THE ASSOCIATION OF ANTERIOR CINGULATE CORTEX GLUTAMATE AND CLOZAPINE ELIGIBILITY IN AN EARLY PSYCHOSIS SAMPLE

by

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Submitted in partial fulfillment of the requirements
For the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
May 2023

Dalhousie University is located in Mi'kma'ki,
the ancestral and unceded territory of the
Mi'kmaq.
We are all Treaty people.

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ABSTRACT

Background: The ability to identify patients who may benefit from clozapine at the earliest opportunity is important for maximizing long term outcomes in schizophrenia. Patients that meet criteria for treatment resistance demonstrate poor antipsychotic medication response from the onset of illness. This poor response to dopamine blocking medications may be due to an inherent lack of dopaminergic elevation. It has been speculated that treatment resistance may be associated with elevated glutamatergic metabolites. Despite individuals meeting criteria, clozapine initiation is frequently delayed. There are currently no objective biomarkers available to support clinicians in accurately identifying individuals who may benefit from a clozapine trial. We hypothesized that elevated glutamate in the Anterior Cingulate Cortex (ACC) would be associated with clozapine eligibility in an early psychosis sample.

Methods: The present study compared glutamate levels in the ACC between clozapine-eligible (CE) patients with early phase psychosis, and those who have responded to traditional treatment (Treatment Responders, TR) using 3T proton magnetic resonance spectroscopy (^{1}H-MRS). Clozapine eligible patients continued to have psychotic symptoms after two antipsychotic medication trials, while treatment responders had minimal symptoms and were taking a single antipsychotic medication. The sample consisted of individuals with non-affective psychotic disorders who were followed by the Nova Scotia Early Psychosis Program (NSEPP) and were within the first five years of their initial presentation of psychotic symptoms.

Results: A total sample size of 30 patients participated (TR= 19 and CE= 11). The TR group (M=15, F=4) had an average age of 26 and PANSS score of 36.63. The CE group (M=8, F=3), had an average age of 26 and PANSS score of 68.64. There was not a significant difference between the two groups for glutamate levels, 10.28 (SD= 0.77) and 9.85 (SD= 0.83) for TR and CE respectively, with an effect size of R^2= 0.138. PANSS was significantly different between the two groups and this significance carried over into all 3 categories of symptomatology on the scale. Antipsychotic dose was also significantly different between the two groups, 270.57mg and 532.39mg for CE and TR.

Discussion: This is the first study to investigate the association of brain glutamate with clozapine eligibility in an early psychosis sample. While we did not find an association between ACC glutamate and clozapine eligibility, we did replicate trends from previous studies. While elevated ACC glutamate has been associated with poor antipsychotic response, it is unclear whether elevated glutamate is associated with clozapine eligibility, a clinically meaningful outcome. It is important that future studies are sufficiently powered to assess this potential association reliably. The ability to prospectively identify clozapine eligibility could lead to reformulation of current guidelines regarding clozapine use and may inform the development of additional treatment options for individuals with treatment resistant schizophrenia.

LIST OF ABBREVIATIONS USED

^{1}H-MRS	Magnetic Resonance Spectroscopy
5-HT_{2A}	serotonin
ACC	anterior cingulate cortex
BIOTIC	Biomedical Translational Imaging center
BPRS	brief psychiatric rating scales
CAITE	Clinical Antipsychotic Trials of Intervention Effectiveness
CE	Clozapine-eligible
CGI-S	clinical global impressions scores
COMT	catechol-O-methyl transferase
CPZ	Chlorpromazine
CRLB	Cramer-Rao Lower Bounds
CSF	cerebrospinal fluid
CUTLASS	Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia
DUP	duration of untreated psychosis
EPP	early phase psychosis
EPS	Extrapyramidal symptoms
FEP	first episode of psychosis
FGAs	First-generation antipsychotics
FWHM	full width at half maximum
Glx	glutamate + glutamine
LAIs	Long-acting injectables
LCModel	Linear Combination of Model
MR	magnetic resonance
MRS	magnetic resonance spectroscopy
NAcc	nucleus accumbens
NMDA	N-methyl-d-aspartate
NSEPP	Nova Scotia Early Psychosis Program
PANSS	Positive and Negative Syndrome Scale
PCP	phencyclidine
PRESS	Point RESolved Spectroscopy
SGAs	second-generation antipsychotics
SNR	signal to noise ratio
SOFAS	Social and Occupational Functioning Assessment Scale
T1	longitudinal relaxation time
TGAs	Third-generation antipsychotics
TR	Treatment Responder
TRRIP	treatment response and resistance in psychosis
TRS	Treatment resistant schizophrenia
VTA	ventral tegmental area

ACKNOWLEDGEMENTS

Graduate school has been one of the toughest journeys I have embarked on so far in my life. With many ups and downs, I could not have done this alone.

I would like to thank my mother, Olanike Jalade and my father Dr. Oloruntoba Oluboka for all the late night calls, cheering me on and supporting me every step of the way and believing in me and reminding me of what I am capable of even when I didn't believe in myself. Although on the other side of the country, you are both with me always.

I would like to thank my classmates' turned friends, Hailey Burns, Haley Bernusky and Tulayla Katmeh who made graduate school bearable and fun in the toughest of moments. I am so glad we got to laugh, suffer and cry together. I am so excited to see what the future holds for each of you, and I am so excited to cheer you on.

I would also like to thank my friends, Cassandra Bagnell, Andre Hendricks, Shannon Hilland, Paige Minicola, Amanda Mcleod, Ashley Osa-Peters, Maya Reynolds and Sana Fashir, for being my biggest cheerleaders and rooting for me from close and afar. Having your support during my masters meant more to me then you will ever know.

To the Nova Scotia Early Psychosis Program staff, thank you for the support of this research study and for allowing me to come bother you during your busy work hours.

Lastly, I would like to thank my supervisors and mentors. Drs. Kara Dempster, and Phil Tibbo, Dr. Candice Crocker, Dr. Tomas Hajek and Dr. Martin Alda. You have all played pivotal roles not only in my thesis but also in my experience in my Masters program. My achievements in this program would not have been possible without you and I thank you for your support of me and my extracurriculars, even when they seemed to get in the way of research :). Thank you for inspiring and mentoring me.

CHAPTER 1 INTRODUCTION

1.1 WHAT IS SCHIZOPHRENIA?

Schizophrenia is a chronic psychotic disorder with several potential functional impacts. Characterized by positive symptoms of psychosis such as hallucinations, delusions, disorganized speech and behaviors, and negative symptoms such as anhedonia and alogia, schizophrenia can have profound impacts on multiple facets of daily living (McCutcheon, Reis Marques, et al., 2020). There is a spectrum of psychotic disorders which includes schizophrenia, delusional disorder, substance induced psychosis, brief psychotic disorder, schizophreniform and schizoaffective disorder (American Psychiatric Association & American Psychiatric Association, 2013). Meeting criteria for schizophrenia requires at least two of the symptoms of psychosis as described above, for at least a month's time with associated functional impairment and attenuated symptoms or a gradual decline in functioning occurring over a 6-month period. Furthermore, the symptoms must not be attributed to drug use or other medical illnesses, and schizoaffective and bipolar disorder must both be ruled out as more suitable explanations for the clinical presentation (American Psychiatric Association & American Psychiatric Association, 2013). In addition to the positive and negative symptoms, impairments in cognitive functioning are commonly observed in roughly 55% of people with schizophrenia (Mascio et al., 2021; Mihaljević-Peleš et al., 2019). Multiple domains of cognition are moderately to severely impacted in schizophrenia including, attention, working memory, verbal learning and memory as well as executive functioning (Bowie & Harvey, 2006; Falkenberg et al., 2014; Griffiths et al., 2022; Huang et al., 2022;

Mihaljević-Peleš et al., 2019; O'Carroll, 2000). To note, both cognitive and negative symptoms have been associated with functional outcomes (Bowie & Harvey, 2006; Eack & Keshavan, 2020; Mihaljević-Peleš et al., 2019; O'Carroll, 2000). Additionally, these cognitive impairments are often observed in patients prior to the onset of the disorder (Harvey, 2009; Woodberry et al., 2008).

Approximately 1% of the global population has schizophrenia at any time (Saha et al., 2005). Recent research has found that 0.9% of Canadians and 0.6% of Nova Scotians have been diagnosed with schizophrenia, (Canadian Chronic Disease Surveillance System (CCDSS), 2019), in line with the global prevalence figures. In Canada, about 54 individuals per 100,000 people are diagnosed with schizophrenia every year (Canadian Chronic Disease Surveillance System (CCDSS), 2019; 2020), which is comparable to the incidence rates in the literature (Saha et al., 2005). The economic burden of this disease is immense. In 2004, the estimated direct healthcare and non-healthcare costs of schizophrenia in Canada were $2.02 billion dollars (Goeree et al., 2005). Schizophrenia is associated with productive morbidity and mortality losses costing up to $4.83 billion dollars (Goeree et al., 2005); schizophrenia is associated with a decreased life expectancy of approximately 15-20 years (Colton & Manderscheid, 2006; Correll et al., 2018; Hjorthøj et al., 2017; Nordentoft et al., 2013).

More recently, a study found that the costs of schizophrenia in Canada have now increased up to $10 billion dollars annually, an approximately $3 billion dollar increase from 2004 (Goeree et al., 2005; Lecomte et al., 2022). On a personal level, schizophrenia not only has individual and societal costs (economic burden), but it can also significantly impact the lives of relatives and friends of those diagnosed, including lost wages in

caring for their loved ones.

1.1.1 THE BIOLOGICAL HYPOTHESES OF SCHIZOPHRENIA

1.1.2 NEURODEVELOPMENTAL VS NEURODEGENERATIVE THEORIES

The neurodevelopmental hypothesis of schizophrenia describes that the disorder originates from a disturbance that occurs during the development of the nervous system, and as such symptoms manifests as neural development progresses (Harrison, 1999; McGrath et al., 2003; Murray & Lewis, 1987; Nasrallah & Weinberger, 1986; Owen et al., 2011). Thomas Clouston spearheaded the idea that schizophrenia may be a neurodevelopmental disorder by calling it a cortical developmental disease (Gupta & Kulhara, 2010; Sadock et al., 2000). Genetic research supports the neurodevelopmental hypothesis by identifying potential genes that are associated with the development of schizophrenia, such as the catechol-O-methyl transferase (COMT) gene (Craddock et al., 2006, 2007; Henriksen et al., 2017; Owen et al., 2011; Riglin et al., 2017; Werner & Coveñas, 2021). As well, one of the largest risk factors for developing schizophrenia is having a relative that has the disorder (Gejman et al., 2010; Kennedy et al., 1999; McClure & Lieberman, 2003; Sacker et al., 1996).

Another factor that supports the neurodevelopmental hypothesis is the finding that patients with schizophrenia are more likely to experience adverse events that impact the intrauterine environment such as fetal malnutrition and hypoxia which have been connected to an increased risk of schizophrenia (Cannon et al., 2002; Dalman et al., 1999; Geddes & Lawrie, 1995; McClure & Lieberman, 2003; Sacker et al., 1996; Verdoux et

al., 1997). Lastly, the two-hit hypothesis of schizophrenia uniquely supports the neurodevelopmental hypothesis. The hypothesis discusses the impact of the genetic vulnerability to schizophrenia that then makes the individual more susceptible to a later environmental impact that then triggers the onset of the disorder (Bayer et al., 1999; Guerrin et al., 2021; Maynard et al., 2001). The two-hit hypothesis describes that neither the genetic vulnerability nor the environmental factors alone can trigger the onset of the disorder, however, when combined, the combination induces schizophrenia in the individual (Maynard et al., 2001). The first hit is hypothesized to occur during prenatal development, while the second hit occurs in the outside environment of the individual (Bayer et al., 1999; Guerrin et al., 2021; Maynard et al., 2001).

Somewhat contrary to the neurodevelopmental hypothesis, the neurodegenerative hypothesis has also been positioned as a biological hypothesis to schizophrenia development. Schizophrenia was first referred to as "dementia praecox" by Emil Kraepelin, when it was then believed that schizophrenia is a progressive disorder that an individual is unable to recover from. The neurodegenerative hypothesis posits that schizophrenia is caused by a process or processes that leads to degeneration of the brain (Gupta & Kulhara, 2010). Unlike the neurodevelopmental hypothesis where the nervous system was disrupted during development, the neurodegenerative hypothesis refers to the degeneration of the brain that occurs over the course of life (Gupta & Kulhara, 2010; Kraepelin, 1919). The progressive nature of schizophrenia is why many believe that the disorder could be considered neurodegenerative (Huber et al., 1980; Kraepelin, 1919; McClure & Lieberman, 2003; McGlashan, 1988; Pfohl & Winokur, 1982). Studies have investigated the course of schizophrenia over the lifespan and it has been found that the

likelihood of deterioration is associated with the number of episodes and duration of episodes with positive symptoms (Gupta & Kulhara, 2010; McClure & Lieberman, 2003; Wyatt, 1991). Additionally, the longer the duration of untreated psychosis, the more likely a patient is to develop negative and catatonic symptoms (Fenton & McGlashan, 1994; Kraepelin, 1919; McClure & Lieberman, 2003; McGlashan & Fenton, 1993; Pfohl & Winokur, 1982). While there is still debate regarding schizophrenia's classification as neurodevelopmental or neurodegenerative, both perspectives have been useful in furthering our understanding of this complex disorder.

1.1.3 NEUROTRANSMITTER THEORIES

Other hypotheses of schizophrenia focus on neurotransmitters and how their functioning plays a role in the presentation of schizophrenia. Of these hypotheses, the dopamine hypothesis has been the most popular and implicated in the pathophysiology of schizophrenia. The dopamine hypothesis implicates excessive dopamine at the D2 dopamine receptors in the mesolimbic pathway, including the ventral tegmental area (VTA) which projects to the nucleus accumbens (NAcc) (Meltzer & Stahl, 1976; Stahl, 2018). The excess of dopamine at the D2 receptors is linked to the presentation of the positive symptoms observed in schizophrenia (Brisch et al., 2014; Kesby et al., 2018; McCutcheon, Reis Marques, et al., 2020; Meltzer & Stahl, 1976; Stahl, 2018). Dopamine has also been connected to negative and cognitive symptoms seen in schizophrenia, including the anhedonia and lack of motivation observed in some patients (Abi-Dargham, 2004; Abi-Dargham et al., 2000; Arnsten, 1998; Brisch et al., 2014; Patel et al., 2010; Shen et al., 2012; Slifstein et al., 2015). Additionally, reduced D1 receptors in the caudate nucleus have been implicated in the production of these negative symptoms seen

in schizophrenia (Brisch et al., 2014; O'Donnell & Grace, 1998).

Alternative hypotheses to the dopamine hypothesis have been presented, providing a wider clinical view of patients with schizophrenia. Another neurotransmitter implicated in the pathophysiology of schizophrenia is serotonin. The serotonin hypothesis is unique as it has found ties between both Parkinson's and Alzheimer's disease to schizophrenia, specifically in patients that have a secondary diagnosis of schizophrenia (Fénelon et al., 2010; Ravina et al., 2007; Stahl, 2016, 2018). This hypothesis focuses on the activation of 5HT2A receptors from excess serotonin on glutamate neurons in the cerebral cortex (Eggers, 2013; Stahl, 2018). This has been connected to hallucinations observed in patients with primary diagnoses of Alzheimer's and Dementia with psychotic symptoms (Ballanger et al., 2010; Huot et al., 2010).

The glutamate hypothesis is described by the low functioning of glutamatergic N-methyl-d-aspartate (NMDA) receptors in the prefrontal cortex, which leads to an increased release of glutamate (Egerton et al., 2020; Goff & Coyle, 2001; Heckers et al., 2000; Javitt & Zukin, 1991; Moghaddam & Javitt, 2012; Stahl, 2018; Uno & Coyle, 2019). This theory was spearheaded by the observation that ketamine and phencyclidine (PCP) induce the psychotic symptoms, cognitive impairments and negative symptoms seen in schizophrenia in healthy individuals (Goff & Coyle, 2001; Javitt & Zukin, 1991; Krystal, 1994; Krystal et al., 1999; Lahti, 1995; Malhotra, 1997; Mei et al., 2018). Additional to the prefrontal cortex, glutamatergic alterations have also been seen in the thalamus, and the temporal lobe, which are areas that are impaired in patient's performances on cognitive tasks (Gao et al., 2000; Goff & Coyle, 2001; Heckers et al., 1999, 2000; Ibrahim et al., 2000).

As ketamine and PCP are NMDA receptor antagonists, this observation was key in the identification of the glutamatergic system's contribution to the presentation of schizophrenia (Goff & Coyle, 2001; Javitt & Zukin, 1991; Krystal, 1994; Krystal et al., 1999; Lahti, 1995; Malhotra, 1997; Mei et al., 2018). With the observation that medications that improve NMDA receptor functioning improves symptoms in some patients (Goff & Coyle, 2001; Lidow, 2000), there have been efforts made towards the development of adjunctive treatments that specifically target the NMDA receptors. However, the results of such studies have been inconsistent, with most studies not finding significant improvement in patients while other studies that observe an improvement only see a small effect (Singh & Singh, 2011; Tsai & Lin, 2010; Wu et al., 2021). As research continues in this area, it is important to consider the role of the glutamatergic system and factors that impact glutamate transmission.

The glutamatergic system helps regulate neuronal migration, synaptic pruning and the development of new synapses, and thus has been implicated in some of the brain architectural abnormalities reported in schizophrenia, for example reduced synaptic connection (Akbarian, 1993a, 1993b; Choi, 1988; Goff & Coyle, 2001; Kerwin, 1993; Komuro & Rakic, 1993; McDonald & Johnston, 1990; McGlashan & Hoffman, 2000). Another factor to consider that may play a role in glutamate level variability is sex differences (Martins-de-Souza et al., 2010; Wickens et al., 2018). Previous research has found that in women, glutamate levels are higher than they are in men. This is explained by an enzyme called glutamine synthetase, an enzyme involved in glutamate metabolism (Martins-de-Souza et al., 2010). This enzyme is important for cell proliferation, apoptosis, cell signalling and is involved in the maitenance of glutamate levels (Martins-

de-Souza et al., 2010). This enzyme has been found to be upregulated in women with schizophrenia, suggesting that this could be the reason why there is a potential sex differnce in glutamate levels between individuals with schizophrenia (Martins-de-Souza et al., 2010; Wickens et al., 2018). This research has also been supported by examining sex differences in in vivo brain imaging of anterior cingulate cortex (ACC) glutamate, where this same finding was supported (Martins-de-Souza et al., 2010).

All of these hypotheses paint different parts of the picture of the pathogenesis of schizophrenia. Although there is more to learn, it is evident that the development of the disorder goes beyond the dopamine hypothesis and, if anything showcases that all of these pathways and hypotheses are in fact interrelated (Stahl, 2018). Gaining a wider view of the biological causes of the disorder assists in the development of treatments for this disorder. As we continue to learn more about schizophrenia, the clearer it is that there is an important role that early recognition and early intervention can play in improving the outcomes for patients diagnosed with schizophrenia.

1.1.4 EARLY PHASE PSYCHOSIS & IMPORTANCE OF EARLY INTERVENTION

Outcomes for individuals with schizophrenia have historically been suboptimal, with decreased life expectancies and increased mortality rates (Colton & Manderscheid, 2006; Correll et al., 2018; Hjorthøj et al., 2017; Nordentoft et al., 2013), and this has led to the prioritization of interventions in the early phase of psychosis (Correll et al., 2018; Kahn et al., 2015). Intervening at the first episode of psychosis (FEP) can have a significant impact on an individual's long term outcomes (Albert & Weibell, 2019; Cheng & Schepp, 2016; Correll et al., 2018; O'Connell et al., 2021). The term early

phase psychosis (EPP) refers to the first 5 years of psychosis, which is a crucial period of time for intervention (Birchwood et al., 1998). Duration of untreated psychosis (DUP) is defined as the period of time between the initial onset of psychotic symptoms and the initiation of pharmacological treatment (Marshall et al., 2005). Previous work has shown that early intervention decreases the DUP, leading to a significant decrease in both positive and negative symptoms (Albert & Weibell, 2019; Verma et al., 2012).

Early intervention services work by addressing potential barriers to early identification and treatment, including improving early access to care, by promoting and educating around early detection in health care (in mental health and primary care) as well as the general public, and utilizing open referrals and rapid response services (Bechardevans et al., 2007; Birchwood et al., 1998; Iyer et al., 2015; Malla et al., 2003). Early interventions increase the odds of patients returning to their pre-morbid functioning, decreasing the potential of negative long-term outcomes. Early intervention services are also cost-effective when compared to standard treatment (Hastrup et al., 2013; McGorry, 2015). This is also important when considering the disease burden and schizophrenia's economic consequences. Lastly, early intervention is particularly important for a sub-population of patients that may have a more difficult illness to treat, particularly those that will be diagnosed as treatment resistant (treatment resistant schizophrenia (TRS)). Patients that are treatment resistant often have worse outcomes than treatment responders, and are in need of more intensive mental health services, available through early intervention programs (Kanahara et al., 2018). Individuals that develop TRS often have a longer DUP than patients that are responsive to treatment (Kanahara et al., 2018), underscoring the need for an early intervention approach to

schizophrenia. Early identification of TRS and subsequent effective TRS specific treatments in this population are a priority to maximize outcomes (Demjaha et al., 2017; Kanahara et al., 2018; Siskind et al., 2022).

1.1.5 TREATMENT RESPONSE IN EARLY PSYCHOSIS

Treatment response is defined as the degree to which a patient improves in the presence or absence of symptoms (Leucht & Kane, 2006; Schennach et al., 2012; Schennach-Wolff et al., 2010). Most patients respond to treatment in their first episode or early phase of psychosis and evidence shows that intervening in the FEP leads to improved long term outcomes for these individuals (Harrison et al., 2001; Loebel et al., 1992; Schennach et al., 2012; Schennach-Wolff et al., 2010). However, it is challenging to identify treatment response in early psychosis. For example, in anti-psychotic naïve individuals, they will likely respond initially, however this response may taper off if individuals are partial responders or non-responders to the medication (Jäger et al., 2007; Robinson et al., 1999; Schennach et al., 2012). Additionally, it takes time to identify treatment response and it requires patience to identify response in these individuals when they first present and undergo treatment.

1.2 PHARMACOLOGICAL INTERVENTIONS FOR SCHIZOPHRENIA

Pharmacological treatments for schizophrenia have evolved over the years. Known as typical antipsychotics, first-generation antipsychotics (FGAs) were developed in the 1950s and act as dopamine receptor antagonists, specifically at the D2 receptor (Abou-Setta et al., 2012). Although effective in treating psychotic symptoms, FGAs increase the

risk of developing extrapyramidal symptoms (EPS) (Abou-Setta et al., 2012; Kane & Correll, 2010; Remington et al., 2021; Rosenbaum, 2005). This negative side-effect is what inevitably motivated the development of second-generation antipsychotics (SGAs). Known as atypical antipsychotics, SGAs are effective in treating psychosis while carrying a lower risk of developing EPS, however, SGAs are known to cause more weight gain in patients and have adverse cardiometabolic effects (Leucht et al., 2009; Zhang et al., 2013).

Clozapine, an SGA is the most effective antipsychotic in treating schizophrenia (Gammon et al., 2021; Kane, 1988; Remington et al., 2013, 2016). Clozapine use has been associated with decreasing suicidality in patients with a high risk of dying by suicide. These findings are consistent between both treatment resistant patients and non-resistant patients with schizophrenia (Meltzer, 2003; Meltzer & Okayli, 1995; Reid et al., 1998; Reinstein et al., 2002; Walker et al., 1997). Additionally, when compared to other SGAs, clozapine use is associated with a decrease in hospitalization risk as well as all-cause discontinuation, a measure of the medication's effectiveness (Land et al., 2017; Masuda et al., 2019). As included in the measurement of all-cause discontinuation, clozapine demonstrates a strong positive association with improved outcomes with decreases in symptoms and illness severity (Masuda et al., 2019).

Despite clozapine's efficacy in treating schizophrenia, the medication is underutilized. There are many reasons that factor into the underutilization of this medication. A large barrier to clozapine prescribing is clinician's lack of experience with the drug as well as clinician's negative view of the medication (Cirulli, 2005; Mistry & Osborn, 2011; Nielsen, Dahm, et al., 2010). This negative attitude towards clozapine has

stemmed from an overestimation of the negative side effects of taking the medication (Hodge & Jespersen, 2008; Mistry & Osborn, 2011; Nielsen, Dahm, et al., 2010). For example, clozapine carries a risk of agranulocytosis which requires patients on clozapine to undergo routine blood monitoring (Nielsen, Dahm, et al., 2010; Rosenbaum, 2005; Schulte, 2006). Although the risk of developing this condition runs low at a rate of 0.68%, many clinicians view this as a significant barrier to using clozapine. Meanwhile, their patients do not find this to be a significant barrier to taking the medication (Mistry & Osborn, 2011; Taylor et al., 2000). To improve the rates of clozapine use and clozapine prescribing, it is important that clinicians are well informed on the medication and have a realistic view of the severity of the medication's side effects.

The third generation of antipsychotics (TGAs) also known as partial dopamine agonists, act uniquely in comparison to FGAs and SGAs. A partial agonist is a drug that can bind and activate a receptor however, their activity at the receptor is partial in comparison to the activity of a full agonist at a receptor (Lieberman, 2004). Examples of TGAs include aripiprazole, cariprazine, and brexpiprazole. These medications decrease dopamine hyperactivity similar to previous generations, however, they also promote dopamine activity in the cortical regions of the brain that play a role in cognitive and negative symptoms in schizophrenia (Stahl, 2001). Additionally, these medications support dopamine in areas of the brain that mediate motor control, minimizing the development of EPS (Lieberman, 2004; Remington et al., 2021; Stahl, 2001).

Long-acting injectables (LAIs) are another pharmacological option in the treatment of schizophrenia. Many patients struggle to remember to take their medications with nonadherence rates ranging from 26% to 44% in this clinical population (Kaplan et

al., 2013; Kikkert & Dekker, 2017; Remington et al., 2021). Additionally, two thirds of patients are partially nonadherent to their medications, making medication adherence a large barrier to treatment (Kaplan et al., 2013). LAIs alleviate the stress and pressure associated with remembering to take an oral pill daily while also assisting in keeping the patients active in receiving their treatment (Correll et al., 2021; Kaplan et al., 2013). Initially, these LAIs consisted of FGAs and thus had similar negative side effect profiles of their FGA oral formulations. However, with the more recent development of SGA LAIs there exist LAI options with better side effect profiles, offering one month, two month and three month LAI options. (Correll et al., 2021; Remington et al., 2021).

LAIs not only support medication compliance but also have additional benefits. Previous research has found that SGA LAI use is associated with increases in patient satisfaction, functioning and quality of life (Lloyd et al., 2010; Macfadden et al., 2011; Marinis et al., 2007). Additionally, SGA LAIs are associated with decreases in relapse and hospitalizations (Kane et al., 2020; Kaplan et al., 2013; Zhornitsky & Stip, 2012). Lastly, there is also an economic benefit with the use of LAIs in the clinic. Given the costs associated with hospitalizations when patients relapse, when LAIs are consistently used, the risk of relapse and hospitalizations decrease, easing up on the pressure hospitalizations place on the healthcare system economically (Furiak et al., 2012). On the other hand, this economic benefit is not only viewed from the system's perspective but also from the patient's perspective as well. When patients have improved functioning and outcomes, this alleviates the economic cost and disease burden associated with schizophrenia (Furiak et al., 2012).

Both the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and

Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUTLASS) studies compared the effectiveness of SGAs in comparison to FGAs (Lally & MacCabe, 2015; Lieberman et al., 2005). The CATIE study did not find evidence to support the idea that SGAs are superior to FGAs in terms of reducing both cognitive and negative symptoms (Jones et al., 2006; Lally & MacCabe, 2015; Lieberman et al., 2005). As response to medications vary between generations, it is tough to produce a general rate of response to all antipsychotics. However, approximately 30% of patients are treatment resistant, meaning that these patients still have symptoms despite adequate treatment (Kennedy et al., 2014). This means that about 70% of patients respond to medications (Kennedy et al., 2014). For these patients with TRS, about 50-60% of them will respond to clozapine (Lally & MacCabe, 2015; Meltzer, 1992).

Remission in psychiatry is defined by both a time and symptom criteria. Symptoms must be minimal and below a set threshold across all symptom domains (Andreasen et al., 2005; Yeomans et al., 2010). Additionally, this state must be sustained for at least six months before an individual is in remission. It is estimated that about 20%-60% of people with schizophrenia achieve remission (Yeomans et al., 2010). Recovery can be defined from a clinical perspective as meeting a timepoint with sustained functioning and symptom remission (Emsley et al., 2011; Leucht & Lasser, 2006; Ponce-Correa et al., 2023). Achieving recovery is associated with a significant increase in functioning and quality of life (Andreasen et al., 2005; Yeomans et al., 2010). Although schizophrenia can be an incredibly debilitating disorder, it is important to note that remission and recovery are still possible.

1.3 TREATMENT RESISTANT SCHIZOPHRENIA (TRS) & ITS CLINICAL RELEVANCE

Treatment Resistant Schizophrenia (TRS) affects about 20-30% of individuals with schizophrenia, leading to poor symptomatic and functional outcomes (Kennedy et al., 2014). Criteria for identifying TRS was originally created by Kane in 1988 (Kane, 1988). Kane described meeting criteria as meeting three main elements: 1) 3 or more periods of treatment with at least 2 antipsychotics for a minimum of 6 weeks, 2) no period of good functioning within the last 5 years and, 3) the persistence of severe symptoms according to the brief psychiatric rating scales (BPRS) and the clinical global impressions scores (CGI-S) (Kane, 1988). Although this criterion is helpful in identifying TRS, the criteria left room for other patients to not meet these criteria when arguably they were treatment resistant. For example, a patient could be treatment resistant in their FEP yet not meet the Kane criteria of TRS as they did not meet the 5 year criteria of poor functioning (Howes et al., 2017). Additionally, with the further development of LAIs, the original criteria regarding adequate antipsychotic treatment did not include LAIs. With the increased frequency of LAI use, treatment guidelines for TRS should include this treatment as it could impact the criteria for an adequate medication trial.

Additionally, these criteria left room for personal interpretation as to what certain terms mean, for example the terms “treatment resistant” and “good functioning” can be subjective, and this terminology has been used and applied inconsistently. This sparked the creation of the treatment response and resistance in psychosis (TRRIP) working group and their work towards operationally defining TRS with set criteria. The TRRIP working group reformulated the criteria as follows: Criteria requires individuals to have

moderately severe symptoms, moderate to severe impairment in functioning, at least 2 prior antipsychotic trials at a therapeutic dose (≥ 6 weeks with a dose equivalent to 600mg Chlorpromazine) and a 80% adherence to medications (Howes et al., 2017). Specifically for patients on LAIs, the TRRIP guidelines suggest one failed trial (at least 6 weeks) on an LAI following the achieved steady state, which is generally 4 months after beginning treatment (Howes et al., 2017).

It is important to note that identifying TRS can be difficult as there are many factors that can explain a patient's lack of response to treatment other than TRS. For example, patients that are not taking their medications consistently can be viewed as TRS clinically, when really they are not adhering to their medication (Altamura et al., 2005; Dold & Leucht, 2014; Kane et al., 2019; Potkin et al., 2020). Another example is the use of recreational drugs, which can inhibit and impact a patient's ability to respond to pharmaceutical treatment (Potkin et al., 2020). Additionally, some patients may need a higher dose than others to elicit a response to medications (Potkin et al., 2020). All these factors make it easy to mistake a patient as TRS when they might not be. This is known as pseudo-resistance (Altamura et al., 2005; Dold & Leucht, 2014; Kane et al., 2019; Potkin et al., 2020). In order to avoid pseudo-resistance, it is important that when we classify a patient as TRS, that the patient is actually meeting those requirements in absence of recreational drug use or medication nonadherence (Altamura et al., 2005; Dold & Leucht, 2014; Potkin et al., 2013).

As is, schizophrenia can be an incredibly debilitating disorder, with the life expectancy decreased by 10-15 years for this population (Laursen et al., 2014; Tiihonen et al., 2009). There is a critical need to understand the pathogenesis and underlying

mechanisms of TRS to treat these patients rather than just focusing on management. As dopamine has been the primary target in pharmacological treatments, when patients do not respond to these treatments, it is clear that the dopamine hypothesis does not fully represent these individuals. This has opened a space to think critically about what pharmaceutical medications may be able to aid this group of patients.

1.3.1 PHARMACOLOGICAL TREATMENT IN TRS

Clozapine has been shown to be an effective pharmacological treatment for this clinical sub-group (Juul Povlsen et al., 1985; Kane et al., 1988; Kane, 1992; León, 1979; Meltzer et al., 1989; Nielsen, Dahm, et al., 2010; Remington et al., 2016; Rosenheck et al., 1997; Small et al., 1987). However, current guidelines suggest not using clozapine until a patient has failed at least two drug trials (≥ 6 weeks) (Kane et al., 2019). The Canadian schizophrenia guidelines echo these guidelines by suggesting clozapine to individuals who have failed 2 adequately dosed antipsychotic trials (Remington et al., 2017). Additionally, these guidelines refer to the Positive and Negative Syndrome Scale (PANSS) for identifying treatment response (Kay et al., 1987; Remington et al., 2017). It is suggested that individuals with a 20% reduction in positive symptoms after 2 antipsychotic trials continue to struggle in treatment (Remington et al., 2017). Clozapine is viewed as a last resort medication by some clinicians due to its negative side effects such as myocarditis, seizures, blurred vision, EPS, weight gain, hypoglycemia, sedation, fatigue, hypersalivation, agitation, dizziness, as well as tachycardia (Gianfrancesco et al., 2002; Gürcan et al., 2017; Iqbal et al., 2020; Nielsen, Skadhede, et al., 2010).

For patients with TRS this means that the process of receiving a potentially effective treatment through clozapine is prolonged. A previous study found that on

average it took participants 6.7 years before their first trial on clozapine (Doyle et al., 2017). Previous work suggests that the earlier clozapine is initiated, the more robust the response to treatment is (Correll et al., 2022; Leclerc et al., 2021; Schneider et al., 2014; Thien et al., 2018; Williams et al., 2017). Although there is not an exact estimate, a study by Demjaha et al., (2017) found that of the 23% of individuals with TRS, 84% of them were resistant to treatment from the onset of illness (Demjaha et al., 2017). Research on clozapine use in poor responders in their FEP support the early use of clozapine, with patients showing clinical improvement after clozapine initiation (Agid et al., 2007; Doyle et al., 2017; Leclerc et al., 2021; Szymanski et al., 1994; Williams et al., 2017). This highlights the importance of utilizing an effective treatment such as clozapine as early as possible for patients who are poor responders in their EPP.

1.3.2 THE NEED FOR BIOMARKERS IN TRS

There is a lack of understanding as to why some individuals fail to respond to first-line pharmacological options including at early stages of illness when generally individuals respond quicker and with lower doses to pharmacologic treatments. There is a need for biomarkers to further support clinicians in identifying patients who could benefit from clozapine use in early psychosis. As this would identify patients for clozapine use in the earlier stage of illness, this could potentially improve outcomes for patients who need clozapine.

1.4 MAGNETIC RESONANCE SPECTROSCOPY (MRS)

Although magnetic resonance spectroscopy (MRS) was historically used in studying brain tumors, the technique has found a place in clinical research in psychiatry, helping to understand the pathophysiology of illness (Mullins et al., 2003). MRS is a safe

and non-invasive method used to measure neurometabolites in the brain (Ross & Bluml, 2001). MRS is a technique that is conducted using an MRI, and as such follows the same scientific prinicples, utilizing the spinning of charged atomic nuclei. A magnetic field is created by the charged nuclei that align themselves with the magnetic field created by the external magnet in the MRI. A voxel is then used to specify the location of the brain that is of interest. The voxel isolates the area of interest from external noise outside the voxel, allowing for precise measurement. MRS uses pulse sequences to acquire the signal of the metabolites of interest and this generates a spectra. The acquired spectra shows the displayed concentration levels of the neurometabolites in the region of interest.

One of the most commonly used pulse sequences in MRS is the Point RESolved Spectroscopy (PRESS) sequence. The PRESS sequence uses one 90° and two 180° refocusing pulses to create a spin echo (Zhu & Barker, 2011), whereas another sequence known as STEAM uses three 90° pulses to create a a stimulated echo (Moonen et al., 1992; van Zijl et al., 1989; Zhu & Barker, 2011). Although both sequences are similar, they differ in that the PRESS sequence has double the signal than STEAM, making it prefeable in some research (Moonen et al., 1989; Zhu & Barker, 2011). As different metabolites vary in their peaks, different sequences are better than others at optimizing the signal of different biochemicals. For example, research has allowed the development of sequences that optimizes for specific metabolites, including glutamate (Mullins et al., 2008; Zhu & Barker, 2011). Glutamate is unique in that it cycles together with glutamine and in MRIs less than 3 Tesla, the glutamate signal is highly contaminated by glutamine, making it difficult to isolate and receive an accurate measurement of glutamate concentration (Kandel et al., 2000; Kreis et al., 1992; Tkác et al., 2001; Zhu & Barker,

2011).

As MRIs are relatively accessible in middle to high income countries, this tool can potentially be used to inform clinicians and play a potential key role in treatment (Egerton, 2021; Matrone et al., 2022; Merritt et al., 2019). Previous research in TRS has demonstrated this, with research furthering our understanding of the potential role of glutamate in TRS. The ability to measure neurometabolites can provide support and guidance to clinicians in treating their patients.

1.5 MRS STUDIES ON GLUTAMATE IN SCHIZOPHRENIA & TRS

The connection between glutamate and schizophrenia has been well researched. Previous research has found that individuals with an established diagnosis of schizophrenia have higher levels of glutamate, glutamine and glutamate + glutamine (Glx) in different areas of the brain in comparison to healthy controls (Merritt et al., 2016, 2023; Nakahara et al., 2022). The study samples included in these meta-analyses varied from comparing individuals with FEP to healthy controls; to other studies that compared individuals with established schizophrenia or TRS to healthy controls (Merritt et al., 2016, 2023). The areas in the brain implicated in these studies include the basal ganglia, the thalamus (Merritt et al., 2016; Nakahara et al., 2022) and the medial prefrontal cortex (Bartha et al., 1997). These meta-analyses support the hypothesized role of glutamate dysfunction in the pathophysiology of both schizophrenia and potentially TRS (McCutcheon, Krystal, et al., 2020; Merritt et al., 2023).

Understanding the potential variables that may impact glutamate levels is also important in understanding the relationship between this neurometabolite and

schizophrenia. A study by Merritt et al., (2021) examined whether clinical and demographic characteristics are associated with brain glutamate or Glx levels in schizophrenia (Merritt et al., 2021). The results from this mega-analysis suggests that lower levels of glutamate in patients may be associated with antipsychotic medication exposure, whereas higher levels of glutamate may be a biomarker for illness severity in schizophrenia (Merritt et al., 2021). In the study there was no support for age being a factor relating to a decrease in brain glutamate (Merritt et al., 2021). Previous work to this study has conflicting findings to this study's finding on the impact of antipsychotic treatment. Some studies have seen an impact of antipsychotic treatment on glutamate levels while other studies do not see this association (Egerton et al., 2017; Egerton et al., 2021; Kubota et al., 2020). The most recently published study on this topic found no major effect of antipsychotic treatment on glutamate levels (Zahid et al., 2022). Further research stemming from these findings has sparked the investigation of glutamate functioning within poor responding patients to further understand this relationship between glutamate dysfunction and treatment response (Egerton et al., 2012, 2018; Egerton et al., 2023; Merritt et al., 2021).

As many patients with TRS are poor responders to medications in their FEP, it is important to explore how a lack of response to treatment is reflected in MRS glutamate. Egerton et al., (2012) found that FEP patients that were still symptomatic following antipsychotic treatment had higher glutamate levels in the ACC than patients that responded to treatment in their FEP (Egerton et al., 2012). Following this study, in 2018, Egerton et al., investigated whether response to antipsychotic medication was related to glutamate levels in the ACC prior to treatment (Egerton et al., 2018). The study found

that higher levels of glutamate in the ACC prior to treatment was associated with more severe symptoms at initial presentation and a decrease in likelihood of remission (Egerton et al., 2018). Lastly, a more recent study examined ACC glutamate in FEP as a predictor for treatment response in schizophrenia (Egerton et al., 2023). Similar to previous studies, the authors found that higher levels of ACC glutamate predicted non-response to antipsychotic treatment (Egerton et al., 2023). This research focus is important as it informs us that treatment responsiveness in EPP may be reflected in glutamate levels (Egerton et al., 2023; Kanahara et al., 2018).

In moving from treatment responsiveness to defined TRS, previous literature shows support for a relationship between glutamate and TRS (Demjaha et al., 2014; Mouchlianitis E et al., 2016). A study completed by Demjaha et al (2014) looked at both dopamine synthesis capacity and ACC glutamate levels between TRS patients, treatment responders and healthy controls (Demjaha et al., 2014). The authors found that patients with TRS had normal dopamine synthesis capacity; however, they also had elevated glutamate levels in the ACC in comparison to both treatment responders and healthy controls (Demjaha et al., 2014). A separate study in 2016 also investigated the relationship between TRS and glutamate levels in the ACC (Mouchlianitis et al., 2016). The authors found that TRS patients had higher levels of glutamate in the ACC than treatment responders (Mouchlianitis et al., 2016). These two studies implicate glutamate in the ACC and make a direct connection between the neurometabolite and TRS, making glutamate a potential biomarker for this clinical sub-population. While informative regarding the underlying pathophysiology of schizophrenia, a focus on the glutamate system has not yet generated successful alternative management approaches for

individuals who may have underlying glutamatergic abnormalities. To date, there has not been a study on connecting ACC glutamate and the need for clozapine in an early psychosis sample.

As this literature focuses on glutamate alterations in the ACC, it is important to understand the role of the ACC and how it is impaired in schizophrenia. The ACC plays an important role in our cognition, attention and emotional processing (Allman et al., 2006; Bush et al., 2000; Nelson et al., 2015). These domains are often impaired in individuals with schizophrenia. For example, studies have found that the ACC is hypoactive during the processing of emotions in patients with schizophrenia (Fallgatter et al., 2003; Reske et al., 2009; Taylor et al., 2012). Additionally, patients with schizophrenia perform poorly on cognitive tasks in comparison to healthy controls (Hofer et al., 2003; Meyer-Lindenberg et al., 2001; Rubia et al., 2001). As the literature suggests that elevated glutamate in the ACC could potentially explain TRS and poor response to treatment (Demjaha et al., 2014; Egerton et al., 2012, 2018, 2023; Iwata et al., 2019; McQueen et al., 2021; Merritt et al., 2021; Mouchlianitis et al., 2016), it is important that further research is conducted to consider alternative treatment options for these patients.

1.5.1 THE CONNECTION BETWEEN CLOZAPINE AND GLUTAMATE IN TRS

As clozapine is the only medication to be efficacious in the treatment of TRS, and the only medication suggested for treatment in various schizophrenia clinical treatment guidelines (Correll et al., 2022; Howes et al., 2017), it is understandable that building on research examining glutamate, treatment responsiveness, and clozapine effects on this system has become a focus of research. However, only three studies have investigated

glutamatergic neurometabolite levels in individuals with TRS on clozapine. The first study compared glutamatergic metabolite levels in healthy controls, treatment responders, TRS, and ultra-treatment resistant groups (defined in the study as having failed a trial on clozapine) (Goldstein et al., 2015). The TRS group had higher levels of Glx in the putamen, while no group differences were seen in the ACC (Goldstein et al., 2015). In the second study, TRS patients had a reduction of Glx in the caudate nucleus but not in the ACC after 12 weeks on clozapine (McQueen et al., 2021). In the third study, higher ACC Glx was found in the clozapine-resistant group in comparison to the healthy control group. However, there was no significant differences found between other patient groups (Iwata et al., 2019).

While dampening of glutamate has been hypothesized as one of the pathways leading to symptom improvement in patients treated with clozapine (Evins et al., 1997), to date McQueen et al is the only study that has compared ACC glutamate levels in TRS patients before and after clozapine initiation (McQueen et al., 2021). Continuing to investigate ACC glutamate in clozapine-eligible patients who have not yet been exposed to clozapine could provide information on glutamatergic alterations in individuals with TRS without the confounding influence of this medication.

1.6 STUDY RATIONALE

To date, no studies have investigated the association of elevated glutamate with clozapine-eligibility (patients who are symptomatic despite adequate treatment) in an EPP sample, a group in which clozapine initiation may have the most profound effects on long-term outcomes. For the purposes of this study, glutamate was measured in the ACC as there is considerable support for this region playing a key role in the

pathophysiology of schizophrenia (Demjaha et al., 2014; Egerton et al., 2021; Egerton et al., 2012, 2020, 2023; Griffiths et al., 2022; Martins-de-Souza et al., 2010; Mouchlianitis et al., 2016; Roberts et al., 2015). Given the key role the ACC plays in attention and cognition, it is thought that the ACC is essential for executive functioning tasks (Braver et al., 2001; Griffiths et al., 2022). Additionally, it has been previously demonstrated that individuals with schizophrenia display impairment during cognitive processing tasks (Fallgatter et al., 2003; Kerns et al., 2005; Nelson et al., 2015; Reske et al., 2009; Taylor et al., 2012). Additionally, elevated ACC glutamate has been associated with poor treatment response in schizophrenia (Egerton et al., 2012, 2018; Mouchlianitis et al., 2016; Szulc et al., 2013).

1.6.1 RESEARCH QUESTION AND HYPOTHESIS

The primary purpose of this study is to answer the following question: Do glutamatergic metabolites in the ACC differentiate clozapine eligibility from treatment responsiveness in patients with EPP? Given that elevated ACC glutamate has been associated with poor treatment response in EPP (Bojesen et al., 2020; Egerton et al., 2012, 2018; Mouchlianitis et al., 2016; Szulc et al., 2013), it was hypothesized that clozapine-eligible patients will have higher ACC glutamate levels than treatment responsive patients.

CHAPTER 2 METHODS

2.1 STUDY DESIGN

In this cross-sectional study, clozapine eligible and treatment responsive patients with EPP underwent Magnetic Resonance Spectroscopy (MRS) to measure glutamate in the ACC. In our study we used an analytical cross-sectional design, allowing us to assess the association between glutamate levels in the ACC and clozapine eligibility. The project received Research Ethics Board approval in 2019 and has been successfully renewed each year since the study started in January 2021.

2.2 SAMPLE SIZE

To estimate the sample size for our study, a power analysis was done using G*Power (Version 3.1). Two prior studies that assessed glutamate levels and treatment response in early psychosis were consulted for our power analysis (Egerton et al., 2012, 2018). Egerton et al. (2012) used an a priori sample size analysis estimate of 19 participants per group with Cohen's d of 1.11 and alpha set at 0.05. Egerton et al (2018) had a repeated measures design power analysis with an effect size of 0.3515, giving them an estimated sample size of 21 per group for their 3 groups (treatment responders, non-responders, and healthy controls). With an alpha of 0.05 and Cohen's d of 1.05, our calculations estimated a sample of 21 participants per group (clozapine-eligible and treatment responders) being suitable for this study.

2.3 PARTICIPANTS

Participants were recruited from the Nova Scotia Early Psychosis Program (NSEPP). NSEPP is a mental health program for youths and young adults ranging from ages 19 to

35 who have experienced a first episode of psychosis. NSEPP utilizes a team-based approach, with staff including a psychiatrist, a nurse case-manager, and access to social worker and an occupational therapy as required. After acceptance to the program, individuals are followed by the NSEPP for 5 years. All participants read and signed the study's consent form to participate in the study (Please see Appendix K). All current patients of the NSEPP were eligible to participate in this study, however, exclusion criteria included:

1. Primary diagnosis of a substance-induced psychosis
2. Diagnosis of substance dependence in the last 6 months (Except nicotine)
3. History of head trauma leading to loss of consciousness for >30mins
4. Inability to provide informed consent
5. Unstable medical/neurological illness
6. MRI contraindications

Participants were clinically assessed by a psychiatrist (KD) to separate them into either of the two naturally occurring subgroups (clozapine eligible or treatment responders). Individuals were classified as clozapine-eligible based on the modified treatment response and resistance in psychosis working group consensus criteria from Howes et al (Howes et al., 2017) and were naïve to clozapine. Our clozapine-eligible criteria were thus:

1. Have undergone two trials of antipsychotic treatment for at least 6 weeks with at least 600mg chlorpromazine equivalents (calculated using (Gardner et al., 2010))
2. Illness severity rating of greater than or equal to 4 (moderate) on the CGI-S and greater than or equal to 4 (moderate) on 2 PANSS positive symptom items, or at

least one symptom with a severe rating (despite treatment with adequate antipsychotic medication)

3. Moderate (<60) functional impairment on the SOFAS
4. Estimated medication compliance of 80% of prescribed doses during the 6-week treatment trials (compliance will consider patient's report, family input as relevant and input from the patient's care team)
5. Have not yet undergone a trial of clozapine

Our second group in this study consisted of treatment responders who were also recruited from the NSEPP. Criteria for the treatment responders' group was:

1. Having ratings of no more than mild severity on all PANSS items for at least 12 consecutive weeks with mild or better functional impairment (60 or greater on SOFAS)
2. Currently receiving treatment with a single antipsychotic medication
3. Estimated medication compliance of 80% of prescribed doses during the 6-week treatment trials (compliance considered patient's report, family input as relevant and input from the patient's care team)

The Positive and Negative Syndrome Scale (PANSS; (Kay et al., 1987) please see Appendix L) was used to assess severity of psychotic symptoms. The PANSS has been widely used and has sufficient validity and internal reliability for its subsections on positive, negative and general psychopathology (α coefficients= 0.73, 0.83 and 0.79) (Kay et al., 1987) and test-retest reliability (r= 0.80, 0.68 and 0.60) (Kay et al., 1987). The Clinical Global Impression-Severity scale (CGI-S, please see Appendix M) was used to assess illness severity (Haro et al., 2003). The CGI-S has strong reliability (intraclass

correlation (ICC) >0.70) amongst all dimensions except for the depressive dimension (0.64) and shows strong concurrent validity with the PANSS scale, with correlation coefficients ranging from 0.75-0.86 (Haro et al., 2003). And lastly the Social and Occupational Functioning Assessment Scale (SOFAS, please see Appendix N) (Goldman et al., 1992) was used to assess functioning. The SOFAS scale is also a valid measure with strong reliability (ICC >0.74) (Hilsenroth et al., 2000).

Demographic information collected at entry to the study included: age, biological sex, gender, ethnicity, employment status as well as education completed and family history of psychosis (Please see Appendix O). The participant's treatment history such as medication trials, current antipsychotic dose, DUP as well as their recreational drug use both in the past and present was collected. DUP was calculated based on the time in-between the patient's initial onset of symptoms and the start of the patient's treatment. All participants remained on their presently prescribed antipsychotic medications and their antipsychotic dose was recorded and converted to chlorpromazine (CPZ) equivalents (calculated using (Gardner et al., 2010) . Patients were screened for contraindications prior to their magnetic resonance imaging scan and signed the consent form for participation.

2.4 MAGNETIC RESONANCE SPECTROSCOPY (^{1}H-MRS)

Neuroimaging took place at the Biomedical Translational Imaging Center (BIOTIC) in the Halifax Infirmary using a 3 Tesla GE Healthcare Model MR750 whole-body magnetic resonance (MR) imager (GE Healthcare MR750, Milwaukee, WI, USA). To acquire brain images, we used a 3D T1-weighted IR-FSPGR sequence (slice thickness of 1mm, isometric) in sagittal plane (TE=1.34ms, TR= 4.056ms, FOV= 256mm and flip

angle= 9). For the purposes of this study, glutamate was measured in the ACC as the previous literature supports this region as being intricately important in the pathophysiology of schizophrenia (Egerton et al., 2012, 2018; Mouchlianitis et al., 2016; Roberts et al., 2015; Szulc et al., 2013).

2.5 MRS DATA COLLECTION PARAMETERS

A 2.0 x 2.0 x 2.0 cm ($8cm^3$) ^{1}H-MRS voxel was placed in the bilateral dorsal ACC (see Figure 1). The posterior end of the voxel was set to coincide with the precentral gyrus and caudal face of the voxel coinciding with the most caudal location that is not part of the corpus callosum. Additionally, we had a coding script that allowed us to check the voxel placement after the scan to ensure our placements were as consistent as possible amongst the scans. The duration of the scan lasted approximately 30 minutes. We utilized the Point RESolved Spectroscopy (PRESS) sequence (TE= 40ms;TR= 3000ms; 128 averages) in ^{1}H-MRS acquisition (Frahm et al., 1989; Mullins et al., 2008). Previous studies have shown this PRESS sequence to be highly reliable in repeated measurement of glutamate (Mullins et al., 2008).

The quality criteria for spectral profiles was set at an LCModel signal to noise ratio (SNR) greater than 15**,** a scanner full width at half maximum (FWHM) of the water peak less than 10HZ, and a percent error (technical error) in the estimation of ^{1}H-MRS concentration levels lower than 18% standard deviation/Cramer- Rao lower bounds (%SD) around the mean value for each specific signal as reported by LCModel.

To account for potential drift we tested the ^{1}H-MRS Spectroscopy phantom (GE Healthcare, Milwaukee, WI, USA) monthly over the course of the study (Woo et al., 2009). This testing allows us to evaluate quality assurance and avoid potential mistakes

or defects made by the machine (Woo et al., 2009).

MRS data was fit with Linear Combination of Model (LCModel) (Provencher, 2001). The LCModel is an automatic and objective measure. The program analyzes the spectra and produces a maximum likelihood estimate of metabolite concentrations as well as a %SD for each estimate (Provencher, 2001). The unsuppressed water spectrum was used for internal referencing of the glutamate concentration. The T1-weighted sequence was used for tissue type segmentation and glutamate concentration levels were adjusted for partial volumes of cerebrospinal fluid (CSF), grey matter and white matter in each voxel of interest.

We have previously tested the above parameters to acquire a 1H spectrum from a single participant (Please see Figure 2).

2.6 MRI SOFTWARE UPGRADES

It is important to note that the 3 Telsa MRI machine underwent a software upgrade during data collection. The update occurred from November 2022 to December 2022. Prior to this upgrade we performed a pre-scan with our protocol to compare with a post-scan after the upgrade was completed. We used the same individual for both the pre and post scans and used the 32 channel head coil that had been used in all of our previous research study scans. The pre and post scans did not indicate any problems with the spectral data. The SNR was 18 pre-upgrade and 36 post-upgrade, the FWHM was 4.9 Hz pre-upgrade and 5.5 Hz post-upgrade. Additionally, the Cramer-Rao Lower Bounds (CRLB) were met for glutamate measurement before (6%) and after the upgrade (4%). CRLB for glutamine was 26% pre-upgrade and 12% post-upgrade. For Glx CRLB were 5% pre-upgrade and 5% post-upgrade. For consistency, we continued to use the same 32

channel head coil after the scanner upgrade. Out of our completed study sample, 3 of those participants were scanned after the MRI software upgrade.

2.7 STATISTICAL ANALYSIS

All statistical analyses were performed using IBM SPSS version 28. A P-value of <0.05 was the threshold for establishing statistical significance. Descriptive statistics were used to describe the patient population with means ± standard deviation for continuous variables, and frequency and counts for categorical variables. Baseline demographics are described and compared between the two groups (clozapine eligible patients and treatment responders). To decide what variables to include in the model, baseline demographics were compared using an independent t-test between the two groups to see if they were significantly different. If these variables were significantly different between the two groups, the variable was then included in the regression analysis as a covariate. In this model, glutamate was the dependent variable, and our groups were the independent variable. Covariates were then entered in the covariate section in the settings of the linear regression model parameters. Once all the parameters were entered in their respective sections, the analysis was then completed.

Glutamate is the primary outcome and was compared between the two groups using linear regression. Glx and glutamine were also be reported as following the previous research that reports both of these metabolites (Egerton et al., 2012; Egerton et al., 2018, 2023; Iwata et al., 2019; McQueen et al., 2021; Mouchlianitis et al., 2016).

CHAPTER 3 RESULTS

3.1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF STUDY SAMPLE

A total of 31 participants consented for research participation; 30 completed both the clinical assessment and MRI. 19 treatment responders (TR, 15 males, 4 females) and 11 clozapine eligible participants (CE, 8 males, 3 females) with non-affective psychotic disorders were recruited from the Nova Scotia Early Psychosis Program (NSEPP). Please see Figure 3 for a breakdown of study recruitment. 24 participants had a diagnosis of schizophrenia, 2 had a diagnosis of schizoaffective disorder. 1 individual had schizophrenia and comorbid OCD and opiate use disorder that was in remission at the time of participation. 1 individual had a diagnosis of schizophrenia and comorbid social anxiety disorder, 1 individual had a diagnosis of unspecified psychotic disorder, and 1 individual had a diagnosis of schizophreniform disorder. Of the 30 participant's ethnicities, 26 were Caucasian-north American, 1 was Caucasian-European, 2 were Black-African and 1was Middle Eastern. 15 of our 30 participants reported smoking cigarettes. 10 participants reported marijuana use. 17 of our 30 participants were on LAIs while 13 were on oral medications, please see Table 1 for the list of medications patients were on at the time of study participation.

Only one participant did not complete the study participation due to concerns about undergoing an MRI scan after consenting to participate. As the participant did not complete the clinical assessment or the MRI scan, they are not included in our clinical or demographic data..

The average age for the study was (mean ± SD) 25.63 ± 3.98 years. The average

number of weeks since participants were accepted into the NSEPP was 136.63 ± 85.18 weeks. The average DUP in our overall sample was 15.20 ± 12.45 weeks. For the PANSS scale, the average total PANSS score was 48.37 ± 17.75, average PANSS positive score was 12.60 ± 6.23, average PANSS negative score was 12.37 ± 5.30, average PANSS general score was 23.07 ± 7.84 in our overall sample. The average SOFAS score for the entire sample was 57.83 ± 20.74. The average antipsychotic dose in CPZ equivalents was 366.57mg ± 248.98mg for participants.

The average age per group were 25.74 years ± 3.26 (TR) and 25.54 ± 5.17 years (CE). Age was not significantly different between the two groups (t-statistic (degrees of freedom), p-value), t=-0.184 (28), p-value= 0.855. The average number of weeks in the NSEPP clinic were 152 ± 85 weeks for the CE group and 128 ± 86 weeks for the TR group. The number of weeks since accepted into the NSEPP did not significantly different between the two groups, t=0.764 (28), p-value= 0.451. Average DUP was 20.45± 9.67 weeks for CE and 12.16 ± 13.08 weeks for TR. DUP did not differ between the two groups t=1.829 (28), p-value=0.078.

The average PANSS score was 68.64 ± 13.89 for the CE group and 36.63 ± 3.42 for the TR group. These were significantly different between the two groups t= 9.988(28), p-value= <0.001. The average PANSS positive scores were 19.91 ± 3.99 for CE and 8.37 ± 1.50 for TR. The PANNS positive scores were significantly different between the two groups, t=11.417(28), p-value= <0.001. The average PANSS negative scores were 17.27 ± 5.37 for CE and 9.53 ± 2.46 for TR. The PANSS negative scores were significantly different between the two groups, t= 5.431(28), p-value= <0.001. The average PANSS general scores were 31.45 ±6.47 for CE and 18.21 ± 2.78 for TR. These

were significantly different between the two groups, t= 7.831(28), p-value=<0.001.

The average SOFAS scores were 40.00 ± 14.66 for CE and 68.16 ± 16.32. The SOFAS scores significantly differed between the two groups, t= -4.719(28), p-value= <0.001. The average CPZ equivalent dose was 532.40 mg ± 322mg for the CE group and 270.16mg ± 125.40mg for the TR group. Antipsychotic dose differed significantly between the two groups, t= 3.183(28), p-value=0.004. The CE group had an average of 2.18 ± 0.751 medication trials while the TR group had an average of 1.05 ± 0.621 medication trials. Please see Table 2 for a breakdown of the mean/SD of clinical and demographic variables between the two groups and the T-value/p-value from comparing the 2 groups across the clinical and demographic.

3.2 1H-MRS SPECTRAL QUALITY

Of the 30 spectral data collected, all were included in the analysis. As forementioned, we had spectral quality criteria to assess the inclusion of the data, the average SNR for all scans were 25.2, above our >15 SNR threshold. The average FWHM for all the spectral data were 6.09Hz, meeting our criteria of <10Hz. Our Cramer- Rao lower bounds (CRLB)/ %SD for the estimated glutamate concentration values were 5%, meeting our <18% threshold. The mean glutamate CRLB did significantly differ between the two groups, t= 2.64 (28), p-value= 0.013. The average CRLB for glutamate were (mean (SD) in %) 6.2% (0.02) for CE; 4.8% (0.01) for TR.

The mean Glx CRLB met the criteria as well with an average %SD of 5%. CRLB also did not differ between the two groups for Glx, t= 1.66(28), p-value= 0.11. The average CRLB per group were 5.4% (0.02) for CE; 4.7% (0.07) for TR.

The mean glutamine CRLB for the CE group did not meet our criteria with an

average of 19.27% (0.07 SD). The average CRLB for the TR group was 16.16% (0.05 SD). The mean CRLB did not significantly differ between the two groups, 1.41(28), p-value= 0.171. Please see Table 3 for the quality data for both groups.

3.3 LINEAR REGRESSION ANALYSIS RESULTS

All glutamate measurements were included in the analysis. As the PANSS total, PANSS positive, PANSS negative, PANSS general, SOFAS scores and antipsychotic dose (in CPZ equivalents) were significantly different between the two study groups, these variables were included in our linear regression model as covariates.

The linear regression showed no significant differences between the two groups glutamate levels (mean difference= -0.324, std error =0.751, p-value = 0.670, CI: [-1.883, 1.234]). The overall model had an F-statistic of 0.503, a degree of freedom of 7 and p-value= 0.822. The linear regression model had an R^2 of 0.138, with an adjust R^2 of -0.136.

The PANSS total score did not have a significant association to glutamate levels in our participants (F= 0. 069 (1), p= 0.795). The PANSS positive, negative, and general scores also did not have a significant association with glutamate levels (F= 0.296(1), p-value= 0.592, F= 0.016(1), p-value= 0.0.902 and F= 0.028(1), p-value= 0.869 respectively). The SOFAS scale did not have an association to glutamate levels (F= 0.986(1), p-value= 0.332) and antipsychotic dose also was not significant in the model (F= 0.215(1), p-value= 0.647 Please see Table 4 for the linear regression results.

All Glx measurements were included in the analysis. Similarly, to our regression model for glutamate, we included PANSS total, PANSS positive, PANSS negative, PANSS general, SOFAS scores and antipsychotic dose as covariates.

Our linear regression analysis showed no significant difference between the two group's Glx levels. The average Glx concentrations did not significantly differ between the two groups (mean difference= 1.276, std error = 1.145 p-value = 0.277, CI: [-1.099, 3.650]). The overall model had an F-statistic of 0.638, a degree of freedom of 7 and p-value= 0.638. The linear regression model had an R^2 of 0.169 and an adjusted R^2 of -0.096.

The PANSS score did not have a significant association with Glx (F= 0.138 (1), p= 0.714). The PANSS positive, negative, and general scores also did not have a significant association with Glx levels (F= 0.082(1), p-value= 0.777, F= 0.417 (1), p-value= 0.526 and F= 0.014(1), p-value= 0.907 respectively). The SOFAS scale did not have an association to Glx levels (F= 0.560(1), p-value= 0.462) and antipsychotic dose also was not significant in the model (F= 0.030(1), p-value= 0.864). Please see Table 5 for the linear regression results and Figures 4 and 5 for box plots of glutamate and Glx for each group.

Although the average CRLB for glutamine was 17.3%, only 18 scans out of our 30 met the CRLB quality criteria. As we would need to exclude approximately half of our data set to do a complete analysis, we decided to not do a regression analysis on our glutamine data due to the number of scans that did not meet our quality criteria.

After the analysis was completed, it was brought to our attention that including the PANSS scores as covariates could potentially washout the likelihood of observing a difference in our 2 study samples. As this difference in PANSS scores were already likely to exist, we decided to run the linear regression without the PANSS scores as covariates and report the results of this model for both glutamate and Glx.

All glutamate measurements were included in the analysis. We included the SOFAS scores and antipsychotic dose as covariates. The linear regression showed no significant difference in glutamate levels between the two groups (mean difference= -0.189, std error =0.441, p-value = 0.651, CI: [-1.040, 0.662]). The overall model had an F-statistic of 0.729, a degree of freedom of 3 and a p-value= 0.544. The linear regression model had an R^2 of 0.078 and an adjusted R^2 of -0.029. The SOFAS scale did not have an association to glutamate levels (F= 0.690(1), p-value= 0.414) and antipsychotic dose also was not significant in the model (F= 0.225(1), p-value= 0.639).

All Glx measurements were included in the analysis. We included the SOFAS scores and antipsychotic dose as covariates. The linear regression showed no significant difference in Glx levels between the two groups (mean difference= 0.042, std error =0.645, p-value = 0.948, CI: [-1.283, 1.367]). The overall model had an F-statistic of 0.673, a degree of freedom of 3 and a p-value= 0.576. The linear regression model had an R^2 of 0.072 and an adjusted R^2 of -0.035. The SOFAS scale did not have an association to Glx levels (F= 1.126 (1), p-value= 0.298) and antipsychotic dose also was not significant in the model (F= 0.020(1), p-value= 0.889).

CHAPTER 4 DISCUSSION

The current study is the first to investigate whether there is an association between glutamate levels in the ACC and clozapine eligibility in an early psychosis sample. We hypothesized that clozapine-eligible patients would have higher ACC glutamate levels than treatment responsive patients. This hypothesis was not supported by our results. Despite having two clinically different groups, we did not find any difference in our primary metabolite of interest, glutamate. We also found that Glx levels did not significantly differ between the two groups.

4.1 ASSOCIATION BETWEEN GLUTAMATERGIC METABOLITES AND CLOZAPINE ELIGIBILITY

Contrary to our expectations, our results did not demonstrate an association between ACC glutamatergic metabolites and clozapine eligibility. Our hypothesis was based on previous findings demonstrating an association between elevated glutamate and poor antipsychotic treatment response (Demjaha et al., 2014; Egerton et al., 2012, 2018, 2021; Iwata et al., 2019; Mouchlianitis et al., 2016). Several methodological differences may explain the lack of a significant association in contrast to other studies (Demjaha et al., 2014, 2017; Egerton et al., 2012, 2018, 2021; Goldstein et al., 2015; Iwata et al., 2019; McQueen et al., 2021; Merritt et al., 2021; Mouchlianitis et al., 2016; Zahid et al., 2022).

Stage of illness may have a significant impact on glutamate levels. While others found an association between elevated glutamate and indices of treatment response in chronic schizophrenia (Demjaha et al., 2014; Iwata et al., 2019; Mouchlianitis et al., 2016), our sample consisted of patients in the first 5 years of illness. We are unable to

directly compare our findings to patients that have already been established as treatment resistant or ultra-resistant (resistant to all first line medications and clozapine) (Iwata et al., 2019). The patients in these studies have been unwell for a longer period of time as they have met criteria for TRS or ultra-resistant schizophrenia (Demjaha et al., 2014; Iwata et al., 2019; Mouchlianitis et al., 2016). Since our study is in a different clinical population (EPP patients), there could be some differences between our study sample and the previous studies sample of patients with chronic schizophrenia (Demjaha et al., 2014; Iwata et al., 2019; Mouchlianitis et al., 2016).

Of the studies that included patients with TRS, one study (McQueen et al., 2021) inferred TRS in patients based off medical records and the patient's psychiatrists, unlike the other studies that used modified Kane et al criteria or the TRIPP guidelines for TRS (Demjaha et al., 2014, 2017; Goldstein et al., 2015; Howes et al., 2017; Iwata et al., 2019; Kane et al., 2019; Mouchlianitis et al., 2016). The variability in defining TRS could be a reason for the variance in study findings. As previously mentioned, identifying TRS can be confounded by variables such as recreational drug use and medication adherence. As this study showed an association between clozapine use and decrease glutamate levels 3 months after clozapine initiation, it is important that the literature is consistent in defining TRS in research studies.

Several other studies noting a relationship between glutamate and treatment response have focused on assessing antipsychotic response at a single, finite point in time (Egerton et al., 2018, 2023). In the 2018 study, ACC glutamate was measured before and after 4 weeks on a medication. Although this study connected ACC glutamate to treatment response at 4 weeks, ultimate designation of TRS and clozapine-eligibility is more

nuanced. An individual who does not respond by 4 weeks does not necessarily go on to meet criteria for TRS. Additionally, this study included patients who were likely not severely unwell, as they excluded patients who were receiving compulsory treatment which could have impacted the results as well (Egerton et al., 2018). Additionally, all participants were receiving amisulpride a medication that is not available in Canada, while in our study half of our sample is on LAIs which could also potentially explain the differences in our findings. Although not fully understood, the use of antipsychotics could potentially alter glutamate levels. Although the findings on this topic have been conflicting, they are solely focused on oral medications (Merritt et al., 2021; Zahid et al., 2022). The potential impact of LAI use on glutamate levels is unknown but could potentially explain the differences in our results. Lastly treatment response in this study was decided based on the changes in participant's PANSS scores (Egerton et al., 2018). When measuring response, especially in an unwell population, you will almost always see an initial response to treatment, making it more likely that you will observe a relationship that would not otherwise be observed (Egerton et al., 2018). As well, individuals who do not score high on the PANSS scale at baseline would have a harder time in meeting the cutoff to be considered a responder to treatment, which could also play a part in this study's findings (Egerton et al., 2018).

The second study looked at response at 2 and 6 weeks, this study also found a connection between ACC glutamate and treatment response, however, the same concern arises. Although, they measured response at 6 weeks, this timepoint is the bare minimum to indicate response and may still be too soon to observe response in some patients (Egerton et al., 2023). Similar to the previously discussed study, how treatment response

is defined is important to consider when comparing our study to this previous literature (Egerton et al., 2018, 2023).

Another factor that could have impacted the results in the previous studies is the voxel placement. We placed the voxel in the bilateral dorsal ACC while the Egerton and McQueen et al., studies placed their voxel in the ventral part of the ACC (Egerton et al., 2012, 2018, 2023; McQueen et al., 2021). We chose the dorsal ACC as it plays a key role in our attention and cognition, which are impaired in individuals with schizophrenia and within resistant patients cognition is further impaired (Heilbronner & Hayden, 2016; Kerns et al., 2005; Paus, 2001; Roberts et al., 2015; Shenhav et al., 2016). This could potentially have a strong impact on the results in their studies versus our own and could explain why elevated and changes in glutamate levels are being observed in their studies.

Similarly to glutamate, our study was not successful in finding an association between ACC Glx metabolites and clozapine eligibility (Egerton et al., 2021). Although this contradicts some studies findings, it is consistent with others (Egerton et al., 2012; Goldstein et al., 2015; Iwata et al., 2019; Mouchlianitis et al., 2016). In the singular study that found a difference in Glx between responders and non-responder, non-responders were classified as having at least 2 antipsychotic trials for at least >4 weeks (Egerton et al., 2021). This threshold is below the recommended length of an antipsychotic trial of at least or greater than 6 weeks (Howes et al., 2017). Four weeks is not a sufficient trial on an antipsychotic trial and can result in misclassifying patients as non-responders, when they may need an increased dose of a medication to illicit a response or adherence to their medication (Altamura et al., 2005; Dold & Leucht, 2014; Kane et al., 2019; Potkin et al., 2020).

4.2 ASSOCIATION BETWEEN PANSS AND GLUTAMATERGIC METABOLITES

We did not find an association between the PANSS scores and glutamate levels, nor did we find an association between the PANSS scores and Glx levels. The relationship between the PANSS score and ACC glutamate has been quite variable in previous literature (Egerton et al., 2012, 2018, 2021, 2023; Goldstein et al., 2015; Iwata et al., 2019; McQueen et al., 2021; Mouchlianitis et al., 2016). Our results contradict some of the previous literature (Egerton et al., 2012, 2018) but finds support from other previous literature as well (Egerton et al., 2021, 2023; Goldstein et al., 2015; Iwata et al., 2019; McQueen et al., 2021; Mouchlianitis et al., 2016).

Previous work by Egerton et al. (2012), found a significant association between PANSS negative scores and ACC glutamate (Egerton et al., 2012). A later study by Egerton et al., (2018) also found that higher baseline ACC glutamate was associated with a greater PANSS total score (Egerton et al., 2018). However, a study by Mouchlianitis et al (2016) did not find an association between PANSS total score (Mouchlianitis et al., 2016). Additionally, a study by McQueen et al (2016) also did not find a correlation between PANSS score and ACC glutamate at baseline in their study (McQueen et al., 2021). However, this study's sample consisted of only TRS patients, (McQueen et al., 2021), whereas our study included both poor responders and treatment responders. Two other studies by Egerton et al., did not find an association between ACC glutamate and PANSS total scores (Egerton et al., 2021, 2023). Goldstein et al., (2015) also did not find a relationship between ACC glutamate and PANSS scores (Goldstein et al., 2015) and lastly, a study by Iwata et al., did not find relationships between glutamate levels and

PANSS total score however, similarly to McQueen's study, this study only included a specific group of patients, those with ultra-resistant schizophrenia (Iwata et al., 2019).

For Glx, our study's lack of relationship between the metabolite and PANSS scores seems to align with most of the previous findings in the literature (Egerton et al., 2023; Goldstein et al., 2015; Iwata et al., 2019; McQueen et al., 2021; Mouchlianitis et al., 2016). This excludes the findings from Egerton et al (2012) that reported an association between Glx and the PANSS negative score in their study sample. A potential reason as to why this study saw a relationship and the other studies did not was that the sample included FEP patients, whereas all the other studies were in patients who were classified as non-responders or TRS. Both the 2018 and 2021 studies by Egerton et al., did not report on the relationship between Glx and PANSS scores (Egerton et al., 2018, 2021).

As it stands, the relationship between glutamatergic metabolites and symptoms is highly variable from study to study, further research is needed to better understand this relationship.

4.3 THE ASSOCIATION BETWEEN ANTIPSYCHOTIC DOSE AND GLUTAMATERGIC METABOLITES

Although the clozapine-eligible group had a significantly higher average antipsychotic dose than our treatment responders, we did not find an association between antipsychotic dose and glutamate levels. Nor did we find an association between antipsychotic dose and Glx levels. Our findings are concurrent with the previous literature on this relationship with studies not observing a relationship between antipsychotic dose and glutamatergic metabolites (Egerton et al., 2021; Mouchlianitis et

al., 2016; Zahid et al., 2022). Egerton et al., (2021) did not find a significant association between ACC glutamate levels and antipsychotic dose (Egerton et al., 2021). This finding was also observed when examining ACC Glx and antipsychotic dose (Egerton et al., 2021). A second study found similar findings with a non-significant relationship between ACC glutamate and Glx and antipsychotic dose (Zahid et al., 2022). Another study by Mouchlianitis et al (2016) also had similar findings to these studies with no relationship being observed between the two neurometabolites and antipsychotic dose (Mouchlianitis et al., 2016).

Although we did not study the impact of antipsychotic exposure in our study, it is important to note that there is literature on the change in glutamatergic metabolites before and after antipsychotic exposure (McQueen et al., 2021; Merritt et al., 2021; Zahid et al., 2022). Future studies should also consider this potential relationship when looking at the relationship between antipsychotic dose and glutamatergic metabolites.

4.4 LIMITATIONS

There are limitations of this study that need to be considered when discussing the findings. The first being that our study is underpowered. As forementioned, our power analysis indicated that a sample size of 21 participants per group would suffice for our design. Our completed sample size includes 19 treatment responders and 11 clozapine eligible, below the power threshold of 21 participants per group. Furthermore, the group numbers were quite uneven with almost double the number of treatment responders than clozapine eligible. These factors unfortunately decrease the reliability and validity of our results.

Although we foresaw recruiting poor responders as a potential barrier, we did our

due diligence in supporting these patients with reminder calls, rescheduling and meeting with patients. However, one of the toughest factors in recruiting this population was that there was less of them than we expected. When considering who would be eligible many individuals were screened out due to drug use and medication nonadherence. Another barrier for recruiting these patients for our study was mental health, as this is a clinical population, it is tough to recruit for research when patients are not doing well. Additionally, many potential clozapine eligible patients were less likely to agree to study participation and were uninterested in research. Lastly, of the few potential clozapine eligible patients that were identified to us, some were hard to reach or did not respond to us when contacted for research participation.

Another limitation to our study is its design. As a cross-sectional study we are only getting a snapshot of patients and are unable to account for what occurs in their treatment prior and after their study participation. As we are trying to establish a relationship between glutamate levels and clozapine eligibility, a longitudinal prospective study design would be helpful in answering our research question. Having the ability to follow patients would allow us to reliably investigate whether there is in fact a relationship between glutamate levels and clozapine eligibility. There is a longitudinal aspect to the study that was outside the scope of the present paper but will hopefully shed light on the relationship between glutamate levels and clozapine use.

Another fundamental limitation to our study to consider is that we were unable to establish a relationship between glutamate levels and clozapine eligible patients in an early psychosis sample. As our sample size may be a main contributor to this, it is important that future studies have a large enough sample size in order to determine

whether this relationship between ACC glutamate and clozapine eligibility does in fact exist in early psychosis.

4.4.1 1H-MRS & GLUTAMATE MEASUREMENT

A methodological limitation for this study stemmed from our MRS acquisition. It is important to consider that a practical limitation from using 3T ^{1}H-MRS is that the glutamate signal has contamination from both glutamine and GABA. Although the contamination estimation is rather small (8%, 7% respectively), it could still impact our measuring of glutamate concentrations. In the Mouchliantis et al., (2016) and McQueen et al (2021) studies, this was viewed as a limitation in their study, as we also used a 3T scanner this is a practical limitation in our study as well (McQueen et al., 2021; Mouchlianitis et al., 2016).

Additionally, we had a MRS related limitation that we did not correct for. Partial saturation occurs when TR is shorter than 4-5 times the longitudinal relaxation time (T1). This makes the magnetization unable to recover before the next excitation, which leads to a reduced steady-state longitudinal magnetization. Incomplete T1 relaxation reduces the signal amplitude. This can lead to generating images with an increased contrast between the regions with different relaxation times. Partial saturation could be accounted for with the use of a MATLAB toolkit called FID Appliance (FID-A) (Simpson et al., 2017). This program allows you to compare the MRS data and correct frequency and phase drifts (Near et al., 2015; Simpson et al., 2017). Additionally, FID-A implements higher level processing to automatically detect and remove motion-corrupted scans (Simpson et al., 2017). The FID-A toolkit cleans up our raw data and corrects the MRS data by accounting for error across the scans. We initially applied FID-A to our data after using

LCModel, however, we found that it did not improve our data quality measurements. It decreased our SNR and increased our FWHM and CRLB. Due to this, we decided to report and analyze the data we received from LCModel without the use of FID-A. It is important that future studies account for the limitations of MRS at 3T with the use of processing software that can improve the quality of the data.

Another limitation to our spectroscopy is the fact that our protocol may not have optimized for the glutamate signal. In the study that used the protocol that we use in the present study, J-editing techniques were used to assist in the optimizing of glutamate, a J-coupled metabolite at 3T and to test the reliability with repeated measuring of glutamate (Mullins et al., 2008). Their findings showed that standard PRESS was sufficient in the measurement of glutamate, thus we used the same parameters as this study (TE= 40ms; TR= 2000ms; 128 averages). However it is important to note that previous MRS studies in our research area utilized PRESS but with various sequences: TE =30 msec; TR =3000 msec; 96 averages; TE = 35 ms, TR = 2000 ms, 128 averages; TE = 35ms; TR = 3000ms; 64 averages; TE =30; TR =2000ms; 80 averages (Egerton et al., 2012, 2018, 2023; Goldstein et al., 2015; McQueen et al., 2021; Mouchlianitis et al., 2016). The varying sequences could potentially account for the varying results we observe between our study and the previous literature. This includes the results between samples for the glutamatergic metabolites but also the observed associations between the metabolites and symptoms and antipsychotic dose. As this area of research develops, it will be important to consider a more unified approach in spectroscopy at 3T. This would improve the ability to compare studies and to improve the reliability and validity of reported findings across these studies.

4.5 FUTURE DIRECTIONS

Although we were unsuccessful in finding a connection between ACC glutamate and clozapine eligibility, we had a few limitations that strongly impacted our ability to accurately characterize any potential relationship. It is important that future studies establish sufficient power to produce reliable findings. Additionally, future studies should investigate the relationship between antipsychotic dose and glutamate levels, as the previous literature in this area is highly variable, more research is needed in understanding this relationship. Future studies should also be clear in how they identify TRS, poor responders and non-responders. The use of clinical guidelines such as the TRRIP's modified criteria is important to use to minimize subjectivity and extraneous variables in identifying poor clinical response in patients with schizophrenia. Standardizing these definitions in research will improve our understanding of the role of glutamate and clinical treatment. As well, this will improve our ability to compare study findings.

In studies assessing response in patients, it is important that these studies consider sufficient response in the long term and not just the short term such as 4-6 weeks. Especially when classifying participants in research studies, assessing an individual once at 4-6 weeks after initiating treatment is not sufficient in identifying whether a patient is a strong enough responder to treatment. Again, utilizing the TRIPP guidelines would be useful in these studies to improve the standardization across the studies in this area.

Lastly, with the use of spectroscopy, specifically at 3T, it is important that there is a unified approach on the parameters surrounding MRS sequences. With the varying types of sequences used, we are unable to tease apart whether results are due to the

differences in parameters or if there is in fact a different effect being observed in these studies. The more consistent the approach to MRS acquisition is across studies, the easier it will be to observe the potential relationships between glutamate and clinical variables in schizophrenia.

4.6 CONCLUSION

There is research suggesting that glutamate could be a potential biomarker for clozapine use. Although our study was not successful in identifying this relationship, it is important that this topic undergoes further investigation. For patients with TRS, their care is centered around management and if we could identify a relationship between glutamate levels and the need for clozapine earlier then patients could go one to use the medication. Additionally, understanding the pathophysiology of schizophrenia, provides the opportunity to improve treatment options for patients. This would not only restore hope in some patients but also change clinician's approach to care for these patients. As there is clear literature to back up and support the use of clozapine in EPP, it is important that we use this to identify individuals who will benefit the most from that medication and establish a system of care that supports these individuals in receiving this medication. Deciphering clozapine eligibility in the early phase of illness could allow us to improve the course of outcome and prognosis of many patients that struggle with this chronic yet treatable disorder.

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APPENDIX A TABLE 1

Medications

	N	%
Abilify	4	13.3%
Abilify & Trinza	1	3.3%
Clozapine	1	3.3%
Lurasidone & Olanzapine	1	3.3%
Lurasidone & Quetiapine	1	3.3%
Maintena	9	30.0%
Maintena & Abilify	1	3.3%
Olanzapine	4	13.3%
Quetiapine	1	3.3%
Risperidone	1	3.3%
Sustenna	2	6.7%
Sustenna & Maintena	1	3.3%
Sustenna & Olanzapine	1	3.3%
Trinza	2	6.7%

APPENDIX B TABLE 2

Clinical and Demographic Data by Total Samples and Groups

	Total group (mean/SD)	CE (mean/SD)	TR (mean/SD)	T-value/P-value
Age (yrs)	25.63/3.98	25.54/5.17	25.74/3.26	0.184/0.855
Sex (M/F)	23/7	8/3	15/4	N/A
Diagnosis (SCZ/Other)	24/6	10/1	14/5	N/A
Cigarette Smoker (Y/N)	15/15	7/4	8/11	N/A
Cannabis Use (Y/N)	10/20	2/9	8/11	N/A
Medication (LAI/Oral)	17/13	6/5	11/8	N/A
Medication Trials	1.47/0.86	2.18/0.75	1.05/0.62	4.446/<0.001
DUP (in weeks)	15.20/12.45	20.45/9.67	12.16/13.08	1.829/0.078
Weeks in NSEPP	136.63/85.18	152/ 85	128/86	0.764/0.451
PANSS total	48.37/17.75	68.64/13.39	36.63/3.42	9.988/<0.001
PANSS positive	12.60/6.23	19.91/3.98	8.37/1.50	11.417/<0.001
PANSS negative	12.37/5.30	17.27/5.37	9.53/2.46	5.431/<0.001
PANSS general	23.07/7.84	31.45/6.47	18.21/2.78	7.831/<0.001
SOFAS	57.83/20.74	40.00/14.66	68.16/16.32	-4.719/<0.001
Antipsychotic Dose (CPZ equivalents in mg)	366.57/248.98	532.39/322.04	270.57/125.39	3.183/0.004

Note. Schizophrenia (SCZ), Long Acting Injectables (LAIs), Duration of Untreated Psychosis (DUP), Positive & Negative Syndrome Scale (PANSS), Social & Occupational Functioning Assessment Scale (SOFAS), Chlorpromazine (CPZ), Nova Scotia Early Psychosis Program (NSEPP)

APPENDIX C TABLE 3

MRS Quality Criteria by Groups

Group		N	Mean	Std. Deviation
CE	Glu.CRLB (%)	11	6.18	1.991
	Gln.CRLB(%)	11	19.27	6.915
	Glx.CRLB (%)	11	5.45	1.635
	FWHM (Hz)	11	6.18	0.405
	SNR	11	25.6364	6.05430
TR	Glu.CRLB (%)	19	4.84	0.765
	Gln CRLB (%)	19	16.16	5.167
	Glx.CRLB (%)	19	4.74	0.733
	FWHM (Hz)	19	6.00	0.577
	SNR	19	24.9474	7.22245

Note. cramer-rao lower bounds (CRLB), glutamate + glutamine (Glx), full width at half maximum (FWHM), single to noise ratio (SNR), clozapine eligible (CE) and treatment responders (TR).

APPENDIX D TABLE 4

Glutamate Linear Regression Data by Total Samples and Groups

	Total group (mean/SD)	CE (mean/SD)	TR (mean/SD)	F-value/P-value
Glutamate (μmol/g)	9.34 /0.73	9.13 /0.80	9.45 /0.69	0.186/0.670
PANSS total	48.37/17.75	68.64/13.39	36.63/3.42	0.069/0.795
PANSS positive	12.60/6.23	19.91/3.98	8.37/1.50	0.296/0.592
PANSS negative	12.37/5.30	17.27/5.37	9.53/2.46	0.016/0.902
PANSS general	23.07/7.84	31.45/6.47	18.21/2.78	0.028 /0.869
SOFAS	57.83/20.74	40.00/14.66	68.16/16.32	0.986/0.332
Antipsychotic Dose (CPZ equivalents in mg)	366.57/248.98	532.39/322.04	270.57/125.39	0.215/0.647

Note. Positive & Negative Syndrome Scale (PANSS), Social & Occupational Functioning Assessment Scale (SOFAS), Chlorpromazine (CPZ)

APPENDIX E TABLE 5

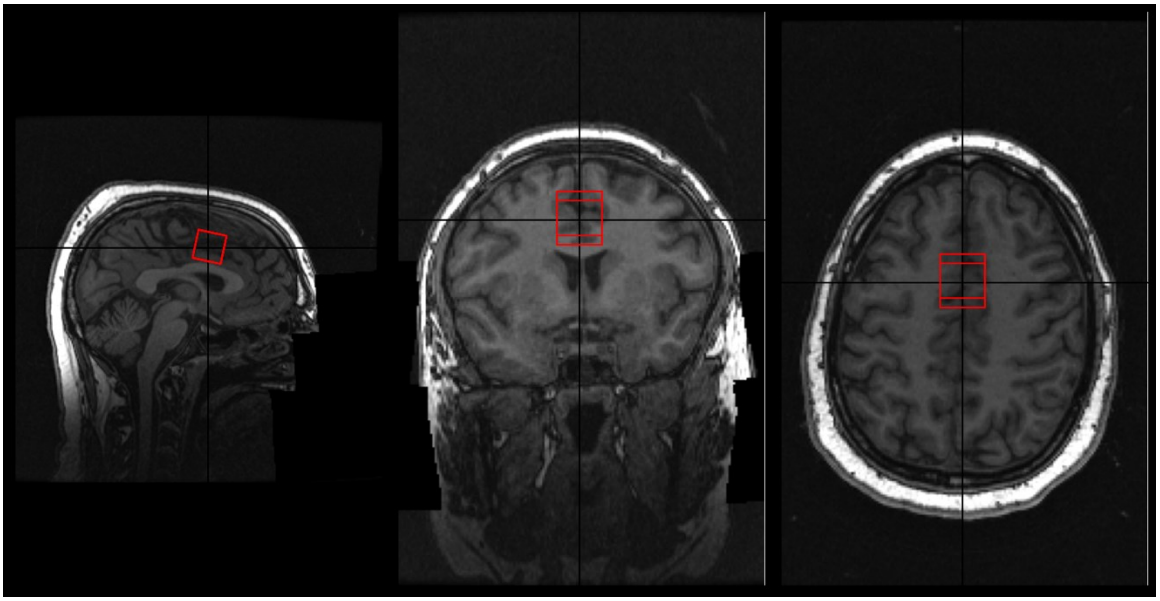
Glutamate + Glutamine Linear Regression Data by Total Samples and Groups

	Total group (mean/SD)	CE (mean/SD)	TR (mean/SD)	F-value/P-value
Glx (μmol/g)	12.91/1.14	12.65/1.22	13.06/1.09	0.638/0.720
PANSS total	48.37/17.75	68.64/13.39	36.63/3.42	0.138 /0.714
PANSS positive	12.60/6.23	19.91/3.98	8.37/1.50	0.082/0.777
PANSS negative	12.37/5.30	17.27/5.37	9.53/2.46	0.416 /0.526
PANSS general	23.07/7.84	31.45/6.47	18.21/2.78	0.014 /0.907
SOFAS	57.83/20.74	40.00/14.66	68.16/16.32	0.560/0.462
Antipsychotic Dose (CPZ equivalents in mg)	366.57/248.98	532.39/322.04	270.57/125.39	0.030/0.864

Note. Glutamate + Glutamine (Glx), Positive & Negative Syndrome Scale (PANSS), Social & Occupational Functioning Assessment Scale (SOFAS), Chlorpromazine (CPZ).

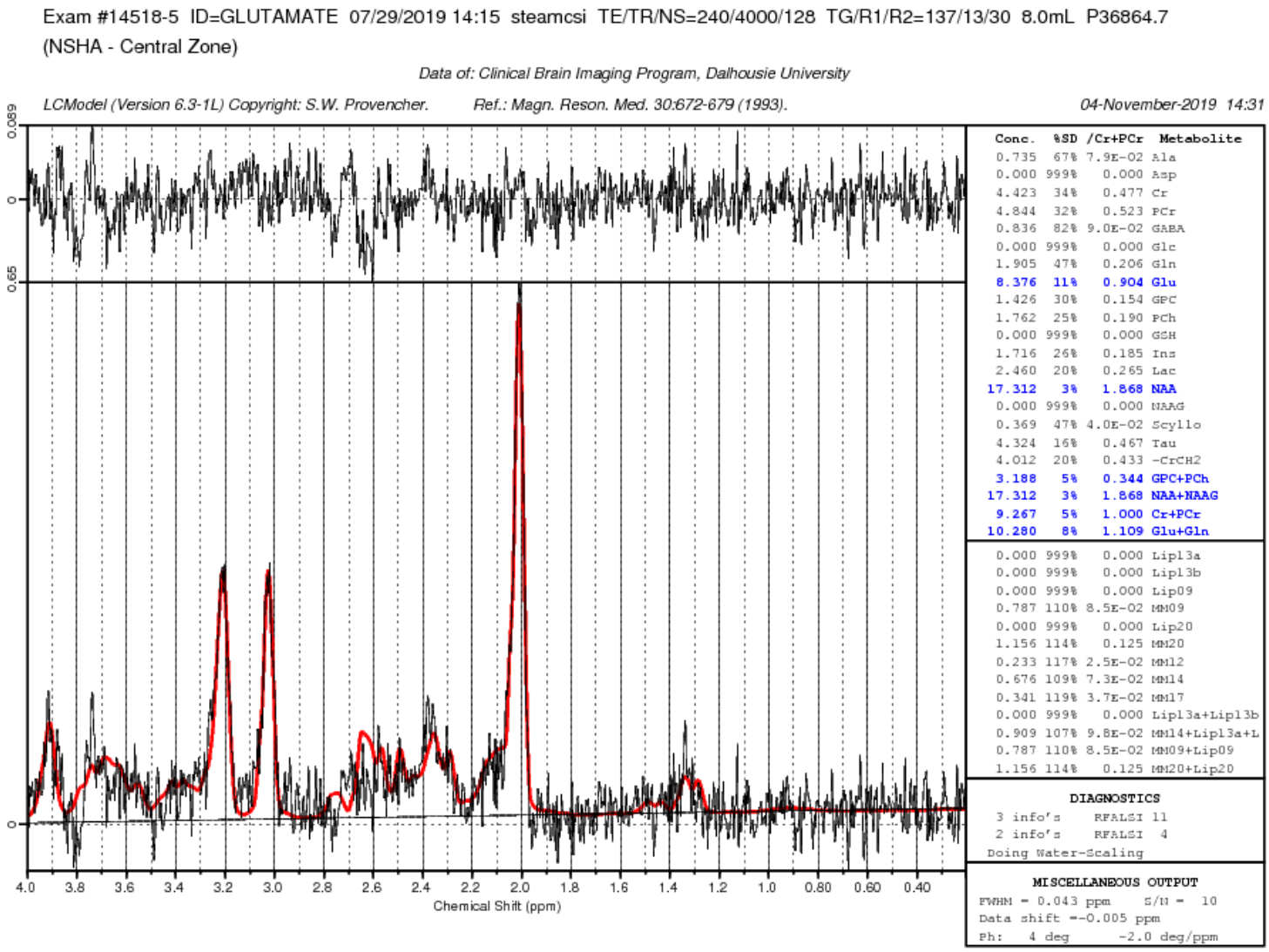
APPENDIX F FIGURE 1

Dorsal ACC Voxel placement for Glutamate Estimation



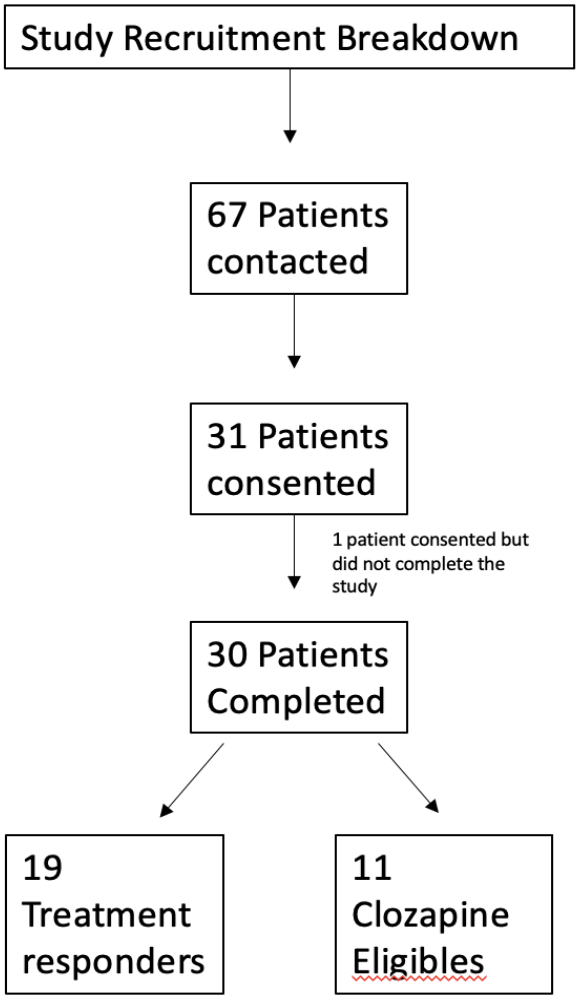
APPENDIX G FIGURE 2

Example of Fitted Spectrum from a single participant



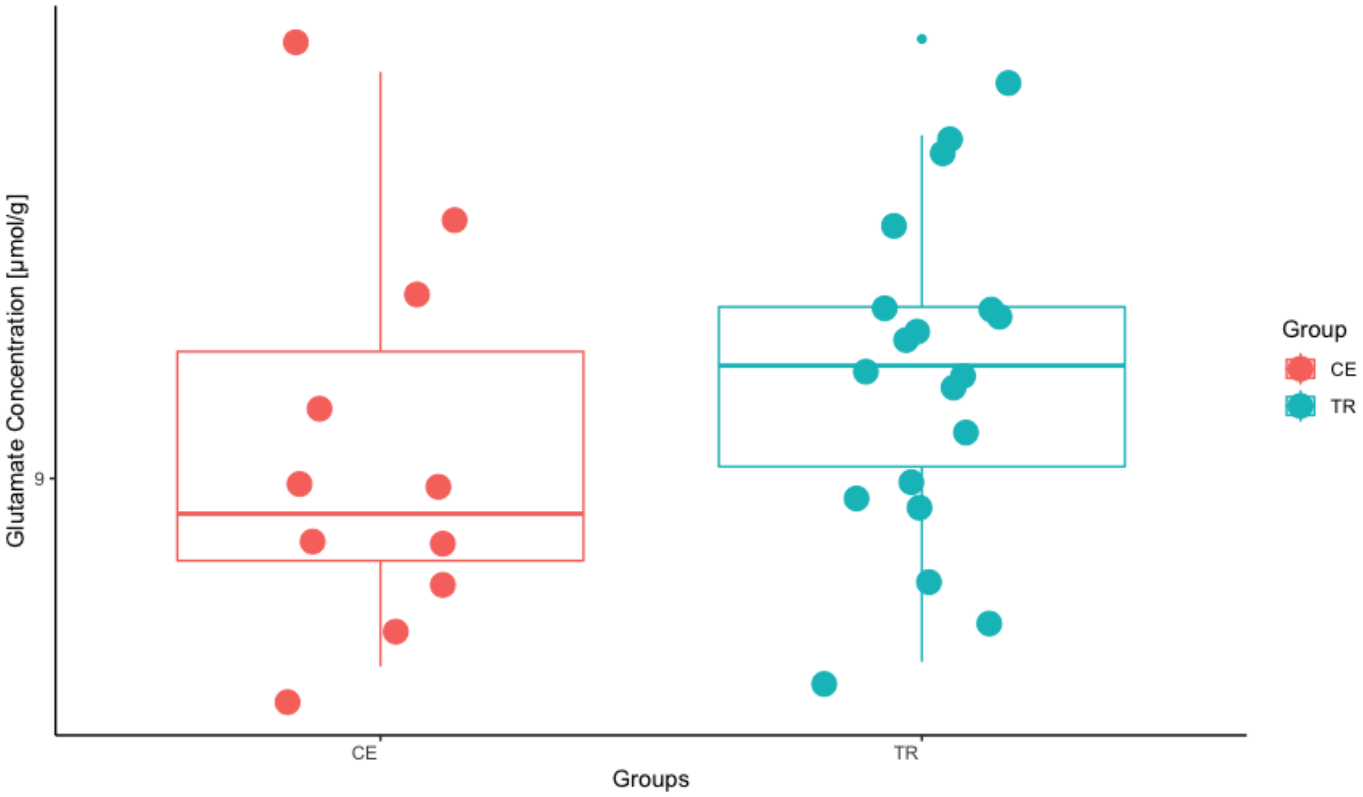
APPENDIX H FIGURE 3

Study Recruitment Breakdown



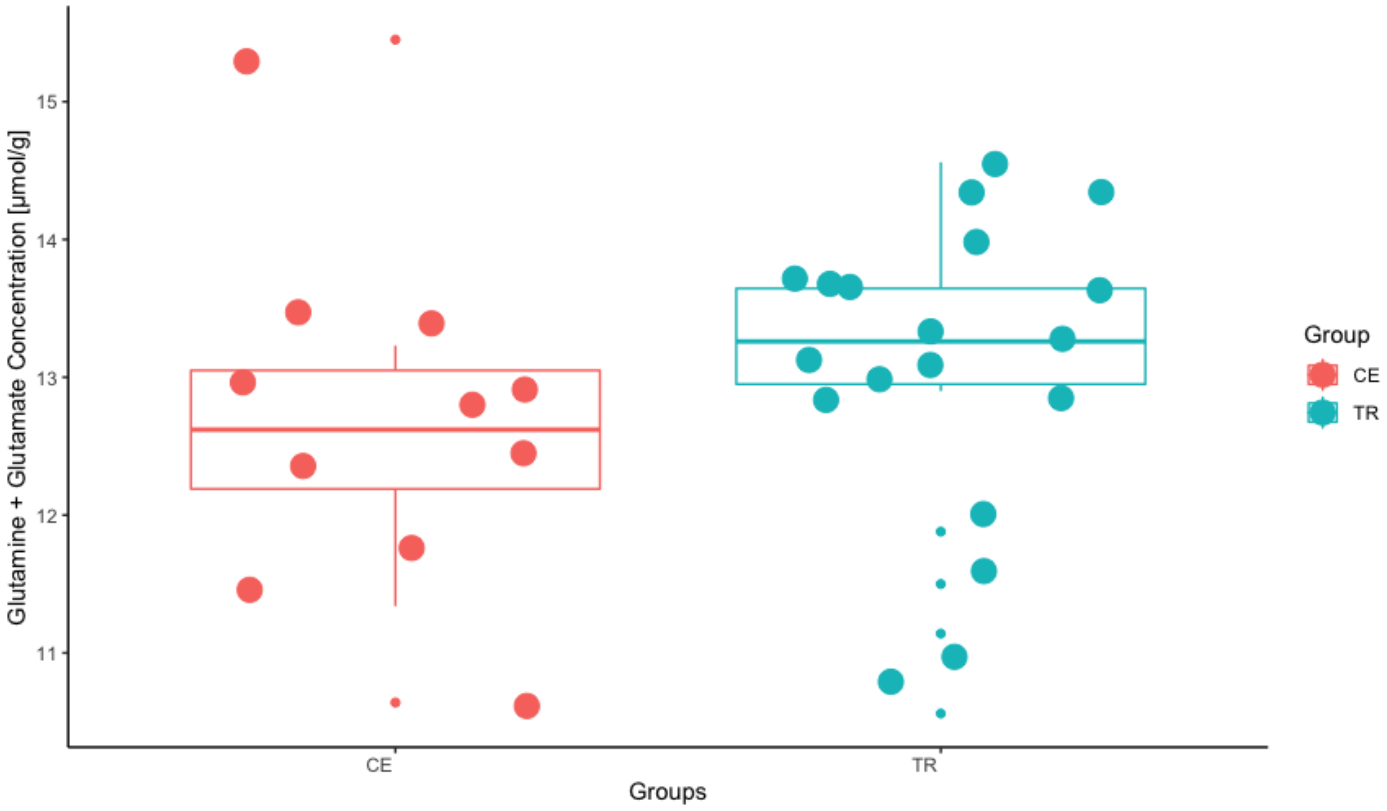
APPENDIX I FIGURE 4

Box Plot of Glutamate



APPENDIX J FIGURE 5

Box Plot of Glutamate + Glutamine



APPENDIX K CONSENT FORM

STUDY TITLE: Investigating Anterior Cingulate Cortex Glutamate as a Biomarker of Clozapine Eligibility in First Episode Psychosis

PRINCIPAL INVESTIGATOR: Dr. Kara Dempster, Department of Psychiatry, Nova Scotia Early Psychosis Program, 3rd Floor Abbie J Lane Building, 5909 Veteran's Memorial Lane, Halifax NS, B3H 2E2

FUNDER: Nova Scotia Health Authority Research Fund

Introduction
You have been invited to take part in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study. You may take as much time as you wish to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor.

Please ask the research team to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

The researchers will:
Discuss the study with you
Answer your questions
Be available during the study to deal with problems and answer questions

You are being asked to consider participating in this study because you are an active patient of the Nova Scotia Early Psychosis Program.

If you decide not to take part or if you leave the study early, your usual health care will not be affected.
2. Why is there a need for this study?
Some patients experiencing symptoms of psychosis do not improve with traditional antipsychotic medications. Clozapine is a very effective medication for many patients who do not improve with first-line antipsychotic medications, however, doctors have no ability to predict who is going to need clozapine treatment, and who will get better with traditional antipsychotic medications. The aim of this study is to investigate whether some brain differences can help predict who would benefit from clozapine treatment. Specifically, we will be measuring a chemical called glutamate in the brain as this chemical has previously been found to be elevated in patients with psychosis who did not respond well to treatment. No one has looked at whether elevated glutamate in early psychosis is associated with clozapine-eligibility. The results of this study may help doctors predict earlier who may benefit from clozapine treatment. It may also help develop other treatment options for individuals with psychosis who do not respond to traditional antipsychotic medications.

3. How Long Will I Be In The Study?
All participants who choose to be involved in this study will undergo an hour long assessment with a researcher, and have an MRI done (this will take approximately one hour). If desired, the assessment and MRI can be scheduled on the same day. If not on the same day, they will be scheduled within the same week.

Those participants who later decide (with their psychiatrist and treatment team) to take clozapine will be invited back for an additional MRI scan 6 months after starting clozapine treatment. This MRI scan will also take approximately one hour. Participants will have the option to choose to participate in this follow-up MRI scan or not without any effect on their care.

The entire study is expected to take about 3 years to complete and the results of the study should be available at that time.
4. How Many People Will Take Part In This Study?
It is anticipated that about 50 patients of the Nova Scotia Early Psychosis Program will participate in this study.

5. What Will Happen If I Take Part In This Study?
If you agree to participate in this research study you will be asked to undergo an initial assessment with a researcher, and then have a Magnetic Resonance Imaging (MRI) scan which will also take approximately one hour. Specifically, you would have a Functional Magnetic Resonance Imaging Scan (fMRI). Using a technique called Magnetic Resonance Spectroscopy, we are able to use data from your MRI scan to measure biochemicals in the brain.

During the assessment with the researcher, you will be asked questions about symptoms of psychosis, and your general mental health. You will also be asked questions about the medication treatments you have received in the past. If you are unable to recall some details about previous medication treatments, you will be asked permission for the research team to access this information by speaking with your treatment team, or looking it up in your medical record. The research team will not access any other details of your personal health information. The assessment with the researcher will take place in the Nova Scotia Early Psychosis Program.

For the MRI scan, you will meet with an MRI technician who will ensure that an MRI scan is safe for you by asking you some screening questions. You will lie on a table that will then move inside the MRI machine. You will be asked to lie still while the machine collects data. This process will take approximately one hour. During this time you will be exposed to magnetic fields, which you will not feel, and are not harmful. The MRI makes repetitive tapping noises as it is collecting data. You will be required to wear earplugs to dampen these sounds and protect your hearing. The MRI scan will take place at the Biomedical Translational Imaging Centre (BIOTIC) located at the QEII Health Sciences Centre.

Several precautions are being taken to ensure your safety from a COVID-19 perspective. When you go for your MRI scan, surgical masks will be work by all staff when they are in the same room as you. Physical distancing will be maintained with the exception of when the MR technologist helps you get into the MRI machine. They will be wearing a mask during this time. During your clinical assessment, physical distancing will be maintained at all times. If at all possible, your clinical assessment will be coordinated with a regular clinic visit to minimize the number of times you have to come to the hospital.

At any point during this study (during the assessment or the MRI), you can decide to withdraw from the study without penalty, and this will have no effect on the care you receive with the Nova Scotia Early Psychosis Program.

Study participants who later chose to be treated with the antipsychotic medication clozapine will be invited

back to participate in a follow-up MRI scan 6 months after starting treatment with clozapine. This follow-up MRI scan will take approximately one hour and the process will be the same as for the initial MRI scan. Participants who choose to take clozapine can decide not to participate in this follow-up MRI without any penalties, and their care with the Nova Scotia Early Psychosis Program will not be effected by this decision.

6. Are There Risks To The Study?

As with all research, there is a chance that confidentiality could be compromised; however, we are taking precautions to minimize this risk. To protect your information, we will not keep your name or other information that may identify you with the sample; only a code number. Files that link your name to the code number will be kept in a secure place. Although no one can absolutely guarantee confidentiality, using a code number makes the chance much smaller that someone other than the research staff or other authorized groups or persons (discussed later in the consent form) will ever be able to link your name to your other study information. We will only be obtaining information that is necessary for the purposes of this study, and ensuring all data remains locked and is only accessible to research team members who require its use for the purposes of the study.

During the assessment with the researcher, you may find some of the questions asked upsetting or distressing. Answering questions about the symptoms you have experienced may cause you to feel sad or anxious. You have the option to choose not to answer questions that you find too distressing, or stop the interview process at any time.

There are no biological risks associated with having an MRI scan. Individuals with any type of metal in their bodies cannot have MRI scans. Some examples of metal implants include heart pacemakers, artificial heart valves, metal ear implants, bullet pieces, insulin pumps, or any metal clips or rings. An MRI technician will conduct a screening questionnaire to ensure that you can safely have an MRI scan. While in the MRI scan, some individuals experience a feeling of claustrophobia (fear of being in an enclosed space) or anxiety. If you feel too uncomfortable, you can ask to stop the scan without penalty at any time.

The MRI scans done for the purposes of this study are performed for research purposes. The data collected is not intended to make clinical diagnoses, and the research team involved is not trained to make medical diagnoses. Nevertheless, there is a small possibility that the scan may find an incidental finding, or anatomical abnormality, unrelated to the research study. If this occurs, you will be notified by the Principal Investigator of the study who will ensure that you are referred for appropriate medical care/further investigation of this incidental finding.

Due to the current COVID-19 pandemic, there is a small risk that you may be exposed to the virus by virtue of coming to the hospital. Several measures have been put in place to minimize your risk. Everyone presenting to the hospital will be screened at entrance for COVID-19 symptoms. Individuals who screen positive for any screening questions are given a medical mask. Anyone who wishes to wear a mask (regardless of screening symptoms) has the option to choose to do so. In accordance with NSHA policies, physical distancing will be maintained throughout your clinical assessment. When you come for your MRI scan, the number of individuals who can be present will be limited to only those who are necessary (this would include the MR technologist and one other research staff). Physical distancing will be maintained at all times with the exception of when you are loaded into the MRI scanner. The MR technologist will be wearing a surgical mask during this time. A 30-minute time period is scheduled between all research participants to ensure distancing, as well as leave ample time for cleaning and disinfecting. All surfaces will be cleaned and disinfected prior to your arrival.

7. Are There Benefits Of Participating In This Study?

We cannot guarantee or promise that you will receive any direct benefits from this research. However, possible benefits include a feeling of satisfaction related to having made a difference in our understanding of the treatment of psychosis. Your participation will help the study researchers investigate whether the brain

chemical glutamate may predict clozapine use. If this were the case, it is possible that in the future, doctors could decide who needs clozapine much earlier through the use of brain imaging. This study will also contribute to our understanding of why some individuals respond well to antipsychotic treatment and some do not. The findings may lead to further investigations of alternative treatment options for psychosis.

8. What Happens at the End of the Study?

It is anticipated that the results of this study will be published and or presented in a variety of forums. In any publication and/or presentation, study results will be provided in such a way that you cannot be identified.

9. What Are My Responsibilities?

As a study participant you will be expected to:
Follow the directions of the research team;
Report all medications being taken or that you plan on taking;
Report any changes in your health to the research team;
Report any problems that you experience that you think might be related to participating in the study;
10. Can My Participation in this Study End Early?

Yes. If you chose to participate and later change your mind, you can say no and stop the research at any time. If you wish to withdraw your consent please inform the research team. If you choose to withdraw from this study, your decision will have no effect on your current or future medical treatment and healthcare. At any point in time, you can choose to have all data collected as a result of your participation withdrawn from the study, and not included in overall data analyses. If you choose to withdraw from the study, nothing further will be expected or asked of you. You will continue to receive your care as usual with your medical team at the Nova Scotia Early Psychosis Program.

Also, the Nova Scotia Health Authority Research Ethics Board and the principal investigator have the right to stop patient recruitment or cancel the study at any time.

11. What About New Information?

You will be told about any other new information that might affect your health, welfare, or willingness to stay in the study and will be asked whether you wish to continue taking part in the study or not.

12. Will It Cost Me Anything?

Compensation

You will receive compensation of $25.00/hour for your time, as well as to cover other costs that may be associated with participating in this research including travel, parking, etc. If you choose to withdraw from the MRI scan on site for any reason, you will still receive the standard compensation of $25.00. If you choose to withdraw from the MRI scan prior to showing up for the scan, you will not receive compensation for this aspect of the study (but would receive compensation for the time you spend doing the clinical assessment with the researcher).

Research Related Injury

If you become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. Your signature on this form only indicates that you have understood to your satisfaction the information regarding your participation in the study and agree to participate as a

subject. In no way does this waive your legal rights nor release the principal investigator, the research staff, the study sponsor or involved institutions from their legal and professional responsibilities.
13. What About My Privacy and Confidentiality?
Protecting your privacy is an important part of this study. Every effort to protect your privacy will be made. If the results of this study are presented to the public, nobody will be able to tell that you were in the study.

However, complete privacy cannot be guaranteed. For example, the principal investigator may be required by law to allow access to research records.

If you decide to participate in this study, the research team may look at your personal health information and collect only the information they need for this study. Personal health information" is health information about you that could identify you because it includes information such as your:
Name,
Age or month/year of birth (MM/YY),
Information from the study interviews and questionnaires;
New and existing medical records, or
The types, dates and results of previous medication treatments.

Access to Records

Other people may need to look at your personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines. These people might include:
The Nova Scotia Health Authority Research Ethics Board (NSHA REB) and people working for or with the NSHA REB because they oversee the ethical conduct of research studies within the Nova Scotia Health Authority;

Use of Your Study Information

The research team and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying you from the study data is very small, it can never be completely eliminated.

The research team will keep any personal health information about you in a secure and confidential location for 7 years and then destroy it according to NSHA policy. Your personal health information will not be shared with others without your permission.

You have the right to be informed of the results of this study once the entire study is complete.

The REB and people working for or with the REB may also contact you personally for quality assurance purposes.

Your access to records

You have the right to access, review, and request changes to your study data.

14. Declaration of Financial Interest

The Nova Scotia Health Authority Research Fund is reimbursing the principal investigator and/or the principal investigator's institution to conduct this study. The amount of payment is sufficient to cover the

costs of conducting the study.
15. What About Questions or Problems?
For further information about the study you may call the principal investigator, who is the person in charge of this study.

The principal investigator is Dr. Kara Dempster
Telephone: 902-473-2768
16. What Are My Rights?

You have the right to all information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction before you make any decision. You also have the right to ask questions and to receive answers throughout this study. You have the right to withdraw your consent at any time.

If you have questions about your rights as a research participant, and/or concerns or complaints about this research study, you can contact the Nova Scotia Health Authority Research Ethics Board manager at 902-473-8426 or Patient Relations at (902) 473-2133 or 1-855-799-0990 or healthcareexperience@nshealth.ca.

In the next part you will be asked if you agree (consent) to join this study. If the answer is "yes", please sign the form.
17. Consent Form Signature Page

I have reviewed all of the information in this consent form related to the study called:
Investigating Anterior Cingulate Cortex Glutamate as a Biomarker of Clozapine Eligibility in First Episode Psychosis

I have been given the opportunity to discuss this study. All of my questions have been answered to my satisfaction.

I authorize access to my personal health information and research study data as explained above.

This signature on this consent form means that I agree to take part in this study. I understand that I am free to withdraw at any time without affecting my future care.

_____	_____	_____ / _____ / ____
Signature of Participant	Name (Printed)	Year Month Day*
_____	_____	_____ / _____ / ____
Signature of Person Conducting Consent Discussion	Name (Printed)	Year Month Day*
_____	_____	_____ / _____ / ____
Signature of Principal Investigator	Name (Printed)	Year Month Day*

If the consent discussion has been conducted in a language other than English, please indicate:

_____
Language

_____ _____ _____ / _____ / ____
Signature of Translator Name (Printed) Year Month Day*

I will be given a signed copy of this consent form.

Consent Section for Optional Clozapine Sub Study (for those who independently elect to take clozapine treatment).

_____ _____ _____ / _____ / ____
Signature of Participant Name (Printed) Year Month Day*

_____ _____ _____ / _____ / ____
Signature of Person Conducting Consent Discussion Name (Printed) Year Month Day*

_____ _____ _____ / _____ / ____
Signature of Principal Investigator Name (Printed) Year Month Day*

If the consent discussion has been conducted in a language other than English, please indicate:

_____
Language

_____ _____ _____ / _____ / ____
Signature of Translator Name (Printed) Year Month Day*

APPENDIX L PANSS SCALE

SCI–PANSS BOOKLET

Structured Clinical Interview for the Positive and Negative Syndrome Scale

SCI-PANSS

L. A. Opler, M.D., Ph.D. S. R. Kay, Ph.D. J. P. Lindenmayer, M.D. A. Fiszbein, M.D.

Patient Name or ID: _____
Interviewer: _____ Date: _____ / _____ / _____

Data on "Lack of Spontaneity and Flow of Conversation" (N6), "Poor Rapport" (N3), and "Conceptual Disorganization" (P2)

Hi, I'm ... We're going to be spending the next 30 to 40 minutes talking about you and your reasons for being here. Maybe you can start out by telling me something about yourself and your background?

(Instruction to interviewer: Allow at least 5 minutes for a non-directive phase serving to establish rapport in the context of an overview before proceeding to the specific questions listed below.)

Data on "Anxiety" (G2)

1. Have you been feeling worried or nervous in the past week?
   _____

   IF YES, skip to question 3. IF NO, continue.

2. Would you say that you're usually calm and relaxed?
   _____

   IF YES, skip to question 8. IF NO, continue.

3. What's been making you feel nervous (worried, not calm, not relaxed)?
   _____
4. Just how nervous (worried, etc.) have you been feeling?
   _____
5. Have you been shaking at times, or has your heart been racing?
   _____
6. Do you get into a state of panic?
   _____
7. Has your sleep, eating, or participation in activities been affected?
   _____

Data on "Delusions (General)" (P1) and "Unusual Thought Content" (G9)

8. Have things been going well for you?

   _____

9. Has anything been bothering you lately?

   _____

10. Can you tell me something about your thoughts on life and its purpose?

    _____

SCI–PANSS BOOKLET

11. Do you follow a particular philosophy (any special rules, teachings, or religious doctrine)?

    _____

12. Some people tell me they believe in the Devil; what do you think?

    _____

    IF NO (i.e., he/she doesn't believe in the Devil), skip to question 14. IF YES (i.e., he/she does believe), continue.

13. Can you tell me more about this?

    _____

14. Can you read other people's minds?

    _____

    IF NO, skip to question 16. IF YES, continue.

15. How does that work?

    _____

16. Can others read your mind?

    _____

    IF NO, skip to question 19. IF YES, continue.

17. How can they do that?

    _____

18. Is there any reason that someone would want to read your mind?

    _____

19. Who controls your thoughts?

    _____

Data on "Suspiciousness/Persecution" (P6) and "Poor Impulse Control" (GI4)

20. How do you spend your time these days?

    _____

21. Do you prefer to be alone?

    _____

22. Do you join in activities with others?

    _____

    IF YES, skip to question 25. IF NO, continue.

23. Why not? ... Are you afraid of people, or do you dislike them?

    _____

    IF NO, skip to question 26. IF YES, continue.

24. Can you explain? _____

    Skip to question 26.

25. Tell me about it. _____
26. Do you have many friends?

    _____

    IF YES, skip to question 30. IF NO, continue.

27. Just a few? _____

    IF YES, skip to question 29. IF NO, continue.

MHS

28. Any? .... Why? _____

    Skip to question 32.

29. Why just a few friends?

    _____

30. Close friends? _____

    IF YES, skip to question 32. IF NO, continue.

31. Why not? _____

32. Do you feel that you can trust most people?

    _____

    IF YES, skip to question 34. IF NO, continue.

33. Why not? _____
34. Are there some people in particular who you don't trust?

    _____

    IF NO to question 34 and YES to question 32, skip to question 41. IF NO to question 34 and NO to question 32, skip to question 36. IF YES to question 34, continue.

35. Can you tell me who they are?

    _____

36. Why don't you trust people (or name specific person)?

    _____

    IF "DON'T KNOW" OR "DON'T WANT TO SAY," continue. Otherwise, skip to question 41.

37. Do you have a good reason not to trust ...?

    _____

38. Is there something that .... did to you?

    _____

39. Perhaps something that ... might do to you now?

    _____

    IF NO, skip to question 41. IF YES, continue.

40. Can you explain to me?

    _____

41. Do you get along well with others?

    _____

    IF YES, skip to question 43. IF NO, continue.

42. What's the problem?

    _____

43. Do you have a quick temper?

    _____

44. Do you get into fights?

_____

IF NO, skip to question 48. IF YES, continue.

45. How do these fights start?

_____

46. Tell me about these fights.

_____

47. How often does this happen?

_____

48. Do you sometimes lose control of yourself?

_____

IF NO, skip to question 50. IF YES, continue.

49. What happens when you lose control of yourself?

_____

50. Do you like most people?

_____

IF YES, skip to question 52. IF NO, continue.

51. Why not? _____

52. Are there perhaps some people who don't like you?

_____

IF NO, skip to question 54. IF YES, continue.

53. For what reason? _____

54. Do others talk about you behind your back?

_____

IF NO, skip to question 57. IF YES, continue.

55. What do they say about you?

_____

56. Why?_____

__

57. Does anyone ever spy on you or plot against you?

_____

58. Do you sometimes feel in danger?

_____

IF NO, skip to question 64. IF YES, continue.

59. Would you say that your life is in danger?

    _____

60. Is someone thinking of harming you or even perhaps thinking of killing you?

    _____

61. Have you gone to the police for help?

    _____

62. Do you sometimes take matters into your own hands or take action against those who might harm you?

    _____

    __

    IF NO, skip to question 64. IF YES, continue.

63. What have you done? _____

Data on "Hallucinatory Behavior" (P3) and associated delusions

64. Do you once in a while have strange or unusual experiences?

    _____

65. Sometimes people tell me that they can hear noises or voices inside their head that others can't hear. What about you? _____

    IF YES, skip to question 68. IF NO, continue.

66. Do you sometimes receive personal communications from the radio or TV?

    _____

    IF YES, skip to question 68. IF NO, continue.

67. From God or the Devil?:

    _____

    IF NO, skip to question 83. IF YES, continue.

68. What do you hear?

    _____

69. Are these as clear and loud as my voice?

    _____

70. How often do you hear these voices, noises, messages, etc.?
    _____
71. Does this happen at a particular time of day or all the time?
    _____

IF HEARING NOISES ONLY, skip to question 80. IF HEARING VOICES, continue.

72. Can you recognize whose voices these are?
    _____
73. What do the voices say?
    _____
74. Are the voices good or bad?
    _____
75. Pleasant or unpleasant?
    _____
76. Do the voices interrupt your thinking or your activities?
    _____
77. Do they sometimes give you orders or instructions?
    _____

IF NO, skip to question 80. IF YES, continue.

78. For example? _____
79. Do you usually obey these orders (instructions)?
    _____
80. What do you make of these voices (or noises); where do they really come from?
    _____
81. Why do you have these experiences?
    _____

82. Are these normal experiences?
    _____
83. Do ordinary things sometimes look strange or distorted to you?_____
84. Do you sometimes have “visions” or see things that others can’t see?
    _____

IF NO, skip to question 88. IF YES, continue.

85. For example? _____
86. Do these visions seem very real or life-like?

_____

87. How often do you have these experiences?

_____

88. Do you sometimes smell things that are unusual or that others don't smell?

_____

IF NO, skip to question 90. IF YES, continue.

89. Please explain. _____
90. Do you get any strange or unusual sensations from your body?

_____

IF NO, skip to question 92. IF YES, continue.

91. Tell me about this.

_____

Data on "Somatic Concern" (GI)

92. How have you been feeling in terms of your health?

_____

IF OTHER THAN "GOOD," skip to question 94. IF "GOOD," continue.

93. Do you consider yourself to be in top health?

_____

IF YES, skip to question 95. IF NO, continue.

94. What has been troubling you?

_____

95. Do you have any medical illness or disease?

_____

96. Has any part of your body been troubling you?

_____

IF YES, skip to question 98. IF NO, continue.

97. How is your head? Your heart? Stomach? The rest of your body? _____
98. Could you explain?

_____

99. Has your head or body changed in shape or size?

_____

IF NO, skip to question 102. IF YES, continue.

100. Please explain.

_____

101. What is causing these changes?

_____

Data on "Depression" (G6)

102. How has your mood been in the past week: mostly good, mostly bad?

_____

IF "MOSTLY BAD," skip to question 104. IF "MOSTLY GOOD," continue.

103. Have there been times in the past week when you were feeling sad or unhappy? _____

IF NO, skip to question 114. IF YES, continue.

104. Is there something in particular that is making you sad?

_____

105. How often do you feel sad?

_____

106. Just how sad have you been feeling?

_____

107. Have you been crying lately?

_____

108. Has your mood in any way affected your sleep?

_____

109. Has it affected your appetite?

_____

110. Do you participate less in activities on account of your mood? _____

111. Have you had any thoughts of harming yourself?

_____

IF NO, skip to question 114. IF YES, continue.

112. Any thoughts about ending your life?

_____

IF NO, skip to question 114. IF YES, continue.

113. Have you attempted suicide?

_____

Data on "Guilt Feelings" (G3) and "Grandiosity" (P5)

114. If you were to compare yourself to the average person, how would you come out: a little better, maybe a little worse, or about the same?

_____

IF "BETTER," skip to question 117.
IF "ABOUT THE SAME," skip to question 118. IF "WORSE," continue.

115. Worse in what ways?

_____

116. Just how do you feel about yourself?

_____

Skip to question 120.

117. Better in what ways?

_____

Skip to question 120.

118. Are you special in some ways?

_____

IF NO, skip to question 120. IF YES, continue.

119. In what ways?

_____

120. Would you consider yourself gifted?

_____

121. Do you have talents or abilities that most people don't have?

_____

IF NO, skip to question 123. IF YES, continue.

122. Please explain.

_____

123. Do you have any special powers?

_____

IF NO, skip to question 126. IF YES, continue.

124. What are these?

_____

125. Where do these powers come from?

_____

126. Do you have extrasensory perception (ESP), or can you read other people's minds?_____

127. Are you very wealthy?

_____

IF NO, skip to question 129. IF YES, continue.

128. Explain please.

_____

129. Can you be considered to be very bright?

_____

IF NO, skip to question 131. IF YES, continue.

130. Why would you say so?

_____

131. Would you describe yourself as famous?_____

132. Would some people recognize you from TV, radio, or the newspaper?

_____

IF NO, skip to question 134. IF YES, continue.

133. Can you tell me about it?

134. Are you a religious person?

IF NO, skip to question 140. IF YES, continue.

135. Are you close to God?

IF NO, skip to question 140. IF YES, continue.

136. Did God assign you some special role or purpose?

137. Can you be one of God's messengers or angels?

IF NO, skip to question 139. IF YES, continue.

138. What special powers do you have as God's messenger (angel)?

139. Do you perhaps consider yourself to be God?

140. Do you have some special mission in life?

IF NO, skip to question 143. IF YES, continue.

141. What is your mission?

142. Who assigned you to that mission?

143. Did you ever do something wrong — something you feel bad or guilty about?

IF NO, skip to question 149. IF YES, continue.

144. Just how much does that bother you now?

145. Do you feel that you deserve punishment for that?

IF NO, skip to question 149. IF YES, continue.

146. What kind of punishment would you deserve?

_____

147. Have you at times thought of punishing yourself?

_____

IF NO, skip to question 149. IF YES, continue.

148. Have you ever acted on those thoughts of punishing yourself?

_____

Data on "Disorientation" (GlO)

149. Can you tell me today's date (i.e., the day, month, and year)?

_____

IF YES, skip to question 151. IF NO, continue.

150. Can you tell me what day of the week it is? _____

151. What is the name of the place that you are in now?_____

IF NOT HOSPITALIZED, skip to question 154. IF HOSPITALIZED, continue.

152. What ward are you on?_____

153. What is the address of where you're now staying?

_____

IF ABLE TO TELL, skip to question 155. IF NOT ABLE TO TELL, continue.

154. Can you tell me your home address?

_____

IF NOT HOSPITALIZED, skip to question 156. IF HOSPITALIZED, continue.

155. If someone had to reach you by phone, what number would that person call?

_____

156. If someone had to reach you at home, what number would that person call?

_____

157. What is the name of the doctor who is treating you?

_____

IF NOT HOSPITALIZED, skip to question 159. IF HOSPITALIZED, continue.

158. Can you tell me who else is on the staff and what they do?

_____

159. Do you know who is currently the president (prime minister, etc.)?

_____

160. Who is our governor (premier, etc.)?

_____

161. Who is the mayor (town supervisor, etc.) of this city (town, etc.)?

_____

MHS

Data on "Difficulty in Abstract Thinking" (N5)

I'm going to now say a pair of words, and I'd like you to tell me in what important way they're alike. Let's start, for example, with the words "apple" and "banana." How are they alike — what do they have in common? **IF THE RESPONSE IS THAT "THEY'RE BOTH FRUIT", THEN SAY:** Good. Now what about ...? *(Select three other items from the Similarities list at varying levels of difficulty from Appendix A.)*

IF AN ANSWER IS GIVEN THAT IS CONCRETE, TANGENTIAL, OR IDIOSYNCRATIC (E.G., "THEY BOTH HAVE SKINS,"

"YOU CAN EAT THEM," "THEY'RE SMALL," OR "MONKEYS LIKE THEM"), THEN SAY: OK, but they're both fruit. Now how about ... and ... : how are these alike? *(Select three other items from the Similarities list at varying levels of difficulty from Appendix A.)*

APPENDIX A
Items for Similarities in the evaluation of "Difficulty in Abstract Thinking"

1. How are a ball and an orange alike? 2. Apple and banana ?
3. Pencil and pen?
4. Nickel and dime?

_____

5. Table and chair?
6. Tiger and elephant? 7. Hat and shirt?
8. Bus and train?

_____

9. Arm and leg?

10. Rose and tulip?

11. Uncle and cousin?
12. The sun and the moon?

_____

13. Painting and poem?
14. Hilltop and valley?
15. Air and water?
16. Peace and prosperity?

Note on Appendix A: Similarities are generally assessed by sampling four items at different levels of difficulty (i.e., one item selected from each quarter of the full set). When using the PANSS longitudinally, items should be systematically altered with successive interviews so as to provide different selections from the various levels of difficulty and thus minimize repetition.

Notes on Similarities responses: _____
_____
_____
_____

You've probably heard the expression, "Carrying a chip on the shoulder." What does that really mean? There's a very old saying, "Don't judge a book by its cover." What is the deeper meaning of this proverb? *(Select two other proverbs from the list in Appendix B at varying levels of difficulty.)*

APPENDIX B
Items for assessing PROVERB INTERPRETATION in the evaluation of "Difficulty in Abstract Thinking"

What does the saying mean:
1. "Plain as the nose on your face"
2. "Carrying a chip on your shoulder" 3. "Two heads are better than one"
4. "Too many cooks spoil the broth"

_____

5. "Don't judge a book by its cover"
6. One man's food is another man's poison"
7. "All that glitters is not gold"
8. "Don't cross the bridge until you come to it"

_____

9. "What's good for the goose is good for the gander" 10. "The grass always looks greener on the other side" 11. "Don't keep all your eggs in one basket"
12. "One swallow does not make a summer"

_____

Note on Appendix B: Proverb interpretation is generally assessed by sampling four items at different levels of difficulty (i.e., one item selected from each quarter of the full set). When using the PANSS longitudinally, items should be systematically altered with succes- sive interviews so as to provide different selections from the various levels of difficulty and thus minimize repetition.

Notes on Proverb responses: _____
_____
_____

_____

13. “A stitch in time saves nine”
14. “A rolling stone gathers no moss”
15. “The acorn never falls far from the tree”
16. “People who live in glass houses should not throw stones at others”

MHS

Circle the Proverbs Used Circle the Similarities Used

SCI–PANSS BOOKLET

Data on “Lack of Judgment and Insight” (G12)

162. How long have you been in the hospital (clinic, etc.)?

_____

163. Why did you come to the hospital (clinic, etc.)?

_____

164. Did you need to be in a hospital (clinic, etc.)?

_____

IF YES, skip to question 167. IF NO, continue.

165. Did you have a problem that needed treatment?

_____

IF NO, skip to question 169. IF YES, continue.

166. Would you say that you had a psychiatric or mental problem?

_____

IF NO, skip to question 169. IF YES, continue.

167. Why?....would you say that you had a psychiatric or mental problem? _____

IF NO, skip to question 169. IF YES, continue.

168. Can you tell me about it and what it consisted of? _____

169. In your own opinion, do you need to be taking medicine?

_____

IF YES, skip to question 171.

IF NO and unmedicated, skip to question 172. IF NO and medicated, continue.

170. Why then are you taking medicines?

_____

Skip to question 172.

171. Why?... Does the medicine help you in any way?

_____

172. Do you at this time have any psychiatric or mental problems?

_____

IF YES, skip to question 174. IF NO, continue.

173. For what reason are you at the hospital (clinic, etc.)?

_____

Skip to question 175.

174. Please explain

_____

SCI–PANSS BOOKLET

175. Just how serious are these problems? _____

IF UNHOSPITALIZED, skip to question 178. IF HOSPITALIZED, continue.

176. Are you ready yet for discharge from the hospital?

_____

177. Do you think you'll be taking medicine for your problems after discharge?

_____

178. What are your future plans?

_____

179. What about your longer-range goals?

_____

Well, that's about all I have to ask of you now. Are there any questions that you might like to ask of me? Thank you for your cooperation.

APPENDIX M CGI-S

Clinical Global Impression (CGI)

1. Severity of illness

Considering your total clinical experience with this particular population, how mentally ill is the patient at this time?

0 = Not assessed
1 = Normal, not at all ill 2 = Borderline mentally ill 3 = Mildly ill

4 = Moderately ill
5 = Markedly ill
6 = Severely ill
7 = Among the most extremely ill patients

2. Global improvement: Rate total improvement whether or not, in your judgement, it is due entirely to drug treatment. Compared to his condition at admission to the project, how much has he changed?

0 = Not assessed
1 = Very much improved 2 = Much improved
3 = Minimally improved

4 = No change
5 = Minimally worse 6 = Much worse
7 = Very much worse

3. Efficacy index: Rate this item on the basis of **drug effect only**.
Select the terms which best describe the degrees of therapeutic effect and side effects and record the number in the box where the two items intersect.
EXAMPLE: Therapeutic effect is rated as 'Moderate' and side effects are judged 'Do not significantly interfere with patient's functioning'.

Therapeutic effect

Side effects

None Do not significantly interfere with

patient's functioning

01 02 05 06 09 10 13 14

Marked **Moderate** **Minimal** Vast improvement. Complete or nearly complete remission of all symptoms Decided improvement. Partial remission of symptoms Slight improvement which doesn't alter status of care of patient *Significantly interferes Outweighs with patient's therapeutic functioning effect* 03 04 07 08 11 12 15 16 **Unchanged or worse** Not assessed = 00
Reproduced from Guy W, editor. ECDEU Assessment Manual for Psychopharmacology. 1976. Rockville, MD, U.S. Department of Health, Education, and Welfare

APPENDIX N SOFAS SCALE

Name: _____
Date: _____

Social and Occupational Functioning Assessment Scale (SOFAS)

Consider social and occupational functioning on a continuum from excellent functioning to grossly impaired. Include impairments in functioning due to physical limitations, as well as those due to mental impairments. To be counted, impairment must be a direct consequence of mental and physical health problems; the effects of lack of opportunity and other environmental limitations are not to be considered.

91-100	Superior functioning in a wide range of activities
81-90	Good functioning in all areas, occupationally and socially effective
71-80	No more than slight impairment in social, occupational, or school functioning (e.g., infrequent interpersonal conflict, temporarily falling behind in schoolwork)
61-70	Some difficulty in social, occupational, or school functioning, but generally functioning well, has some meaningful interpersonal relationship
51-60	Moderate difficulty in social, occupational, or school functioning (e.g., few friends, conflicts with peers or co-workers)
41-50	Serious impairment in social, occupational, or school functioning (e.g., no friends, unable to keep a job)
31-40	Major impairment in several areas, such as work or school, family relations (e.g., depressed man avoids friends, neglects family, and is unable to work; child frequently beats up younger children, is defiant at home, and is failing at school)
21-30	Inability to function in almost all areas (e.g., stays in bed all day; no job, home, or friends)
11-20	Occasionally fails to meet minimal personal hygiene; unable to function independently
1-10	Persistent inability to maintain minimal personal hygiene. Unable to function without harming self or others or without considerable external support (e.g., nursing care and supervision)

Score: _____

Clinician Signature: _____

APPENDIX O DEMOGRAPHICS FORM

Demographics Form:

Participant number: Date of assessment:
Date of MRI Scan:

General exclusion criteria (if meets any of these not eligible for study):

- Primary diagnosis of a substance-induced psychosis
- Diagnosis of substance dependence in the past 6 months (excluding nicotine)
- History of head trauma leading to loss of consciousness for >30mins
- Inability to provide informed consent
- Unstable medical/neurological illness
- Contraindications to undergoing MRI

Sex: M F Transgender Prefer not to answer

Age: Date of birth:

Gender: M F Other: _____ Prefer not to answer

Ethnicity (check one only):

- Asian-East (e.g., Chineese, Japaneese, Korean)
- Asian-South (e.g., Indian, Pakistani, Sri Lanken)
- Asian-South East (e.g., Malaysian, Fillipino, Vietnamese)
- Black-African (e.g., Ghanaian, Kenyan, Somali)
- Black-Caribbean (e.g., Barbadian, Jamaican)
- Black-North American (e.g., Canadian, American, Afridan Nova Scotian)
- First Nation-Mi'kmaq
- First Nation-Other
- Metis
- Indian-Caribbean (e.g., Guyanese with origins in India)
- Indigenous/Aboriginal not included elsewhere
- Inuit
- Latin American (e.g., Argentinean, Chilean, Salvadoran)
- Middle Eastern (e.g., Egyptian, Iranian, Lebanese)
- White- European (e.g., English, Italian, Portuguese, Russian)
- White- North American (e.g., Canadian, American)
- Other(s) (Specify): _____
- Prefer not to answer
- Do not know

Employment: Y N
If Y, specify employment: _____

Currently enrolled in academics: Y N
If Y, specify program: _____
Years of education completed:

DSM 5 Diagnoses:
1.
2.
3.
4.
5.

Family history of psychosis: Y N
If Y, specify relationship and diagnosis (if known): _____

Treatment history:
Date of acceptance to NSEPP:
Estimated Duration of illness (weeks):
Estimated duration of untreated psychosis (weeks):
Inpatient admissions (# and approximate duration):

Current Medications (include all psychiatric medications):

Medication	Max Dose	Total weeks on medication	Estimated compliance (0-100%)	Effect	Side effects

Medication trials (include only antipsychotic medication trials):

Medication	Max Dose	Total weeks on medication	Estimated compliance (0-100%)	Effect	Side effects

Drug use (current):
Cigarette smoker: Y N
If Y, specify duration of smoking:

Drug	Frequency of use

Drug use (past):

Drug	Frequency of use	Time since last use

PANSS score: SOFAs score: CGI score:
Positive:
Negative:
General:

Subgroup criteria (see below):
Clozapine eligible Treatment responder

Criteria for Clozapine-Eligibility:

- Have undergone two trials of antipsychotic treatment for at least 6 weeks with at least 600mg chlorpromazine equivalents daily
- Illness severity rating of greater than or equal to 4 (moderate) on the CGI-S, and greater than or equal to 4 (moderate) on 2 PANSS symptom items, or at least one symptom with a 6 (severe) rating (despite treatment with adequate antipsychotic medication)
- <60 on SOFAs
- Estimated medication compliance of 80% of prescribed doses during the 6-week treatment trials.
- Have not yet undergone a trial of clozapine

Criteria for Treatment-Responders:

- Having ratings of no more than mild severity on all PANSS items sustained for a minimum of 12 weeks
- 60 or greater SOFAS score.
- Currently receiving treatment with a single antipsychotic medication.
- Estimated medication compliance of 80% of prescribed doses.