2), 291–299. https://doi.org/10.1093/schbul/sbq104

Blyler, C. R., Gold, J. M., Iannone, V. N., & Buchanan, R. W. (2000). Short form of the WAIS-III for use with patients with schizophrenia. *Schizophrenia Research*, *46*(2), 209–215. https://doi.org/10.1016/S0920-9964(00)00017-7

Constantinidis, C., & Klingberg, T. (2016). The neuroscience of working memory capacity and training. *Nature Reviews Neuroscience*, *17*(7), Article 7. https://doi.org/10.1038/nrn.2016.43

Davies, N. M., Hill, W. D., Anderson, E. L., Sanderson, E., Deary, I. J., & Davey Smith, G. (2019). Multivariable two-sample Mendelian randomization estimates of the effects of intelligence and education on health. *eLife*, *8*, e43990. https://doi.org/10.7554/eLife.43990

Deakin, B., Suckling, J., Barnes, T. R. E., Byrne, K., Chaudhry, I. B., Dazzan, P., Drake, R. J., Giordano, A., Husain, N., Jones, P. B., Joyce, E., Knox, E., Krynicki, C., Lawrie, S. M., Lewis, S., Lisiecka-Ford, D. M., Nikkheslat, N., Pariante, C. M., Smallman, R., … Dunn, G. (2018). The benefit of minocycline on negative symptoms of schizophrenia in patients with recent-onset psychosis (BeneMin): A randomised, double-blind, placebo-controlled trial. *The Lancet Psychiatry*, *5*(11), 885–894. https://doi.org/10.1016/S2215-0366(18)30345-6

Del Giudice, M., & Gangestad, S. W. (2018). Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. *Brain, Behavior, and Immunity, 70*, 61–75. https://doi.org/10.1016/j.bbi.2018.02.013

Dinga, R., Schmaal, L., Penninx, B. W. J. H., van Tol, M. J., Veltman, D. J., van Velzen, L., Mennes, M., van der Wee, N. J. A., & Marquand, A. F. (2019). Evaluating the evidence for biotypes of depression: Methodological replication and extension of. *NeuroImage. Clinical*, *22*, 101796. https://doi.org/10.1016/j.nicl.2019.101796

Fan, Y., Gao, Y., Ma, Q., Zhao, B., He, X., Zhu, F., Wang, W., Ma, X., & Li, Y. (2022). Grey matter volume and its association with cognitive impairment and peripheral cytokines in excited individuals with schizophrenia. *Brain Imaging and Behavior*, *16*(6), 2618–2626. https://doi.org/10.1007/s11682-022-00717-7

Feng, Z., Zhang, Y., You, X., Zhang, W., Ma, Y., Long, Q., Liu, Z., Hao, W., Zeng, Y., & Teng, Z. (2020). Effects of risperidone on blood levels of interleukin-6 in schizophrenia. *Medicine*, *99*(15), e19694. https://doi.org/10.1097/MD.0000000000019694

Fillman, S. G., Sinclair, D., Fung, S. J., Webster, M. J., & Shannon Weickert, C. (2014). Markers of inflammation and stress distinguish subsets of individuals with schizophrenia and bipolar disorder. *Translational Psychiatry*, *4*(2), Article 2. https://doi.org/10.1038/tp.2014.8

Fillman, S. G., Weickert, T. W., Lenroot, R. K., Catts, S. V., Bruggemann, J. M., Catts, V. S., & Weickert, C. S. (2016). Elevated peripheral cytokines characterize a subgroup of people with schizophrenia displaying poor verbal fluency and reduced Broca's area volume. *Molecular Psychiatry*, *21*(8), Article 8. https://doi.org/10.1038/mp.2015.90

Fourrier, C., Singhal, G., & Baune, B. T. (2019). Neuroinflammation and cognition across psychiatric conditions. *CNS Spectrums*, *24*(1), 4–15. https://doi.org/10.1017/S1092852918001499

Frodl, T., & Amico, F. (2014). Is there an association between peripheral immune markers and structural/functional neuroimaging findings? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *48*, 295–303. https://doi.org/10.1016/j.pnpbp.2012.12.013

García-Juárez, M., & Camacho-Morales, A. (2022). Defining the Role of Anti- and Pro-inflammatory Outcomes of Interleukin-6 in Mental Health. *Neuroscience*, *492*, 32–46. https://doi.org/10.1016/j.neuroscience.2022.03.020

Gascoyne, L. E., Brookes, M. J., Rathnaiah, M., Katshu, M. Z. U. H., Koelewijn, L., Williams, G., Kumar, J., Walters, J. T. R., Seedat, Z. A., Palaniyappan, L., Deakin, J. F. W., Singh, K. D., Liddle, P. F., & Morris, P. G. (2020). Motor-related oscillatory activity in schizophrenia according to phase of illness and clinical symptom severity. *NeuroImage : Clinical*, *29*, 102524. https://doi.org/10.1016/j.nicl.2020.102524

Gaser, C., Dahnke, R., Thompson, P. M., Kurth, F., Luders, E., & Initiative, A. D. N. (2022). *CAT – A Computational Anatomy Toolbox for the Analysis of Structural MRI Data* (p. 2022.06.11.495736). bioRxiv. https://doi.org/10.1101/2022.06.11.495736

Girgis, R. R., Ciarleglio, A., Choo, T., Haynes, G., Bathon, J. M., Cremers, S., Kantrowitz, J. T., Lieberman, J. A., & Brown, A. S. (2018). A Randomized, Double-Blind, Placebo-Controlled Clinical Trial of Tocilizumab, An Interleukin-6 Receptor Antibody, For Residual Symptoms in Schizophrenia. *Neuropsychopharmacology*, *43*(6), Article 6. https://doi.org/10.1038/npp.2017.258

Goldsmith, D. R., Bekhbat, M., Mehta, N. D., & Felger, J. C. (2023). Inflammation-Related Functional and Structural Dysconnectivity as a Pathway to Psychopathology. *Biological Psychiatry*, *93*(5), 405–418. https://doi.org/10.1016/j.biopsych.2022.11.003

Goldsmith, D. R., Haroon, E., Miller, A. H., Strauss, G. P., Buckley, P. F., & Miller, B. J. (2018). TNF-α and IL-6 are Associated with the Deficit Syndrome and Negative

Symptoms in Patients with Chronic Schizophrenia. *Schizophrenia Research*, *199*, 281–284. https://doi.org/10.1016/j.schres.2018.02.048

Herold, D., Spengler, S., Sajonz, B., Usnich, T., & Bermpohl, F. (2016). Common and distinct networks for self-referential and social stimulus processing in the human brain. *Brain Structure and Function*, *221*(7), 3475–3485. https://doi.org/10.1007/s00429-015-1113-9

Hu, B., Rao, J., Li, X., Cao, T., Li, J., Majoe, D., & Gutknecht, J. (2017). Emotion Regulating Attentional Control Abnormalities In Major Depressive Disorder: An Event-Related Potential Study. *Scientific Reports*, *7*(1), Article 1. https://doi.org/10.1038/s41598-017-13626-3

Jacomb, I., Stanton, C., Vasudevan, R., Powell, H., O'Donnell, M., Lenroot, R., Bruggemann, J., Balzan, R., Galletly, C., Liu, D., Weickert, C. S., & Weickert, T. W. (2018). C-Reactive Protein: Higher During Acute Psychotic Episodes and Related to Cortical Thickness in Schizophrenia and Healthy Controls. *Frontiers in Immunology*, *9*. https://www.frontiersin.org/articles/10.3389/fimmu.2018.02230

Jeppesen, R., Christensen, R. H. B., Pedersen, E. M. J., Nordentoft, M., Hjorthøj, C., Köhler-Forsberg, O., & Benros, M. E. (2020). Efficacy and safety of anti-inflammatory agents in treatment of psychotic disorders – A comprehensive systematic review and meta-analysis. *Brain, Behavior, and Immunity*, *90*, 364–380. https://doi.org/10.1016/j.bbi.2020.08.028

Kalmady, S. V., Venkatasubramanian, G., Shivakumar, V., Gautham, S., Subramaniam, A., Jose, D. A., Maitra, A., Ravi, V., & Gangadhar, B. N. (2014). Relationship between Interleukin-6 Gene Polymorphism and Hippocampal Volume in Antipsychotic-Naïve Schizophrenia: Evidence for Differential Susceptibility? *PLOS ONE*, *9*(5), e96021. https://doi.org/10.1371/journal.pone.0096021

Kay, S. R., Fiszbein, A., & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*, *13*(2), 261–276. https://doi.org/10.1093/schbul/13.2.261

Kelly, K. M., Smith, J. A., & Mezuk, B. (2021). Depression and interleukin-6 signaling: A Mendelian Randomization study. *Brain, Behavior, and Immunity*, *95*, 106–114. https://doi.org/10.1016/j.bbi.2021.02.019

Khandaker, G. M., Stochl, J., Zammit, S., Goodyer, I., Lewis, G., & Jones, P. B. (2018). Childhood inflammatory markers and intelligence as predictors of subsequent persistent depressive symptoms: A longitudinal cohort study. *Psychological Medicine*, *48*(9), 1514–1522. https://doi.org/10.1017/S0033291717003038

J.S. Kreutzer, J. DeLuca, B. Caplan (Eds.), Encyclopedia of Clinical Neuropsychology, Springer New York, New York, NY (2011), p. 2667

Lalousis, P. A., Wood, S. J., Schmaal, L., Chisholm, K., Griffiths, S. L., Reniers, R. L. E. P., Bertolino, A., Borgwardt, S., Brambilla, P., Kambeitz, J., Lencer, R., Pantelis, C., Ruhrmann, S., Salokangas, R. K. R., Schultze-Lutter, F., Bonivento, C., Dwyer, D., Ferro, A., Haidl, T., … PRONIA Consortium. (2021). Heterogeneity and Classification of Recent Onset Psychosis and Depression: A Multimodal Machine Learning Approach. *Schizophrenia Bulletin*, *47*(4), 1130–1140. https://doi.org/10.1093/schbul/sbaa185

Leucht, S., Leucht, C., Huhn, M., Chaimani, A., Mavridis, D., Helfer, B., Samara, M., Rabaioli, M., Bächer, S., Cipriani, A., Geddes, J. R., Salanti, G., & Davis, J. M. (2017).

Sixty Years of Placebo-Controlled Antipsychotic Drug Trials in Acute Schizophrenia: Systematic Review, Bayesian Meta-Analysis, and Meta-Regression of Efficacy Predictors. *American Journal of Psychiatry*, *174*(10), 927–942. https://doi.org/10.1176/appi.ajp.2017.16121358

Li, H., Jia, X., Li, Y., Jia, X., & Yang, Q. (2021). Aberrant Amplitude of Low-Frequency Fluctuation and Degree Centrality within the Default Mode Network in Patients with Vascular Mild Cognitive Impairment. *Brain Sciences*, *11*(11), 1534. https://doi.org/10.3390/brainsci11111534

Lin, C., Chen, K., Yu, J., Feng, W., Fu, W., Yang, F., Zhang, X., & Chen, D. (2021). Relationship between TNF-α levels and psychiatric symptoms in first-episode drug-naïve patients with schizophrenia before and after risperidone treatment and in chronic patients. *BMC Psychiatry*, *21*(1), 561. https://doi.org/10.1186/s12888-021-03569-5

Lizano, P., Lutz, O., Xu, Y., Rubin, L. H., Paskowitz, L., Lee, A. M., Eum, S., Keedy, S. K., Hill, S. K., Reilly, J. L., Wu, B., Tamminga, C. A., Clementz, B. A., Pearlson, G. D., Gershon, E. S., Keshavan, M. S., Sweeney, J. A., & Bishop, J. R. (2021). Multivariate relationships between peripheral inflammatory marker subtypes and cognitive and brain structural measures in psychosis. *Molecular Psychiatry*, *26*(7), Article 7. https://doi.org/10.1038/s41380-020-00914-0

Mennigen, E., Jiang, W., Calhoun, V. D., van Erp, T. G. M., Agartz, I., Ford, J. M., Mueller, B. A., Liu, J., & Turner, J. A. (2019). Positive and general psychopathology associated with specific gray matter reductions in inferior temporal regions in patients with schizophrenia. *Schizophrenia Research*, *208*, 242–249. https://doi.org/10.1016/j.schres.2019.02.010

Meyer, U. (2019). Neurodevelopmental Resilience and Susceptibility to Maternal Immune Activation. *Trends in Neurosciences*, *42*(11), 793–806. https://doi.org/10.1016/j.tins.2019.08.001

Misiak, B., Stańczykiewicz, B., Kotowicz, K., Rybakowski, J. K., Samochowiec, J., & Frydecka, D. (2018). Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: A systematic review. *Schizophrenia Research*, *192*, 16–29. https://doi.org/10.1016/j.schres.2017.04.015

Morrens, M., Overloop, C., Coppens, V., Loots, E., Van Den Noortgate, M., Vandenameele, S., Leboyer, M., & De Picker, L. (2022). The relationship between immune and cognitive dysfunction in mood and psychotic disorder: A systematic review and a meta-analysis. *Molecular Psychiatry*, *27*(8), Article 8. https://doi.org/10.1038/s41380-022-01582-y

Murray, A. J., Rogers, J. C., Katshu, M. Z. U. H., Liddle, P. F., & Upthegrove, R. (2021). Oxidative Stress and the Pathophysiology and Symptom Profile of Schizophrenia Spectrum Disorders. *Frontiers in Psychiatry*, *12*. https://www.frontiersin.org/articles/10.3389/fpsyt.2021.703452

Nettis MA, Lombardo G, Hastings C, Zajkowska Z, Mariani N, Nikkheslat N et al. The interaction between kynurenine pathway, suicidal ideation and augmentation therapy with minocycline in patients with treatment-resistant depression. *Journal of Psychopharmacology*. 2023;37(6):531-538. doi: 10.1177/02698811231173588

Onitsuka, T., Shenton, M. E., Salisbury, D. F., Dickey, C. C., Kasai, K., Toner, S. K., Frumin, M., Kikinis, R., Jolesz, F. A., & McCarley, R. W. (2004). Middle and Inferior Temporal Gyrus Gray Matter Volume Abnormalities in Chronic Schizophrenia: An MRI Study. *The American Journal of Psychiatry*, *161*(9), 1603–1611. https://doi.org/10.1176/appi.ajp.161.9.1603

Pape, K., Tamouza, R., Leboyer, M., & Zipp, F. (2019). Immunoneuropsychiatry—Novel perspectives on brain disorders. *Nature Reviews Neurology*, *15*(6), Article 6. https://doi.org/10.1038/s41582-019-0174-4

Penadés, R., García-Rizo, C., Bioque, M., González-Rodríguez, A., Cabrera, B., Mezquida, G., & Bernardo, M. (2015). The search for new biomarkers for cognition in schizophrenia. *Schizophrenia Research: Cognition*, *2*(4), 172–178. https://doi.org/10.1016/j.scog.2015.10.004

Peng, Y., Zhang, S., Zhou, Y., Song, Y., Yang, G., Hao, K., Yang, Y., Li, W., Lv, L., & Zhang, Y. (2021). Abnormal functional connectivity based on nodes of the default mode network in first-episode drug-naive early-onset schizophrenia. *Psychiatry Research*, *295*, 113578. https://doi.org/10.1016/j.psychres.2020.113578

Probert, L. (2015). TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects. *Neuroscience*, *302*, 2–22. https://doi.org/10.1016/j.neuroscience.2015.06.038

Rose-John, S., Winthrop, K., & Calabrese, L. (2017). The role of IL-6 in host defence against infections: Immunobiology and clinical implications. *Nature Reviews Rheumatology*, *13*(7), 399–409. https://doi.org/10.1038/nrrheum.2017.83

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in Immunology*, *9*. https://www.frontiersin.org/articles/10.3389/fimmu.2018.00754

Sun, Y., Koyama, Y., & Shimada, S. (2022). Inflammation From Peripheral Organs to the Brain: How Does Systemic Inflammation Cause Neuroinflammation? *Frontiers in ageing Neuroscience*, *14*, 903455. https://doi.org/10.3389/fnagi.2022.903455

Tu, P.-C., Li, C.-T., Lin, W.-C., Chen, M.-H., Su, T.-P., & Bai, Y.-M. (2017). Structural and functional correlates of serum soluble IL-6 receptor level in patients with bipolar disorder. *Journal of Affective Disorders*, *219*, 172–177. https://doi.org/10.1016/j.jad.2017.04.036

Ullah, I., Awan, H. A., Aamir, A., Diwan, M. N., de Filippis, R., Awan, S., Irfan, M., Fornaro, M., Ventriglio, A., Vellante, F., Pettorruso, M., Martinotti, G., Di Giannantonio, M., & De Berardis, D. (2021). Role and Perspectives of Inflammation and C-Reactive Protein (CRP) in Psychosis: An Economic and Widespread Tool for Assessing the Disease. *International Journal of Molecular Sciences*, *22*(23), 13032. https://doi.org/10.3390/ijms222313032

Upthegrove, R., Manzanares-Teson, N., & Barnes, N. M. (2014). Cytokine function in medication-naive first episode psychosis: A systematic review and meta-analysis. *Schizophrenia Research*, *155*(1), 101–108. https://doi.org/10.1016/j.schres.2014.03.005

Upthegrove, R., Marwaha, S., & Birchwood, M. (2017). Depression and Schizophrenia: Cause, Consequence, or Trans-diagnostic Issue? *Schizophrenia Bulletin*, *43*(2), 240–244. https://doi.org/10.1093/schbul/sbw097

Varol, E., Sotiras, A., & Davatzikos, C. (2017). HYDRA: Revealing heterogeneity of imaging and genetic patterns through a multiple max-margin discriminative analysis framework. *NeuroImage*, *145*, 346–364. https://doi.org/10.1016/j.neuroimage.2016.02.041

Wang, Y., Zou, L., Xie, W., Yang, Z., Zhu, X., Cheung, E. F. C., Sørensen, T. A., Møller, A., & Chan, R. C. K. (2019). Altered Functional Connectivity of the Default Mode Network in Patients With Schizo-obsessive Comorbidity: A Comparison Between Schizophrenia and Obsessive-compulsive Disorder. *Schizophrenia Bulletin*, *45*(1), 199–210. https://doi.org/10.1093/schbul/sbx194

Watson, A. J., Giordano, A., Suckling, J., Barnes, T. R. E., Husain, N., Jones, P. B., Krynicki, C. R., Lawrie, S. M., Lewis, S., Nikkheslat, N., Pariante, C. M., Upthegrove, R., Deakin, B., Dazzan, P., & Joyce, E. M. (2023). Cognitive function in early-phase schizophrenia-spectrum disorder: IQ subtypes, brain volume and immune markers. *Psychological Medicine*, *53*(7), 2842–2851. https://doi.org/10.1017/S0033291721004815

Williams, J. A., Burgess, S., Suckling, J., Lalousis, P. A., Batool, F., Griffiths, S. L., Palmer, E., Karwath, A., Barsky, A., Gkoutos, G. V., Wood, S., Barnes, N. M., David, A. S., Donohoe, G., Neill, J. C., Deakin, B., Khandaker, G. M., Upthegrove, R., & PIMS Collaboration. (2022). Inflammation and Brain Structure in Schizophrenia and Other Neuropsychiatric Disorders: A Mendelian Randomization Study. *JAMA Psychiatry*, *79*(5), 498–507. https://doi.org/10.1001/jamapsychiatry.2022.0407

World Health Organization. ICD-10 classifications of mental and behavioural disorder: clinical descriptions and diagnostic guidelines. Geneva: World Health Organization; 1992.

Wu, D., Lv, P., Li, F., Zhang, W., Fu, G., Dai, J., Hu, N., Liu, J., Xiao, Y., Li, S., Shah, C., Tao, B., Zhao, Y., Gong, Q., & Lui, S. (2019). Association of peripheral cytokine levels with cerebral structural abnormalities in schizophrenia. *Brain Research*, *1724*, 146463. https://doi.org/10.1016/j.brainres.2019.146463

Xie, W., Kong, C., Luo, W., Zheng, J., & Zhou, Y. (2024). C-reactive protein and cognitive impairment: A bidirectional Mendelian randomization study. *Archives of Gerontology and Geriatrics*, *121*, 105359. https://doi.org/10.1016/j.archger.2024.105359

Zajkowska Z, Nikkheslat N, Manfro PH, Souza L, Rohrsetzer F, Viduani A et al. Sex-specific inflammatory markers of risk and presence of depression in adolescents. *Journal of Affective Disorders.* 2023;342:69-75. doi: 10.1016/j.jad.2023.07.055

Zhao, Z., Li, X., Feng, G., Shen, Z., Li, S., Xu, Y., Huang, M., & Xu, D. (2018). Altered Effective Connectivity in the Default Network of the Brains of First-Episode, Drug-Naïve Schizophrenia Patients With Auditory Verbal Hallucinations. *Frontiers in Human Neuroscience*, *12*, 456. https://doi.org/10.3389/fnhum.2018.00456

7.5 Probing the Biological Underpinnings of Advanced Brain Ageing in Schizophrenia – References

Ballester, P. L., Romano, M. T., de Azevedo Cardoso, T., Hassel, S., Strother, S. C., Kennedy, S. H., & Frey, B. N. (2022). Brain age in mood and psychotic disorders: A systematic review and meta-analysis. *Acta Psychiatrica Scandinavica*, *145*(1), 42–55. https://doi.org/10.1111/acps.13371

Ballester, P. L., Suh, J. S., Ho, N. C. W., Liang, L., Hassel, S., Strother, S. C., Arnott, S. R., Minuzzi, L., Sassi, R. B., Lam, R. W., Milev, R., Müller, D. J., Taylor, V. H., Kennedy, S. H., Reilly, J. P., Palaniyappan, L., Dunlop, K., & Frey, B. N. (2023). Gray matter volume drives the brain age gap in schizophrenia: A SHAP study. *Schizophrenia*, *9*(1), Article 1. https://doi.org/10.1038/s41537-022-00330-z

Beheshti, I., Nugent, S., Potvin, O., & Duchesne, S. (2019). Bias-adjustment in neuroimaging-based brain age frameworks: A robust scheme. *NeuroImage. Clinical*, *24*, 102063. https://doi.org/10.1016/j.nicl.2019.102063

Blake, K. V., Ntwatwa, Z., Kaufmann, T., Stein, D. J., Ipser, J. C., & Groenewold, N. A. (2023). Advanced brain ageing in adult psychopathology: A systematic review and meta-analysis of structural MRI studies. *Journal of Psychiatric Research*, *157*, 180–191. https://doi.org/10.1016/j.jpsychires.2022.11.011

Castellano, G., Dias, C. S. B., Foerster, B., Li, L. M., & Covolan, R. J. M. (2012). NAA and NAAG variation in neuronal activation during visual stimulation. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas E Biologicas*, *45*(11), 1031–1036. https://doi.org/10.1590/s0100-879x2012007500128

Cole, J. H., Poudel, R. P. K., Tsagkrasoulis, D., Caan, M. W. A., Steves, C., Spector, T. D., & Montana, G. (2017). Predicting brain age with deep learning from raw imaging data results in a reliable and heritable biomarker. *NeuroImage*, *163*, 115–124. https://doi.org/10.1016/j.neuroimage.2017.07.059

Constantinides, C., Han, L. K. M., Alloza, C., Antonucci, L. A., Arango, C., Ayesa-Arriola, R., Banaj, N., Bertolino, A., Borgwardt, S., Bruggemann, J., Bustillo, J., Bykhovski, O., Calhoun, V., Carr, V., Catts, S., Chung, Y.-C., Crespo-Facorro, B., Díaz-Caneja, C. M., Donohoe, G., … Walton, E. (2023). Brain ageing in schizophrenia: Evidence from 26 international cohorts via the ENIGMA Schizophrenia consortium. *Molecular Psychiatry*, *28*(3), Article 3. https://doi.org/10.1038/s41380-022-01897-w

Cui, L.-B., Wang, X.-Y., Fu, Y.-F., Liu, X.-F., Wei, Y., Zhao, S.-W., Gu, Y.-W., Fan, J.-W., Wu, W.-J., Gong, H., Lin, B. D., Yin, H., Guan, F., & Chang, X. (2023). Transcriptional level of inflammation markers associates with short-term brain structural changes in first-episode schizophrenia. *BMC Medicine*, *21*(1), 250. https://doi.org/10.1186/s12916-023-02963-y

Das, T. K., Javadzadeh, A., Dey, A., Sabesan, P., Théberge, J., Radua, J., & Palaniyappan, L. (2019). Antioxidant defense in schizophrenia and bipolar disorder: A meta-analysis of

MRS studies of anterior cingulate glutathione. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *91*, 94–102. https://doi.org/10.1016/j.pnpbp.2018.08.006

DeMayo, M. M., Lv, J., Duffy, S. L., D'Souza, A., Mowszowski, L., Naismith, S. L., & Calamante, F. (2023). Hippocampal Neuronal Integrity and Functional Connectivity Within the Default Mode Network in Mild Cognitive Impairment: A Multimodal Investigation. *Brain Connectivity*, *13*(3), 143–153. https://doi.org/10.1089/brain.2022.0050

Dempster, K., Jeon, P., MacKinley, M., Williamson, P., Théberge, J., & Palaniyappan, L. (2020). Early treatment response in first episode psychosis: A 7-T magnetic resonance spectroscopic study of glutathione and glutamate. *Molecular Psychiatry*, *25*(8), Article 8. https://doi.org/10.1038/s41380-020-0704-x

Do, K. Q., Trabesinger, A. H., Kirsten-Krüger, M., Lauer, C. J., Dydak, U., Hell, D., Holsboer, F., Boesiger, P., & Cuénod, M. (2000). Schizophrenia: Glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *European Journal of Neuroscience*, *12*(10), 3721–3728. https://doi.org/10.1046/j.1460-9568.2000.00229.x

Egerton, A., Grace, A. A., Stone, J., Bossong, M. G., Sand, M., & McGuire, P. (2020). Glutamate in schizophrenia: Neurodevelopmental perspectives and drug development. *Schizophrenia Research*, *223*, 59–70. https://doi.org/10.1016/j.schres.2020.09.013

Franke, K., & Gaser, C. (2019). Ten Years of BrainAGE as a Neuroimaging Biomarker of Brain ageing: What Insights Have We Gained? *Frontiers in Neurology*, *10*, 789. https://doi.org/10.3389/fneur.2019.00789

Hajek, T., Franke, K., Kolenic, M., Capkova, J., Matejka, M., Propper, L., Uher, R., Stopkova, P., Novak, T., Paus, T., Kopecek, M., Spaniel, F., & Alda, M. (2019). Brain Age in Early Stages of Bipolar Disorders or Schizophrenia. *Schizophrenia Bulletin*, *45*(1), 190–198. https://doi.org/10.1093/schbul/sbx172

Hardy, C. J., Tal, A., Babb, J. S., Perry, N. N., Messinger, J. W., Antonius, D., Malaspina, D., & Gonen, O. (2011). Multivoxel Proton MR Spectroscopy Used to Distinguish Anterior Cingulate Metabolic Abnormalities in Patients with Schizophrenia. *Radiology*, *261*(2), 542–550. https://doi.org/10.1148/radiol.11110675

Kaufmann, T., van der Meer, D., Doan, N. T., Schwarz, E., Lund, M. J., Agartz, I., Alnæs, D., Barch, D. M., Baur-Streubel, R., Bertolino, A., Bettella, F., Beyer, M. K., Bøen, E., Borgwardt, S., Brandt, C. L., Buitelaar, J., Celius, E. G., Cervenka, S., Conzelmann, A., … Westlye, L. T. (2019). Common brain disorders are associated with heritable patterns of apparent ageing of the brain. *Nature Neuroscience*, *22*(10), 1617–1623. https://doi.org/10.1038/s41593-019-0471-7

Kay, S. R., Fiszbein, A., & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*, *13*(2), 261–276. https://doi.org/10.1093/schbul/13.2.261

Kolenic, M., Franke, K., Hlinka, J., Matejka, M., Capkova, J., Pausova, Z., Uher, R., Alda, M., Spaniel, F., & Hajek, T. (2018). Obesity, dyslipidemia and brain age in first-episode psychosis. *Journal of Psychiatric Research*, *99*, 151–158. https://doi.org/10.1016/j.jpsychires.2018.02.012

Koutsouleris, N., Davatzikos, C., Borgwardt, S., Gaser, C., Bottlender, R., Frodl, T., Falkai, P., Riecher-Rössler, A., Möller, H.-J., Reiser, M., Pantelis, C., & Meisenzahl, E. (2014).

Accelerated brain ageing in schizophrenia and beyond: A neuroanatomical marker of psychiatric disorders. *Schizophrenia Bulletin*, *40*(5), 1140–1153. https://doi.org/10.1093/schbul/sbt142

Kumar, J., Liddle, E. B., Fernandes, C. C., Palaniyappan, L., Hall, E. L., Robson, S. E., Simmonite, M., Fiesal, J., Katshu, M. Z., Qureshi, A., Skelton, M., Christodoulou, N. G., Brookes, M. J., Morris, P. G., & Liddle, P. F. (2020). Glutathione and glutamate in schizophrenia: A 7T MRS study. *Molecular Psychiatry*, *25*(4), 873–882. https://doi.org/10.1038/s41380-018-0104-7

Lalousis, P., Schmaal, L., Wood, S. J., L.E.P Reniers, R., Cropley, V. L., Watson, A., Pantelis, C., Suckling, J., Barnes, N. M., Pariante, C., Jones, P. B., Joyce, E., Barnes, T. R. E., Lawrie, S. M., Husain, N., Dazzan, P., Deakin, B., Shannon Weickert, C., & Upthegrove, R. (2023). Inflammatory subgroups of schizophrenia and their association with brain structure: A semi-supervised machine learning examination of heterogeneity. *Brain, Behavior, and Immunity*, *113*, 166–175. https://doi.org/10.1016/j.bbi.2023.06.023

Limongi, R., Jeon, P., Théberge, J., & Palaniyappan, L. (2021). Counteracting Effects of Glutathione on the Glutamate-Driven Excitation/Inhibition Imbalance in First-Episode Schizophrenia: A 7T MRS and Dynamic Causal Modeling Study. *Antioxidants (Basel, Switzerland)*, *10*(1), 75. https://doi.org/10.3390/antiox10010075

Lizano, P., Lutz, O., Xu, Y., Rubin, L. H., Paskowitz, L., Lee, A. M., Eum, S., Keedy, S. K., Hill, S. K., Reilly, J. L., Wu, B., Tamminga, C. A., Clementz, B. A., Pearlson, G. D., Gershon, E. S., Keshavan, M. S., Sweeney, J. A., & Bishop, J. R. (2021). Multivariate Relationships Between Peripheral Inflammatory Marker Subtypes and Cognitive and Brain Structural Measures in Psychosis. *Molecular Psychiatry*, *26*(7), 3430–3443. https://doi.org/10.1038/s41380-020-00914-0

Lundberg, S., & Lee, S.-I. (2017). *A Unified Approach to Interpreting Model Predictions* (arXiv:1705.07874). arXiv. https://doi.org/10.48550/arXiv.1705.07874

MacKinley, M., Ford, S. D., Jeon, P., Théberge, J., & Palaniyappan, L. (2022). Central Oxidative Stress and Early Vocational Outcomes in First Episode Psychosis: A 7-Tesla Magnetic Resonance Spectroscopy Study of Glutathione. *Schizophrenia Bulletin*, *48*(4), 921–930. https://doi.org/10.1093/schbul/sbac012

Madre, M., Canales-Rodríguez, E. J., Fuentes-Claramonte, P., Alonso-Lana, S., Salgado-Pineda, P., Guerrero-Pedraza, A., Moro, N., Bosque, C., Gomar, J. J., Ortíz-Gil, J., Goikolea, J. M., Bonnin, C. M., Vieta, E., Sarró, S., Maristany, T., McKenna, P. J., Salvador, R., & Pomarol-Clotet, E. (2020). Structural abnormality in schizophrenia versus bipolar disorder: A whole brain cortical thickness, surface area, volume and gyrification analyses. *NeuroImage: Clinical*, *25*, 102131. https://doi.org/10.1016/j.nicl.2019.102131

Malaspina, D., Kranz, T. M., Heguy, A., Harroch, S., Mazgaj, R., Rothman, K., Berns, A., Hasan, S., Antonius, D., Goetz, R., Lazar, M., Chao, M. V., & Gonen, O. (2016). Prefrontal neuronal integrity predicts symptoms and cognition in schizophrenia and is sensitive to genetic heterogeneity. *Schizophrenia Research*, *172*(1), 94–100. https://doi.org/10.1016/j.schres.2016.02.031

Merritt, K., McGuire, P. K., Egerton, A., 1H-MRS in Schizophrenia Investigators, Aleman, A., Block, W., Bloemen, O. J. N., Borgan, F., Bustillo, J. R., Capizzano, A. A., Coughlin, J. M., De la Fuente-Sandoval, C., Demjaha, A., Dempster, K., Do, K. Q., Du, F., Falkai,

P., Galinska-Skok, B., Gallinat, J., … Yamasue, H. (2021). Association of Age, Antipsychotic Medication, and Symptom Severity in Schizophrenia With Proton Magnetic Resonance Spectroscopy Brain Glutamate Level: A Mega-analysis of Individual Participant-Level Data. *JAMA Psychiatry*, *78*(6), 667–681. https://doi.org/10.1001/jamapsychiatry.2021.0380

Murray, A. J., Humpston, C. S., Wilson, M., Rogers, J. C., Zia Ul Haq Katshu, M., Liddle, P. F., & Upthegrove, R. (2024). Measurement of brain glutathione with magnetic Resonance spectroscopy in Schizophrenia-Spectrum disorders—A systematic review and Meta-Analysis. *Brain, Behavior, and Immunity*, *115*, 3–12. https://doi.org/10.1016/j.bbi.2023.09.017

Palaniyappan, L., Park, M. T. M., Jeon, P., Limongi, R., Yang, K., Sawa, A., & Théberge, J. (2021). Is There a Glutathione Centered Redox Dysregulation Subtype of Schizophrenia? *Antioxidants*, *10*(11), 1703. https://doi.org/10.3390/antiox10111703

Sanford, N., Ge, R., Antoniades, M., Modabbernia, A., Haas, S. S., Whalley, H. C., Galea, L., Popescu, S. G., Cole, J. H., & Frangou, S. (2022). Sex differences in predictors and regional patterns of brain age gap estimates. *Human Brain Mapping*, *43*(15), 4689–4698. https://doi.org/10.1002/hbm.25983

Schnack, H. G., van Haren, N. E. M., Nieuwenhuis, M., Hulshoff Pol, H. E., Cahn, W., & Kahn, R. S. (2016). Accelerated Brain ageing in Schizophrenia: A Longitudinal Pattern Recognition Study. *American Journal of Psychiatry*, *173*(6), 607–616. https://doi.org/10.1176/appi.ajp.2015.15070922

van Erp, T. G. M., Walton, E., Hibar, D. P., Schmaal, L., Jiang, W., Glahn, D. C., Pearlson, G. D., Yao, N., Fukunaga, M., Hashimoto, R., Okada, N., Yamamori, H., Bustillo, J. R., Clark, V. P., Agartz, I., Mueller, B. A., Cahn, W., de Zwarte, S. M. C., Hulshoff Pol, H. E., … Turner, J. A. (2018). Cortical Brain Abnormalities in 4474 Individuals With Schizophrenia and 5098 Control Subjects via the Enhancing Neuro Imaging Genetics Through Meta Analysis (ENIGMA) Consortium. *Biological Psychiatry*, *84*(9), 644–654. https://doi.org/10.1016/j.biopsych.2018.04.023

Wang, J., Kochunov, P., Sampath, H., Hatch, K. S., Ryan, M. C., Xue, F., Neda, J., Paul, T., Hahn, B., Gold, J., Waltz, J., Hong, L. E., & Chen, S. (2021). White matter brain ageing in relationship to schizophrenia and its cognitive deficit. *Schizophrenia Research*, *230*, 9–16. https://doi.org/10.1016/j.schres.2021.02.003

Williams, J. A., Burgess, S., Suckling, J., Lalousis, P. A., Batool, F., Griffiths, S. L., Palmer, E., Karwath, A., Barsky, A., Gkoutos, G. V., Wood, S., Barnes, N. M., David, A. S., Donohoe, G., Neill, J. C., Deakin, B., Khandaker, G. M., Upthegrove, R., & PIMS Collaboration. (2022). Inflammation and Brain Structure in Schizophrenia and Other Neuropsychiatric Disorders: A Mendelian Randomization Study. *JAMA Psychiatry*, *79*(5), 498–507. https://doi.org/10.1001/jamapsychiatry.2022.0407

Wilson, M. (2021). spant: An R package for magnetic resonance spectroscopy analysis. *Journal of Open Source Software*, *6*(67), 3646. https://doi.org/10.21105/joss.03646

Yazaji, M., Battas, O., Agoub, M., Moussaoui, D., Gutknecht, C., Dalery, J., d'Amato, T., & Saoud, M. (2002). Validity of the depressive dimension extracted from principal component analysis of the PANSS in drug-free patients with schizophrenia. *Schizophrenia Research*, *56*(1–2), 121–127. https://doi.org/10.1016/s0920-9964(01)00247-x

7.6 General Discussion – References

Akhondzadeh, S., Tabatabaee, M., Amini, H., Ahmadi Abhari, S. A., Abbasi, S. H., & Behnam, B. (2007). Celecoxib as adjunctive therapy in schizophrenia: A double-blind, randomized and placebo-controlled trial. *Schizophrenia Research*, *90*(1), 179–185. https://doi.org/10.1016/j.schres.2006.11.016

Aldaham, S., Foote, J. A., Chow, H.-H. S., & Hakim, I. A. (2015). Smoking Status Effect on Inflammatory Markers in a Randomized Trial of Current and Former Heavy Smokers. *International Journal of Inflammation*, *2015*, 439396. https://doi.org/10.1155/2015/439396

Altuntas, I., Aksoy, H., Coskun, I., Cayköylü, A., & Akçay, F. (2000). Erythrocyte superoxide dismutase and glutathione peroxidase activities, and malondialdehyde and reduced glutathione levels in schizophrenic patients. *Clinical Chemistry and Laboratory Medicine*, *38*(12), 1277–1281. https://doi.org/10.1515/CCLM.2000.201

Bordt, E. A., & Polster, B. M. (2014). NADPH oxidase- and mitochondria-derived reactive oxygen species in proinflammatory microglial activation: A bipartisan affair? *Free Radical Biology & Medicine*, *76*, 34–46. https://doi.org/10.1016/j.freeradbiomed.2014.07.033

Bryll, A., Skrzypek, J., Krzyściak, W., Szelągowska, M., Śmierciak, N., Kozicz, T., & Popiela, T. (2020). Oxidative-Antioxidant Imbalance and Impaired Glucose Metabolism in Schizophrenia. *Biomolecules*, *10*(3), Article 3. https://doi.org/10.3390/biom10030384

Chitty, K. M., Lagopoulos, J., Hickie, I. B., & Hermens, D. F. (2015). A longitudinal proton magnetic resonance spectroscopy study investigating oxidative stress as a result of alcohol and tobacco use in youth with bipolar disorder. *Journal of Affective Disorders*, *175*, 481–487. https://doi.org/10.1016/j.jad.2015.01.021

Collste, K., Plavén-Sigray, P., Fatouros-Bergman, H., Victorsson, P., Schain, M., Forsberg, A., Amini, N., Aeinehband, S., Karolinska Schizophrenia Project (KaSP) consortium, Erhardt, S., Halldin, C., Flyckt, L., Farde, L., & Cervenka, S. (2017). Lower levels of the glial cell marker TSPO in drug-naive first-episode psychosis patients as measured using PET and [11C]PBR28. *Molecular Psychiatry*, *22*(6), 850–856. https://doi.org/10.1038/mp.2016.247

Conen, S., Gregory, C. J., Hinz, R., Smallman, R., Corsi-Zuelli, F., Deakin, B., & Talbot, P. S. (2021). Neuroinflammation as measured by positron emission tomography in patients with recent onset and established schizophrenia: Implications for immune pathogenesis. *Molecular Psychiatry*, *26*(9), 5398–5406. https://doi.org/10.1038/s41380-020-0829-y

Conus, P., Seidman, L. J., Fournier, M., Xin, L., Cleusix, M., Baumann, P. S., Ferrari, C., Cousins, A., Alameda, L., Gholam-Rezaee, M., Golay, P., Jenni, R., Woo, T.-U. W., Keshavan, M. S., Eap, C. B., Wojcik, J., Cuenod, M., Buclin, T., Gruetter, R., & Do, K. Q. (2018). N-acetylcysteine in a Double-Blind Randomized Placebo-Controlled Trial: Toward Biomarker-Guided Treatment in Early Psychosis. *Schizophrenia Bulletin*, *44*(2), 317–327. https://doi.org/10.1093/schbul/sbx093

Coughlin, J. M., Yang, K., Marsman, A., Pradhan, S., Wang, M., Ward, R. E., Bonekamp, S., Ambinder, E. B., Higgs, C. P., Kim, P. K., Edwards, J. A., Varvaris, M., Wang, H., Posporelis, S., Ma, S., Tsujimura, T., Edden, R. A. E., Pomper, M. G., Sedlak, T. W., … Sawa, A. (2021). A multimodal approach to studying the relationship between peripheral glutathione, brain glutamate, and cognition in health and in schizophrenia. *Molecular Psychiatry*, *26*(7), 3502–3511. https://doi.org/10.1038/s41380-020-00901-5

Da Silva, T., Hafizi, S., Andreazza, A. C., Kiang, M., Bagby, R. M., Navas, E., Laksono, I., Truong, P., Gerritsen, C., Prce, I., Sailasuta, N., & Mizrahi, R. (2018). Glutathione, the Major Redox Regulator, in the Prefrontal Cortex of Individuals at Clinical High Risk for Psychosis. *International Journal of Neuropsychopharmacology*, *21*(4), 311–318. https://doi.org/10.1093/ijnp/pyx094

Das, T. K., Javadzadeh, A., Dey, A., Sabesan, P., Théberge, J., Radua, J., & Palaniyappan, L. (2019). Antioxidant defense in schizophrenia and bipolar disorder: A meta-analysis of MRS studies of anterior cingulate glutathione. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *91*, 94–102. https://doi.org/10.1016/j.pnpbp.2018.08.006

Deakin, B., Suckling, J., Barnes, T. R. E., Byrne, K., Chaudhry, I. B., Dazzan, P., Drake, R. J., Giordano, A., Husain, N., Jones, P. B., Joyce, E., Knox, E., Krynicki, C., Lawrie, S. M., Lewis, S., Lisiecka-Ford, D. M., Nikkheslat, N., Pariante, C. M., Smallman, R., … Dunn, G. (2018). The benefit of minocycline on negative symptoms of schizophrenia in patients with recent-onset psychosis (BeneMin): A randomised, double-blind, placebo-controlled trial. *The Lancet Psychiatry*, *5*(11), 885–894. https://doi.org/10.1016/S2215-0366(18)30345-6

Del Giudice, M., & Gangestad, S. W. (2018). Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. *Brain, Behavior, and Immunity*, *70*, 61–75. https://doi.org/10.1016/j.bbi.2018.02.013

Detcheverry, F., Senthil, S., Narayanan, S., & Badhwar, A. (2023). Changes in levels of the antioxidant glutathione in brain and blood across the age span of healthy adults: A systematic review. *NeuroImage. Clinical*, *40*, 103503. https://doi.org/10.1016/j.nicl.2023.103503

Fendri, C., Mechri, A., Khiari, G., Othman, A., Kerkeni, A., & Gaha, L. (2006). [Oxidative stress involvement in schizophrenia pathophysiology: A review]. *L'Encephale*, *32*(2 Pt 1), 244–252. https://doi.org/10.1016/s0013-7006(06)76151-6

Ferrucci, L., Corsi, A., Lauretani, F., Bandinelli, S., Bartali, B., Taub, D. D., Guralnik, J. M., & Longo, D. L. (2005). The origins of age-related proinflammatory state. *Blood*, *105*(6), 2294–2299. https://doi.org/10.1182/blood-2004-07-2599

Fisher, E., Gillam, J., Upthegrove, R., Aldred, S., & Wood, S. J. (2020). Role of magnetic resonance spectroscopy in cerebral glutathione quantification for youth mental health: A systematic review. *Early Intervention in Psychiatry*, *14*(2), 147–162. https://doi.org/10.1111/eip.12833

Fisher, E., Wood, S. J., Elsworthy, R. J., Upthegrove, R., & Aldred, S. (2020). Exercise as a protective mechanism against the negative effects of oxidative stress in first-episode

psychosis: A biomarker-led study. *Translational Psychiatry*, *10*(1), 1–11. https://doi.org/10.1038/s41398-020-00927-x

Gunes, M., Altindag, A., Bulut, M., Demir, S., Ibiloglu, A. O., Kaya, M. C., Atli, A., & Aksoy, N. (2017). Oxidative metabolism may be associated with negative symptoms in schizophrenia. *Psychiatry and Clinical Psychopharmacology*, *27*(1), 54–61. https://doi.org/10.1080/24750573.2017.1293243

Hafizi, S., Tseng, H.-H., Rao, N., Selvanathan, T., Kenk, M., Bazinet, R. P., Suridjan, I., Wilson, A. A., Meyer, J. H., Remington, G., Houle, S., Rusjan, P. M., & Mizrahi, R. (2017). Imaging Microglial Activation in Untreated First-Episode Psychosis: A PET Study With [18F]FEPPA. *The American Journal of Psychiatry*, *174*(2), 118–124. https://doi.org/10.1176/appi.ajp.2016.16020171

Han, Y., Ding, L., Cheng, X., Zhao, M., Zhao, T., Guo, L., Li, X., Geng, Y., Fan, M., Liao, H., & Zhu, L. (2020). Hypoxia Augments Cerebral Inflammation in a Dextran Sulfate Sodium-Induced Colitis Mouse Model. *Frontiers in Cellular Neuroscience*, *14*, 611764. https://doi.org/10.3389/fncel.2020.611764

Iskusnykh, I. Y., Zakharova, A. A., & Pathak, D. (2022). Glutathione in Brain Disorders and ageing. *Molecules (Basel, Switzerland)*, *27*(1), 324. https://doi.org/10.3390/molecules27010324

Jeon, P., Limongi, R., Ford, S. D., Mackinley, M., Dempster, K., Théberge, J., & Palaniyappan, L. (2021). Progressive Changes in Glutamate Concentration in Early Stages of Schizophrenia: A Longitudinal 7-Tesla MRS Study. *Schizophrenia Bulletin Open*, *2*(1), sgaa072. https://doi.org/10.1093/schizbullopen/sgaa072

Jeppesen, R., Christensen, R. H. B., Pedersen, E. M. J., Nordentoft, M., Hjorthøj, C., Köhler-Forsberg, O., & Benros, M. E. (2020). Efficacy and safety of anti-inflammatory agents in treatment of psychotic disorders – A comprehensive systematic review and meta-analysis. *Brain, Behavior, and Immunity*, *90*, 364–380. https://doi.org/10.1016/j.bbi.2020.08.028

Khandaker, G. M., Stochl, J., Zammit, S., Goodyer, I., Lewis, G., & Jones, P. B. (2018). Childhood inflammatory markers and intelligence as predictors of subsequent persistent depressive symptoms: A longitudinal cohort study. *Psychological Medicine*, *48*(9), 1514–1522. https://doi.org/10.1017/S0033291717003038

Koga, M., Serritella, A. V., Sawa, A., & Sedlak, T. W. (2016). Implications for reactive oxygen species in schizophrenia pathogenesis. *Schizophrenia Research*, *176*(1), 52–71. https://doi.org/10.1016/j.schres.2015.06.022

Lalousis, P., Schmaal, L., Wood, S. J., L.E.P Reniers, R., Cropley, V. L., Watson, A., Pantelis, C., Suckling, J., Barnes, N. M., Pariante, C., Jones, P. B., Joyce, E., Barnes, T. R. E., Lawrie, S. M., Husain, N., Dazzan, P., Deakin, B., Shannon Weickert, C., & Upthegrove, R. (2023). Inflammatory subgroups of schizophrenia and their association with brain structure: A semi-supervised machine learning examination of heterogeneity. *Brain, Behavior, and Immunity*, *113*, 166–175. https://doi.org/10.1016/j.bbi.2023.06.023

Limongi, R., Jeon, P., Théberge, J., & Palaniyappan, L. (2021). Counteracting Effects of Glutathione on the Glutamate-Driven Excitation/Inhibition Imbalance in First-Episode Schizophrenia: A 7T MRS and Dynamic Causal Modeling Study. *Antioxidants*, *10*(1), Article 1. https://doi.org/10.3390/antiox10010075

Lin, C., Chen, K., Yu, J., Feng, W., Fu, W., Yang, F., Zhang, X., & Chen, D. (2021). Relationship between TNF-α levels and psychiatric symptoms in first-episode drug-naïve patients with schizophrenia before and after risperidone treatment and in chronic patients. *BMC Psychiatry*, *21*(1), 561. https://doi.org/10.1186/s12888-021-03569-5

Liu, F., Guo, X., Wu, R., Ou, J., Zheng, Y., Zhang, B., Xie, L., Zhang, L., Yang, L., Yang, S., Yang, J., Ruan, Y., Zeng, Y., Xu, X., & Zhao, J. (2014). Minocycline supplementation for treatment of negative symptoms in early-phase schizophrenia: A double blind, randomized, controlled trial. *Schizophrenia Research*, *153*(1), 169–176. https://doi.org/10.1016/j.schres.2014.01.011

Lugrin, J., Rosenblatt-Velin, N., Parapanov, R., & Liaudet, L. (2014). The role of oxidative stress during inflammatory processes. *Biological Chemistry*, *395*(2), 203–230. https://doi.org/10.1515/hsz-2013-0241

Mandal, P. K., Tripathi, M., & Sugunan, S. (2012). Brain oxidative stress: Detection and mapping of anti-oxidant marker 'Glutathione' in different brain regions of healthy male/female, MCI and Alzheimer patients using non-invasive magnetic resonance spectroscopy. *Biochemical and Biophysical Research Communications*, *417*(1), 43–48. https://doi.org/10.1016/j.bbrc.2011.11.047

McKim, D. B., Weber, M. D., Niraula, A., Sawicki, C. M., Liu, X., Jarrett, B. L., Ramirez-Chan, K., Wang, Y., Roeth, R. M., Sucaldito, A. D., Sobol, C. G., Quan, N., Sheridan, J. F., & Godbout, J. P. (2018). Microglial recruitment of IL-1β-producing monocytes to brain endothelium causes stress-induced anxiety. *Molecular Psychiatry*, *23*(6), 1421–1431. https://doi.org/10.1038/mp.2017.64

Menezes, A. M. B., Oliveira, P. D., Wehrmeister, F. C., Assunção, M. C. F., Oliveira, I. O., Tovo-Rodrigues, L., Ferreira, G. D., & Gonçalves, H. (2019). Association of modifiable risk factors and IL-6, CRP, and adiponectin: Findings from the 1993 Birth Cohort, Southern Brazil. *PLoS ONE*, *14*(5), e0216202. https://doi.org/10.1371/journal.pone.0216202

Misiak, B., Stańczykiewicz, B., Kotowicz, K., Rybakowski, J. K., Samochowiec, J., & Frydecka, D. (2018). Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: A systematic review. *Schizophrenia Research*, *192*, 16–29. https://doi.org/10.1016/j.schres.2017.04.015

Müller, N., Krause, D., Dehning, S., Musil, R., Schennach-Wolff, R., Obermeier, M., Möller, H.-J., Klauss, V., Schwarz, M. J., & Riedel, M. (2010). Celecoxib treatment in an early stage of schizophrenia: Results of a randomized, double-blind, placebo-controlled trial of celecoxib augmentation of amisulpride treatment. *Schizophrenia Research*, *121*(1), 118–124. https://doi.org/10.1016/j.schres.2010.04.015

Murray, A. J., Humpston, C. S., Wilson, M., Rogers, J. C., Zia Ul Haq Katshu, M., Liddle, P. F., & Upthegrove, R. (2024). Measurement of brain glutathione with magnetic

Resonance spectroscopy in Schizophrenia-Spectrum disorders—A systematic review and Meta-Analysis. *Brain, Behavior, and Immunity*, *115*, 3–12. https://doi.org/10.1016/j.bbi.2023.09.017

Murray, A. J., Rogers, J. C., Katshu, M. Z. U. H., Liddle, P. F., & Upthegrove, R. (2021). Oxidative Stress and the Pathophysiology and Symptom Profile of Schizophrenia Spectrum Disorders. *Frontiers in Psychiatry*, *12*. https://doi.org/10.3389/fpsyt.2021.703452

Nettis, M. A., Lombardo, G., Hastings, C., Zajkowska, Z., Mariani, N., Nikkheslat, N., Worrell, C., Enache, D., McLaughlin, A., Kose, M., Sforzini, L., Bogdanova, A., Cleare, A., Young, A. H., Pariante, C. M., & Mondelli, V. (2021). Augmentation therapy with minocycline in treatment-resistant depression patients with low-grade peripheral inflammation: Results from a double-blind randomised clinical trial. *Neuropsychopharmacology*, *46*(5), 939–948. https://doi.org/10.1038/s41386-020-00948-6

Ottoy, J., De Picker, L., Verhaeghe, J., Deleye, S., Wyffels, L., Kosten, L., Sabbe, B., Coppens, V., Timmers, M., van Nueten, L., Ceyssens, S., Stroobants, S., Morrens, M., & Staelens, S. (2018). 18F-PBR111 PET Imaging in Healthy Controls and Schizophrenia: Test-Retest Reproducibility and Quantification of Neuroinflammation. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *59*(8), 1267–1274. https://doi.org/10.2967/jnumed.117.203315

Palaniyappan, L., Park, M. T. M., Jeon, P., Limongi, R., Yang, K., Sawa, A., & Théberge, J. (2021). Is There a Glutathione Centered Redox Dysregulation Subtype of Schizophrenia? *Antioxidants*, *10*(11), Article 11. https://doi.org/10.3390/antiox10111703

Palaniyappan, L., Sabesan, P., Li, X., & Luo, Q. (2021). Schizophrenia Increases Variability of the Central Antioxidant System: A Meta-Analysis of Variance From MRS Studies of Glutathione. *Frontiers in Psychiatry*, *12*, 796466. https://doi.org/10.3389/fpsyt.2021.796466

Patlola, S. R., Donohoe, G., & McKernan, D. P. (2023). Anti-inflammatory effects of 2nd generation antipsychotics in patients with schizophrenia: A systematic review and meta-analysis. *Journal of Psychiatric Research*, *160*, 126–136. https://doi.org/10.1016/j.jpsychires.2023.01.042

Probert, L. (2015). TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects. *Neuroscience*, *302*, 2–22. https://doi.org/10.1016/j.neuroscience.2015.06.038

Raffa, M., Atig, F., Mhalla, A., Kerkeni, A., & Mechri, A. (2011). Decreased glutathione levels and impaired antioxidant enzyme activities in drug-naive first-episode schizophrenic patients. *BMC Psychiatry*, *11*, 124. https://doi.org/10.1186/1471-244X-11-124

Rampino, A., Marakhovskaia, A., Soares-Silva, T., Torretta, S., Veneziani, F., & Beaulieu, J. M. (2019). Antipsychotic Drug Responsiveness and Dopamine Receptor Signaling; Old Players and New Prospects. *Frontiers in Psychiatry*, *9*. https://doi.org/10.3389/fpsyt.2018.00702

Reddy, R., Keshavan, M., & Yao, J. K. (2003). Reduced plasma antioxidants in first-episode patients with schizophrenia. *Schizophrenia Research*, *62*(3), 205–212. https://doi.org/10.1016/s0920-9964(02)00407-3

Sarafian, T. A., Magallanes, J. A., Shau, H., Tashkin, D., & Roth, M. D. (1999). Oxidative stress produced by marijuana smoke. An adverse effect enhanced by cannabinoids. *American Journal of Respiratory Cell and Molecular Biology*, *20*(6), 1286–1293. https://doi.org/10.1165/ajrcmb.20.6.3424

Selvaraj, S., Bloomfield, P. S., Cao, B., Veronese, M., Turkheimer, F., & Howes, O. D. (2018). Brain TSPO imaging and gray matter volume in schizophrenia patients and in people at ultra high risk of psychosis: An [11C]PBR28 study. *Schizophrenia Research*, *195*, 206–214. https://doi.org/10.1016/j.schres.2017.08.063

Sydnor, V. J., & Roalf, D. R. (2020). A meta-analysis of ultra-high field glutamate, glutamine, GABA and glutathione 1HMRS in psychosis: Implications for studies of psychosis risk. *Schizophrenia Research*, *226*, 61–69. https://doi.org/10.1016/j.schres.2020.06.028

Tenório, M. C. dos S., Graciliano, N. G., Moura, F. A., de Oliveira, A. C. M., & Goulart, M. O. F. (2021). N-Acetylcysteine (NAC): Impacts on Human Health. *Antioxidants*, *10*(6), 967. https://doi.org/10.3390/antiox10060967

Wang, M., Barker, P. B., Cascella, N. G., Coughlin, J. M., Nestadt, G., Nucifora, F. C., Sedlak, T. W., Kelly, A., Younes, L., Geman, D., Palaniyappan, L., Sawa, A., & Yang, K. (2023). Longitudinal changes in brain metabolites in healthy controls and patients with first episode psychosis: A 7-Tesla MRS study. *Molecular Psychiatry*, *28*(5), 2018–2029. https://doi.org/10.1038/s41380-023-01969-5

Watson, A. J., Giordano, A., Suckling, J., Barnes, T. R. E., Husain, N., Jones, P. B., Krynicki, C. R., Lawrie, S. M., Lewis, S., Nikkheslat, N., Pariante, C. M., Upthegrove, R., Deakin, B., Dazzan, P., & Joyce, E. M. (2023). Cognitive function in early-phase schizophrenia-spectrum disorder: IQ subtypes, brain volume and immune markers. *Psychological Medicine*, *53*(7), 2842–2851. https://doi.org/10.1017/S0033291721004815

Xin, L., Mekle, R., Fournier, M., Baumann, P. S., Ferrari, C., Alameda, L., Jenni, R., Lu, H., Schaller, B., Cuenod, M., Conus, P., Gruetter, R., & Do, K. Q. (2016). Genetic Polymorphism Associated Prefrontal Glutathione and Its Coupling With Brain Glutamate and Peripheral Redox Status in Early Psychosis. *Schizophrenia Bulletin*, *42*(5), 1185–1196. https://doi.org/10.1093/schbul/sbw038

Yao, J. K., Reddy, R. D., & van Kammen, D. P. (2001). Oxidative Damage and Schizophrenia. *CNS Drugs*, *15*(4), 287–310. https://doi.org/10.2165/00023210-200115040-00004

Yolland, C. O., Hanratty, D., Neill, E., Rossell, S. L., Berk, M., Dean, O. M., Castle, D. J., Tan, E. J., Phillipou, A., Harris, A. W., Barreiros, A. R., Hansen, A., & Siskind, D. (2020). Meta-analysis of randomised controlled trials with N-acetylcysteine in the treatment of schizophrenia. *Australian & New Zealand Journal of Psychiatry*, *54*(5), 453–466. https://doi.org/10.1177/0004867419893439

Zheng, W., Zhang, Q.-E., Cai, D.-B., Yang, X.-H., Qiu, Y., Ungvari, G. S., Ng, C. H., Berk, M., Ning, Y.-P., & Xiang, Y.-T. (2018). N-acetylcysteine for major mental disorders: A

systematic review and meta-analysis of randomized controlled trials. *Acta Psychiatrica Scandinavica*, *137*(5), 391–400. https://doi.org/10.1111/acps.12862

Appendices

Appendix A. Chapter 3 Supplement

Supplementary Table 1: Study Quality Assessment Checklist

Category 1: Sample characteristics (9)
1. Patients were evaluated with specific standardized diagnostic criteria (1)
2. Important demographic data (age and gender) were reported with mean (or median) and standard deviations (or range)) (2)
3. Healthy comparison subjects were evaluated to exclude psychiatric and medical illnesses and demographic data was reported (1)
4. Important clinical variables (e.g. illness duration, medication status, mood status [for bipolar studies] and psychosis severity [for schizophrenia]) were reported with mean (or median) and standard deviations (or range)) (3)
5. Sample size per group > 10 (2)
Category 2: MRS Methodology and reporting (11)
1. Field strength >1.5 T (1)
2. Pulse sequence reported (1)
3. Volume of interest (VOI) locations reported (1)
4. Nominal VOI size [cm3, mm3] reported (1)
5. Repetition time (*T* R), echo time (*T* E) [ms, s] reported (2)
6. Total number of excitations or acquisitions per spectrum reported (1)
7. Analysis software fitting reported (1)
8. Output measure (eg absolute concentration, institutional units, ratio) (1)
9. Quality measures of postprocessing model fitting (eg CRLB, goodness of fit, SD of residual) (2)

A maximum score of 20 for each study, allocated as per the criteria specified above.

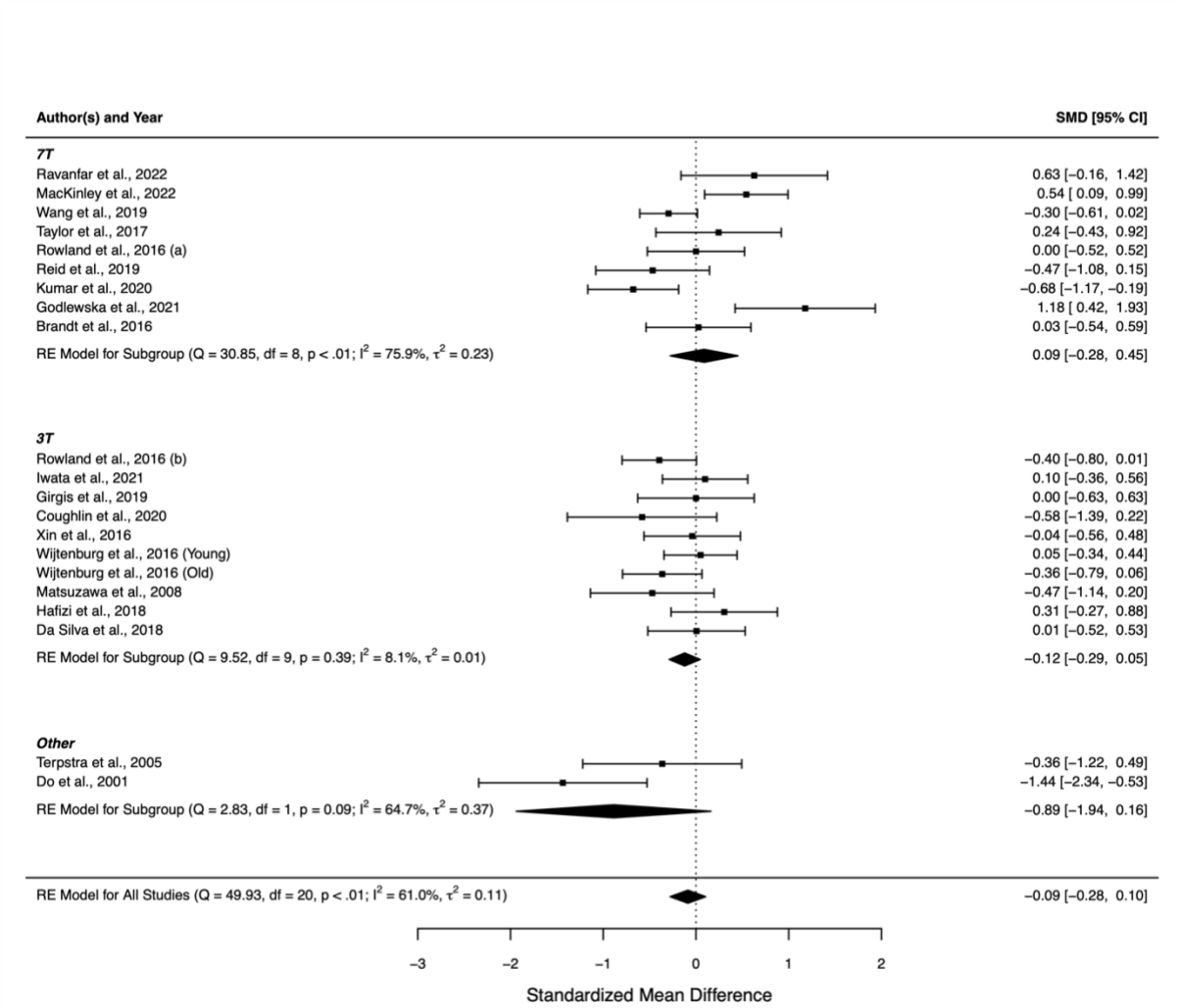
Supplementary Table 2: Leave One Out Analysis of All "Medial Prefrontal" Studies.

Study Removed	estimate	se	zval	pval	ci.lb	ci.ub	Q	Qp	tau2	I2	H2
Brandt et al., 2016	-0.09	0.10	-0.89	0.37	-0.29	0.11	49.76	0.00	0.13	63.71	2.76
Coughlin et al., 2020	-0.05	0.09	-0.56	0.58	-0.23	0.13	41.50	0.00	0.08	54.20	2.18
Da Silva et al., 2018	-0.11	0.10	-1.06	0.29	-0.30	0.09	47.91	0.00	0.12	61.75	2.61
Do et al., 2001	-0.07	0.10	-0.70	0.48	-0.27	0.13	48.72	0.00	0.12	62.31	2.65
Girgis et al., 2019	-0.07	0.10	-0.68	0.50	-0.27	0.13	48.39	0.00	0.12	62.06	2.64
Godlewska et al., 2021	-0.10	0.10	-0.92	0.36	-0.30	0.11	49.31	0.00	0.13	63.02	2.70
Hafizi et al., 2018	-0.09	0.10	-0.86	0.39	-0.29	0.11	49.88	0.00	0.13	63.80	2.76
Iwata et al., 2021	-0.09	0.10	-0.90	0.37	-0.29	0.11	49.72	0.00	0.13	63.69	2.75
Kumar et al., 2020	-0.07	0.10	-0.70	0.48	-0.26	0.13	48.54	0.00	0.12	62.00	2.63
Lesh et al., 2019	-0.09	0.10	-0.89	0.38	-0.29	0.11	49.83	0.00	0.13	63.76	2.76
MacKinley et al., 2022	-0.13	0.09	-1.52	0.13	-0.30	0.04	38.66	0.00	0.07	49.13	1.97
Matsuzawa et al., 2008	-0.10	0.10	-0.95	0.34	-0.30	0.10	49.15	0.00	0.13	63.10	2.71
Ravanfar et al., 2022	-0.05	0.10	-0.56	0.58	-0.24	0.13	44.34	0.00	0.10	56.80	2.31
Reid et al., 2019	-0.07	0.10	-0.69	0.49	-0.27	0.13	48.52	0.00	0.12	62.12	2.64
Rowland et al., 2016 (a)	-0.09	0.10	-0.89	0.38	-0.29	0.11	49.78	0.00	0.13	63.72	2.76
Rowland et al., 2016 (b)	-0.07	0.10	-0.66	0.51	-0.27	0.13	47.69	0.00	0.12	61.36	2.59
Taylor et al., 2017	-0.10	0.10	-1.00	0.32	-0.30	0.10	48.90	0.00	0.12	62.72	2.68
Terpstra et al., 2005	-0.08	0.10	-0.77	0.44	-0.28	0.12	49.57	0.00	0.12	63.13	2.71
Wang et al., 2019	-0.07	0.10	-0.69	0.49	-0.28	0.13	48.20	0.00	0.13	61.39	2.59
Wijtenburg et al., 2016	-0.13	0.09	-1.37	0.17	-0.31	0.05	41.48	0.00	0.09	53.46	2.15
Xin et al., 2016	-0.11	0.10	-1.16	0.25	-0.30	0.08	46.58	0.00	0.11	59.93	2.50

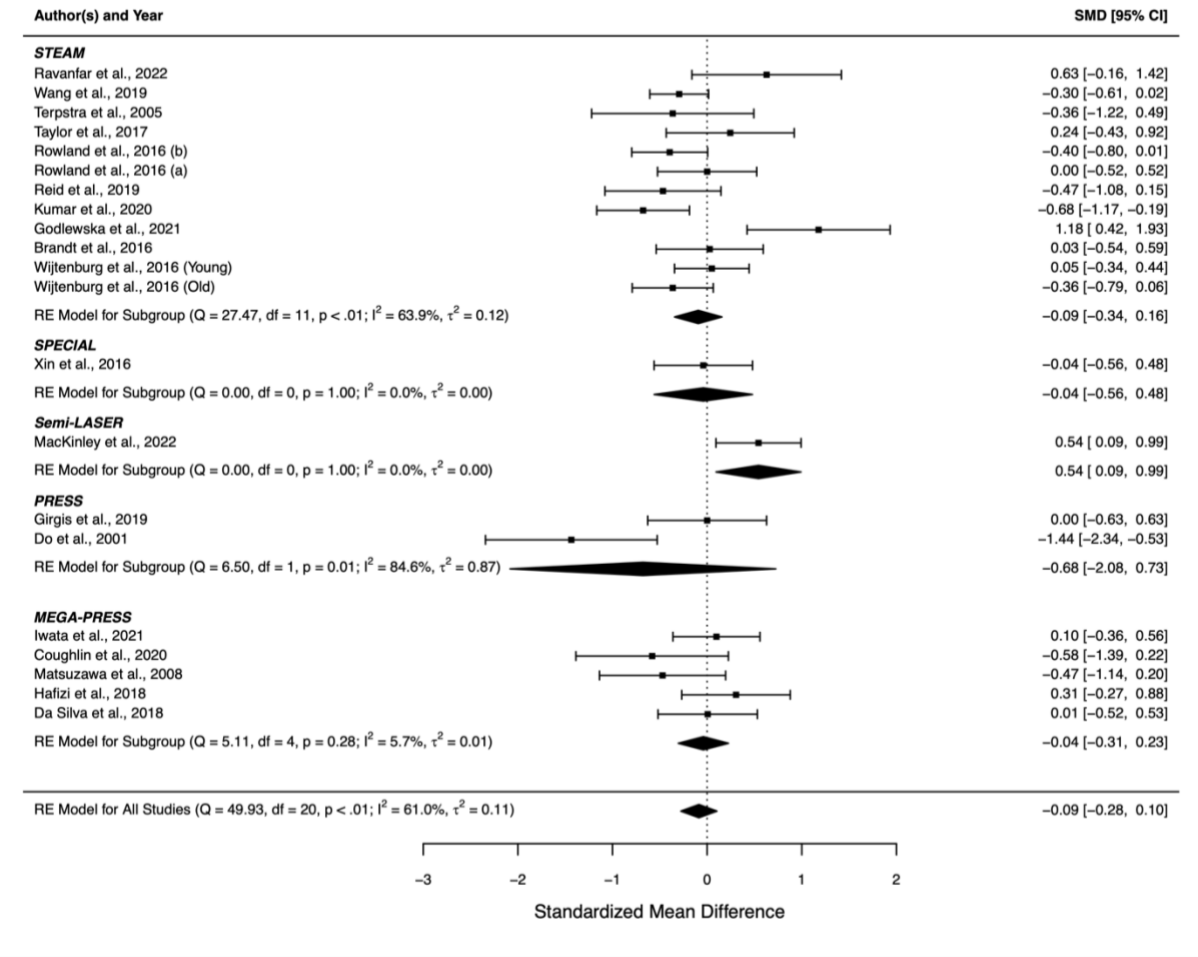
Supplementary Table 3: Leave One Out Analysis of "Medial Prefrontal" Stable Schizophrenia

Study Removed	estimate	se	zval	pval	ci.lb	ci.ub	Q	Qp	tau2	I2	H2
Do et al., 2001	-0.15	0.09	-1.68	0.09	-0.34	0.03	17.10	0.15	0.03	28.19	1.39
Matsuzawa et al., 2008	-0.18	0.11	-1.70	0.09	-0.40	0.03	23.77	0.02	0.07	45.54	1.84
Wijtenburg et al., 2016 (Old)	-0.18	0.11	-1.63	0.10	-0.41	0.04	23.79	0.02	0.07	46.56	1.87
Wijtenburg et al., 2016 (Young)	-0.23	0.11	-2.07	0.04	-0.45	-0.01	22.76	0.03	0.07	43.33	1.76
Brandt et al., 2016	-0.22	0.11	-1.99	0.05	-0.44	0.00	23.84	0.02	0.07	46.46	1.87
Coughlin et al., 2020	-0.18	0.11	-1.72	0.09	-0.39	0.03	23.56	0.02	0.06	44.44	1.80
Girgis et al., 2019	-0.22	0.11	-1.96	0.05	-0.43	0.00	24.08	0.02	0.07	47.03	1.89
Iwata et al., 2021	-0.23	0.11	-2.12	0.03	-0.44	-0.02	22.73	0.03	0.06	42.73	1.75
Kumar et al., 2020	-0.15	0.10	-1.58	0.11	-0.34	0.04	20.38	0.06	0.03	28.64	1.40
Rowland et al., 2016 (a)	-0.22	0.11	-1.96	0.05	-0.44	0.00	23.89	0.02	0.07	46.81	1.88
Rowland et al., 2016 (b)	-0.18	0.11	-1.59	0.11	-0.40	0.04	23.36	0.02	0.07	45.17	1.82
Taylor et al., 2017	-0.23	0.11	-2.17	0.03	-0.43	-0.02	22.77	0.03	0.06	42.49	1.74
Terpstra et al., 2005	-0.19	0.11	-1.80	0.07	-0.41	0.02	24.32	0.02	0.07	46.58	1.87
Ravanfar et al., 2022	-0.24	0.10	-2.43	0.01	-0.43	-0.05	20.15	0.06	0.04	34.86	1.54

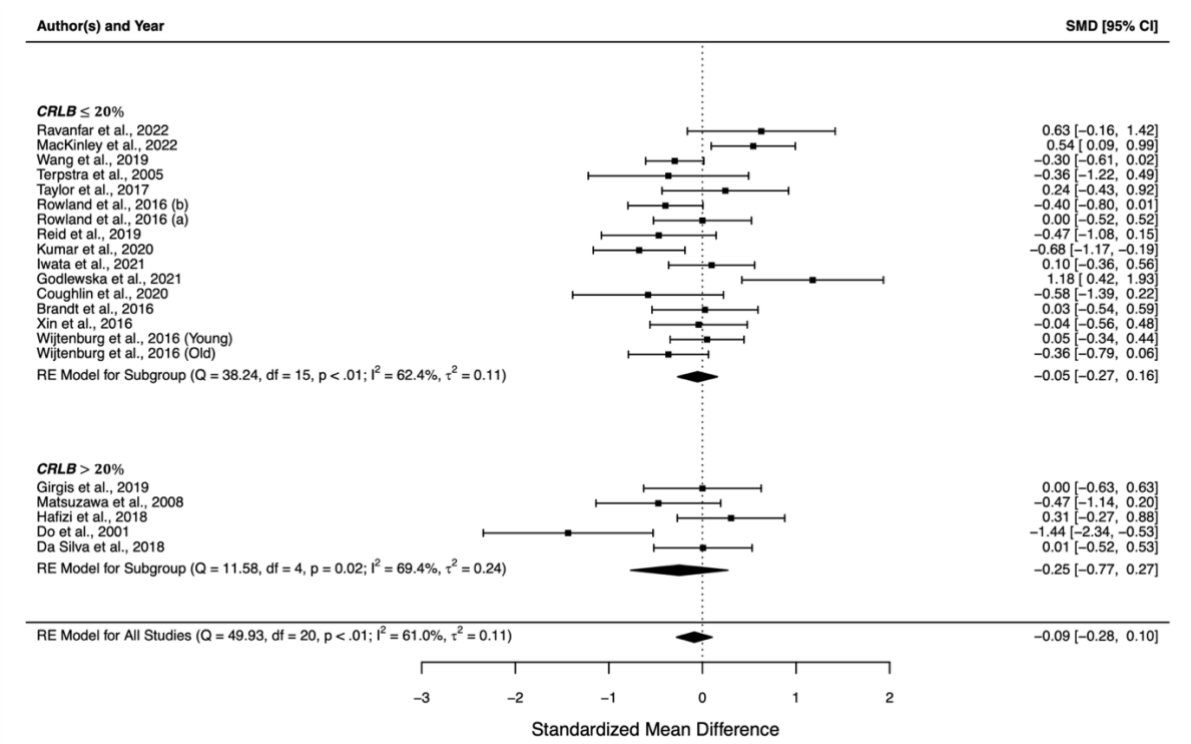
Supplementary Figure S1: Subgroup Analysis by Scanner Field Strength



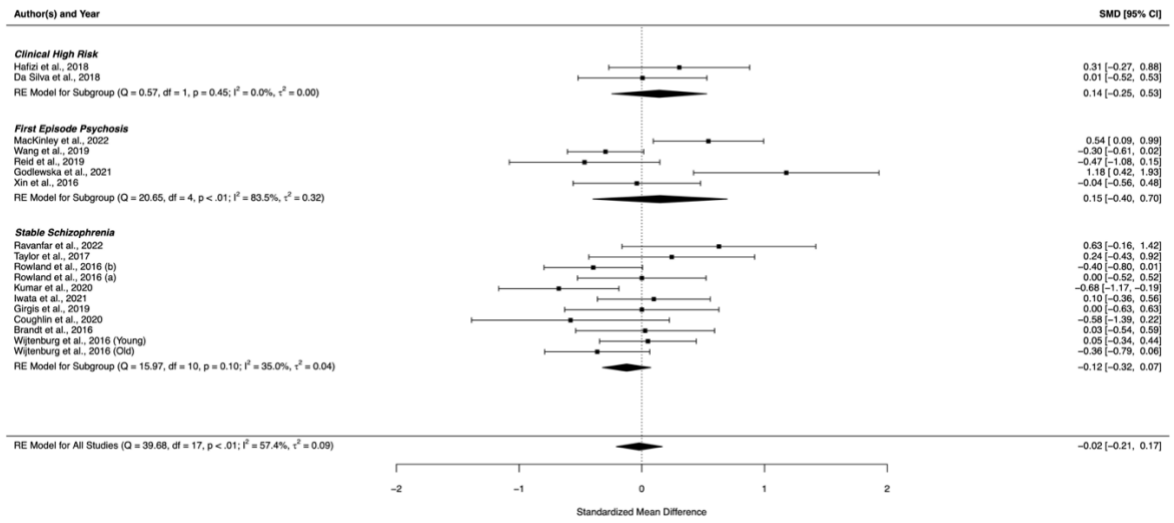
Supplementary Figure S2: Subgroup Analysis by MRS Pulse Sequence



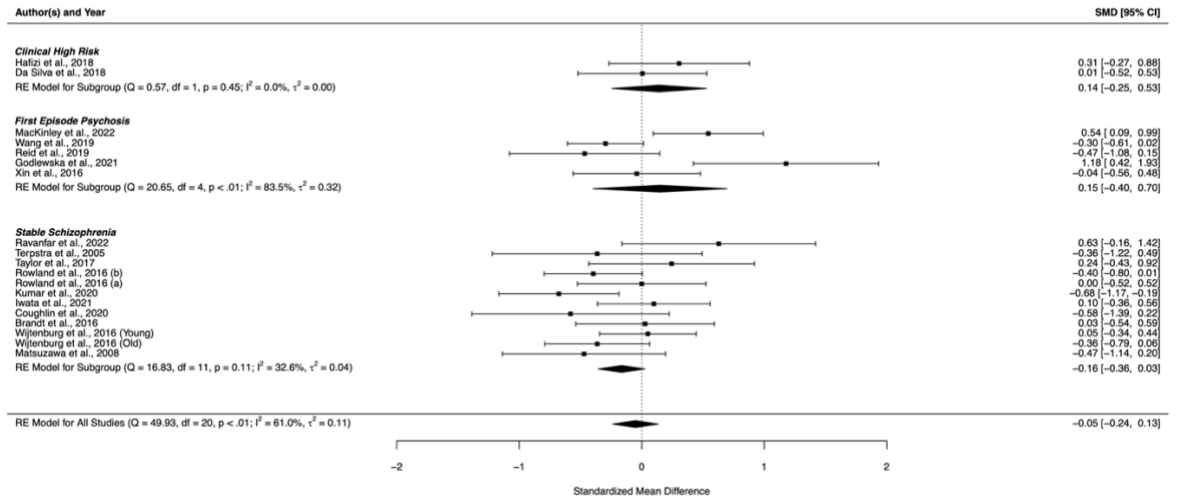
Supplementary Figure S3: Subgroup Analysis by CRLB Cut-off



Supplementary Figure S4: Sensitivity Analysis with Older Studies Removed.



Supplementary Figure S5: Sensitivity Analysis with PRESS Studies Removed.



Peripheral and Central GSH:

Some researchers suggest peripheral antioxidant capacity may provide insight into oxidative stress levels within the brain. However, others have argued that peripheral status is simply indirect evidence for what may be occurring centrally, and traces of oxidative damage may arise from a variety of areas within the body.

Only 6 studies (Coughlin et al., 2020; Da Silva et al., 2018; Xin et al., 2016; Bryll et al., 2020; Conus et al., 2018; Fisher et al., 2020) reported both peripheral and central measures of GSH, thus it was not possible to conduct a quantitative analysis. Peripheral measures came from blood cells (Xin et al., 2016; Conus et al., 2018) and plasma (Coughlin et al., 2020; Da Silva et al., 2018; Bryll et al., 2020; Fisher et al., 2020), tested using ferric-reducing ability of plasma (FRAP), thiobarbituric acid-reactive substances (TBARS), malondialdehyde test, GSH concentration and or GPx activity.

Of the six studies, three directly measured the correlation between peripheral and central GSH, all finding no significant correlation. A further study assessed the combined central measure of GSH + GLN + GLU and found a significant relationship with FRAP (Bryll et al., 2020). The remaining studies did not complete an analysis of peripheral and

central measures, however three found significant markers of oxidative stress in the periphery reported at the same time decreased central GSH.

This non-quantitative analysis of peripheral oxidative stress markers compared to central markers indicates that peripheral and central levels of GSH may not be interrelated, and peripheral markers of oxidative stress such as enzymatic activity and lipid peroxidation may not consistently reflect redox status within the brain. It has been suggested that there is a lack of a significant transport system that allows GSH to cross the blood brain barrier. It is also worth noting that blood measures of GSH are susceptible to artefactual oxidation during preparation and long-term storage of samples and as such may not necessarily reflect true redox status unless caution is taken.

Appendix B. Chapter 4 Supplement

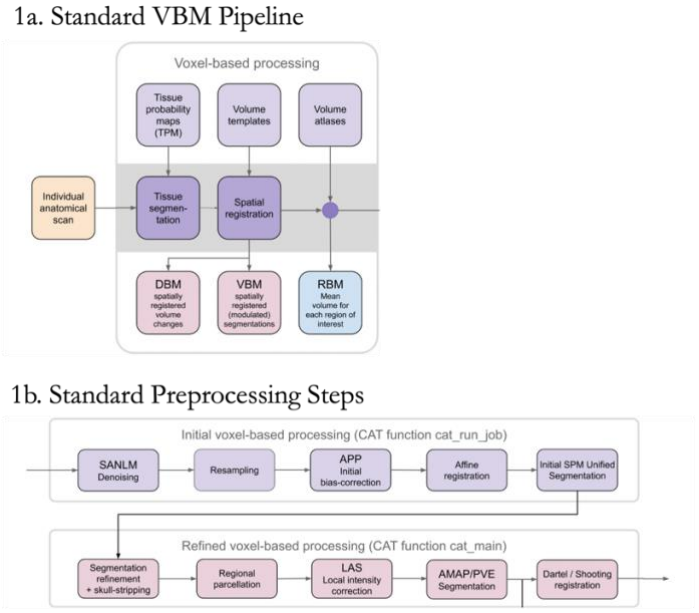
Supplementary Methods:

1.1) Data Acquisition Protocol

A structural, T1-weighted MRI was acquired with either a 3T GE scanner (Cardiff) or 3T Phillips scanner (Manchester). Alongside a battery of 1H-MRS scans (not presented here). All data for each participant was acquired in one day, with a morning of cognitive tests and interviews, then blood acquisition, followed by an afternoon of imaging procedures. T1 data was acquired using a standard MPRAGE SENSE acquisition sequence. Resolution matrix=256×256, slices = 180, voxel dimensions = 0.9375 mm x 0.9375 mm x 0.9 mm, TR = 1s, slice thickness = 0.9 mm.

1.2) VBM Pipeline

This study followed the standard cat-12 VBM pipeline presented here: https://neuro-jena.github.io/cat12-help/#major process



sFigure 1. Standard cat-12 processing pipeline (adapted from the cat-12 manual).

Within this study the major processing steps were followed as such:

1. Denoising: Images were initially denoised by employing Spatially Adaptive Non-Local Means (SANLM) filtering - removing noise while maintaining edges (Manjon et al., 2010).
2. Bias Correction: Imaged are then bias-corrected and global intensities are normalised to improve the following steps.
3. Affine Pre-processing/Registration (APP): To improve the initial SPM segmentation, an initial affine registration is applied to a bias-corrected image and the intensity range is limited to avoid problems in special protocols.
4. Initial Segmentation: Images are segmented using the standard SPM "unified segmentation" (Ashburner and Friston, 2005).
5. Parcellation: The unified segmentation output is then skull-stripped and the brain is parcellated into left and right hemisphere, subcortical areas, and the cerebellum.
6. Local Adaptive Segmentation (LAS): LAS addresses inhomogeneities in white and gray matter intensities. This is crucial, especially in regions with differing iron content, such as cortical and subcortical structures.
7. Adaptive Maximum A Posterior (AMAP) Segmentation: AMAP segmentation models local intensity variations as slowly varying spatial functions. This approach ensures a consistent segmentation across cortical and subcortical structures.
8. Partial Volume Segmentation: A partial volume segmentation algorithm models tissues with intensities between GM and WM, as well as GM and cerebrospinal fluid (CSF). This algorithm is specifically employed on the tissue segments generated by AMAP.
9. Spatial Normalisation: The tissue segmentations undergo spatial normalization to a standardized reference space through Geodesic Shooting registrations (Ashburner and Friston in 2011).
10. Quality Assurance Check: Image quality was assessed using the Quality Assurance framework of CAT12 to assess the quality of the GMV maps.
11. Smooth: Images were smoothed to 6mm FWHM
12. Modelling: Statistical models were built to compare across each cluster and healthy controls. TIV was corrected for via ANCOVA and age and sex were added into the model as covariates.

Supplementary Results

sTable 1. Demographic and Clinical Characteristics of the Sample – Split by Schizophrenia Subtype

	FEP (N=196)	EST (N=33)	HC (N=38)	F/t/X^2	*p*
Demographics					
Age	25.46 (5.25)	42.98 (8.28)	35.04 (12.18)	104.10	<.001***
Sex [a]	55 Female	9 Female	8 Female	0.80	0.67
BMI	27.10 (6.08)	29.45 (5.38)	25.33 (4.19)	4.52	0.01*
Cytokines					
IFN-γ	4.64 (5.57)	4.29 (3.95)	3.33 (2.24)	1.08	0.34
IL-6	0.68 (0.46)	0.78 (0.50)	0.50 (0.24)	4.02	0.02*
TNF-α	2.52 (0.68)	2.72 (1.08)	2.29 (0.43)	3.23	0.04*
CRP	2.57 (2.79)	3.34 (3.63)	2.34 (3.28)	1.14	0.32
Clinical Symptoms					
PANSS Positive [b]	16.37 (5.11)	13.18 (5.57)		3.28	<.001***
PANSS Negative [b]	16.23 (5.93)	13.55 (6.53)		2.37	0.01*
PANSS General [b]	32.53 (7.69)	25.97 (6.62)		4.62	<.001***
PANSS Total [b]	65.18 (15.06)	52.7 (14.19)		4.44	<.001***
Cognitive Tests					
WAIS Digit	6.73 (2.56)	7.48 (2.32)	10.42 (3.26)	30.55	<.001***
WAIS Arithmetic	8.41 (3.19)	8.09 (3.19)	10.00 (3.21)	4.42	0.01*
WAIS Information	10.07 (3.19)	10.42 (2.89)	11.92 (3.06)	5.50	<.001***
WAIS Block Design	9.63 (3.15)	9.55 (2.76)	11.47 (3.19)	5.78	<.001***
WAIS Sum of scales	33.61 (9.59)	35.55 (7.77)	43.82 (9.84)	18.68	<.001***
WAIS Full Scale IQ	91.69 (15.21)	92.33 (12.68)	105.09 (20.88)	11.47	<.001***
Calculated WTAR	97.51 (18.54)	102.76 (14.85)	106.89 (15.03)	5.10	<.001***

FEP first episode psychosis, *EST* established schizophrenia, *HC* healthy controls, *BMI* body mass index, *IFN-γ* interferon gamma, *IL-6* interleukin 6, *TNF-α* tumour necrosis factor alpha, *CRP* C-reactive protein, *PANSS* positive and negative syndrome scale, *WAIS* Wechsler adult intelligence scale, *WTAR* Wechsler test of adult reading. *F* ANOVA F test statistic, *t* t-test statistic, X^2 Chi-Square test statistic.

* = <0.05, *** = <0.001

[a] Chi-square tests

[b] t-test

sTable 2: Tukey's Post-Hoc Comparisons:

Dependent Variable	(I) Clusters	(J) Clusters	Mean Difference (I-J)	Std. Error	Sig.
Negative Dimension	CRP	IL-6	1.092	1.777	0.927
		Noninflamed	2.209	1.602	0.514
		TNFa	0.009	1.880	1.000
	IL-6	CRP	-1.092	1.777	0.927
		Noninflamed	1.117	1.292	0.823
		TNFa	-1.083	1.624	0.909
	Noninflamed	CRP	-2.209	1.602	0.514
		IL-6	-1.117	1.292	0.823
		TNFa	-2.200	1.431	0.417
	TNFa	CRP	-0.009	1.880	1.000
		IL-6	1.083	1.624	0.909
		Noninflamed	2.200	1.431	0.417
Positive Dimension	CRP	IL-6	0.222	1.089	0.997
		Noninflamed	0.065	0.983	1.000
		TNFa	0.267	1.153	0.996
	IL-6	CRP	-0.222	1.089	0.997
		Noninflamed	-0.157	0.793	0.997
		TNFa	0.045	0.996	1.000
	Noninflamed	CRP	-0.065	0.983	1.000
		IL-6	0.157	0.793	0.997
		TNFa	0.202	0.878	0.996
	TNFa	CRP	-0.267	1.153	0.996
		IL-6	-0.045	0.996	1.000
		Noninflamed	-0.202	0.878	0.996
Anxio-depressive Dimension	CRP	IL-6	-0.434	0.842	0.955
		Noninflamed	1.408	0.759	0.251
		TNFa	1.187	0.891	0.543
	IL-6	CRP	0.434	0.842	0.955
		Noninflamed	1.842*	0.612	0.015*
		TNFa	1.622	0.770	0.154
	Noninflamed	CRP	-1.408	0.759	0.251
		IL-6	-1.842*	0.612	0.015*
		TNFa	-0.221	0.678	0.988
	TNFa	CRP	-1.187	0.891	0.543
		IL-6	-1.622	0.770	0.154
		Noninflamed	0.221	0.678	0.988
Excitement Dimension	CRP	IL-6	-0.405	0.469	0.823
		Noninflamed	-0.628	0.422	0.448
		TNFa	-0.347	0.496	0.897
	IL-6	CRP	0.405	0.469	0.823
		Noninflamed	-0.222	0.341	0.914
		TNFa	0.058	0.428	0.999
	Noninflamed	CRP	0.628	0.422	0.448
		IL-6	0.222	0.341	0.914
		TNFa	0.280	0.377	0.880
	TNFa	CRP	0.347	0.496	0.897
		IL-6	-0.058	0.428	0.999
		Noninflamed	-0.280	0.377	0.880
Disorganisation	CRP	IL-6	-0.040	0.821	1.000

Other Dimension		Noninflamed	-0.088	0.741	0.999
		TNFa	-0.355	0.869	0.977
	IL-6	CRP	0.040	0.821	1.000
		Noninflamed	-0.048	0.597	1.000
		TNFa	-0.314	0.751	0.975
	Noninflamed	CRP	0.088	0.741	0.999
		IL-6	0.048	0.597	1.000
		TNFa	-0.266	0.661	0.978
	TNFa	CRP	0.355	0.869	0.977
		IL-6	0.314	0.751	0.975
		Noninflamed	0.266	0.661	0.978
WAIS Digit scaled	CRP	IL-6	-1.298	0.597	0.133
		Noninflamed	-1.707*	0.539	0.009**
		TNFa	-1.315	0.634	0.165
	IL-6	CRP	1.298	0.597	0.133
		Noninflamed	-0.409	0.433	0.780
		TNFa	-0.017	0.546	1.000
	Noninflamed	CRP	1.707*	0.539	0.009**
		IL-6	0.409	0.433	0.780
		TNFa	0.392	0.483	0.848
	TNFa	CRP	1.315	0.634	0.165
		IL-6	0.017	0.546	1.000
		Noninflamed	-0.392	0.483	0.848
WAIS Arithmetic scaled	CRP	IL-6	-0.930	0.739	0.591
		Noninflamed	-1.261	0.664	0.232
		TNFa	-1.223	0.788	0.409
	IL-6	CRP	0.930	0.739	0.591
		Noninflamed	-0.331	0.539	0.928
		TNFa	-0.293	0.687	0.974
	Noninflamed	CRP	1.261	0.664	0.232
		IL-6	0.331	0.539	0.928
		TNFa	0.038	0.605	1.000
	TNFa	CRP	1.223	0.788	0.409
		IL-6	0.293	0.687	0.974
		Noninflamed	-0.038	0.605	1.000
WAIS Information scaled	CRP	IL-6	-1.210	0.740	0.361
		Noninflamed	-1.142	0.667	0.320
		TNFa	-0.936	0.784	0.631
	IL-6	CRP	1.210	0.740	0.361
		Noninflamed	0.069	0.534	0.999
		TNFa	0.274	0.674	0.977
	Noninflamed	CRP	1.142	0.667	0.320
		IL-6	-0.069	0.534	0.999
		TNFa	0.205	0.593	0.986
	TNFa	CRP	0.936	0.784	0.631
		IL-6	-0.274	0.674	0.977
		Noninflamed	-0.205	0.593	0.986
WAIS Block Design scaled	CRP	IL-6	-0.884	0.740	0.631
		Noninflamed	-1.157	0.664	0.305
		TNFa	-1.139	0.786	0.470
	IL-6	CRP	0.884	0.740	0.631
		Noninflamed	-0.273	0.532	0.956
		TNFa	-0.255	0.678	0.982
	Noninflamed	CRP	1.157	0.664	0.305
		IL-6	0.273	0.532	0.956
		TNFa	0.019	0.594	1.000
	TNFa	CRP	1.139	0.786	0.470

		IL-6	0.255	0.678	0.982
		Noninflamed	-0.019	0.594	1.000
WAIS Sum of Scaleds	CRP	IL-6	-4.313	2.137	0.184
		Noninflamed	-6.006*	1.927	0.011*
		TNFa	-3.928	2.261	0.307
	IL-6	CRP	4.313	2.137	0.184
		Noninflamed	-1.693	1.554	0.696
		TNFa	0.385	1.953	0.997
	Noninflamed	CRP	6.006*	1.927	0.011*
		IL-6	1.693	1.554	0.696
		TNFa	2.078	1.720	0.623
	TNFa	CRP	3.928	2.261	0.307
		IL-6	-0.385	1.953	0.997
		Noninflamed	-2.078	1.720	0.623
WAIS Full Scale IQ	CRP	IL-6	-7.662	3.415	0.115
		Noninflamed	-9.183*	3.057	0.016*
		TNFa	-9.073	3.608	0.060
	IL-6	CRP	7.662	3.415	0.115
		Noninflamed	-1.521	2.499	0.929
		TNFa	-1.411	3.149	0.970
	Noninflamed	CRP	9.183*	3.057	0.016*
		IL-6	1.521	2.499	0.929
		TNFa	0.111	2.756	1.000
	TNFa	CRP	9.073	3.608	0.060
		IL-6	1.411	3.149	0.970
		Noninflamed	-0.111	2.756	1.000
Calculated WTAR	CRP	IL-6	-11.881*	4.120	0.022*
		Noninflamed	-12.907*	3.720	0.003**
		TNFa	-13.006*	4.344	0.016*
	IL-6	CRP	11.881*	4.120	0.022*
		Noninflamed	-1.026	3.028	0.987
		TNFa	-1.125	3.768	0.991
	Noninflamed	CRP	12.907*	3.720	0.003**
		IL-6	1.026	3.028	0.987
		TNFa	-0.099	3.326	1.000
	TNFa	CRP	13.006*	4.344	0.016*
		IL-6	1.125	3.768	0.991
		Noninflamed	0.099	3.326	1.000

sTable 3: Demographics Across BeneMin Sites

Please note, all participants recruited in BeneMin were first episode patients.

	Manchester (n = 90)	**Edinburgh (n = 12)**	**Cambridge (n = 25)**	**KCL (n = 8)**	**UCL (n = 10)**	**Birmingham (n = 16)**	**F/X²**	***p***
Demographics								
Age	25.70 (5.21)	27.58 (5.42)	25.32 (6.16)	28.25 (5.01)	25.30 (24.31)	24.13 (4.46)	0.98	0.43
Sex[a]	28 Female	4 Female	7 Female	2 Female	3 Female	0 Female	6.95	0.22
BMI	27.74 (6.84)	28.51 (7.49)	25.19 (3.37)	26.97 (5.28)	27.50 (6.37)	28.82 (6.26)	0.90	0.48
Inflammatory Markers								
IFN-y (pg/ml)	5.26 (7.28)	5.46 (4.52)	4.52 (4.17)	3.89 (1.88)	2.93 (1.00)	4.22 (3.93)	0.40	0.85
IL-6 (pg/ml)	0.80 (0.52)	0.83 (0.55)	0.52 (0.25)	0.54 (0.27)	0.49 (0.27)	0.64 (0.31)	2.64	0.03*
TNF-a (pg/ml)	2.49 (0.60)	2.97 (0.73)	2.55 (0.67)	2.74 (1.29)	2.20 (0.45)	2.64 (0.52)	1.88	0.10
CRP (mg/L)	2.62 (2.95)	2.13 (2.18)	2.81 (2.58)	0.71 (0.45)	2.71 (3.24)	4.01 (2.61)	1.67	0.15
Clinical Symptoms								
PANSS Total Positive	17.03 (4.87)	18.00 (3.07)	18.60 (4.02)	11.88 (3.91)	14.70 (3.74)	15.81 (5.76)	3.32	0.01*
PANSS Total Negative	16.57 (5.92)	18.08 (4.23)	18.72 (5.63)	19.00 (5.35)	16.70 (5.62)	17.06 (5.42)	0.82	0.54
PANSS Total General	33.36 (7.27)	36.75 (6.88)	36.00 (6.92)	28.38 (3.70)	32.50 (7.56)	32.38 (7.73)	2.06	0.07
PANSS Total	67.08 (13.89)	72.83 (9.89)	73.32 (13.50)	59.25 (8.46)	63.90 (15.41)	65.25 (15.95)	2.08	0.07
Cognitive Tests								
WAIS Digit Symbol	6.89 (2.51)	7.25 (2.77)	6.23 (2.01)	5.00 (1.83)	5.50 (2.42)	5.56 (2.28)	2.03	0.08
WAIS Arithmetic	8.35 (2.86)	9.75 (3.55)	9.58 (3.22)	7.63 (3.29)	7.10 (3.51)	7.50 (3.12)	1.93	0.09
WAIS Information	9.72 (3.03)	11.75 (3.47)	10.58 (2.90)	9.75 (3.65)	11.00 (3.59)	9.13 (3.52)	1.49	0.20
WAIS Block	9.22 (3.05)	11.00 (3.19)	11.04 (2.87)	9.38 (3.85)	7.60 (2.17)	8.06 (2.17)	3.59	<0.01**

Design								
WAIS Sum of scales Scores	32.90 (9.04)	39.75 (8.99)	32.76 (10.70)	31.13 (10.13)	31.20 (8.88)	30.25 (8.22)	1.70	0.14
WAIS Current IQ	90.05 (13.29)	100.08 (15.99)	98.67 (15.29)	87.25 (17.56)	85.40 (14.58)	83.81 (13.35)	3.75	<0.01**
WTAR Premorbid IQ	95.14 (18.86)	101.00 (15.24)	107.17 (14.04)	91.00 (16.20)	101.78 (9.80)	92.44 (22.75)	2.36	0.04*

BMI body mass index, *IFN-γ* interferon gamma, *IL-6* interleukin 6, *TNF-α* tumour necrosis factor alpha, *CRP* C-reactive protein, *PANSS* positive and negative syndrome scale, *WAIS* Wechsler Adult Intelligence Scale, *WTAR* Wechsler Test of Adult Reading, *F* ANOVA F test statistic, X^2 Chi-Square test statistic.

* p = <0.05, ** p = <0.01

[a] Chi-square tests

sTable 4: Demographics Across SPRING Sites

4a. SPRING First Episode Psychosis Cohort

	Cardiff (n = 15)	**Manchester (n = 20)**	**t/X²**	***p***
Demographics				
Age	24.21 (4.31)	24.23 (5.07)	-0.01	0.99
Sex				
BMI	26.52 (5.58)	24.66 (3.59)	1.20	0.24
Inflammatory Markers				
IFN-y (pg/ml)	3.83 (2.21)	3.62 (3.35)	0.21	0.84
IL-6 (pg/ml)	0.50 (0.31)	0.56 (0.46)	-0.40	0.69
TNF-a (pg/ml)	2.66 (0.82)	2.25 (0.68)	1.60	0.12
CRP (mg/L)	1.82 (1.62)	2.42 (3.46)	-0.62	0.54
Clinical Symptoms				
PANSS Total Positive	13.47 (6.46)	14.90 (5.68)	-0.70	0.49
PANSS Total Negative	11.67 (5.98)	11.90 (4.46)	-0.13	0.90

PANSS Total General	24.67 (8.93)	29.65 (6.18)	-1.95	0.06
PANSS Total	49.80 (17.73)	56.45 (11.86)	-1.33	0.19
Cognitive Scores				
WAIS Digit Symbol	7.13 (3.11)	7.95 (2.40)	-0.88	0.39
WAIS Arithmetic	8.07 (3.56)	8.40 (3.73)	-0.27	0.79
WAIS Information	9.47 (3.29)	10.90 (3.18)	-1.30	0.20
WAIS Block Design	10.67 (4.08)	10.50 (2.74)	0.14	0.89
WAIS Sum of scales Scores	35.33 (11.75)	37.75 (8.78)	-0.70	0.49
WAIS Current IQ	92.40 (20.33)	96.25 (14.67)	-0.65	0.52
WTAR Premorbid IQ	95.60 (19.15)	100.20 (20.76)	-0.67	0.51

4b. SPRING Established Schizophrenia Cohort

	Cardiff (n = 14)	**Manchester (n = 19)**	**t/X^2**	***p***
Demographics				
Age	38.06 (9.56)	46.60 (6.59)	-3.37	<0.01**
Sex				
BMI	29.48 (5.44)	29.42 (5.49)	0.03	0.98
Inflammatory Markers				
IFN-y (pg/ml)	3.59 (1.59)	4.81 (5.02)	-0.88	0.39
IL-6 (pg/ml)	0.96 (0.61)	0.65 (0.35)	1.86	0.07
TNF-a (pg/ml)	3.24 (1.30)	2.33 (0.70)	2.58	0.02*
CRP (mg/L)	3.54 (3.42)	3.19 (3.87)	0.27	0.79
Clinical Symptoms				
PANSS Total Positive	10.93 (3.08)	14.84 (6.44)	-2.10	0.44
PANSS Total Negative	15.14 (8.33)	12.37 (4.71)	1.22	0.23

PANSS Total General	24.64 (6.83)	26.95 (6.50)	-0.99	0.33
PANSS Total	50.71 (15.58)	54.16 (13.33)	-0.68	0.50
Cognitive Scores				
WAIS Digit Symbol	7.00 (2.57)	7.84 (2.12)	-1.03	0.31
WAIS Arithmetic	7.50 (3.06	8.53 (3.30)	-0.91	0.37
WAIS Information	10.21 (2.72)	10.58 (3.08)	-0.35	0.73
WAIS Block Design	9.07 (2.59)	9.89 (2.90)	-0.84	0.41
WAIS Sum of scales Scores	33.79 (7.59)	36.84 (7.84)	-1.12	0.27
WAIS Current IQ	89.50 (12.31)	94.42 (12.86)	-1.11	0.28
WTAR Premorbid IQ	102.00 (14.04)	103.32 (15.78)	-0.25	0.81

4c. SPRING Healthy Control Cohort

	Cardiff (n = 20)	**Manchester (n = 18)**	**t/X^2**	***p***
Demographics				
Age	32.75 (11.83)	37.58 (12.39)	-1.23	0.23
Sex				
BMI	24.09 (3.31)	26.70 (4.70)	-1.96	0.05
Inflammatory Markers				
IFN-y (pg/ml)	3.23 (1.73)	3.44 (2.75)	-0.28	0.78
IL-6 (pg/ml)	0.49 (0.21)	0.51 (0.28)	-0.15	0.88
TNF-a (pg/ml)	2.26 (0.38)	2.33 (0.49)	-0.51	0.62
CRP (mg/L)	2.02 (2.69)	2.70 (3.88)	-0.63	0.53

Cognitive Scores				
WAIS Digit Symbol	9.45 (3.14)	11.50 (3.13)	-2.01	0.05
WAIS Arithmetic	8.60 (3.33)	11.56 (2.28)	-3.16	<0.01**
WAIS Information	10.85 (3.53)	13.11 (1.91)	-2.42	0.02*
WAIS Block Design	10.45 (3.19)	12.61 (2.85)	-2.19	0.04*
WAIS Sum of scales Scores	39.35 (9.58)	48.78 (7.64)	-3.33	<0.01**
WAIS Current IQ	95.43 (21.01)	115.83 (14.94)	-3.42	<0.01**
WTAR Premorbid IQ	100.75 (17.47)	113.72 (7.54)	-2.91	<0.01**

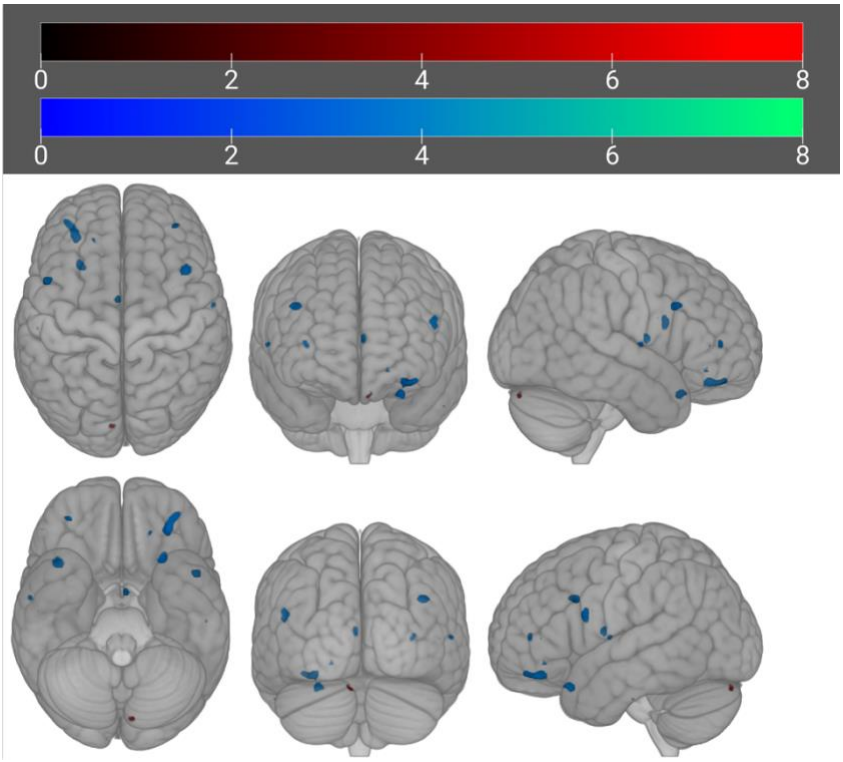
sTable 5: Distribution of Study Participants into Each Cluster

	BeneMin	**SPRING**
CRP	22	7
IL-6	40	12
Noninflamed	72	37
TNF-a	27	12

X^2 (3, N = 229) = 2.49, p = 0.48

sFigure 2: Across-Cluster VBM Comparisons

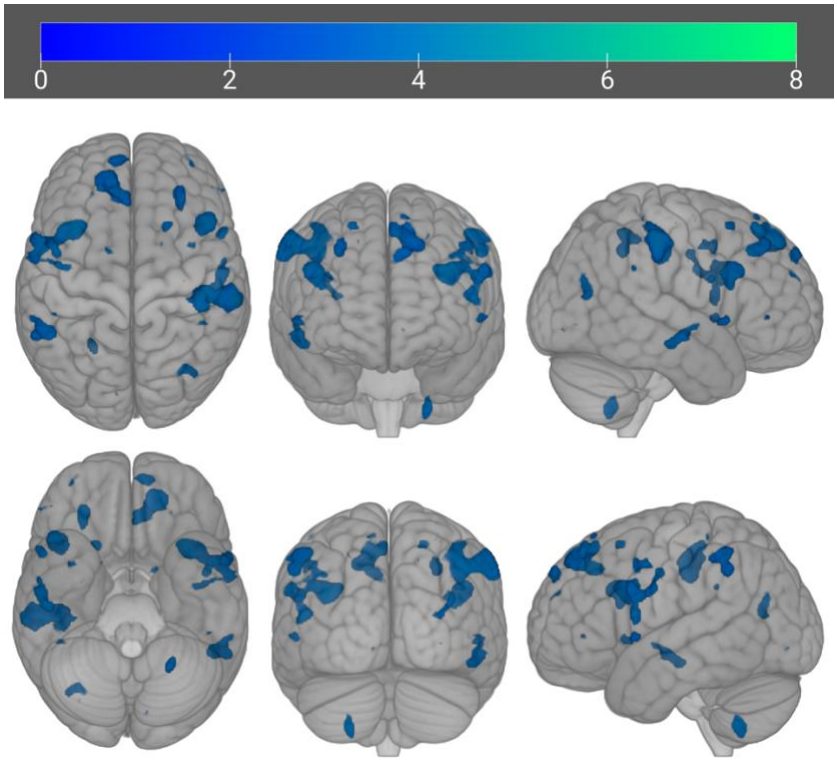
2a: CRP vs IL-6



Blue = decreases in CRP cluster

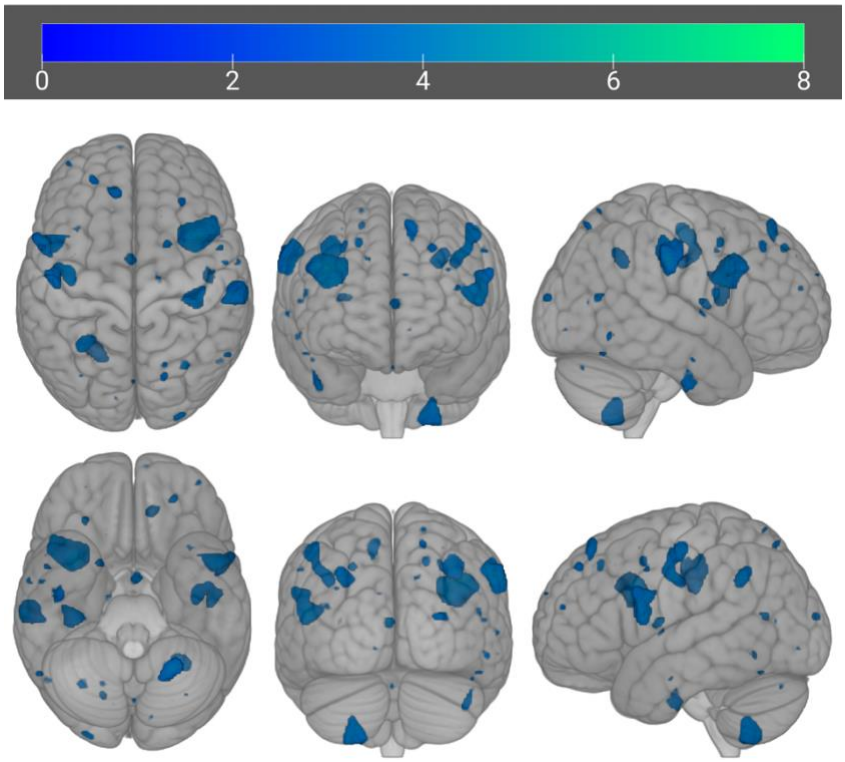
Red = decreases in IL-6 cluster

2b. CRP vs Noninflamed



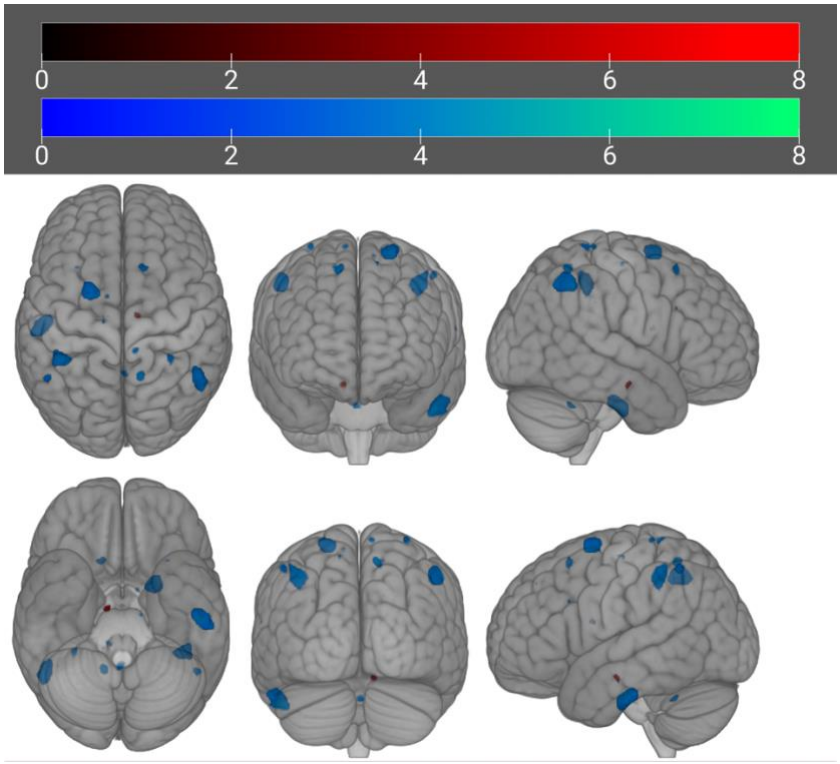
Blue = decreases in CRP cluster

2c. CRP vs TNFa



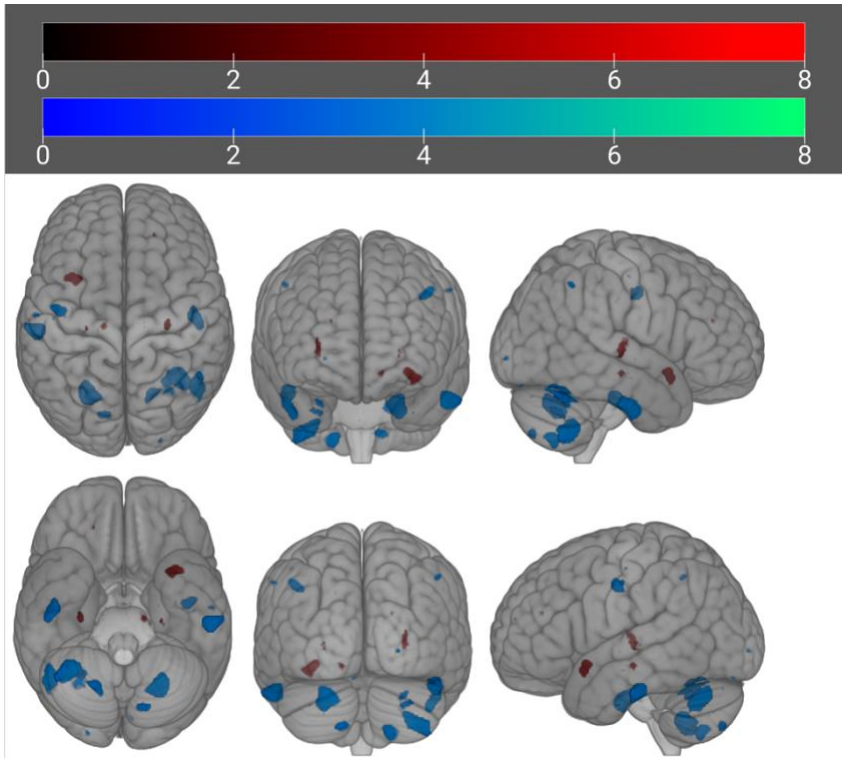
Blue = decreases in CRP cluster

2d. IL-6 vs Noninflamed



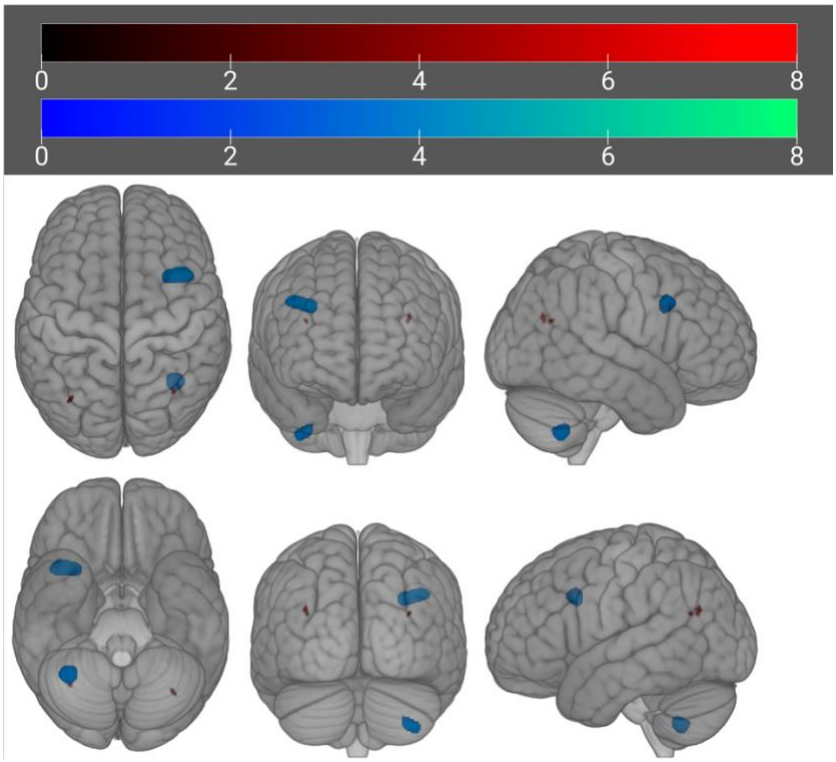
Blue = decreases in IL-6 cluster
Red = decreases in noninflamed cluster

2e. IL-6 vs TNFa



Blue = decreases in IL-6 cluster
Red = decreases in TNFa cluster

2f. Noninflamed vs TNFa



Blue = decreases in noninflamed cluster

Red = decreases in TNFa cluster

Appendix C. Chapter 5 Supplement

Supplementary Methods:

Data Acquisition Protocol:

A structural, T1-weighted MRI was acquired with either a 3T GE scanner (Cardiff) or 3T Phillips scanner (Manchester). Alongside a battery of 1H-MRS scans A single voxel was placed in the bilateral dorsal anterior cingulate cortex (dACC) superior to the genu of the corpus collosum. A point resolved spectroscopy (PRESS) 1H-MRS sequence (TE/TR = 35/2000 ms, bandwidth = 2000 Hz, Navg = 1 Voxel size = 35 x 40 x 20 mm3) was used to acquire water-suppressed spectra as well as a water-unsuppressed spectrum for spectral editing and quantification. Additional Mescher-Garwood (MEGA)-edited PRESS data for glutathione was collected from the same voxel (TE/TR = 130/2000 ms, bandwidth = 2000 Hz, Navg = 64 Voxel size = 35 x 40 x 20 mm3). All data for each participant was acquired in one day, with a morning of cognitive tests and interviews, then blood acquisition, followed by an afternoon of imaging procedures. T1 data was acquired using a standard MPRAGE SENSE acquisition sequence. Resolution matrix=256×256, slices = 180, voxel dimensions = 0.9375 mm x 0.9375 mm x 0.9 mm, TR = 1s, slice thickness = 0.9 mm.

sFigure 1: MRS Voxel Placement.

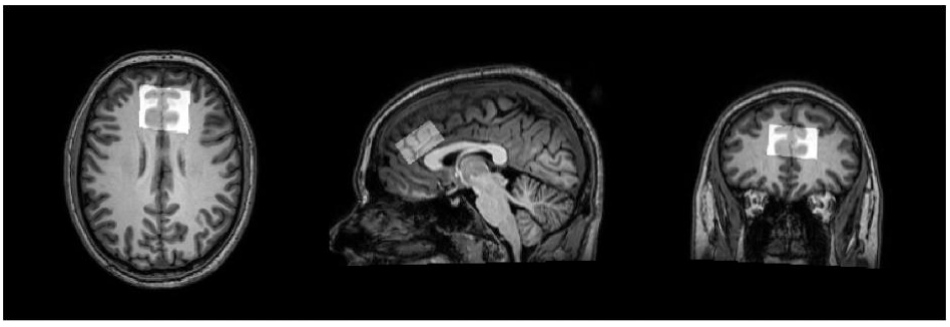
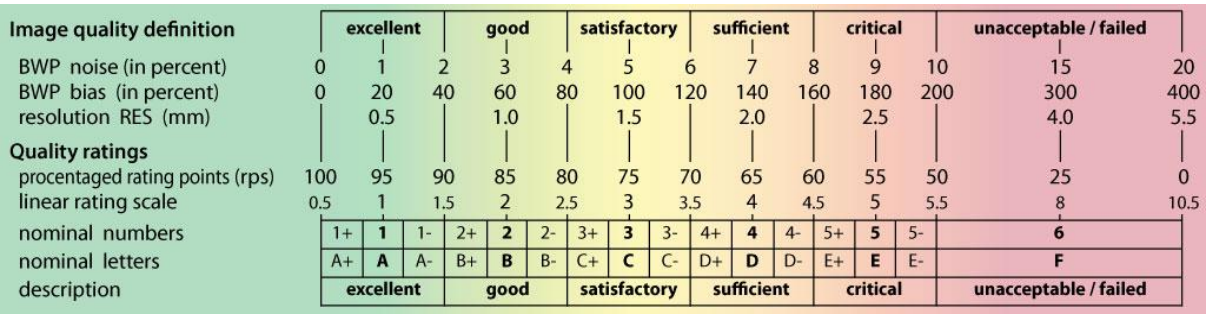


Image Quality Rating:

After segmentation cat-12 will automatically output image quality ratings for each T1 file. The image quality measures describe the properties of the image before CAT12 processing.

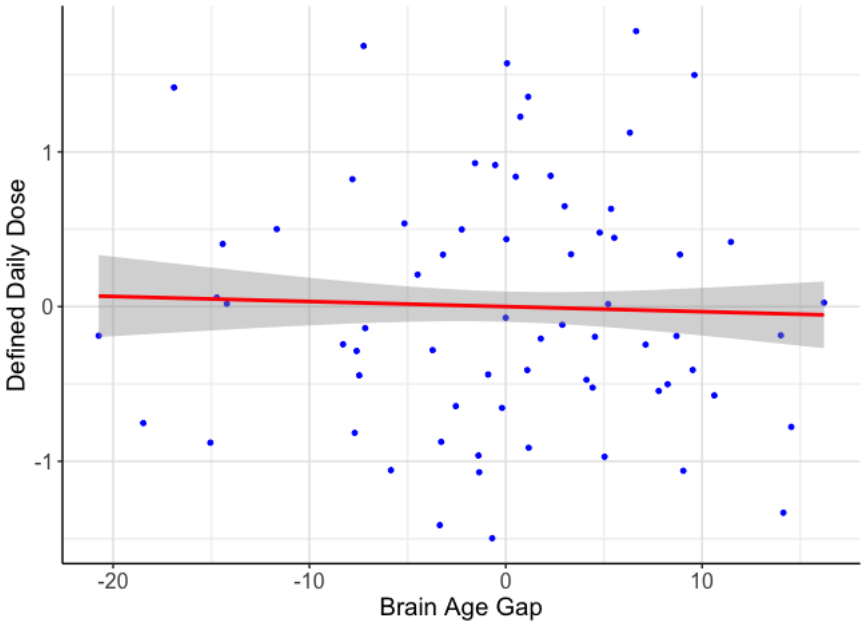
They were estimated by using the tissue segmentation and were (nearly) independent of subject sex and health status.

sFigure 2: CAT12 Image Quality Ratings

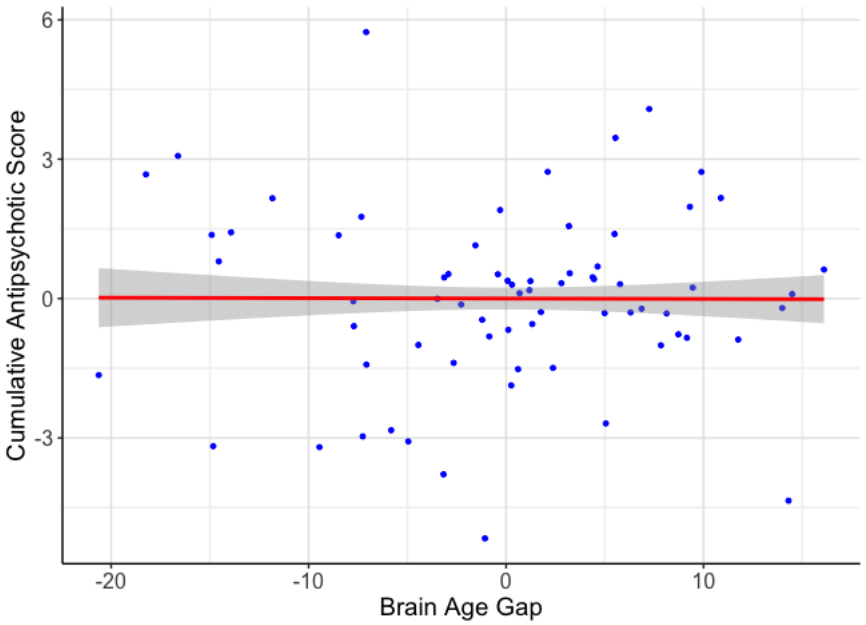


Supplementary Results:

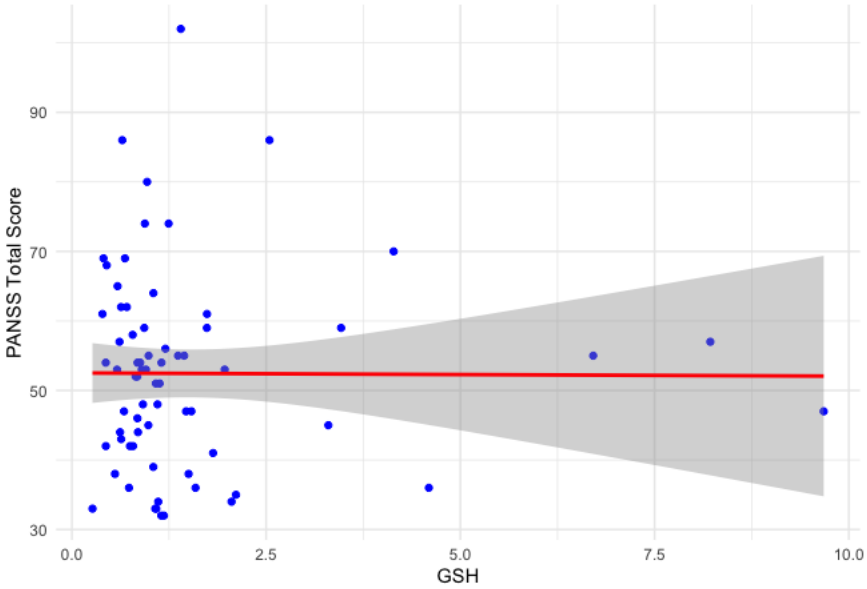
sFigure 3: Relationship Between BAG and Defined Daily Dose



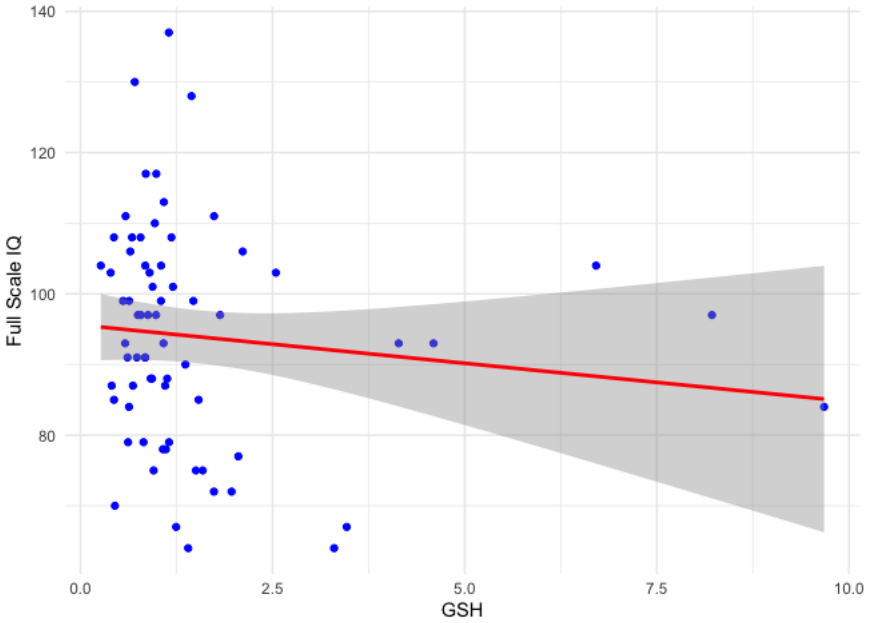
sFigure 4: Relationship Between BAG and Cumulative Antipsychotic Score



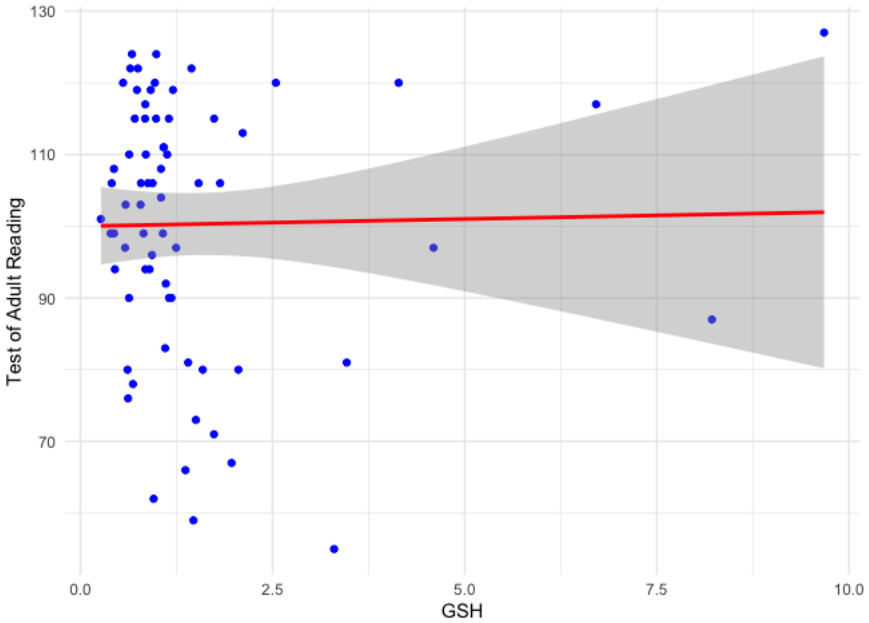
sFigure 5: Relationship Between Glutathione and PANSS Total Score



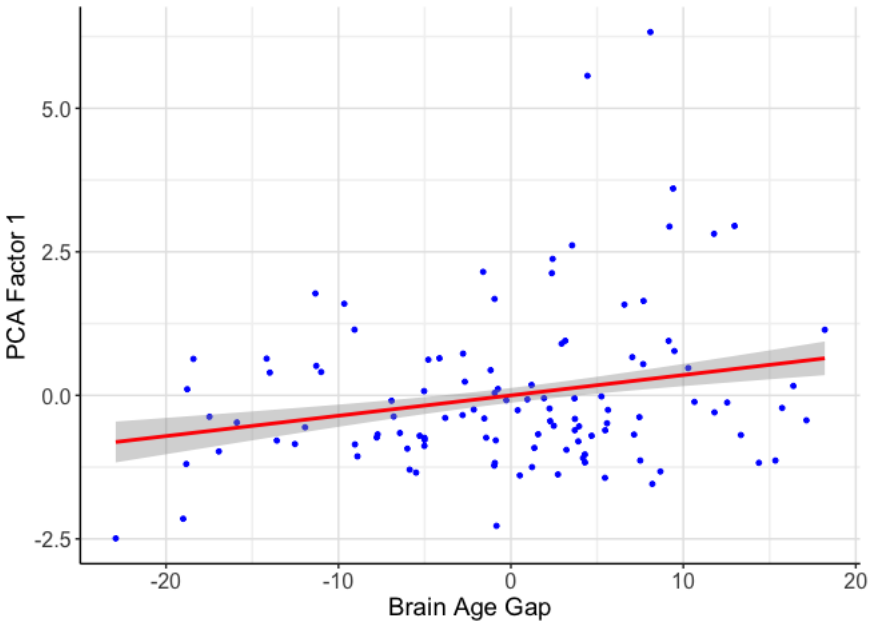
sFigure 6: Relationship Between Glutathione and Full Scale IQ



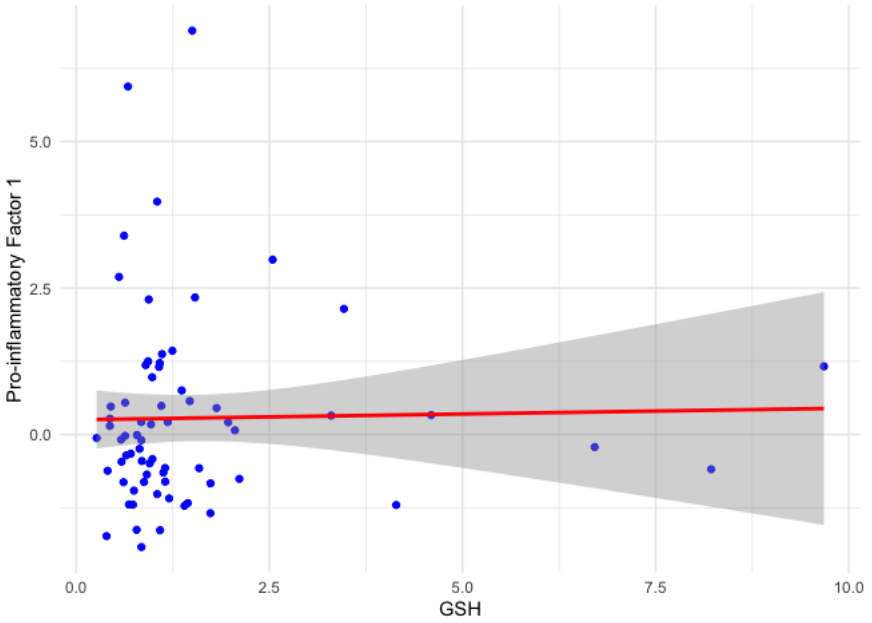
sFigure 7: Relationship Between Glutathione and Weschler Test of Adult Reading



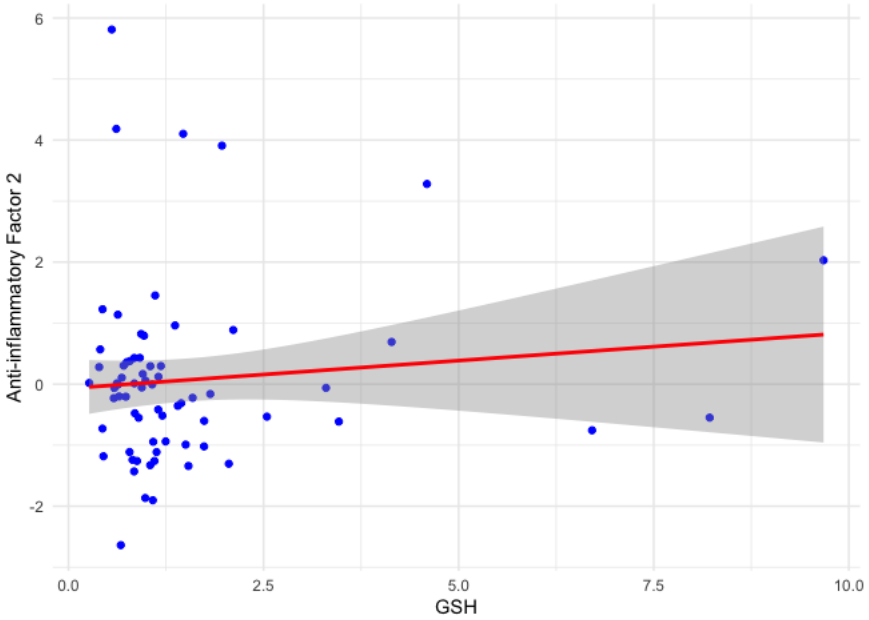
sFigure 3: Relationship Between BAG and Pro-inflammatory Factor 1 in total dataset



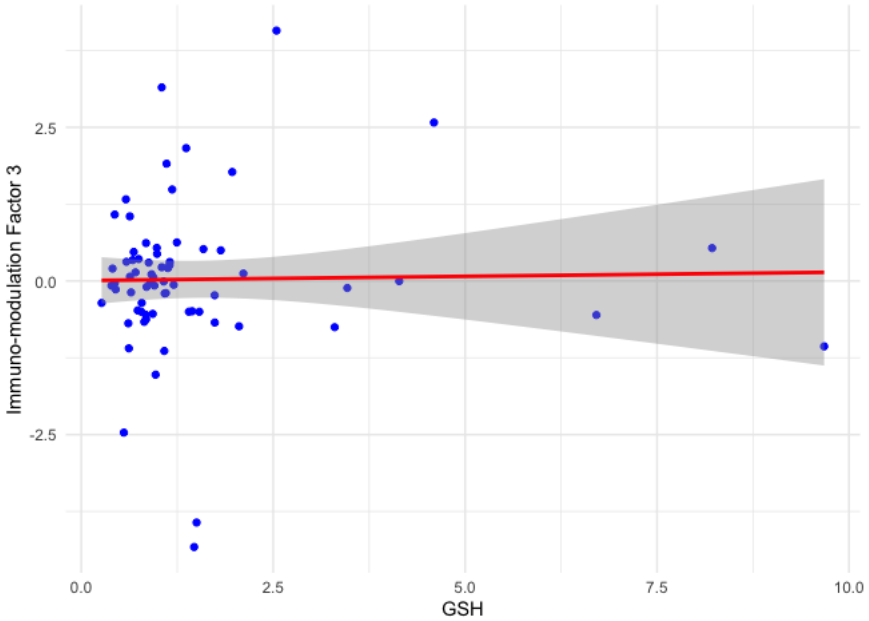
sFigure 9: Relationship Between Glutathione and Pro-inflammatory Factor 1



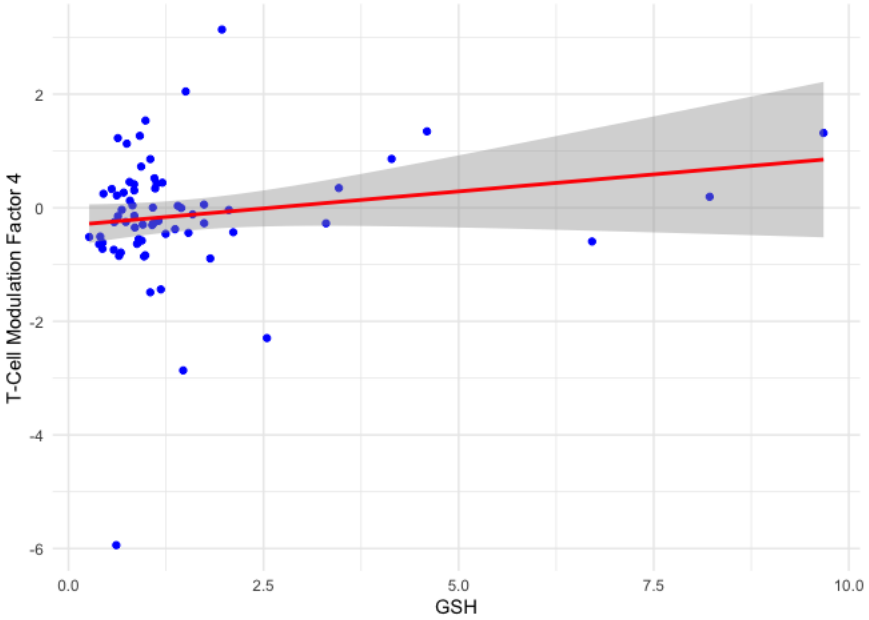
sFigure 10: Relationship Between Glutathione and Anti-inflammatory Factor 2



sFigure 11: Relationship Between Glutathione and Immuno-Modulation Factor 3



sFigure 12: Relationship Between Glutathione and T-Cell Modulation Factor 4



sFigure 13: Relationship Between Glutathione and Th1 Immune Response Factor 5